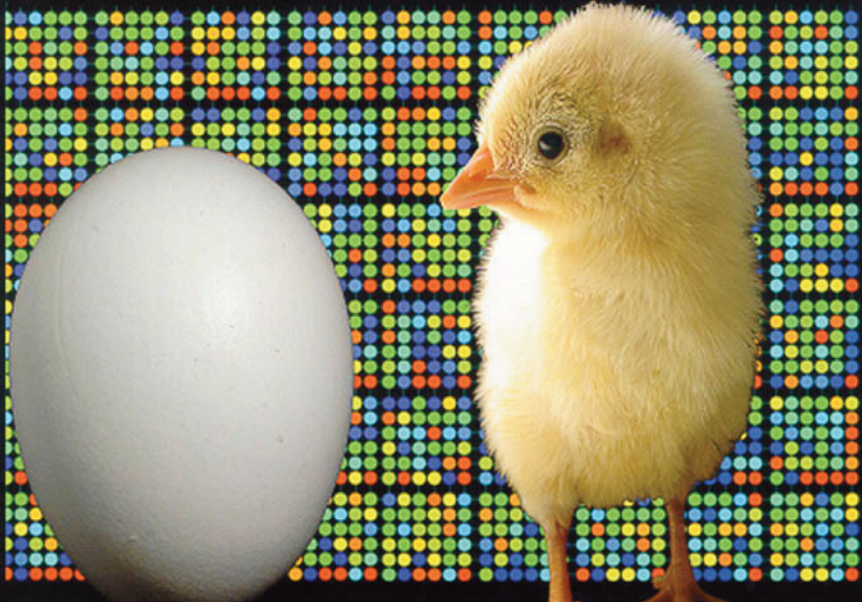# Poultry Genetics, Breeding and Biotechnology

Edited by W.M. Muir and S.E. Aggrey

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Poultry Genetics, Breeding and Biotechnology

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Preface

The wheel on the axis of science rotates every couple of years with new innovative basic ideas that revolutionize traditional thinking and direct scientists to areas unimaginable to them in the past. Improvements in poultry and other livestock species have been pursued through empirical statistical approaches with underlying genetic principles. Since the publication of *Poultry Breeding and Genetics* (1990), new ideas have emerged in molecular genetics, computation strategies and bioinformatics with concurrent breeding-related problems in poultry. It was the combination of the rotating axis of science coupled with emerging new problems in poultry breeding that led to the birth of this book.

This book represents the first complete integration of the state of the art in quantitative and molecular genetics as applied to poultry breeding. Our approach is first to define problems encountered in poultry breeding in 'Problems and Issues Associated with Poultry Breeding'. Then methods to address these issues are examined, including both quantitative and molecular genetics, which are simply different tools to address these problems with differing strengths and weakness. Quantitative approaches are examined in 'Breeding Strategies and Objectives' while molecular approaches and integration with quantitative ones are examined in 'Use of Genomics and Bioinformatics in Poultry'.

Coverage of genomics includes structural, comparative and functional. Use of transgenic technology in poultry is also examined. Transgenic technologies offer the promise of being able to address issues by creating new genetic variability, rather than being restricted to existing variation as with quantitative and genomics methods. In addition, transgenic technology can develop new uses for egg products, particularly as a bioreactor for other applications. One of the greatest issues in the poultry industry is that of disease resistance and transmission. A special section is devoted to the genetics of disease resistance.

We feel that we have achieved our goal of producing an outstanding book, with the top scientists in their field addressing each subtopic. Although the field of molecular genetics is progressing rapidly, we feel the issues and methods outlined in this book will be with us for a long time. We thank all the authors for their outstanding contributions to what will surely be a standard of excellence against which all future books will be measured.

We also wish to thank Tim Hardwick (CABI Publishing) for giving us the opportunity to put this book together, and Marian Kaiser for her kind assistance and technical skills in editing the final drafts.

William M. Muir, PhD
Samuel E. Aggrey, PhD

1 Industrial Perspective on Problems and Issues Associated with Poultry Breeding

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Consumption of poultry meat and eggs is increasing steadily. It has moved from a combined total of 85 million tonnes in 1992 to 117 million tonnes in 2000 (*Executive Guide to World Poultry Trends*, 2001). Of the current total, 8% is produced from turkeys, ducks or poultry species other than chickens. This chapter focuses on developments in chicken breeding. There are unique concerns for each of the other species, but developments in the breeding of the other species have in general paralleled those in the breeding of chickens.

Egg-type Chickens

Since the early 20th century, the breeding of egg-type chickens has seen significant changes. The genetic performance of the bird has improved substantially over this time. In order to be able to continue the improvement of the laying hen, further changes will need to be made. In the following discussion, consideration will be given to traits, methods of selection and industry structure.

Traits

Breeders today must select for, or at least monitor, the age at sexual maturity, rate of lay before and after moult, livability in the growing and laying house, egg weight, body weight, feed conversion, shell colour, shell strength, albumen height, egg inclusions (blood and meat spots) and temperament, plus traits affecting the productivity of the parent. Since the early 1980s, the increasing proportion of eggs broken out for further processing has added additional traits, including percentage solids and lipids in the egg.

Egg production per hen housed will continue to be the single most important trait under selection. However, the emphasis has been shifting from peak rate of lay to persistency of lay (Preisinger and Flock, 1998). As flocks maintain high rates of lay for longer periods of time, they can be kept to advanced ages without being moulted. While much is now known about the physiology of age-related changes, the elements that trigger these events remain elusive (Ottinger, 1992). A better understanding of these processes is necessary for more effective selection, and may allow the identification of specific genes influencing ageing.

There is increasing use of induced moulting to extend the laying life of the hen in much of the world, despite opposition to this practice by animal welfare activists in some developed countries. It can be expected that breeders will continue to work

for improved post-moult performance for the foreseeable future.

It has been stated that the economic impact of variability in disease resistance is relatively small and that it is not a high priority trait in most breeding schemes (Albers, 1993). However, the emphasis placed on disease resistance varies from one breeding firm to another. An epidemic of a specific disease can increase the importance of that disease in the minds of poultry producers and they may choose not to purchase stocks that are susceptible. For example, this has occurred in the USA (in the 1950s) and in Australia (in the 1990s) for the disease lymphoid leucosis. Relative Marek's disease susceptibility affected buying decisions in the USA in the 1960s. Currently, feather pecking and resultant cannibalism is a problem in chickens housed in alternative systems in Europe and perceived differences among commercially available varieties are affecting sales.

Breeding for resistance to disease is difficult because of low heritabilities and rapid evolution to more virulent forms among disease-causing microorganisms. Heritabilities are generally under 10% for total mortality, but somewhat higher for specific diseases (Gavora, 1990). Some diseases thought to be controlled by vaccination, such as bronchitis and Marek's disease, keep reappearing due to the occurrence of variant viral strains.

Breeding for resistance to specific diseases caused by microorganisms involves exposure of the birds to disease agents in a controlled manner, usually by inoculation of highly pathogenic variants of the organism. This cannot normally be done in the pedigreed population under selection, due to the risk of killing excessive numbers of breeders and reducing effective selection for other traits. For this reason, the disease challenge is sometimes done in siblings or progeny of the birds under selection, at an isolated location, and the selection is done on a family basis. The deliberate exposure of birds to pathogenic agents raises questions from an animal welfare standpoint.

There is need for improved methods of identification of genetically resistant birds. Marker-assisted selection, or better yet the identification and labelling of specific genes for resistance, will enhance progress. One such gene, influencing resistance to salmonella in chickens, has recently been reported (Hu *et al.*, 1997).

An understanding of the genetics of the disease organisms themselves might make possible the use of pathogen-derived genes (Witter, 1998), which, once inserted into the bird's genome, could confer levels of resistance to the disease organisms not currently found in existing populations of poultry. To implement this theoretical strategy, these new constructs would have to be inserted through the use of transgenic technology.

The trait with the most impact on profitability is feed conversion. The conversion of feed into eggs is primarily a function of egg numbers. It is also influenced by egg size and body weight. Breeders improved feed conversion throughout the 20th century, especially in brown egg stock, by selecting for increased egg mass and smaller body size. Since the mid-1980s, commercial poultry geneticists have also been selecting for improvement of that part of feed consumption not explained by egg mass and body weight. This is referred to as residual feed consumption. Incorporation of selection on residual feed consumption will improve feed efficiency at a faster rate than selection on egg mass and body weight alone (Nordskog *et al.*, 1991). To accomplish this, consumption is measured for individual hens. Expected feed consumption for each hen is calculated from the bird's egg mass and body size using a linear model. Residual feed consumption is calculated by subtracting expected intake from the measured intake. Hens with high levels of residual intake are culled.

Feed conversion in the USA and Canada has improved from 2.95 g feed g^{-1} egg in 1960 (Agricultural Research Service, 1960) to 2.01 g g^{-1} in 2001 (R.L. Chilson, California, 2001, in CMC Strain Performance Reports). Further continued improvement will be aided by a better understanding of the factors influencing feed conversion, including feather cover, activity and feed wastage.

Some aspects of egg quality continue to improve, while others remain unchanged. Little change is occurring in overall egg

weight, as most commercial varieties have already been selected to fit the needs of the markets in which they are sold. However, there is selection for attainment of the desirable egg size at an earlier age. This requires concurrent selection against increased egg size at a later age because of the strong genetic correlation between early and late egg size.

Inclusions (so-called blood and meat spots) have been selected to low levels of occurrence in white egg stock, so that little additional response can be achieved. In brown egg populations, there continues to be genetic variability for inclusions, and effective selection is practised to reduce the incidence of blood and meat spots. In brown egg varieties, effective selection also continues for eggshells with a darker brown colour.

Some selection is practised for albumen height, so that Haugh units will remain at acceptable levels in markets where this measure is incorporated into egg grading standards. Shell strength improvement also continues and should improve for the foreseeable future, allowing the hens to produce a lower number of cracked eggs and eggs to be kept for longer periods of time.

New challenges are arising in relation to the use of eggs for further processing. Buyers of liquid egg are setting standards for the percentage of solids or lipids in the liquid egg product. In the USA, buyers of mixed white and yolk require that the mix contain at least 24.2% solids. If the level of solids is below this, processors must add yolk to increase the level. This reduces profits for the processor since yolk generally receives a higher price than albumen. In Italy, where yolks are in high demand for the production of pasta, buyers have established a standard of 10.5% lipid in the yolk. Both solids and lipids vary from one commercial cross to another (Ahn *et al.*, 1997). Breeders can influence these traits by switching parent lines used to produce their commercial cross. However, it is extremely difficult to select within populations, since measurement of solids and lipids for individual birds is time-consuming and expensive. Quicker, cheaper methods are needed for the measurement of solids and lipids.

Another issue affecting further processed eggs is the strength of the vitelline membrane. Egg whites must whip into foam with a good height. Contamination of the white with yolk will reduce the foam height. If the yolk ruptures during separation of the white, the contaminated product must be removed, reducing the speed of the breaking process and reducing the value of the product. Therefore, breeders must maintain vitelline membrane strength.

As more attention is focused on animal welfare, several traits increase in importance. Foremost among these is bird-to-bird aggression, which can lead to cannibalism and which also impacts feather cover. Craig and Muir (1996) have shown that selection in group cages can be used to reduce aggression. At least one commercial breeder has used this practice for over 30 years. Layers from this firm have relatively low levels of cannibalism when their beaks are left untrimmed (Craig and Lee, 1989).

Another welfare-related issue is the increasing demand for eggs produced by floor-housed birds (free-range, organic, etc.). The EU has issued a directive requiring the elimination of conventional cages by 2012. Cannibalism is an important issue for floor-housed birds. Nesting behaviour is another important issue, as birds must search out the nest. Eggs laid on the floor are more likely to be soiled and require special labour for collection.

The EU currently mandates 550 cm^2 per bird in cages. Guidelines of the US United Egg Producers call for 432 cm^2 per bird for all birds placed by the year 2012. Currently most commercial white egg layers in the world are housed in cages at 310–350 cm^2 per bird. Breeders will need to take the changing cage densities into account in their breeding plans.

Other welfare-related factors that are likely to become of increasing concern to breeders include the killing of unneeded cockerels and maintenance of skeletal integrity as the bird ages. If induced moulting is banned, this will also alter optimal breeding strategies.

Intensive animal agriculture has raised concerns among the general public about the

effect on the environment of high concentrations of livestock. Manure output has become a concern and producers are asked to control not only the total amount of manure spread on the land, but also the amount of moisture in the manure (for fly control) and the phosphorus content. Birds that consume less feed will excrete less, so selection for reduced residual feed consumption should also result in less manure to spread on the land. There are genetic differences between varieties in the amount of moisture in the faeces. Varieties with dry droppings are more prone to the development of urolithiasis (Lent and Wideman, 1993). It is possible to select for drier droppings on an individual bird basis (Preisinger *et al.*, 1994) but care should be taken not to increase the incidence of urolithiasis.

The greatest potential for increased egg consumption is in the tropics, where per capita egg consumption levels are low and are increasing. Much research has already been done on resistance to heat stress but, to date, no bird bred specifically for resistance to heat stress has captured much market share in the tropics in general. As a result of depressed feed consumption and poor-quality feed ingredients in tropical areas, there may be a benefit in having a bird for the tropics with a large appetite (Ansah, 2000). However, feed prices are very high in most of the tropical countries, so the ultimate solution for the production of eggs at economical prices in these areas may be the construction of controlled-environment houses to utilize the efficiencies of the modern layer.

Methods

Beginning about 1970, the advent of high-speed computers and the development of sophisticated statistical estimates of genetic value have permitted improved rates of within-line improvement. Questions remain concerning the effect of statistical tools such as best linear unbiased prediction (BLUP) that incorporate family information on the rate of exhaustion of genetic variability (Muir, 1997). Accelerated loss of

genetic variation due to the use of BLUP may necessitate early outcrossing of the pure lines that make up these crosses, to reintroduce genetic variability. Alternatively, selection rules could be considered that would allow for more conservation of genetic variance and optimal balance of long-term vs. short-term response.

The use of marker-assisted selection (MAS) is expected to increase the accuracy of breeding value information and to be especially useful for traits that have low heritabilities or are difficult to measure. MAS will also allow the improved utilization of available 'selection space' (Soller and Medjugorac, 1999). This underutilized 'selection space' is provided by the surplus males that are available in chicken breeding stock. Far more males can be produced than are needed since, at the time of selection, full brothers without progeny tests all have identical predictions of breeding values for traits that can only be measured in females. Vallejo *et al.* (1998) found several markers for genes controlling resistance to Marek's disease. Lamont *et al.* (1996) reported on markers for egg production, and Van Kaam *et al.* (1999) reported on markers for feed efficiency.

The use of transgenesis plays a major role in the breeding of plants. Several companies are now striving to develop transgenic strains of chickens that can be used to produce pharmaceuticals or other valuable proteins in eggs, but this tool has yet to be applied to the breeding of commercial poultry stocks. Since the single-celled chicken zygote is difficult to manipulate and then reintroduce into the egg for further development, transgenic poultry are more difficult to produce than are transgenic plants or mammals. With the developing concern about genetically modified organisms (GMOs), commercial breeding firms are now being required by some consumers to state that they are not using GMOs. This has a chilling effect on the interest of breeders in using the transgenic tool. Eventually transgenesis will prove too valuable to ignore and commercial hens will become available that have enhanced performance due to the introduction of DNA that has

been synthesized in the laboratory or that originates from other species.

Industry structure

Since 1950, breeding firms have become much fewer in number and much larger in size. Three holding companies now control the majority of the breeding work on the commercially available breeding stock for egg-type chickens, though their products are marketed under nine different brand names. The reduction in the number of breeding firms has been due to international competition and to the high cost of maintaining modern breeding, marketing and distribution programmes in comparison with potential income.

The reduced number of breeding firms has raised concerns about reduced competition and an associated reduction in the potential for innovative research and development (Sheldon, 2000). From an insider's perspective, competition is still intense, some of it among companies within the same groups. However, there has been a dramatic reduction in the number of geneticists working for breeding firms and in the total number of chicken populations under selection.

There is also concern about the narrowness of the base of the genetic stock now being marketed. There is danger in this situation due to the potential susceptibility of 'monocultures' to new diseases that could destroy or damage a genetically uniform population, as happened with maize in the southern corn leaf blight epidemic in the USA in 1970 (Duvick, 1978).

There has also been increasing planned and unplanned loss of stock used as resource populations in the public sector (Pisenti *et al.*, 2001). Some of the lost stock was developed over a period of many years, and their loss reduces the scope of future research. From the standpoint of genetic variability for long-term improvement in commercial stock, the important factor is not the preservation of unique research populations or of the degree of heterozygosity within populations, but the maintenance of allelic diversity across the species (Notter, 1999). The combined losses of research and commercial populations formerly held by now defunct breeders can limit the future genetic potential of the chicken.

Conclusions

Since the early 1960s, feed conversion in the USA and Canada has improved by almost 1 g, from 2.96 g feed g^{-1} egg to 2.01 g g^{-1}. It is not possible to know how much of this improvement was genetic and how much was due to management, but it is safe to assume that a major part of the change is due to improved breeding stock.

In 2000, there were 50.4 million tons of eggs produced in the world (*Executive Guide to World Poultry Trends*, 2001). Thus, with full implementation of the changes of the past 40 years (i.e. since the 1960s), there would be a saving of about 50 million tons of feedstuffs per year due to improved feed efficiency. As the population of the world continues to grow, there will be increased demand for feed grains and increased importance of the continued improvement in the efficiency of poultry and other farm animals. For that reason, it is critical that the necessary research be conducted and that breeders have access to all available genetic tools, including marker-assisted selection and transgenesis. In addition, care should be taken that important genetic diversity is not lost.

Meat-type Chickens

Global development of chicken meat production and the role of breeding

Well into the 20th century, most chickens in the world were kept in small non-specialized units. Although many very different breeds already existed, true specialization for egg or meat production hardly existed and most chicken breeds were used for both purposes. In the late

19th and the first half of the 20th century, small specialized production units emerged and these used selection from the available breeds. Some new synthetic breeds were also developed. Some genetic selection was applied for the specific purpose of the local industry.

Around the Second World War, larger and more specialized production units for poultry were being developed in North America and Europe. This triggered the development of more advanced genetic improvement programmes. Stimulated by earlier developments in plant breeding, line specialization and crossbreeding were introduced. With the success of the introduction of crossbreds, the number of breeding programmes reduced very quickly to the small number of units that were able to support such large-scale programmes.

In only 50 years, poultry meat production developed from a side activity of numerous small farms into a specialized global business. An industry that was fragmented at first into many small specialized units for breeding, multiplication, hatching, growing and processing soon developed into large integrated poultry meat production companies, often with live production sites contracted out to private farmers. Especially in the Americas, fully integrated companies were spearheading poultry production right from the beginning. In the last quarter of the 20th century poultry production became a truly global industry with international trade effectively enforcing standardization of production methods and products. Large production companies are even beginning to spread their production facilities around the globe.

World chicken meat production had grown to 56.9 million tonnes in 2000 with 90% of the number slaughtered being young broilers. Output has been growing at an annual rate of 3–5% for many years and this increase is expected to continue. In the last 7 years, poultry's share of total world meat output rose from less than 25% to nearly 29%. Chicken accounts for 86% of total poultry meat output, leaving turkey and duck far behind with 7 and 4%, respectively (*Executive Guide to World Poultry Trends*, 2001).

Poultry breeding has enabled and supported this development of poultry meat production. Breeding companies have been very successful in their efforts to populate the production industry in a logistically efficient manner with increasing numbers of increasingly efficient stock of increasingly high health status. Due to the relatively low cost of breeding programmes for poultry (around 0.5% of live production value), the relative ease of transporting eggs and day-old chicks around the globe, and the fast growth of the industry, the efficiency of chicken meat production has shown a dramatic increase since the 1950s. The joint success of poultry breeding and production is illustrated in Fig. 1.1 by the comparison of the increase of production efficiency in broilers and pigs since 1960. The role of
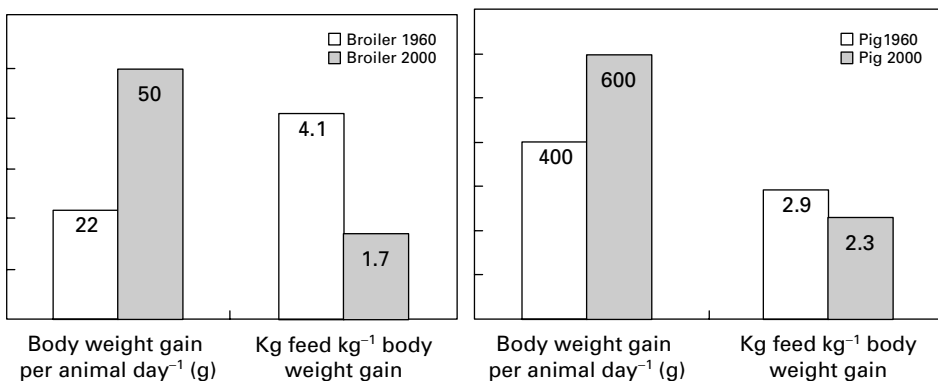
Fig. 1.1. Increase of efficiency of meat production in pigs and poultry over four decades calculated for the entire life of the animal from birth to slaughter.

breeding in this success has been dominant, as has been elegantly shown by Havenstein *et al.* (1994). Some 80% of progress over time has been made possible by improvement of the genetic potential of the birds used.

Evolution of breeding programmes and breeding technologies

Until the beginning of the 20th century there were no other means of selection and breeding than to identify the best breeder candidates by way of phenotype and to mate these for producing the next generation during the breeding season. A number of technologies in controlled management of reproduction, in control of pedigrees and matings and, lastly, in the accuracy and early availability of estimation of true breeding value of breeding candidates were developed and introduced successively from then onwards. Before the 1940s these technologies were exclusively applied in pure-breeding lines for purebred production stock; from then onwards all breeding programmes for meat poultry consisted of several specialized lines, with distinct breeding goals per line, and production animals (broilers) were crossbreds. Today, final product broilers are a three-way or four-way cross of specific closed pure-breeding lines. There are four generations between the pure-breeding line and the final broiler. The generation and multiplication levels from breeding to meat production are as follows.

1. Pure-breeding line. Owned by the breeding company and subjected to the full-scale selection programme. Three or four lines are used for each broiler product. Each breeding company has a range of broiler products and therefore maintains at least ten pure-breeding lines.
2. Great-grandparent stock. Fully controlled by the breeding company, subjected to limited (usually mass) selection for selected traits. This generation is mainly used to multiply the pure lines to the large numbers (at least tens of thousands) needed to produce the grandparent stock.

3. Grandparent stock. In case of a four-way final cross (ABCD) this generation is the first generation of crossbreeding with A males, B females, C males and D females making up the grandparent flocks. Grandparents are distributed throughout the world in at least hundreds of thousands to local operations, which may be integrated production companies or local distributors of parent stock.
4. Parent stock. This is the second generation of crossbreeding with AB hybrid males being mated to CD hybrid females. Parent stock flocks are largely owned by production companies that produce broilers.
5. Broilers. These are the birds that are grown, slaughtered and processed for large-scale chicken meat production.

Table 1.1 gives a short summary of the various critical breeding and selection technologies and the approximate time of their introduction.

Evolution of breeding goals

Breeders set breeding goals as a reflection of their expectations of future market demands. With the ongoing changes of production and consumption trends, breeders have responded by adapting breeding goals continuously. Global trends since the early 1950s have been as follows.

Table 1.1. Critical technologies for poultry breeding.

Technique	Decade of introduction (approximate)
Mass selection	1900
Trapnesting	1930
Hybridization	1940
Pedigreeing	1940
Artificial insemination	1960
Osborne index	1960
Family feed conversion testing	1970
Selection index	1980
Individual feed conversion testing	1980
BLUP breeding value estimation	1990
DNA markers	2000

1. Broiler growth has consistently been the prime selection trait, because of its ease of selection, high heritability and large impact on total meat production cost.

2. There has been increasing emphasis on yield of white (breast) meat, because this was increasingly favoured by consumers.

3. There was also a growing emphasis on efficiency factors, most notably feed efficiency of broiler growth, as a maturing production industry was increasingly focusing on financial bottom lines for integrated production operations.

An overview of the most important selection traits and the changes in their relative importance over time is presented in Table 1.2.

At first, up to the 1980s, the impact of these trends was not clear-cut, but with the increasing globalization of the industry only the most profitable breeding products now remain. Only four independent groups with significant world market shares in 2001 have survived this selection process, by adapting their programmes in a more timely and adequate fashion than the non-survivors. Differences between companies in timely adjustment of breeding goals have played a more important role in this process than differences in the availability and application of up-to-date breeding and selection technologies. Alongside the large breeding programmes that produce the 'commercial white broiler' for large-scale production of chicken meat, a limited number of small breeding programmes have continued to breed specific products for small niche markets. In particular, the French market has continuously used slower-growing and coloured breeds for *Label Rouge* and other types of certified chicken meat production. China has a significant part of its chicken meat production on a similar basis. There is a clear tendency for such markets to increase and this growing interest could well be the start of a new trend of a return to larger product diversification.

Expectations for the future

Future developments in the breeding of meat-type chickens will no doubt be governed by the same factors that have determined developments in the past but with one important difference: the 'chicken itself' is likely to play its own role when it presents the limits of its biological capabilities. It has been speculated that genetic progress at the present rate and for the current main traits will be possible for a limited period of less than two decades (Albers, 1998). Areas of speculation for the future impact of main determinants include: industry developments; consumer demands; breeding technologies; and biological constraints.

Industry developments

Further consolidation of chicken meat production into large integrated national and supranational units is to be expected. More emphasis will therefore be on efficiency of production and on the increasingly efficient translation of consumer demands by way of intimate partnerships between large production companies and large clients

Table 1.2. Trends in relative selection pressure for various traits in broiler breeding programmes.

Selection trait	Relative selection pressure during years		
	1975–1985	1985–1995	1995–2002
Hatching egg production	+++	++	+
Fertility	+	+	+
Broiler growth rate	+++	+++	+++
Broiler feed efficiency	++	+++	+++
Meat yield traits	+	++	+++
Liveability	+	+	++

(retailers and food service companies). Even fewer breeding programmes, probably three, will survive.

Consumer demands

Price will remain the most important buying incentive for consumers and therefore production cost per unit of product will be of prime importance. However, with increasing living standards, secondary consumer demands will increase. General product quality, food safety, further processing and product diversification will complicate the picture. Meat sales will be less through retailers and increasingly through food service companies and therefore processability issues (e.g. meat and bone quality) will become more important. Increasingly wealthy and critical consumers will also set requirements on the production methods used. This will include demand standards for animal welfare (e.g. bird density in the broiler house, feed restriction of breeder birds), safety of products and use of production technologies including genetic techniques (cf. the current GMO debate). The introduction of organic products is an illustration of this trend and it is to be expected that bulk production of chicken meat will at some stage be significantly influenced by this trend. It is not clear whether all trends mentioned will have the same impact worldwide and it is quite possible that there will be regional differences, e.g. due to marked differences in living standards. This could well affect the balance between cost price demands and secondary consumer demands.

Breeding technologies

Reproduction technologies, breeding value evaluation technologies and DNA-based technologies are the three critical groups of technologies in breeding in general. The increase of reproductive capacity in chickens offers very little perspective for increase of genetic progress, as selection pressures are already very high. Increase of reproductive capacity (e.g. by cloning) could only help to speed up the process of multiplication of genetic progress to the production units. However, it is very hard to improve, in a cost-effective way, on the relatively high reproduction rate of chickens and no dramatic developments should therefore be expected in this area.

Theoretically, the ability to reproduce at an earlier age would support the increase of genetic progress by a reduction of the generation interval. The technical problems associated with this, the acceptability of the technologies to be used and the relatively small impact of this make such a new development unlikely.

The only remaining reproduction issue for poultry meat production is the determination of sex of the broiler birds, as the production efficiency of males is significantly higher than of females. With BLUP breeding values now being widely used in meat-type chicken breeding, not too much can be expected from improved mathematical/statistical methodologies for estimation of breeding values. The most important challenges in this area for the foreseeable future are likely to be in the best combination of BLUP procedures with methodology to optimize inbreeding and the inclusion of genomic data in the BLUP-based breeding values.

The most promising new breeding technologies are DNA related: with chicken genomics coming of age, as illustrated by the comprehensive linkage map of the chicken genome (Groenen *et al.*, 2000) and the First Report on Chicken Genes and Chromosomes (Schmid *et al.*, 2000), genomics is now starting to yield for meat-chicken breeding. As the economic value of individual chickens is relatively low, DNA-based genotyping of individual breeding candidates must be done at low cost per bird. Therefore commercial application of genotyping at the DNA level will largely be through direct genotyping for critical genes and not through MAS approaches *per se* that are being designed for larger species. With chicken genomics advancing rapidly a significant impact of this technology on meat-chicken breeding is to be expected, especially for the selection of traits that are not easily dealt with in traditional genetic evaluation

programmes. Such traits will become more important, as indicated in the section on biological constraints, below.

Transgenic technologies are not expected to have a significant effect on commercial meat-chicken breeding in the foreseeable future. Transgenic technologies have not advanced in birds as much as in mammals and it is becoming more and more clear that consumers worldwide are opposing such a development. Breeding companies are not likely to enter into the development process for a transgenic chicken for meat production. As DNA-based selection and breeding technologies are patentable, unlike traditional technologies, the introduction of these novel technologies will also add a new dimension to the competition between breeding companies.

Biological constraints

Growth rate of modern broilers has roughly quadrupled since commercial breeding commenced in the 20th century. The body composition of the birds has changed dramatically, especially the relative size of the pectoral muscles. Although commercial breeding programmes have been successful in counteracting this basic imbalance by genetic improvement of leg strength and other aspects of general livability such as susceptibility to ascites, there is no doubt that commercial broilers today are showing higher mortality and higher susceptibility to suboptimal management of nutrition and environment than broilers that have been selected less extremely for efficiency and meat yield. This is clear from field evidence on slow-growing breeds such as are used in various regional certified broiler production systems in France, but there is also good experimental evidence for higher activity levels and lower mortality rates in such slower-growing genotypes (Lewis *et al.*, 1997).

More constraints will arise on issues such as leg strength, female and male reproduction capacity, metabolic problems in broilers, digestive system functions of broilers and several aspects of carcass and meat quality. Research is urgently needed for better understanding of the biological basis of the consequences of the lack of balance in the modern broiler compared with its wild ancestor. Understanding this biological basis should direct researchers and breeders to design selection approaches aimed at preventing this lack of balance from progressing further. Genomics could well play a key role in this, both in unravelling the biological mechanisms and in supporting the breeders in selection programmes.

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2 Growth and Reproduction Problems Associated with Selection for Increased Broiler Meat Production

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Introduction

The improvement in poultry performance for meat and egg production during the last three-quarters of the 20th century has been tremendous: from 176 eggs per hen per year in 1925 to 309 eggs per hen per year in 1998, while for meat production the days to achieve 1500 g live weight decreased from 120 days in 1925 to 33 days in 1998.

Besides product quality aspects that are dealt with elsewhere in this volume, the main selection goal with laying hens is a high and efficient egg output per hen per year while for poultry meat production the meat output per chick is, or was, the ultimate measure of performance. Since the early 1990s, it has become clear that meat output per breeder has become a more dominant indicator of success (e.g. Pollock, 1999), indicating that both growth and reproduction are important.

As the biological maximum for laying hens is physiologically determined at one egg per day (under normal lighting), a genetic progress of 40–50 eggs per hen per laying year is theoretically possible in the next 15–20 years; this could be realized not by increasing peak production but by improving the persistency of the laying curve, at least if major obstacles to be expected (such as possibilities for mineral mobilization from bone and hence leg problems) are not counterproductive. These problems associated with selection for increased egg production are treated in another chapter, and therefore the focus here will be on growth and reproduction in the broiler sector.

The meat output per breeder is composed of at least three parameters: (i) growth; (ii) feed conversion; and (iii) breeder effectiveness. Any assessment of the efficiency of feed energy utilization for growth in meat animals must consider not only the feed conversion in the slaughter generation, but also the cost of maintaining an effective breeder population.

In prolific species such as poultry, the majority of feed is consumed by the slaughter generation (approximately 95%, vs. 5% for the breeder generation). Therefore, selection goals will be directed predominantly towards traits that favour growth, feed conversion efficiency and carcass quality in the slaughter generation, with much less emphasis on reproductive traits. In slow-reproducing species such as cows,

the metabolizable energy (ME) intake is distributed approximately equally between breeder and slaughter generations and therefore it is theoretically as profitable to manipulate traits relating to the efficiency of the breeding cow as it is to manipulate growth in the slaughter animals (Webster, 1989).

Nevertheless, both the parameters for growth and for reproduction in broiler production will have to be considered. Until now there has been no indication of a reduced genetic variability for the main broiler traits (Pollock, 1999). There are many expectations as to further improvements for broiler meat production but it seems that some of these improvements are linked with dilemmas in the sense that they seem to contradict each other.

Three dilemmas for broiler meat production that have become apparent since about 1980 can be identified: (i) fast growth and feed conversion vs. changes in lean/fat tissue ratio; (ii) ponderal and energetic efficiency of growth and feed conversion ratio (FCR) vs. metabolic disorders; and (iii) fast growth and extreme lean tissue growth vs. reproductive effectiveness of the breeder. From a functional or physiological point of view, the question arises: is there any causal or functional link among and between these dilemmas or are these elements merely correlated to each other without strong causal relationships? From a genetic perspective, this question can be phrased in the context of the genetic and phenotypic correlations among the phenotypes. Have the aforementioned relationships occurred due to genetic correlations (whether due to linkage, epistasis or shared physiological control systems) or environmental influences (associated with fast growth, *per se*) and, of course, which is which? The answer to this question will dictate the path or paths that need to be taken to identify solutions.

The following chapters discuss the physiological and genetic backgrounds of each of these three dilemmas with the aim of clarifying, as much as possible from the scientific evidence available, to what extent these dilemmas were hazards from selection policies as applied until now or were inevitable consequences of the actual selection goals.

Dilemma 1: Fast Growth and Feed Conversion Efficiency vs. Lean/Fat Changes

Discussion of the dilemma of fast growth is complicated by several factors. Growth is clearly a non-linear process (Barbato, 1991) and much of the literature uses the term 'selection for fast growth'. Few, if any, breeders truly select for growth rate. In fact, breeders generally select for body weight at a fixed age, regardless of the time frame of that age relative to the growth curve of the particular species (Anthony *et al.*, 1991). Yet it is clear that the main correlated response to selection for growth at, or near, the inflection point of the growth curve is early exponential growth rate, generally described as occurring during the first 2 weeks post hatch (Ricklefs, 1985; Barbato, 1992a,b). It is also equally clear that there is a very large negative genetic and phenotypic correlation between early exponential growth rate and the subsequent linear phase of growth, regardless of species (Laird, 1966; Barbato, 1991, 1992a,b).

This result is not as surprising as it may at first seem. The negative correlation was probably the result of the intense direct selection for body weight at a fixed age, hence selecting for both early and late growth, resulting in a negative genetic correlation between the traits. Continued selection for body weight at an age near the inflection point of the growth curve selects against the genetic correlation between the exponential and linear phases of growth. It has long been known that selection for two traits having a negative genetic correlation will result in a subsequent reduction in fitness.

How, then, did a change in body weight and associated growth curve of broilers result in an alteration in fatness? The phenomenology of this association has been clearly illustrated by Tzeng and Becker (1981), who showed that abdominal fat pad

weight peaks at or near the inflection point of the cumulative growth curve. Body weight can be considered to be an aggregate selection index of all the body components. Since abdominal fat has a higher heritability than protein, water or skeleton at the age of selection (e.g. Cahaner and Nitsan, 1985), and is proportionately largest during the linear portion of the cumulative growth curve, then fat must increase in a disproportionate manner. From a mechanistic perspective it is interesting to note that the phenomenology is consistent: the large fat pad size at the age of selection can be due to both adipose cell size and adipose cell number, depending on the population (Wyatt *et al.*, 1982; Cartwright, 1994). These data would suggest the possibility of multiple physiological mechanisms for increased fatness, depending on the genetic background and selection history of the lines. Hence, the mechanisms for the relatively high fat content of modern broilers continue to be a subject of controversy (Buyse *et al.*, 1999).

The relative contributions of selection for body weight and dietary regimen on performance and carcass composition of broilers were elegantly assessed by Havenstein *et al.* (1994a,b). In these studies, a typical 1957 broiler strain (Athens-Canadian Randombred Control strain) was compared with a modern 1991 broiler strain (Arbor-Acres) when fed a typical 1957 or a 1991 diet. With respect to carcass fat content, the 1991 broilers were fatter than the 1957 broiler, irrespective of diet or age. Broilers raised on the 1991 diet also had a higher fat content than when reared on the 1957 diet. However, the contribution of diet to carcass fat content was far less than that of genotype. These observations, among many others, clearly indicate that selection for body weight has concomitantly promoted fat accretion.

While selection for body weight at a fixed age has fulfilled the industry goal of increasing protein deposition in the form of skeletal muscle mass, there is very little evidence for genetic improvement of percentage body protein. In a diallelic cross among lines having different growth curves, Barbato (1992a) showed that there is little or

no additive genetic variation for percentage body protein. Further, among lines selected for exponential growth rate at different ages, there was no generational trend in total body protein (Sizemore and Barbato, 2001). On the other hand, it is clear that protein can be redistributed among the muscle groups, and can be influenced pharmacologically by β-agonists.

Research groups from several countries have produced lean and fat lines of broilers by following different selection strategies in order to study more closely the relationship between rapid growth and fatness (Table 2.1). In addition, these divergent lines are excellent models for investigating the regulatory roles of hormones in the intermediary metabolism, which ultimately determines body composition. A non-destructive method to measure leanness and fatness was reported by Whitehead and Griffin (1984). After three cycles of divergent selection using plasma levels of very low density lipoproteins (VLDL), these authors obtained lines that differed by 38% in total lipids and by 49% in abdominal fat pad weight. Selection based on improved feed efficiency has also been shown to be a very effective procedure to obtain lean broiler chickens, because of high negative correlation between feed efficiency and fatness. Indeed, a significantly lower content of body lipids and a reduced proportion of abdominal fat were reported in broiler lines that were selected for improved feed efficiency (Leenstra and Pit, 1987; Sørensen, 1988). Direct selection on the basis of abdominal fat content of siblings has proved to be effective for selecting fatter and leaner broiler chickens. Leclercq (1988) found that, after seven generations, the fat line had four times more abdominal fat and 72% more total lipids than the lean line; Cahaner (1988) obtained similar results. Other divergent selection lines are those of Pym and Solvyns (1979), the 'Siegel' lines selected for high or low body weight gain (Siegel, 1962) and the lines selected by Chambers (1987) and Barbato (1992b). The Barbato lines are particularly interesting since selection for fast early exponential growth rate at 14 days of age resulted in a line of birds having fat pads that

Table 2.1. Experimental broiler lines selected to differ in body composition, feed conversion and/or growth rate from different countries (Buyse *et al.*, 1999).

Country	Line	Selection traits	References
Australia	W	Liveweight gain	Pym and Solvyns, 1979
	F	Food consumption	
	E	Feed efficiency	
	L	Low abdominal fat	
Canada	S1	Body weight + low abdominal fat	Chambers, 1987
	S2	Body weight gain + feed efficiency	
	S3	Combined three traits	
Denmark	GL	High body weight gain	Sørensen, 1988
	FC	Feed conversion	
France	HF	High abdominal fat	Leclercq, 1988
	LF	Low abdominal fat	
Israel	GL	High body weight gain	Cahaner, 1988
	HF	High abdominal fat	
	LF	Low abdominal fat	
The Netherlands	GL	High body weight gain	Leenstra, 1988
	FC	Feed conversion	
UK	FL	High VLDL	Whitehead, 1988
	LL	Low VLDL	
USA	HW	High body weight gain	Siegel, 1962
	LW	Low body weight gain	
	FL	Fat line	Lilburn and Myers-Miller, 1988
	LL	Lean line	
	14L/H	Low/high exponential growth rate to day 14	Barbato, 1992b
	42L/H	Low/high exponential growth rate to day 42	

were 50% smaller than those of birds from a line selected for exponential growth rate to 42 days of age, yet there were no significant differences in body size at 42 days of age (Sizemore and Barbato, 2001).

The efficiency of lean meat deposition can in theory be increased by: (i) an increased proportion of ME above energy requirements for maintenance (ME_m); (ii) an increased proportion of retained energy (RE) as protein (RE_p) with respect to fat (RE_f); and (iii) the efficiency of retention of increments of ME above maintenance as protein and fat (partial energetic efficiency, K, for growth, K_{pf}, as a combination of K_p and K_f). Both ME_m and K_{pf} affect thermogenesis (H) and overall energetic efficiency is (ME – H) /ME. Feed conversion rate is determined by both this overall energetic efficiency and the energetic value of body gains.

From a survey of the energy metabolism of lean and fat broilers as summarized in Table 2.2, gross energy intake (GE) and ME intakes per kg $W^{0.75}$ (body weight W in kilograms raised to the power 0.75) were higher in fat-line chickens (Buyse *et al.*, 1999). However, differences in ME consumption are not very pronounced and also fasting heat production as well as ME requirements for maintenance per kg $W^{0.75}$ were generally found not to be different between lean and fat birds. Apparent metabolizability (ME/GE) values were on average higher for lean compared with fat birds (Jørgensen, 1989; Geraert *et al.*, 1992) but these differences are probably not of such a magnitude that they could account for much of the variation in feed efficiency and body composition.

Therefore, an increased proportion of ME above maintenance cannot be evoked as a major cause for the differences in body composition between lean and fat broiler chickens. Since it is claimed that the efficiency of retention of increments of ME fed above maintenance as protein and fat (K_p and K_f) is less affected by selection pressure (Webster, 1989), the main differences in

Table 2.2. Summary of the survey on energy metabolism of lean and fat broilers (Buyse et al., 1999).

Lines	ME intake per kg $W^{0.75}$	ME/GE	Hp fed per kg $W^{0.75}$	Hp fasting per kg $W^{0.75}$	E retained per kg $W^{0.75}$	E retained as protein	E retained as fat	ME_m per kg $W^{0.75}$	Energetic efficiency
French LF HF[1,2]	LF < HF	LF ≥ HF	LF = HF	LF = HF	LF = HF	LF > HF	LF < HF	LF = HF	LF < HF
Australian E W[3]	E < W	E > W	E = W	E = W	E < W	?	?	E = W	E < W
Danish FC GL[4]	FC < GL	FC > GL	FC = GL	?	FC < GL	FC = GL	FC < GL	?	FC < GL
Scottish LL FL[5]	LL = FL	LL = FL	LL = FL	LL > FL	LL = FL	LL > FL	LL < FL	LL = FL	LL < FL
Dutch FC GL[6]	FC < GL	FC ≥ GL	FC = GL	FC = GL	FC < GL	FC > GL	FC < GL	FC = GL	FC < GL

GE, gross energy; ME_m, metabolizable energy (for maintenance); Hp, heat production.
See Table 2.1 for the description of the lines.
[1]Leclercq and Saadoun, 1982.
[2]Geraert et al., 1988.
[3]Pym et al., 1984.
[4]Jørgensen, 1989.
[5]MacLeod et al., 1988.
[6]Buyse et al., 1998.

energy expenditure must be situated at the level of the amount of energy retained and its partition between RE_p and RE_f. This is indeed corroborated by the observations as summarized in Table 2.2.

Since K_f is much larger than K_p and ME_m levels are similar between lean and fat lines, a higher overall efficiency of increments of ME fed above maintenance should be expected, irrespective of differences in total GE or ME intake. However, heat production per kg $W^{0.75}$ in full-fed birds is not different in lean and fat birds, and therefore a better protein conversion efficiency as found in all lean lines (Geraert *et al.*, 1988; MacLeod *et al.*, 1988; Jørgensen, 1989; Leclercq and Guy, 1991; Leenstra and Ehlhardt, 1994; Buyse *et al.*, 1998) is probably linked with an improved efficiency of retention (K_p) of increments of ME above ME_m as protein (RE_p) and the claim of relative invariability of K_p should be reconsidered. Indeed, protein accretion (the net deposition of protein) is the result of two processes: protein synthesis and protein degradation. It is not always clear which processes are changed, and in what direction, due to selection for leanness.

The higher protein requirements for protein gain as reported by Leclercq and Guy (1991) and the higher breakdown of digested amino acids and urinary nitrogen losses as reported by MacLeod *et al.* (1988) in their fat lines may not only point to a lower dietary protein conversion efficiency, but probably also indicate differences in energetic efficiency (K_p) for RE_p. Indeed, Tomas *et al.* (1991) observed lower fractional myofibril protein degradation rates in chickens selected for food efficiency (line E, Table 2.1) compared with chickens selected on body weight gain (line W, Table 2.1) whereas there were no line differences in fractional synthesis rate. Therefore, increased protein conversion efficiency could very well go together with an increased energetic efficiency of protein accretion, and this could compensate for the much higher K_f vs. K_p in order to neutralize, more or less, the differences in combined K_{pf} values in lean and fat chicken. More research will be needed to elucidate the causal mechanisms underlying the line differences in protein metabolism.

As with protein, lipid accretion (the net deposition of lipids) is the result of several processes but, in contrast to proteins, three processes are involved in lipid accretion: *de novo* lipogenesis, lipolysis and lipid uptake from the blood into tissues (lipid clearance) because lipogenesis and lipolysis are not taking place in the same cells, in contrast to protein synthesis and degradation. With respect to these processes in fat and lean selected lines (as referred to in Table 2.1), lipogenesis was observed to be higher in all fat lines compared with their lean counterparts (Buyse *et al.*, 1999) and differences in lipogenesis are a major contributor to the observed differences in RE_f. Lipolytic activity may be higher (Buyse *et al.*, 1992) or unchanged (Leclercq *et al.*, 1988), depending on the selection strategy used. Differences in lipid clearance were suggested for the lines selected divergently for VLDL (Griffin and Hermier, 1988) while hepatic lipogenesis or abdominal fat lipoprotein lipase activity remained unchanged in these lines. However, low-VLDL chickens demonstrated increased lipoprotein lipase in leg and heart muscle, indicating a difference in tissue-specific lipoprotein clearance rate. Therefore, according to the selection strategy used, similar changes as for energy metabolism were observed, while the underlying mechanisms for decreased or increased fat deposition could be very different according to the lines.

Selection for fast growth, feed conversion efficiency or lean/fat tissue changes also has repercussions on the endocrine physiology as one of the important intermediary signals for realizing these metabolic changes as described above. A review of these changes as a function of the different selection strategies was given by Buyse *et al.* (1999). In short, it turned out that indirect selection for leanness by selecting for low FCR affected particularly the somatotrophic axis and to a much lesser extent the thyrotrophic axis, whereas the opposite was true when direct divergent selection on abdominal fat content was applied.

It can be concluded from these observations on divergently selected experimental broiler lines that hyperphagia is not the

primary cause of the higher fat content in fast-growing or fat-line broilers, nor is the partitioning of ME between maintenance and production energy a major factor. The main difference in energy metabolism is the partitioning of the retained energy between RE_p and RE_f and this explains at the same time the better FCR of the lean lines by either direct or indirect selection, though achieved by triggering other hormonal axes.

Dilemma 2: Ponderal and Energetic Efficiency of Growth vs. Metabolic Disorders (with Reference to Ascites)

No attempt to manipulate the efficiency of growth can be properly assessed without a complete examination of all elements of the energy balance. As has been shown in the first part of this chapter by examining the experimental broiler lines, manipulation of the partition of retained energy between protein and fat is probably the most efficient way of improving FCR as well as energetic efficiency, the latter in an opposite way.

Besides lean/fat tissue ratio, it is nevertheless useful to analyse the components of energy balance on an anatomical as well as a physiological basis. We must be aware that even in fast-growing broilers the principal destination of ME is heat and that, although thermopoiesis is largely linked to essential metabolic functions that are more or less resistant to 'manipulation', ongoing selection may have affected these components of the energy balance as well.

Selection for improved FCR may in this way have resulted in a decreased overall H for a given growth rate, and this may have repercussions on several anatomical or physiological components, which in turn are involved in other functions besides metabolism. Therefore it is not unexpected from an a posteriori viewpoint that an ongoing selection for rapid growth and FCR has resulted in an increased incidence of some metabolic disorders, such as heart failure syndrome (HFS) and ascites (pulmonary hypertension syndrome, PHS).

The following sections try to formulate some mechanistic links between these selection objectives and the increased ascites incidence. This may also be done for other metabolic disorders, such as tibia dyschondroplasia, but this subject is treated elsewhere in this volume.

From an anatomical or structural point of view

Organs such as gut, liver, kidney and heart are major contributors to thermopoiesis but their contribution to body mass is small; therefore, differences between animals in internal organ mass, attributable to their genotype, may have significant effects on thermopoiesis and hence on ME_m. Because hypoxaemia is believed to be the primary and main cause of ascites, circumstances that impose greater metabolic demands (especially during the period of rapid juvenile growth, when the metabolic rate is already very high) increase the incidence of ascites. A higher oxygen demand from the anabolic processes, together with high ME_m requirements, possibly results in the maximal oxygen delivery capacity of the respiratory and cardiovascular system being exceeded, and triggers the events that lead to PHS or ascites syndrome.

If the development of the ascites syndrome is the consequence of structural or morphological/histological defects, these must result in observable physiological changes (Decuypere et al., 2000). Insufficient development of the lungs and/or changed histology of lung tissue or pulmonary blood vessels (such as occurs in primary or idiopathic pulmonary hypertension in mammals) may form the basis of such structural changes. Alterations in proportional growth as a result of selection for greater musculature may have had the effect of producing birds with relatively small respiratory and cardiovascular systems. In addition, alterations in muscle fibre distribution and in the ratio of capillary to fibre size may have occurred as a consequence of selection for growth rate and breast meat

yield in both broilers and turkeys. Selection for leanness in pigs (Landrace, Piétrain) and cattle (double-muscled Belgian Blue) has increased the ratio of glycolytic to oxidative muscle metabolism (Hocquette *et al.*, 1998). This shift from type I (red, slow-fatiguing, oxidative) towards more type IIb (white, fast-fatiguing, glycolytic) muscle fibres has a major impact on energy metabolism post mortem and, hence, on meat quality, including the occurrence of pale soft exudative (PSE) and dark firm dry (DFD) meat.

An increasing incidence of PSE meat has been reported in turkeys (Barbut, 1997a) and broiler chickens (Barbut, 1997b), as well as higher incidences of metabolic diseases such as ascites and sudden-death syndrome in broilers and cardiomyopathy ('round heart' disease) in turkeys. It is therefore intriguing to speculate that these phenomena in poultry are also related to alterations in muscle fibre typology (see Decuypere *et al.*, 2000, for more details). In view of the relative independence of glycolytic white muscle on the requirement for oxygen, it can be speculated that selection for increased breast meat yield will not result in a proportionate increase in heart, blood and lung mass. If this is the case, it will lead to an exacerbation of the disproportion between the cardiopulmonary system on the one hand and muscle mass on the other, and result in an increased susceptibility to metabolic diseases.

From a physiological or functional point of view

The impact of a strong selection pressure on low FCR for obvious economic reasons (since 60–70% of broiler production costs are feed costs) may not just have repercussions on lean/fat ratio. As selection for FCR continues to be shifted to younger ages, before there is a considerable deposit of fat present, this must result in a decreased ME_m. Heart and lungs, as well as gut, liver and kidney, are major contributors to thermopoiesis and have many times higher metabolic rates per unit of weight than

skeletal muscle. This may have strengthened the developmental retardation of these vital oxygen-delivering tissues under the combined selection pressure for growth rate and FCR in broilers. However, these vital tissues have to sustain the high metabolic rate linked to the rapid juvenile growth rate.

A more concave growth curve, as obtained by management measures, may relieve metabolic pressure in birds from selection programmes that emphasize FCR (Buyse *et al.*, 1996; Decuypere *et al.*, 2000). From a genetic perspective, selection for a concave growth curve can be disastrous, as illustrated by the increased susceptibility to ascites (as induced by cool temperatures) of the 14L line chicks (Barbato, 1997). Of the four major selection lines reported by Barbato (1992b), the 14H line, having the most convex growth curve, has the greatest resistance to ascites. These two data sets – one based on environmental manipulation and the other based on genetic selection – suggest that while genes and environment both play a role in ascites susceptibility, they may do so by very different physiological mechanisms.

From interspecies comparisons of energy exchanges during growth, as pointed out by Webster (1989), it can be observed that, when comparing growth in pigs and cattle with that in precocial birds (quail, poultry), the latter mature faster and more efficiently than mammals because their impetus for growth is more sustained, rather than greater at peak.

Taken together, this suggests that from the viewpoints of both efficiency of growth and avoiding susceptibility to HFS and ascites, selection for growth within broiler lines should also be more focused on prolonging the period when the impetus for growth is near-maximal, rather than on increasing maximal growth rate.

Because thyroid function is an important regulatory mechanism of metabolic rate, it is plausible to hypothesize that early selection for FCR could result in functional hypothyroidism, by either decreased thyroid hormone production or changed peripheral metabolism of thyroxine (Decuypere *et al.*, 2000). Buys *et al.* (1993)

and Decuypere et al. (1994) provided direct evidence that susceptibility to ascites is linked to thyroid hormone metabolism. Ascites-sensitive birds are believed to be limited in their thyroxine production (Scheele et al., 1991, 1992). Insufficient thyroid hormone activity to regulate metabolism, related to the genetic background, will therefore become especially apparent at low ambient temperatures (Scheele et al., 1992), indicating that hypothyroidism, observed in lines combining a favourable FCR and fast growth, plays an important part in the reduction of oxygen consumption that leads to anoxia, heart failure and ascites. Although the peak incidence of ascites occurs in the 5th or 6th week of the growing period, the aetiology of the disease may be initiated much earlier, even during the embryonic stage (Coleman and Coleman, 1991). Dewil et al. (1996) showed that ascites-resistant broilers hatched earlier than more sensitive ones, and that this is linked to the higher thyroid activity in the resistant line. These findings at an early developmental stage strengthen the hypothyroidism hypothesis of Scheele et al. (1992) for ascites-sensitive broilers.

Dilemma 3: Fast Growth and Extreme Lean Tissue Growth vs. Reproductive Effectiveness of the Breeder

In domestic poultry, a strong negative relationship exists between body weight and reproductive effectiveness. The strength of this relationship is evidenced by the existence of two types of chickens of commercial significance that show extreme opposites in body weight and reproductive performance. Notably, reproductive effectiveness can be characterized by a combination of phenotypes including egg production, libido, sperm and oocyte quality, sperm storage by the hen, gamete–gamete interactions, genetic compatibility and hatchability – not to mention the potential interactions among the phenotypes. Regardless of mechanism, concomitant with improvements in body weight of broilers,

the ability of meat-type parent stocks to reproduce has been severely reduced. Nevertheless, parents of meat-type poultry must not only have the genetic potential to exhibit fast and efficient growth, but also be capable of reproducing. Empirically, it is known that broiler breeders require dedicated programmes of feed restriction to maximize egg and chick production, and emphasis for improving reproductive performance of broiler breeders has always been more on management than on genetic improvements. An idealized comparison of ad libitum broiler growth compared with feed-restricted breeder growth is presented in Fig. 2.1 (modified from Kerr et al., 2001). This is linked with the low percentage of energy cost for the breeder population in the total efficiency of feed energy for broiler meat production, including slaughter and breeder generation (as mentioned in the introduction to this chapter).

Egg production

Feed restriction of female broiler breeders during the rearing period delays sexual maturity. Factors that are indicated in the literature to govern the attainment of sexual maturity are: body weight (Brody et al., 1984); body fat (Bornstein et al., 1984); body fat-free mass or lean body mass (Soller et al., 1984); photoperiod (Costa, 1981); and age (Brody et al., 1984). Katanbaf et al. (1989) observed that full-fed broiler breeders are dependent upon reaching a critical age to start laying, while feed-restricted hens are dependent upon attaining a critical body weight and carcass fat stores. Yu et al. (1992a,b) suggested that feed intake and changes in body composition (minimum fat level) during the pre-breeding period are the most important factors in the determination of the age of sexual maturity in broiler breeders. A further reduction in fat content as expected by a further selection for FCR may therefore result in later maturity of breeder hens, because the required body composition for sexual maturity is not reached at an acceptable age. This may even
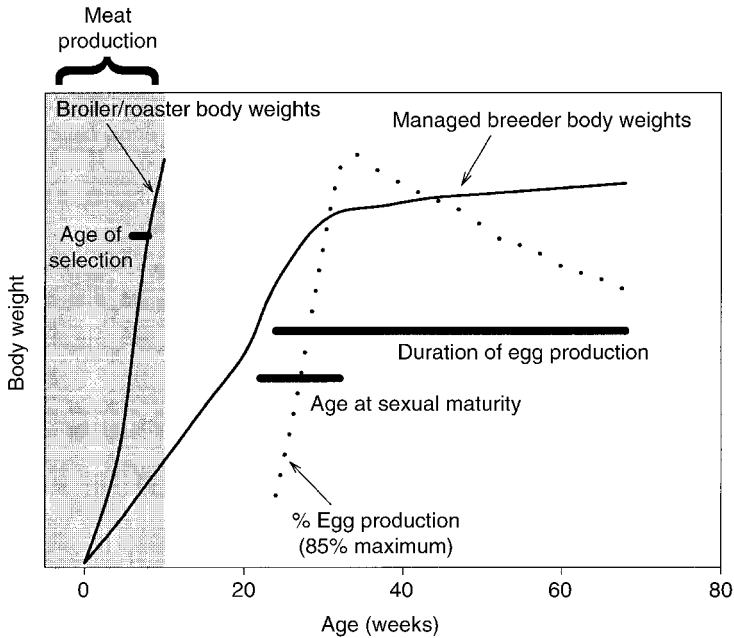
Fig. 2.1. A conceptualized comparison of the growth curve of broilers fed *ad libitum* until processing and the feed-restricted growth curve of the parent breeder stock. An ideal egg production curve is overlaid to provide an estimate of reproductive performance of the breeder hen. (Taken from Kerr *et al.*, 2001.)

be exacerbated because of the need for a more severe and early feed restriction in breeders selected for fast growth, so that both selection for leanness and more stringent feed restriction act together to delay the minimal fat stores needed to reach sexual maturity. On the other hand, in contrast with the age at first egg as negatively affected by excessive leanness, it was found in many experimental selection models that obesity was associated with poor egg production (Leclercq *et al.*, 1985; Cahaner *et al.*, 1986). A negative correlation of about 0.20 has been reported by Maeza (1983) between egg production and fatness. Furthermore, it has been shown by Cahaner *et al.* (1986) that hatchability of eggs produced by lean broiler breeders is higher than that by fat birds.

Comparisons of reproductive performances between fat and lean adult breeder hens are reported in several papers, but a straightforward comparison between selection strategies or even between different lines for similar selection procedures is not an easy task in view of the different feeding

programmes used (Buyse *et al.*, 1999). Since lean and fat lines are characterized by a different appetite, even as adult hens, comparisons of reproductive traits under *ad libitum* feeding conditions can be quite different from restricted conditions. It becomes even more complicated when the restrictions are imposed as a percentage of the *ad libitum* uptake or to the same absolute level for both lines. In the latter case a similar absolute feed allowance (in g day^{-1}) will impose a differential restriction for both lines.

When reproductive characteristics of the French low-fat (FRLF) and high-fat (FRHF) line breeder hens were compared under *ad libitum* conditions or similarly restricted during the laying period (85% of *ad libitum*), the FRLF hens of both feeding regimes were heavier and produced heavier eggs than the FRHF hens (Leclercq and Simon, 1982; Leclercq *et al.*, 1985). The number of eggs produced per hen was more or less similar for both lines and slightly higher in *ad libitum* conditions (over the 140-day experimental period), compared

with restriction for both lines. However, the FRLF hens produced heavier eggs, mainly due to a higher total albumen weight, although the percentage albumen dry matter and percentage protein were lower in eggs from the FRLF line compared with the FRHF line. The proportional weight of the yolk was higher in eggs from FRHF hens, even when corrected for the relationship between egg weight and yolk weight (Leclercq *et al.*, 1985). FRHF birds also differed from FRLF birds in exhibiting elevated plasma lipid and lipoprotein levels, irrespective of the nutritional state (Hermier *et al.*, 1984), which indicates an increased liver lipoprotein production and secretion in the FRHF lines, explaining the higher yolk proportion laid down in eggs of the FRHF lines. A similar number of eggs, but with a higher mean egg weight, resulted in an overall slightly but significantly higher egg output for the FRFL line, although the feed efficiency for egg synthesis for both lines was similar.

The Israeli (IS) lean and fat lines were also compared for their reproductive performances (Cahaner *et al.*, 1986). This study involved a similar feed restriction programme during rearing and laying period (absolute feeding level) for both lines, which may have resulted in a differential restriction for both lines. As for the French LF and HF lines, the IS lines differed significantly with respect to egg weight: the eggs of the ISLF line were heavier than those of the ISHF line. The percentage albumen did not differ between eggs from HF and LF hens but eggs of the latter hens contained more dry matter in the albumen as a percentage, as well as expressed in grams per egg. As for the FRHF line, the proportional yolk weight of eggs from the ISHF line was higher compared with eggs originating from their lean ISLF counterparts. Total number of eggs in the ISLF line tended to be higher and this, together with a higher egg weight, resulted in a significantly increased total egg mass output as well as better feed efficiency in the ISLF line.

While the French LF and HF lines did not differ much in egg number, this discrepancy with the Israeli lines in this correlated response may be due to the feeding strategies in these different experiments. While feed restriction decreased egg numbers in both FRLF and FRHF lines, the same amount of feed given to the ISLF and ISHF broiler hens probably resulted in a differential feed restriction, being more severe for the HF lines. This might explain the slightly higher egg number in the ISLF line, while no differences in number of eggs were found between the FRHF and FRLF lines fed *ad libitum* or at 85% of their *ad libitum*-fed counterparts. On the other hand, selection for leanness by selecting for improved FCR or low plasma lipoprotein concentrations resulted in a higher laying percentage of the lean birds under both restricted (Leenstra, 1988) and *ad libitum* feeding conditions (Whitehead, 1988). When *ad libitum* and restricted feeding were applied for FC (selected for feed efficiency between 3 and 4 weeks of age) and GL (selected for high body weight at 4 weeks of age) lines and compared in a single experiment, differences in laying percentage between both lines were more pronounced under *ad libitum* feeding conditions. This was due to a higher number of GL birds that were intermittently out of lay for a long period, as well as to the lower laying rate (short sequences) of actually laying GL birds. Feed restriction to a level similar to that for commercial broiler breeders (hence differential for GL and FC hens) restored to some extent the reproductive ability of both lines, but this was more pronounced for the GL line. Under both *ad libitum* and restricted feeding conditions, egg weight was significantly higher for the GL line, but total egg mass output was inferior compared with that of FC hens.

Taken together, these data indicate that correlated responses in reproductive abilities with selection for lean or rapidly growing and hence fat birds cannot simply be explained in energetic or mechanistic terms. These selection procedures affect endocrine mechanisms (recently reviewed by Decuypere *et al.*, 1999) that are at least partly involved in the selection goals (N-retention and growth, protein and lipid metabolism) but because of the multifunctional effect of hormonal parameters, and the interrelationship of these hormonal parameters with

other endocrine systems, this may be the basis of a number of correlated responses.

At this point, one aspect of the discussion may be added. After eight generations of divergent selection for exponential growth rate (EGR) from 0 to 14 days (EGR_{14}) of age or 0 to 42 days of age (EGR_{42}) among the previously mentioned Barbato lines (Barbato, 1992b), there was a fourfold difference in EGR_{14} and an eightfold difference in EGR_{42} between the divergently selected lines for each trait (Kerr *et al.*, 2001). Correlated traits contributing to reproductive fitness of the lines included: percentage hen-day egg production (HDP); fertility; and both age (AFE) and weight (WFE) of hens at first egg – estimated under *ad libitum* feeding in each generation. Hens from the line selected for fast EGR_{42} (42H line) were significantly heavier than those from the line selected for slow EGR_{42} (42L line) and came into production 31 days earlier. Among the lines, only the 42H hens exhibited a continual decline in HDP. Both the AFE and WFE were most different among lines divergently selected for EGR_{42}. The AFE and WFE of both EGR_{14} selected lines were the same. Further, the average values for AFE and WFE of the EGR_{14} lines were intermediate to the mean values for the EGR_{42} lines, suggesting a critical period of development between 14 and 42 days of age influencing these traits and the onset of sexual maturation (Kerr *et al.*, 2001). Curiously, fertility diminished rapidly in the line selected for slow EGR_{14} while HDP was unchanged. These observations clearly warrant more study, again suggesting very different physiological mechanisms underlying genetic and nutritional aspects of egg production.

Fertility

It is known that the fertilizing ability of roosters from commercial broiler breeders is declining continuously with each generation (Pollock, 1999). Since the 14L line exhibited a similar decline in fertility, it became a model for identifying genetic factors underlying the poor fertility among commercial poultry. In order to assist in this investigation an *in vitro* assay of sperm–egg binding was developed as an indicator of the potential fertility of roosters (Barbato *et al.*, 1998). Many broiler-type lines (both commercial and experimental) have been tested to determine the relationship between performance of sperm in the *in vitro* assay and fertilizing ability of the sperm. The correlation, across all lines, between the *in vitro* assay and fertility ranges between 0.75 and 0.85, but is not linear and appears to be most predictive of fertility (or, rather, infertility) among poor-binding individuals (Barbato *et al.*, 1998; Barbato, 1999). Curiously, one of the molecules involved in the sperm-binding process has been identified as being related to the saposin family of molecules. Further, a synthetic peptide made to mimic the structure of the native molecule has been shown to increase the binding of sperm from 'bad' males and increase both the fertility and number of chicks that can be obtained from the sperm of that male (Hammerstedt *et al.*, 2001). These data also serve as a reminder that biotechnology exists outside the cell nucleus.

Summarizing Conclusions and Ethics of Further Selection for Performance in Broiler Selection

There are still considerable possibilities for improving broiler production:

- By extending the period for near-maximal growth rate.
- By partitioning towards lean rather than fat, but without structural and/or functional characteristics, probably linked with a reduced ME_m, in order to avoid metabolic disorders and without going so far that excessive leanness together with the necessary feed restriction in broiler breeder raising will result in unacceptable retardation of age of puberty.
- By applying novel biotechnologies to improve reproduction, including the

identification of genes reducing reproductive effectiveness and the development of assisted reproductive techniques to increase chick output.

There is no point in manipulating growth to increase leanness to the point where it leads to deterioration in the quality of the carcass – not as it is perceived by the producer, but as perceived by the consumer. Manipulation of animal production by breeding has to be acceptable in terms of animal welfare. Ethical concern may arise from the manipulation of body size or function in such a way as to increase metabolic diseases.

Therefore, further breeding should consider not only how to increase production and production efficiency, but also how to alleviate correlated side effects by extending or changing selection goals for obvious economic reasons and also because of the unacceptability of some correlated responses to an increasing number of people. Whether the selection goals have to be extended by additional criteria or changed to settle for lower productivity levels will depend on the answer to the questions asked as to what extent the dilemmas discussed here are merely hazards from a selection policy as applied until now, or are causally linked and hence inevitable consequences of the applied selection goals. In the latter case there will be an increasing pressure to settle for lower bird productivity levels. It is of the utmost importance for selection companies to find out the answers to these dilemmas, and breeding companies will need to achieve a better understanding of the biological backgrounds and mechanisms of what they are selecting for as well as the biological obstacles on the road to increasing overall bird performance. This chapter was aiming for these objectives.

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3 Skeletal Problems Associated with Selection for Increased Production

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Introduction

This chapter examines the role of poultry breeding technology as it relates to the skeletal system and how selection for increased productivity has affected: (i) skeletal integrity and mineralization in layers; and (ii) bone, tendon and ligament growth and strength and the development of musculoskeletal disease in meat-type poultry.

Since the early 1950s, poultry breeding has focused on increasing profitability, with little regard for the effect on the skeletal, respiratory or cardiovascular systems or the well-being of the bird. In table-egg laying strains, intensive selection for egg output has resulted in birds of low body weight that lay a large number of eggs on a low food intake. Over a laying year, the amount of calcium these birds can deposit in their shells can be up to 20 times their total body content. Bone acts as a store for much of the calcium deposited in shells, and it is perhaps not surprising that stresses in bone formation and resorption can result in skeletal problems in these birds.

In broilers, the keen competition between commercial breeding companies coupled with the high heritability of growth has resulted in a dramatic increase in the growth potential of broilers over the last 40 years. Thus the daily gain at maximum growth rate has increased from 22 g in 1960 (Sørensen, 1986) to 65 g by the mid- to late 1990s (Havenstein et al., 1994; Emmerson, 1997). Assuming a coefficient variance of 10% and a heritability of 0.4, this means that the modern broiler population has moved 18 genetic standard deviations from its origin in terms of growth capacity. The same pattern can be seen for the turkey (Abourachid, 1991) and, to a lesser extent, for meat-type ducks. As growth rates have risen, skeletal defects manifesting themselves primarily as leg disorders have become increasingly prevalent in broilers and, to a lesser extent, in turkeys.

Two factors are now causing a rethink in the commercial emphasis on intensive selection for growth or egg production. First, the economic cost of lost egg production in layers and mortality and morbidity in broilers resulting from skeletal defects has risen. Secondly, there is an increasing concern in many countries at the welfare implications of these disorders for the birds. As a result, there is now renewed interest in determining the genetic basis for these disorders, establishing the genetic link between these disorders and egg production

or growth and devising genetic or other strategies for minimizing the occurrence or severity of the disorders.

Skeletal Problems in Table-egg Layers

Main causes of abnormality

The main skeletal problems in table-egg laying hens are associated with loss of bone mineral during the laying period, with poor bone quality, bone fracture, paralysis and death being the main consequences. The loss of bone mineral may have two causes. One cause is osteomalacia, which is characterized by defective mineralization of bone tissue, with thick seams of poorly mineralized organic matrix. Osteomalacia is primarily associated with nutritional deficiencies of calcium, phosphorus or vitamin D and has not been shown to have a genetic component. Osteoporosis is the other, more widespread, condition involving bone loss (Randall and Duff, 1988); it has a complex aetiology, with many factors involved.

Osteoporosis in laying hens is defined as a decrease in the amount of fully mineralized structural bone, leading to increased fragility and susceptibility to fracture. Bone loss characteristic of osteoporosis was first described in caged laying hens by Couch (1955), who reported a problem termed 'cage layer fatigue' involving bone brittleness, paralysis and death. Cage layer fatigue can be an extreme consequence of loss of structural bone in the vertebrae that leads to spinal bone collapse and paralysis (Urist and Deutsch, 1960; Bell and Siller, 1962). Generally, however, osteoporosis is not so severe as to result in cage layer fatigue but nevertheless the widespread structural bone loss can lead to high incidences of fractures at various sites throughout the skeleton. There may also be another, more transient, form of cage layer fatigue perhaps related more to an intracellular calcium imbalance that impairs neuromuscular function. This may occur when calcium intake is inadequate in relation to the productive needs of

the bird, resulting in loss of egg production, paralysis and increased mortality and subsequent susceptibility to osteoporosis among survivors (Cransberg *et al.*, 2001). This form of cage layer fatigue contrasts with the more typical chronic osteoporosis, the severity of which does not affect egg output (Rennie *et al.*, 1997).

Gregory and Wilkins (1989) reported results of a survey of end-of-lay battery hens in the UK in which 29% of hens had one or more broken bones during their lifetimes. The fractures occurred during their time in their cages or during depopulation, transport to a processing factory and hanging on shackles. By the time they reached the end of the evisceration line, 98% of carcasses were found to contain broken bones. Ischium, humerus, keel and furculum showed highest fracture incidences, with pubis, ulna, coracoid and femur also breaking frequently. Different fractures can occur at different stages in the bird's life. In a survey of European flocks, Gregory *et al.* (1994) reported that 17% of birds experienced fractures during battery cage life (old breaks) and 10% during depopulation, transport and hanging on shackles (new breaks). Fractures to the humerus and ulna are frequent old breaks. Damage to the keel can arise from contact with the cage front during depopulation and femoral fractures arise commonly during shackling.

The origins of osteoporosis are not well defined. It has been suggested that the problem is partly genetic in origin, resulting from the breeding of lightweight, energetically efficient birds that maintain a high rate of lay over a prolonged period on a low food intake (Whitehead and Wilson, 1992; Cransberg *et al.*, 2001). Most modern hybrid layer strains seem to be susceptible to osteoporosis, but older unimproved strains, such as the Roslin J-line Brown Leghorn, are relatively resistant (Rennie *et al.*, 1997). However, even in susceptible strains there can be wide individual variation, with some hens retaining good bone quality at end of lay. Confining birds in cages with limited opportunity for exercise has undoubtedly contributed to the problem, resulting in a form of disuse osteoporosis.

Characteristics of osteoporosis

Some of the characteristics of laying hen osteoporosis have been reviewed previously (Whitehead and Wilson, 1992; Whitehead and Fleming, 2000). Osteoporotic hens show evidence of widespread loss of structural bone throughout the skeleton. This loss has been shown to start when hens reach sexual maturity and to continue throughout the laying period (Wilson *et al.*, 1992) so that osteoporosis is most severe in hens at the end of lay. The observations are consistent with the theory that at the onset of sexual maturity the rise in circulating oestrogen results in a switch in bone formation from structural to medullary bone. The latter serves as a labile source of calcium for shell formation and has little intrinsic strength, but can contribute to overall fracture resistance of bones (Fleming *et al.*, 1998a). Progressive resorption of structural bone during medullary bone turnover leads to osteoporosis. Evidence for suppression of structural bone formation in laying hens has been provided by Hudson *et al.* (1993) who observed that fluorochrome label was not incorporated into cortical bone. This finding has been confirmed by Whitehead and Fleming (2000), who also observed that loss of reproductive condition induced by forced moulting resulted in rapid loss of medullary bone and resumption of structural bone formation.

As osteoporosis progresses, cortical bone thickness is decreased and there is a less cohesive system of fewer, thinner and less well-connected trabeculae. This is reflected in lower breaking strengths, as measured by the three-point bending test. Amounts of structural bone can also be quantified by computerized histomorphometric analysis, or by radiography of bones that contain little or no medullary bone. Other measures that have been made to assess osteoporosis include cancellous bone volumes of the free thoracic vertebra (FTV) and proximal tarsometatarsus (PTM), radiographic densities of humerus and keel and breaking strengths of humerus and tibia. Whitehead and Wilson (1992) proposed a system for assessing the severity of osteoporosis based on FTV trabecular bone volume, with values above 16% being normal, 14–16% moderate, 8–11% severe and < 8% very severe. Nodulation and deformity of ribs has also been used to assess osteoporosis severity (Cransberg *et al.*, 2001).

Patterns of bone loss with age vary considerably between different bones (Whitehead and Fleming, 2000). Striking changes occur during the first 10 weeks of sexual maturity. There is a marked loss of cancellous bone in both the PTM and FTV suggesting that, in these bones, the major development of osteoporosis occurs within a few weeks of the onset of egg production. Over this period there is also a rapid accumulation of medullary bone in the PTM but at present there is no indication as to whether the loss of cancellous bone is directly linked to the formation of medullary bone. After 25 weeks the further loss of cancellous bone from the FTV is smaller but loss of cancellous bone and the accumulation of medullary bone continues in the PTM, though also at reduced rates. However, there is a continuous net increase in the total amount of bone, measured histomorphometrically, with the result that total bone volume in the PTM can be greatest at the end of lay.

Tibial radiographic densities and breaking strengths, both measured in the midshaft region, increased over the period 15–25 weeks. The laying hen tibia is known to contain appreciable amounts of medullary bone and the increase in radiographic density would be consistent with this occurrence. The small increase in tibial breaking strength between 15 and 25 weeks could thus indicate some accumulation of medullary bone but relatively little loss of structural bone. The major decrease in bone strength between 25 and 50 weeks implies considerable loss of structural bone, which in the midshaft region of the tibia is mainly cortical bone. These changes are illustrated in Fig. 3.1, which shows cross-sections of tibias, with greater cortical thickness in osteoporosis-resistant than in susceptible hens. For comparison, cross-sections from male and 12-week-old pullet tibias are also shown. These observations contrast with the
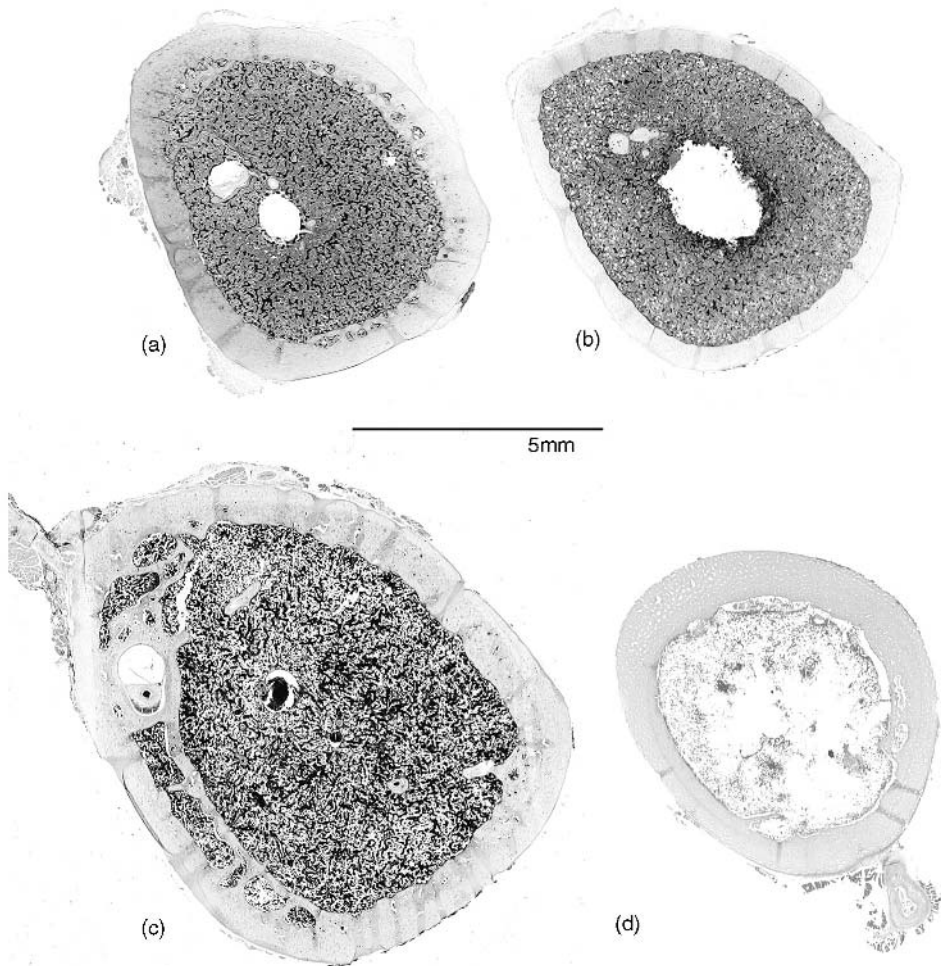
Fig. 3.1. Sections of tibia midshafts from (a) osteoporosis-resistant hen, (b) osteoporotic hen, (c) adult male and (d) 12-week-old pullet.

findings in the PTM that major structural bone loss occurred within 10 weeks of sexual maturity and suggest that different principles may apply to structural bone resorption from epiphyseal and midshaft regions of leg bones. An important factor in this difference may be the greater resorptive surface areas of bone contained in the trabecular structures in epiphyseal regions than in the diaphyseal cortex.

The increases in humerus radiographic density and breaking strength observed between 15 and 25 weeks may be accounted for by the development in some birds of medullary bone in bones that are normally pneumatized, together with the absence of any appreciable loss of structural bone over this period (Fleming *et al.*, 1998a). The subsequent large decline in breaking strength after 25 weeks would suggest that in this bone, as in the tibia, the major onset of osteoporosis occurs at a later age than for the PTM or FTV.

The radiographic density of the keel can also decline, particularly at the leading edge of this bone adjacent to the rest of the sternum. A more general increase in keel radiographic density seen later in the laying period is consistent with the known presence of medullary bone and does not give

any insight into possible changes with time in the structural component.

Environmental Factors in Osteoporosis

Effect of housing

The effects of load-bearing and bio-mechanical forces in stimulating bone formation and remodelling are well established (Lanyon, 1992). Induced inactivity has been shown to accelerate osteoporosis in birds (Nightingale *et al.*, 1972) and the relative lack of activity of battery-caged hens accounts for the severity of the problem in these birds. Effects of exercise and alternative housing systems have been widely studied as potential means of alleviating osteoporosis.

Effects of exercise as a way of stimulating bone growth during rearing have been studied, but neither housing birds in pens nor giving extra exercise through use of a carousel have improved bone quality at start of lay compared with cage rearing (Whitehead and Wilson, 1992).

Changes in bone quality during the laying period are influenced by the nature of the exercise involved. Housing hens in pens has resulted in little change in spinal trabecular bone (Wilson *et al.*, 1993). Fitting perches to cages resulted in small improvements in PTM trabecular bone volume, but no benefit in tibia strength (Hughes *et al.*, 1993). More vigorous exercise than is obtained by walking or hopping on to low perches is needed to improve bone quality markedly. This was demonstrated by Knowles and Broom (1990), who found superior tibia and humerus breaking strengths in birds housed in terrace or perchery systems rather than in cages. The improvement in humerus strength was particularly apparent in the perchery system that allowed birds to fly. Confirmation of these findings came from a more detailed study by Fleming *et al.* (1994). It can be concluded that, as in other species, biomechanical effects on individual bones in hens are dependent upon the degree of strain experienced by the bone.

There is little information on the mechanism by which exercise improves bone characteristics in the hen. However, the finding by Newman and Leeson (1998) that tibial strength increased within 20 days of transfer of hens from cages to an aviary suggests that the mechanism may involve stimulation of structural bone formation, rather than inhibition of resorption.

There have been several studies to determine the welfare impact of the improved bone strength of birds kept in alternative housing systems. Lower incidences of new breaks have been found in birds depopulated from aviary or free-range systems compared with battery cages (Gregory *et al.*, 1990; Van Niekerk and Reuvekamp, 1994), but the incidences of old breaks, particularly in the furculum and keel, were higher with aviary and free-range systems (Gregory *et al.*, 1990). It may be concluded that allowing birds more exercise in alternative systems will improve bone strength, but this does not necessarily improve bird welfare in proportion.

Effect of nutrition

Nutritional deficiencies of calcium, phosphorus or cholecalciferol have been shown to result in bone loss attributable to osteomalacia (Wilson and Duff, 1991) and are likely to lead ultimately to greater severity of osteoporosis. However, there is no evidence that avoidance of osteomalacia can prevent the development of osteoporosis. Rennie *et al.* (1997) reported that none of the dietary factors tested over a laying year had any effect on the proportions of cancellous bone in spinal (FTV) or leg (PTM) bones but that treatments involving a particulate source of calcium (oystershell) or supplementation with fluoride increased the proportions of medullary bone. Providing calcium in particulate form extends the period of calcium absorption later into the night when shell formation is taking place and has been shown to have beneficial effects on shell quality. The finding that provision of calcium with improved

digestive characteristics can increase the amount of medullary bone without having much impact on the loss of structural bone shows that calcium deficiency is not a primary cause of osteoporosis. Fluoride is known to stimulate bone formation in chickens (Lundy *et al.*, 1992) but the increase in synthesis in hens during lay is evidently confined to medullary bone. Confirmation of the practical benefits of particulate calcium sources has been provided by Fleming *et al.* (1998b), who found that feeding limestone particles resulted in improved bone strength in older hens. This effect was probably attributable to the observed increase in medullary bone formation, because the treatment had little effect on cancellous bone volumes. A subsequent study (Fleming *et al.*, unpublished) provided evidence of a beneficial effect of particulate limestone on cortical as well as medullary bone content. Increasing the medullary bone content by nutritional means may thus benefit skeletal quality in two ways: (i) by a direct effect on bone strength; and (ii) from a possible protective effect on cortical bone resorption, as discussed later.

These observations are consistent with the hypothesis that osteoporosis in hens arises as a result of cellular processes rather than nutrient supply and that during the laying period there is continued osteoclastic resorption but little formation of structural bone. The balance of cellular activity can be altered by feeding bisphosphonate, a drug used in human medicine to combat postmenopausal osteoporosis. This acts by inhibiting the action of osteoclasts and has been shown to slow the loss of cancellous bone in hens (Thorp *et al.*, 1993a), but its use is unlikely to be a practical solution for osteoporosis in laying hens.

Genetic Factors in Osteoporosis

Heritability of bone characteristics

A strong genetic component in osteoporosis has been demonstrated by Bishop *et al.*

(2000), who studied the inheritance of characteristics related to osteoporosis over five generations in a commercial pure line of White Leghorns previously selected for high egg production. Initially, measurements were made on a range of morphometric, radiological and strength characteristics of different bones in hens at the end of the laying period to determine heritabilities. Morphometric traits involving cancellous and medullary bone volumes were found to be poorly heritable (FTV cancellous bone volume, $h^2 = 0.19$; PTM cancellous bone volume, $h^2 = 0.0$). This was considered surprising in view of the use of cancellous bone to assess severity of human postmenopausal osteoporosis (Khosla *et al.*, 1994) and as a criterion in earlier laying hen studies (Whitehead and Wilson, 1992; Wilson *et al.*, 1993; Rennie *et al.*, 1997). In contrast, heritabilities of other characteristics were higher (tibia strength, $h^2 = 0.45$; humerus strength, $h^2 = 0.30$; keel radiographic density, $h^2 = 0.39$). There was also a positive correlation between body weight and bone strength.

Selection study

On the basis of the above heritabilities, it has proved possible to select hens for stronger bones (Bishop *et al.*, 2000). A restricted selection index designed to improve bone characteristics, yet hold body weight (BW) constant, was derived from genetic parameters obtained from the preliminary analyses, using standard selection index theory. Three biologically meaningful and moderately to highly heritable traits that could be measured in a short period of time on a large number of hens at the end of lay were included in the bone index (BI), namely keel radiographic density (KRD), humerus strength (HSTR) and tibia strength (TSTR). By including characteristics of wing, leg and axial skeleton, this index gave a wide representation of the overall skeleton. The initial index was: BI = 0.27 × KRD + 0.37 × HSTR + 0.61 × TSTR − 0.25 × BW. The coefficient for body weight was later increased

to 0.35 to counter a slight divergence in this trait that started to appear between the lines. Males were selected on the basis of family breeding values. Selection was performed retrospectively each year, with chickens hatched and raised from all available hens in the experiment.

Genetic parameters for the traits in the BI, and the BI itself, showed that all traits were moderately to highly inherited throughout the study, with the heritability of the BI being 0.4 (Table 3.1). The genetic and phenotypic correlations also show that the three bone measurements in the index were moderately to strongly correlated with each other. Finally, the bone measurements are all positively correlated with body weight, indicating that selection for improved bone strength characteristics alone, without the restriction placed on body weight, would have resulted in considerably heavier birds. Different mean values in the bone strength measurements in different years indicated that these traits were strongly affected by environmental factors, raising the possibility of genotype-by-environment interactions. However, comparison of full-sib flocks reared on different locations gave little evidence for genotype-by-environment interactions, within the range of environments investigated.

From the first year of selection using the index, the high (H) and low (L) BI lines diverged progressively for KRD, HSTR, TSTR and the BI in the desired direction.

The differences were highly significant ($P < 0.01$) from the second generation onwards, with the exception of HSTR in generation 2 where the difference, although in the desired direction, was not significant. Bone characteristics of the two lines are shown in Table 3.2. For the hens, the lines differed by 17% for KRD, 30% for HSTR and 60% for TSTR after five generations. Although selection was based on measurements made on hens, selection was also found to affect bone strength in males, with H-line males being superior to L-line males for all traits. The differences between the lines in the fourth generation were: KRD 13%, HSTR 26% and TSTR 19%. Body weight at slaughter did not differ between the lines in either sex. All bone measurements were strongly correlated with the presence/absence of breakages; the incidences of humeral fractures in hens occurring during the production period and depopulation showed a sixfold difference between the lines after four generations. These findings confirm that genetically improving bone strength will indeed decrease the incidence of bone fractures.

Correlated responses

Performance

Daily feed intake did not differ between the selected lines. Likewise, measurements of

Table 3.1. Heritabilities[a] (with standard errors), phenotypic correlations and genetic correlations (with standard errors) for body weight and bone traits ($n = 1306$) (Bishop *et al.*, 2000).

	Body weight	Keel radiographic density	Humeral strength	Tibial strength	Bone index
Body weight	0.49 (0.06)	0.28	0.21	0.29	−0.10
Keel radiographic density	0.36 (0.06)	0.39 (0.06)	0.33	0.51	0.58
Humeral strength	0.26 (0.11)	0.49 (0.11)	0.30 (0.06)	0.50	0.66
Tibial strength	0.33 (0.10)	0.66 (0.08)	0.77 (0.07)	0.45 (0.06)	0.81
Bone index	−0.12 (0.12)	0.67 (0.08)	0.76 (0.07)	0.84 (0.04)	0.40 (0.06)

[a]Heritabilities on diagonal, phenotypic correlations above diagonal, genetic correlations below diagonal.

rate of egg production and egg weight did not show any differences between the lines. These genetic observations complement the observations of Rennie *et al.* (1997) that the large individual variation observed in the bone characteristics of hens at the end of lay was phenotypically unrelated to egg production in a flock of highly productive hens.

The main production difference between the lines was in shell quality, with H-line hens laying eggs that had thinner, weaker shells. This was reflected in a greater incidence of cracked and second-quality eggs. The difference in shell output per day was very small (0.1 g), but over the laying life of the hen this could represent a difference in calcium output of 12 g. This is large in

relation to the total body bone calcium content of a hen (about 80 g) and could be expected to have an impact on bone content. Negative phenotypic correlations between shell quality and bone (tibia) strength have previously been reported by Orban and Roland (1990), who speculated that this suggested resorption of cortical bone as well as medullary bone during shell calcification.

Bone structure and composition

Differences between the lines in bone characteristics of hens after four generations are shown in Table 3.3. Tibial cortical width differed between the lines at 15 weeks, suggesting that there was more bone formation in the H line during the rearing period.

Table 3.2. Bone characteristics and body weights at the end of the laying period in female and male chickens[a] divergently selected for high (H) or low (L) bone index.

| | Females | | | Males | | |
| | Line | | | Line | | |
	H	L	*P* value	H	L	*P* value
Body weight (kg)	1.80	1.79	NS	2.25	2.21	NS
Keel radiographic density (mm Al equivalent)	0.41	0.35	< 0.001	0.70	0.62	< 0.001
Tibia strength (kg)	38.2	23.7	< 0.001	60.6	51.0	< 0.001
Humerus strength (kg)	17.9	13.6	< 0.001	36.8	29.2	< 0.001

[a]Data for females are from fifth generation of selection, for males from fourth generation.
NS, not significant.

Table 3.3. Bone and plasma characteristics at different ages of H- and L-line hens.

Trait	Age (weeks)	H line	L line	*P* value
Tibia cortical width (mm)	15	0.465	0.448	
	70	0.422	0.365	< 0.001
PTM medullary bone (%)	70	7.83	6.39	< 0.02
Tibia midshaft medullary bone area (mm^2)	70	0.216	0.180	< 0.05
Osteoclasts (/mm^2 medullary bone)	70	979	1175	< 0.10
Collagen content of bone (%)				
Humerus	70	18.68	17.78	NS
Tibia	70	15.54	15.73	NS
Pyrrolic cross-links (mol pyrrole/mol collagen)				
Humerus	70	0.47	0.27	< 0.001
Tibia	70	0.50	0.44	< 0.05
Plasma pyridinoline (pmol μl^{-1})	70	0.39	0.46	< 0.05
Plasma oestradiol (pg ml^{-1})	70	467	438	< 0.10

NS, not significant.

This was consistent with higher plasma concentrations of osteocalcin, a marker of bone formation, during the growing period (Fleming *et al.*, 2001). Tibial cortical thickness declined in both lines over the laying period, but the relative decrease by 70 weeks was almost fourfold greater in the L line. This finding indicates a much higher rate of cortical bone resorption in the L line. This conclusion is supported by the greater plasma concentration in this line of pyridinoline, a breakdown product from the collagenous matrix of bone, and is perhaps explained by the greater number of osteoclasts per unit of bone (Fleming *et al.*, 2001). In contrast, the amount of medullary bone was greater in the H-line. Other differences were also seen in collagen biochemistry. Although total bone collagen did not differ between the lines, the number of pyrrolic cross-links were greater in the bones of the H line hens (Sparke *et al.*, 2002). This could be expected to give a more cohesive and resilient structure in the matrix.

Genetic Basis for Osteoporosis and Future Selection Strategies

It is apparent that there are many changes in the bone composition of osteoporotic hens and thus the condition is likely to be polygenic in origin. The three main differences suggested by the selection study are in rates of bone formation and resorption and composition of bone matrix. Bone quality is a factor in avian osteoporosis, with greater cross-linking in the collagen matrix contributing to improved bone biomechanical properties. Collagen genes (e.g. *COL1A1*) have also been linked to human osteoporosis (Grant *et al.*, 1996). However, factors influencing bone mineralization may be more important. Osteoporosis-resistant hens show increased structural bone formation during growth, suggestive of greater osteoblast activity. A carry-over of this effect may also account for the greater amount of medullary bone accumulated during the laying period. The greater

structural bone resorption in osteoporotic hens over the laying period may be explained by the larger number of osteoclasts. The lower proportion of medullary bone in these birds may contribute to osteoporosis in two ways. Firstly, medullary bone does contribute to overall bone strength (Fleming *et al.*, 1998a). Secondly, medullary bone occurs as spicules within cortical bone cavities and also as a lining on structural bone surfaces. A less complete lining of structural bone will allow more osteoclasts to come directly into contact with, and resorb, structural bone (Fig. 3.2). An imbalance between osteoblast and osteoclast activities may thus explain avian osteoporosis.

Oestrogen is known to affect the coupling between bone formation and resorption and the difference observed between the lines in the circulating concentrations of this hormone (Fleming *et al.*, 2001) may indicate a systemic factor influencing avian as well as human postmenopausal osteoporosis.

At the moment, the best explanation for avian osteoporosis is based mainly on osteoclast activity in the bone. Influences of other organs involved in calcium uptake or excretion can perhaps be discounted on the basis of observations in both males and females. In hens, there is an obvious conflict between bone and shell quality, suggesting a problem in calcium partitioning between these body components. This conflict could be explained by effects in either bone or shell gland. Decreased bone resorption resulting from lower osteoclast activity in the H line could account for the decrease in shell quality observed in this line, since calcium mobilized from bone is an important source of calcium for shell formation, especially towards the end of the period of shell formation in the shell gland when the supply of calcium from digestion has diminished greatly. Alternatively, a genetic defect in calcium transport or deposition in the shell gland could depress the need for bone resorption. However, the finding that parallel bone changes have occurred in males during the selection suggests that an effect in the shell gland might be discounted as the main factor. Likewise, an effect at the level of
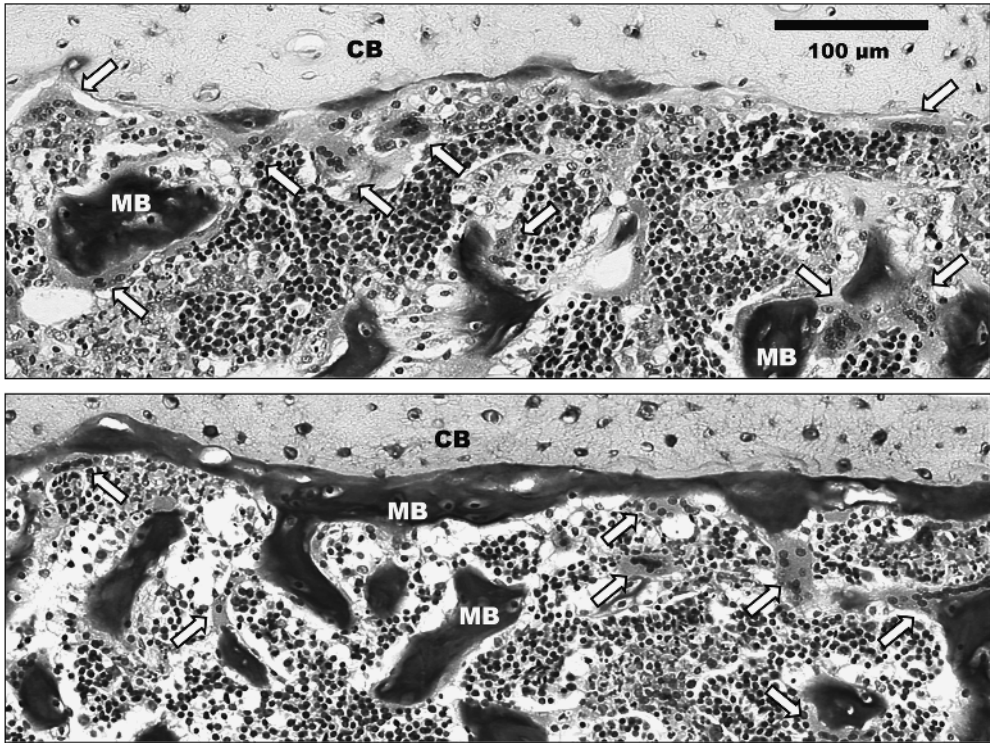
Fig. 3.2. Illustration of the protective 'lining' effect of medullary bone (MB) on endostosteal surfaces of cortical bone (CB) in the tibia midshaft. In section from bird (a), multi-nucleated osteoclasts are arrowed and actively resorb CB surface when there is no lining of MB. In the section from bird (b), osteoclasts are prevented from coming into contact with CB and can only resorb MB. Tartrate-resistant acid phosphatase (TRAP) stain, toluidine blue counterstain.

the gut on calcium absorption or retention also seems unlikely. Better retention could be associated with higher bone mass in males, but would not explain the conflict between partitioning of calcium between bones and shells in hens.

Genetic selection seems to offer the best prospects for improving bone quality and resistance to osteoporosis in hens. The responses to selection show that it is possible to create sizeable differences in bone strength characteristics of hens in a relatively short period of time, using conventional selection techniques. This response can also be made to be independent of body size, should that be the desire of the breeder, though faster progress could be made if body weight were to be allowed to increase. Moreover, the absence of significant or

meaningful genotype-by-environment interactions implies that selection progress seen in a breeding flock should also be expressed under commercial egg production conditions. Selection also gives more far-reaching effects than can be achieved nutritionally. Nevertheless, good nutrition will remain imperative to minimize this component of osteoporosis.

The selection procedure used to date has involved retrospective selection on the basis of hen post-mortem data but this is not practical for application to commercial breeding programmes. A predictive method would be more efficient. A procedure based on digitized fluoroscopy (DF) has shown that it is possible to predict end-of-lay bone characteristics by radiographic methods (Fleming *et al.*, 2000). Radiographic

measures are best made on the humerus because this bone is usually pneumatized and thus free from medullary bone. The DF results showed that measurements made as early as 40 weeks gave a good indication of end-of-lay humeral strength and also some indication of tibia strength. *In vivo* selection on the basis of radiological measures could thus be expected to result in improved bone quality. The equipment used for the DF measurements was rather too bulky for easy on-farm use. Newer, more portable X-ray scanners are becoming available for use in human osteoporosis assessment but initial evaluation of these has suggested that the need for immobilization of a bird over a greater time than was required for the DF method would be a disadvantage.

Biochemical differences were also identified between the lines. However, the differences in plasma oestradiol concentrations between the lines were not sufficiently large to suggest that selection based on this criterion would be sufficiently reliable. Plasma pyridinoline concentration could perhaps be a more effective biochemical criterion among those considered to date, particularly as its likely relationship with bone resorption is central to the basis for osteoporosis. But all of these predictive measures require the bird to be in lay, probably well into the laying period, before the assessment can be made. The future direction of poultry breeding is moving towards the use of marker-assisted selection (MAS). This method of selection depends upon the identification of genetic markers for the desired trait and can be implemented very early in the life of the young chick. As suggested above, it is likely that laying hen osteoporosis is polygenic in origin. Identification of the specific genes associated with osteoporosis and their association with quantitative trait loci for bone quality could lead to the development of markers that could form the basis for commercial MAS for resistance to osteoporosis. It will also be important to establish a method, either genetic or nutritional, of overcoming the apparent inverse relationship between bone and shell quality to encourage poultry breeders to implement this selection.

Skeletal Problems in Meat-type Poultry

Causes of abnormality

Many skeletal defects in meat-type chickens, turkeys and ducks have been related to rapid gain in body weight, particularly during the early growing period (Riddell, 1992; Lilburn, 1994; Velleman, 2000), and are therefore more frequent in male birds. They may be caused by a combination of rapid growth, which produces bone, cartilage, tendon and ligament of poor structural quality and low tensile strength (Rath *et al.*, 2000), and high body weight, which results in mechanical stress. Rupture of the gastrocnemius tendon (Riddell, 1997), rupture or avulsion of the intertarsal ligament (Julian, 1984b) or common reticulum (R. Crespo, 2001, personal communication), femoral fracture in turkeys (Julian and Gazdzinsky, 2000), separation of the proximal femoral epiphysis (Thorp, 1992; Julian, 1998), epiphyselysis and cartilage defects in the femoral head and hip joint (Julian, 1985; Riddell, 1992) and rupture of the peroneal muscle (Julian and Gazdzinsky, 2000) are examples of problems related to rapid growth and stress on tissues with inadequate tensile strength. Leach and Gay (1987) maintained that genetic selection for increased frame size and muscularity produced birds with a posture that prevented them from walking or even standing for extended periods. The stress this caused on the cartilage, bones and tendons of weight-bearing limbs frequently resulted in leg disorders. In the case of tibial dyschondroplasia (TD), it is likely that rapid growth is part of the cause of the initial lesion but that body weight of the bird contributes to the development of bone deformity and subsequent lameness (Riddell, 1975). Rapid growth may be the main factor in defects occurring up to about 4 weeks of age, after which weight produces more stress on the bones, tendons and ligaments, particularly in turkeys.

There may be a number of mechanisms by which rapid growth affects skeletal development. Rapid growth increases the

requirement for oxygen, nutrients, enzymes, hormones, inductive agents, growth factors, etc. The pathogenesis of some rapid growth problems may be related to the high requirement of specific nutrients such as vitamin D_3, since deficiency may develop in the fastest-growing birds in a group, even when an adequate general level is present in the diet. At the cellular level, there may be inadequacies in the production of growth factors (Farquharson and Jefferies, 2000) or in cell receptors or other signalling mechanisms needed for rapid tissue growth or cell proliferation. These cellular problems could affect chondrocyte development and differentiation in the bone growth plate (Thorp *et al.*, 1995; Rath *et al.*, 2000; Reddi, 2000; Velleman, 2000). For instance, decreased numbers of vitamin D receptors on chondrocytes might contribute to TD and explain why dietary 1,25-dihydroxycholicalciferol reduces TD even when there is apparently an adequate amount of vitamin D_3 in the diet (Rennie *et al.*, 1993; Berry *et al.*, 1996). Valgus–varus deformity (VVD) may be primarily a lack of remodelling caused by continuous rapid growth. Rapid growth also reduces immune function and this may explain why skeletal problems associated with bacterial osteomyelitis/synovitis have become more frequent in meat-type poultry.

Nutrition can contribute to skeletal problems. For instance, high-protein diets result in a higher incidence of VVD, even when they do not increase growth (Julian, 1994). However, nutrition is commonly used to counter skeletal problems by slowing growth, particularly in the first 10–14 days of age (Classen and Riddell, 1989; Classen, 1992). Growth rate reduction can be achieved by feeding diets of low nutrient content, particularly metabolizable energy or protein content, or qualitative or quantitative feed restriction. The effectiveness of these nutritional approaches confirms the strong phenotypic relationship between fast growth and many skeletal defects.

Not all skeletal defects are growth related. Scoliosis and other vertebral deformities may be genetic or congenital and they may contribute to VVD. Environmental

factors (improper egg storage, long storage time affecting yolk position, hot or cold incubation temperature) can cause or contribute to skeletal defects. Rotated tibia, splayed legs (straddled, spinners), primary slipped tendon and crooked toes are the result of mechanical or environmental forces associated with sloped or slippery hatch trays or pen conditions, injury or unknown factors. Birds provided with roosts rarely develop crooked toes.

The following sections will describe the nature of skeletal defects that are thought to be associated with fast growth and then consider the genetics of leg disorders in broilers, with a view to addressing two main questions.

1. What type of genetic mechanism should be expected?
2. To what extent are the various leg disorders inherited and genetically correlated with growth rate?

Growth-related skeletal defects

Tibial dyschondroplasia

Dyschondroplasia is the name of a specific form of growth-plate abnormality in which transitional (prehypertrophic) chondrocytes accumulate, forming a mass of avascular cartilage underlying the layer of proliferative chondrocytes. Large lesions are most frequent in the proximal tibia (Walser *et al.*, 1982), hence the condition is usually called tibial dyschondroplasia (TD). However, lesions can also occur beneath the growth plate of other long bones, primarily the proximal metatarsus in broilers and ducks and proximal femur in turkeys, and in articulating vertebrae (Riddell, 1992; Julian, 1998). Lesions develop at about 2–3 weeks of age in broilers but later (about 9–12 weeks) in turkeys. TD is more prominent in males and causes lameness when a large lesion results in: (i) epiphyseal weakness with compression microfractures; (ii) plantar bending resulting from weight bearing or tension from the gastrocnemius muscle; or (iii) ischaemic necrosis, sometimes

following bacterial invasion of damaged cartilage, frequently leading to fractures that may occur on farm or at processing. The accumulation of transitional chondrocytes comprising the lesion will regress as bone growth slows with increasing age and the bone is remodelled, but angular bone deformities will remain and contribute to lameness problems (Lynch *et al.*, 1992).

Rapid growth is part of the pathogenesis of TD (Riddell, 1992, 1993; Lilburn, 1994) perhaps because of a lack of local stimulus (Leach and Twal, 1994; Thorp *et al.*, 1995; Farquharson and Jefferies, 2000; Reddi, 2000; Velleman, 2000) or altered anion–cation ratio in the growth plate (Riddell, 1993). Genetic susceptibility is important (Riddell, 1976; Zhang *et al.*, 1998). Nutritional or toxic factors can also be involved (Riddell, 1992; Thorp, 1992; Cook and Bai, 1994). Research on effective methods of prevention of TD includes selection of resistant breeding lines and nutritional modifications (Riddell, 1993), particularly the use of 1,25-dihydroxyvitamin D (Edwards, 1990; Rennie *et al.*, 1993).

Epiphyseal ischaemic necrosis

Epiphyseal ischaemic necrosis is degeneration or necrosis of epiphyseal cartilage with subsequent tearing, cleft formation, haemorrhage and proliferative changes in the epiphysis. This lesion would be called osteochondrosis in mammals (Riddell, 1996, 1997).

Birds do not have secondary centres of ossification in the epiphysis. The articular cartilage grows from the opposite side of the growth plate. Rapid growth results in thick avascular articular cartilage where, because of excessive thickness, focal ischaemic degenerative changes may occur (Julian, 1998). This lesion is most prominent in the hip joint of heavy turkeys. In older toms and broiler breeders, lesions also develop in other joints (Julian, 1985; Hocking, 1992; Riddell, 1992, 1996). Epiphyseolysis is also often part of the pathogenesis of spondylolisthesis and of spinal cord compression caused by cartilage protrusion into the spinal cord at T4 (Julian, 1998).

Epiphyseal separation

When the legs of young, rapidly growing broiler chickens are disarticulated at necropsy, the articular cartilage is often pulled off the femoral head and trochanter by the joint capsule, leaving the smooth, shiny growth plate. Part of the plate may also pull off, leaving rough, irregular, necrotic-looking subchondral bone. Sometimes this normal post-mortem separation is described incorrectly as 'femoral head necrosis'.

Epiphyseal separation may also occur in broiler chickens that are caught and carried by one leg to be crated for trucking to the processing plant. Heavier birds are more susceptible to this trauma (Gregory and Wilkins, 1992). These broilers bleed from the femur and may die from hypovolaemic shock, or be condemned at processing (Julian, 1998).

Skeletal fracture

Spontaneous fractures are rare in broilers, except in bones weakened by dyschondroplasia or stressed by tension, as when the attached tarsal bones are pulled off the tibia in VVD. Fracture of the femur is seen occasionally in heavy turkeys and avulsion fractures may occur at the hock.

Valgus–varus deformity (VVD); angular bone deformity; twisted leg

VVD is defined as lateral (valgus) or medial deviation of the distal tibiotasus, resulting in a 'hocks-in/feet-out' (valgus) stance or a 'hocks-out/feet-in' (varus) stance. VVD is the most frequent cause of lameness and skeletal deformity in most flocks of broilers and turkeys (Riddell, 1992). It also occurs in meat-type ducks and in a variety of zoo and ornamental birds and ratites when they are fed a commercial turkey or broiler ration. It can be seen as early as day 6 in fast-growing broilers and ducks. As the angulation of the tibia becomes greater and foot placement more abnormal, there are rotational changes of the limb. As the deformity progresses in varus deformity there may be bending and rotation of the metatarsus. However, there is

no rotation of the tibia and 'rotated tibia' is a separate condition, not part of VVD. It is not associated with rapid growth. Radiographs of the tibia in VVD show cortical thickening secondary to loading on the lateral (valgus) or medial (varus) side of the tibia (Julian, 1984a). Growth plates are normal in the distal tibiotarsus but the proximal metatarsus may be enlarged. Intertarsal ligaments become stretched and the joint is slack. The hock may become traumatized by contact with the floor.

VVD in turkeys occurs at an older age (usually after week 6) and the pathogenesis may be different, perhaps more related to weight or poor bone strength. There may be angulation of the proximal metatarsus as well as the tibiotarsus. Rotational changes in the limb are rare in VVD in turkeys.

When the deviation becomes severe, particularly in valgus deformity or when it is bilateral, the bird goes 'off its legs' and is unable to rise. The deformity is then more difficult to recognize as VVD and may be called 'twisted legs' (Thorp, 1992). Twisted legs has also been used to describe other deformities such as rotated tibia and slipped tendon. Slipped tendon is not part of the pathogenesis of VVD, but because of the angle across the hock joint the tendon is often pulled to the side and may occasionally be pulled completely off the chondyles. In these cases the slipped tendon would be secondary to VVD.

The pathogenesis of VVD is not known but the deformity in broilers is related to fast growth under continuous light. Rapid rate of growth may not allow time for remodelling and correction of the alignment of bone that may have started to grow out of line. The cause may have to do with uneven growth of the two attached tarsal bones, or tendon tension on growing bones. VVD is more frequent on wire netting or slat flooring. Similar deformity results from choline, manganese or vitamin B deficiency. When the incidence is above 2% some predisposing cause may have been present. Prevention involves slowing growth or providing long dark periods (Classen, 1992).

Spondylolisthesis (kinky-back)

Ventral dislocation of the anterior end of the only articulating thoracic vertebrae (T4) in poultry with dorsal rotation of the posterior lip, resulting in damage to the spinal cord, is called spondylolisthesis, or 'kinky-back' (Riddell, 1973). Affected broilers are lame, squat, sit on their tail with feet extended or fall sideways (Riddell, 1992). The lesion must be differentiated from scoliosis, as illustrated in Fig. 9.1 of Thorp (1992), which rarely produces clinical signs. The vertebrae must be split medially to show the lesion (Riddell, 1992; Julian, 1998). In many flocks spondylolisthesis is more frequent in females. This condition is caused by rapid growth in broilers that are genetically susceptible (Riddell, 1992).

Osteomyelitis or osteochondrosis at the same vertebrae causing compression of the cord will produce similar clinical signs.

Gastrocnemius tendon rupture

Rupture of the gastrocnemius tendon above the hock is a frequent cause of lameness in heavy meat-type chickens (Gregory and Wilkins, 1992; Riddell, 1997). The rupture is usually primary, caused by excessive stress on tendons with inadequate tensile strength (Julian, 1998). Rupture of the tendon of one leg increases the workload on the other and bilateral rupture is frequent. Affected birds are lame or unable to stand (hock-walkers). Differential diagnosis would include spinal cord injury, and the plantar bending deformity of the proximal tibia secondary to TD. The rupture usually occurs slowly, accompanied by reparative processes that can be palpated as thickening of the tendon on the back of the leg above the hock. Haemorrhage from the injury is visible as red, blue or green discoloration in the tissue above the hock. Rupture of the gastrocnemius tendon is rare in turkeys and ducks.

Rupture of the peroneal muscle in turkeys is seen as a horizontal tear in the muscle on the anterior mid-section of

the drumstick where the tendon attaches to the muscle (Julian and Gazdzinsky, 2000). It occurs, mainly in females, at the age when tendons start to ossify and lose their elasticity. It does not cause lameness.

Genetics of Skeletal Problems in Broilers

Genetic mechanisms

It is possible to discriminate between major skeletal genes that express their effect distinctly, modifier genes that may or may not modify the effect of a major gene, and the combinations of several to many genes interacting with the environment that are sometimes termed the genetic background genes. Johnson (1986) presented a list and description of many of the genes influencing development of the skeleton.

Mutants of major skeletal genes are often harmful to the individual and disappear from the population if their effects are dominant, but recessive mutant genes may remain. A particular situation exists for the creeper (*Cp*) gene. Birds heterozygous for that gene have a shortening of the long bones resulting from delayed cartilage replacement (Johnson, 1986), whereas homozygotes for *Cp* die as embryos. Some breeders of fancy strains like the creeper hens and therefore the gene remains in a few breeds. Another mutant with a major effect is the sex-linked dwarf gene, used by a number of the worldwide broiler breeding companies. Apart from these particular cases, mutants are thought to have disappeared from the populations of strains used for meat-type production because of the strong emphasis on selection for high growth rate.

A number of genes governing the secreting of enzymes, cytokines and hormones may be classified as modifying genes in relation to skeletal development and some of these may have very localized activity (Bain and Watkins, 1993). Mutants of these genes may cause a change in intensity of secretion, time of secretion, tissue specificity, etc. Such mutants may have a positive, negative or neutral effect on the trait at a given time compared with the wild type gene.

Genetic background genes, interacting with the surrounding environment, may be considered as the genes governing the early growth rate. Some of these genes may influence skeletal development, and the extent of this effect can be expressed in terms of a genetic correlation and handled by quantitative genetics (Falconer and Mackay, 1996). However, it has to be realized that the genetic background genes important for today's broiler chickens may be quite different from those important for the chickens of 50 years ago.

To illustrate the changing genetic background genes, consider the early growth pattern of the broiler chickens by looking at their growth rate factor defined as daily gain as percentage of body weight at a given day. In meat-type chickens and turkeys, Bjørnhag (1979) found that the maximum growth rate factors were 13 and 10%, respectively, on the basis of data from 1965 or earlier. Looking at the growth curves of 'year 2000' broiler chickens, the maximum growth rate factor is close to double the earlier value, and has moved closer to the hatching day. As development of the long bone takes place early in the broiler's life, such changes in the population over time mean that metabolic capacity problems may arise. As an example, it has been shown that addition of 1,25-dihydroxyvitamin D to the diet of young broiler chickens reduces the incidence of TD (Thorp *et al.*, 1993b; Mitchell *et al.*, 1997). This suggests that broilers are unable to transform sufficient dietary vitamin D_3 or have not received a sufficient amount through the maternal supply of yolk.

A divergent selection experiment for TD in broilers (Ducro and Sørensen, 1992) was followed by a crossbreeding programme among the experimental lines in order to determine the maternal effect. The results (not yet published) showed that a considerable part of the genetic difference established between the diverse selected lines was of maternal origin and also that this difference was solely additive in genetic origin.

The disturbance of early skeletal development in avian species selected for fast early growth rate over many generations is thus mainly due to changes in the composition of the background genes and interaction between these genes and the modifier genes. The expression of these genes may either influence the individual itself or affect the offspring through the incorporation of important factors in the eggs.

Influence on embryonic development

The development of the skeleton starts during the embryonic stage. Clum *et al.* (1995) demonstrated, using the classical staging techniques, that a commercial male line had a different development from an unselected population. This technique, using developmental landmarks to categorize stage of embryonic maturity, demonstrated that the commercial line was less developed during 5–9 days of incubation. Ossification of long bones in the legs, feather growth and eye development were particularly delayed in the commercial line, but 'caught up' with the unselected line just at time of hatch. These findings suggest that different postnatal growth patterns may be associated with changes in the pattern of embryonic development of different tissues, even though gross morphological differences are not evident at hatch. Furthermore, the authors claimed that these differences in prenatal development represent periods when embryos are experiencing hyperplastic growth of tissues such as muscle as a means of increasing postnatal growth potential.

It is generally accepted that the functional competence of bone is enhanced by mechanisms that involve adaptation to mechanical loading (Lanyon *et al.*, 1982; Rubin and Lanyon, 1984). This adaptation is in part due to the processes of modelling and remodelling of the bones and it has been shown that loading *in vivo* is capable of producing some changes in concentrations of osteogenic metabolites. Pitsillides *et al.* (1999) examined wild-type meat-type and egg-type chickens *in vitro* by culturing tibias

from 18-day-old embryos and then applying a mechanical loading for some time or incubating with prostacyclin. They found some metabolites showing considerable changes in wild-type chick tibia with loading, but no reaction in the meat-type tibia. They concluded that skeletal abnormalities that develop in fast-growing meat-type chickens might reflect a compromised ability to respond to load.

Although the size of the chick at hatch is related solely to the size of its egg, there are thus several indications that selection for high postnatal growth has had a considerable impact on embryonic bone development. Some of these changes may lead to problems for the growing broiler.

Inheritance of Specific Leg Disorders

Several selection experiments and other genetic studies have demonstrated the heritable nature of the various leg disorders. These have been reviewed comprehensively by Sørensen (1992).

Twisted leg

'Twisted leg' is a composite term combining several leg abnormalities that may be further differentiated as valgus–varus or rotational defects, as scored on live chickens. Measurements on bending or rotation of tibia performed on the long bones dissected after slaughter give further information on the nature of the deformities, including information on the plantar bending of the proximal tibia, thought to be a consequence of tibial dyschondroplasia.

Twisted legs were the subject of a selection experiment performed by Leenstra *et al.* (1984) involving the breeding of three experimental lines selected for: (i) high 6-week body weight (C); (ii) full-sib selection for reduced incidence of twisted legs (T); and (iii) individual selection for reduced incidence of twisted legs combined with selection for high 6-week body weight (CT).

Table 3.4. The effect on frequency of twisted legs and body weight from three generations of selection (Leenstra *et al.*, 1984).

Line	Frequency of twisted legs (%)				Body weight at 6 weeks (g)	
	In cages		On litter			
	Male	Female	Male	Female	Male	Female
C	49	28	18	12	1969	1708
T	12	7	5	2	1808	1555
CT	27	12	8	5	1941	1677

Three generations of selection demonstrated strong hereditary relationships, as shown in Table 3.4.

Hartmann and Flock (1979) provided results from family structured data on the incidence of twisted legs showing heritabilities ranging from 0.2 to 0.5. They concluded that a major gene seemed to be involved. Somes (1969) reported on an autosomal recessive gene, incomplete in its expression, responsible for a major part of the genetic variation in the frequency of twisted legs in a control population established in 1955.

Mercer and Hill (1984) carried out a study on a procedure for the estimation of genetic parameters in leg disorders, in floor-raised broilers, using a mixed model for the statistical analysis. They found an average heritability over three lines of 0.20 for leg disorders, considered as a composite trait of leg and keel defects as well as twisted legs.

Le Bihan-Duval *et al.* (1996) reported on a study on family structured data from two commercial broiler strains, including scoring for valgus and varus angulation of the legs in more than 20,000 chickens. For the purpose of statistical analysis, individual broilers were all classified as healthy, varus or valgus. Making use of a multiple logistic model and considering the two deformities as different traits, they obtained the results shown in Table 3.5. The higher values based on the variance component of maternal effects indicates that dominance or maternal effects play an important role. The finding that the genetic correlation between the two abnormalities was very small or even negative indicates that the two abnormalities are

Table 3.5. Estimates of heritabilities for valgus and varus angulation based on sire and mother's grandsire variance components (h^2_S) and mother's variance component within grandsire (h^2_D) (Le Bihan-Duval *et al.*, 1996).

	h^2_S	h^2_D	Incidence (%)
Valgus	0.22	0.37	48.0
Varus	0.23	0.28	9.8

inherited almost independently, an observation also made by Mercer and Hill (1984).

Bending and twisting of the tibia were the subjects of a selection experiment (Sørensen and Harlou, 1984) in which the criterion for selection was family information on either plantar bending of the proximal part of tibia, given as angle (a), or lateral or medial bending of the distal part of tibia, given as angle (c). Two experimental lines were divergently selected for an index of the two measures. The major results are presented in Table 3.6. The angles of the two lines differed significantly for both types and the clinical evaluations also differed between the two lines. The tarsus and hock abnormalities were indications of twisted leg conditions.

Tibial dyschondroplasia (TD)

TD has been the subject of several genetic studies following the finding by Leach and Nesheim (1965) of a genetic involvement. Leach and Nesheim (1972) published the results of 7 years of divergent selection for the incidence of TD in which the chickens fed a commercial starter diet up to 4 weeks

Table 3.6. Means of tibia angles at 25 days and incidence of clinical signs of abnormality at 40 days, after two generations of selection (Sørensen and Harlou, 1984).

Lines	Selected angles			Abnormal (%)	
	Angle (a)	Angle (c)	Crooked toes (%)	Tarsus	Hocks
Low bending	25.6	0.4	5.3	1.3	1.3
High bending	34.6	1.1	30.2	6.4	4.6

of age had incidences of 0 and 30% in the low and high TD lines, respectively.

Riddell (1976) published the results of experiments in which two strains were established from a commercial stock and were divergently selected for TD over three generations. Radiography was used on 6–8-week-old chickens to detect TD, applying a score ranging from 1 to 3. In the third generation, chickens fed a commercial broiler starter and raised on the floor had an incidence of 51% in the high-incidence line, whereas TD was not seen at all in the low-incidence line.

Sheridan *et al.* (1978) published a study of the inheritance of TD in which they had selected a line of broilers for high incidence of TD over four generations. The selection was performed on family information based on scoring of TD after longitudinal section of both tibias from 7-week-old chickens. Feeding the broilers a diet with an added 5 g NH_4Cl kg^{-1} to increase the incidence of TD, they observed TD incidences of 9, 24, 37 and 83% in successive generations. The heritability estimate pooled over four generations was 0.43. By analysing the estimate in more detail, the authors found that a recessive sex-linked gene with a major effect had been present in the basic population and was fixed in the fourth generation.

A device for detecting TD in young living chickens – a portable, handheld low-intensity X-ray apparatus (Lixiscope) – was first described by Bartels *et al.* (1989). The apparatus produces a real-time image similar to that of a fluoroscope and has been shown to provide reliable information on TD. Two selection experiments have so far been reported using the Lixiscope. Wong-Valle *et al.* (1993) and Zhang *et al.* (1998) reported on a divergent selection experiment, started in 1988 in Alabama and run for

ten generations, in which the selection criterion was TD at 7 weeks of age. The realized heritability after four generations was estimated to be 0.44 for the high TD line and close to 0 for the low TD line. Over the ten generations, the response was highly asymmetric, favouring an increased TD incidence. Thus the mean TD incidence had increased by 7.6% in males and 9.1% in females per generation in the high TD line but had not changed significantly in the low TD line. Ducro and Sørensen (1992) reported on a selection experiment carried out in Denmark using the Lixiscope to measure TD at 4 weeks as selection criterion. They found a heritability of 0.33 on the underlying scale. The two divergently selected lines were used at generations five to seven in a crossing programme in which it was demonstrated that a considerable part of the genetic difference established between the lines was of maternal origin (Sørensen and Su, 2001).

The asymmetric response to selection using the Lixiscope can perhaps be partly explained on the basis that it is easier to detect larger lesions with the apparatus. However, it is the larger lesions that are more likely to result in clinical deformity, and commercial experience seems to indicate that removing more severely affected birds from breeding programmes can result in important practical improvements in broiler leg health.

Miscellaneous abnormalities

Crooked toes have been shown to be highly heritable (Hicks and Lerner, 1949) in White Leghorns, and in a commercial male broiler line the frequency of crooked toes was reduced from 30% to less than 15% by

selection over three generation (Sørensen *et al.*, 1980). Spondylolisthesis has been found to have a heritable component, with Riddell (1973) reporting an increase of 9% in clinical spondylolisthesis over a three-generation selection experiment. Abnormalities in the proximal femur have been reported as increasing in recent years, but so far a possible genetic involvement has not been reported.

A bird's walking ability has been quantified on a subjective basis as a 'gait score' (Kestin *et al.*, 1992) to give an overall evaluation that can be related to the welfare of broilers. The gait score thus covers more factors than just skeletal abnormalities. Kestin *et al.* (1999) showed that commercial crosses differed in gait score and also found a significant but low correlation between gait score and TD. The heritability of gait score has been found to be 0.20 in a commercial female line (P. Sørensen and G. Su, unpublished).

Genetic Relationship Between Leg Disorders and Growth Rate

There is no doubt that the rapid growth rate of birds used for meat production is the fundamental cause of skeletal disorders, nor that this situation has been brought about by the commercial selection programmes used over a period of 40–50 generations. In disciplines such as nutrition, veterinary science and physiology there have been many investigations to find methods of decreasing the incidence and severity of the various skeletal disorders. Geneticists have focused mainly on the question of how heritable the traits are and how to measure and analyse the data. This section will deal with assessment of the relationship between leg disorders and growth rate, while the methods of statistical analysis will be covered in Chapters 10 and 11. In considering the relationship between growth and the occurrence of skeletal disorders, a positive correlation is often disadvantageous and may be referred to as an antagonistic correlation.

The genetic correlations between body weight and incidences of crooked toes, bow-leggedness and valgus deformity were estimated to be +0.22, +0.26 and +0.10, respectively, by Mercer and Hill (1984). After pooling all three skeletal disorders into one trait, the genetic correlation between disorders and body weight remainded strongly positive (+0.25). In contrast, Le Bihan-Duval *et al.* (1997), using a multiple logistic model, found minimal genetic correlation between body weight at 3 or 6 weeks of age and either varus or valgus angulation.

There are some contradictory results relating TD and body weight. Sheridan *et al.* (1978) obtained a positive genetic correlation between TD and body weight of 0.29, based on a traditional sire–dam analysis of variance of data from the fourth generation. In the divergent selection experiments for TD carried out in Alabama, Zhang *et al.* (1995) found a substantial negative genetic correlation between TD and body weight based on least-square analysis of data from the seventh generation. Kuhlers and McDaniel (1996) on the same selection experiments found a low genetic correlation (0 to +0.10) between TD and body weight using data from all seven generations using a multiple-trait derivative-free restricted maximum likelihood (DFREML) procedure applying an animal model. This low correlation was later explained by Zhang *et al.* (1998), who found, in a study on responses to ten generations of TD selection, that both of the lines showed decreases in body weight over the generations. This decrease is perhaps due to the relaxing effect on body weight that often occurs in broilers when selection pressure for increased body weight is not maintained. Studies on line differences in growth capacity and TD have been performed on ducks (Wise and Nott, 1975) with a clear indication of higher TD incidence in the faster-growing lines. A similar relationship has been seen in male turkeys, though not in females (Walser *et al.*, 1982).

Inferences on genetic correlations between a continuous trait such as body weight and incidence of TD, often presented as binary data, may be open to doubt. The categorical nature of binary data makes

it sometimes difficult to get unbiased estimates of the genetic correlation with both least-square and REML methods of analysis. Using the Bayesian method, applying the Gibbs sampler on the categorical data transformed to the underlying normally distributed scale will overcome some of the problems. In the Danish selection experiment, investigation of data from seven generations and 7400 birds by the artificial insemination (AI)REML procedure on non-transformed data gave a genetic correlation of −0.02 whereas analysis using the Bayesian method with the Gibbs sampler gave an estimate of +0.17. The latter estimate seems to fit the observed consistent higher body weight of the high TD line during later generations (Sørensen and Su, 2001).

An extremely unfavourable genetic correlation of 0.8 has been found between body weight and overall walking ability, measured as gait score, using various statistical methods including AI-REML and the Bayesian method using Gibbs sampler (Sørensen and Su, 2001). The nature of this relationship has been confirmed by a recent comparison of 12 breed/ breed combinations ranging in growth capacity from modern broiler type to ancient types of breed such as Light Sussex (Kestin *et al.*, 2001). These findings suggest that the more integrated the description of leg health is, the more unfavourable is the genetic correlation with growth.

It can be concluded that for broiler populations an antagonistic genetic correlation exists between growth rate and incidence of skeletal disorders. Although there may be variation among populations and also a variation in degree of the correlation with specific disorders, this antagonistic relationship accounts for the increased susceptibility of broilers to leg disorders over many generations of selection for body weight. Nevertheless, the genetic correlation between body weight and incidence of disorders is generally quite low, so this raises the prospect that appropriate multi-trait selection should permit a genetic improvement in leg health along with a continued, though more modest, improvement in growth rate.

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4 Meat Quality Problems Associated with Selection for Increased Production

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The principle of selection permits to the farmer not only to modify the characters of its flock but also to transform it. It is the magic stick with which he can call to life all the forms and models he pleases.

Charles Darwin (1859)
On the Origin of the Species

Introduction

The quality of a product is a complex notion, which can be summarized as 'the whole of properties of a product that give to it the aptitude to satisfy the requirements of the consumer.' For a meat product, this notion of quality includes the following:

- Organoleptic quality: properties that are linked to the five senses (e.g. colour, taste, flavour, tenderness).
- Dietary quality: properties that make the food suitable for the nutrition requirements.
- Safety quality: the lack of toxicity.
- Technological quality: properties that makes the product interesting for processing.
- Psycho-social quality: properties that fit the hedonic character of the consumer.

Poultry selection has progressed continually since the early 1970s, resulting in a very large increase in growth rate of chickens and turkeys, and to a lesser extent in ducks. In the 1980s, genetic breeders also worked to reduce fatness by direct selection against abdominal fat or indirect selection on weight gain:feed ratio. More recently, and because of the increasing requirement of consumers for processed poultry products and correspondingly less for whole ready-to-roast carcasses, geneticists have also proposed new lines of birds selected for increased yield of breast or thigh muscle.

All these selections have been carried out primarily to reduce breeding costs by improving production efficiency. Less attention has been paid to the influence of such selections on the quality of the meat. However, modifying growth rate, fatness or yield also modifies the growth, structure or overall metabolism of muscle, leading to possible alterations affecting the technological or sensory properties of the meat.

Although poultry carcass grading continues to be based on criteria such as bruising or colour (Dransfield and Sosnicki, 1999), the appearance of meat quality problems (e.g. colour, water-holding capacity, toughness) has highlighted the possible relationship between modern lines and poor meat quality. For example, colour (absolute value and relative uniformity) and water-holding capacity are frequently reported as being poorer in modern poultry flocks.

Colour and water-holding capacity influence perceptions of the acceptability of the meat product but vary with the species, muscle function, age of the animal and storage conditions (Miller, 1994; Berri, 2000). Because most consumers consider more than the price of the product when they buy it, meat producers will probably have to give more attention to the uniformity of their product. For example, Fletcher (1995) reported that in a survey of five commercial broiler-processing plants, breast meat lightness (L*) was found to range between 43.1 and 48.8. This indicates that significant variations in breast meat colour exist and are present at the retail level. It also indicates that much work remains to be done to improve the control of meat quality. Breeders (from private and public companies) will probably have a large role to play in this challenge.

Genetic Selection in Meat-type Poultry

Selection for increased growth rate is still the most efficient and popular way used by breeders to establish new lines of birds. The amount of time required for meat-type broilers to achieve a constant market weight was reduced by 1 day per generation between 1970 and 1990 (Anthony, 1998). For example, in the 1940s, birds took about 16 weeks to reach a marketable weight of 2.0–2.5 kg (Griffin and Goddard, 1994). By the 1990s, improved broiler strains reached the same weight in less than 40 days (Fig. 4.1).

Numerous researchers have shown that selection for increasing body weight at a given age is possible simply by mating the largest male and female birds in a flock to obtain larger and faster-growing birds. This has produced interesting models of divergently selected birds, which are very useful for studying the influence of growth rate on muscle characteristics and meat quality.

Muscle characteristics influencing the quality of the meat are principally linked to the quantitative and qualitative characteristics of the muscle fibres. Quantitative characters are the total number of muscle fibres and their size, generally estimated as the mean cross-sectional area (CSA). In chickens divergently selected on body weight at 8 weeks of age, Rémignon *et al.* (1994) reported that fast-growing chickens have more numerous and bigger muscle fibres than slow-growing birds at a given age. This was also observed in quails (Fowler *et al.*, 1980) and in turkeys by Wilson *et al.* (1990), who compared different lines of birds selected for rapid growth. In selected and
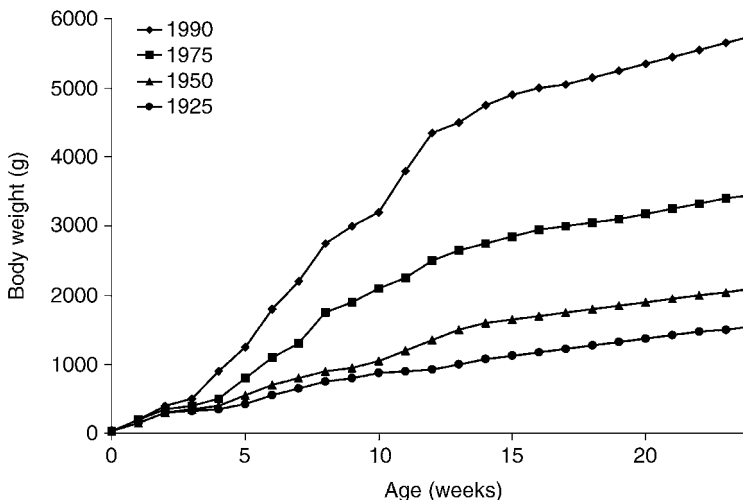

Fig. 4.1. Growth curves for the typical chicken in 1925 and meat-type chicken in 1950, 1975 and 1990 (Anthony, 1995).

unimproved turkeys, Bentley (1999) reported that selection for higher body weight had increased fibre size rather than number. On the contrary, in comparing egg-type and meat-type turkeys, Swatland (1989) did not report differences in muscle fibre number or CSAs. Nevertheless, hypertrophy of the muscle fibres is the assumed dominant model for postnatal growth in poultry (Goldspink, 1980). However, Cherel *et al.* (1994) suggested that, in turkeys, a post-natal muscle cell hyperplasia could exist.

Muscle fibre types are of great importance for meat quality because they are characteristic of white and dark meat. A high percentage of white myofibres (fast twitch, glycolytic, type IIb) means light-coloured meat, with low fat and myoglobin. Dark (or red) meat has a higher content in oxidative (type I) or oxido-glycolytic (type IIa) muscle fibres, which contain more myoglobin and fat because of their higher oxidative metabolism.

In mammals, it has been suggested that selection for increased body weight or muscle mass entails higher conversion from type IIa (fast, oxido-glycolytic) to type IIb (fast, glycolytic) muscle fibres. In chickens and turkeys, the breast muscle is almost entirely composed of type IIb muscle fibres. Despite the fact that muscle fibre types are physiologically able to change, it seems difficult to modify completely the typology of single-type muscle. This is probably the reason why no differences in breast muscle typology have been reported by Aberle and Stewart (1983), Horak *et al.* (1989) or Rémignon *et al.* (1995, 2000) in comparing chickens or turkeys with different growth rates. On the contrary, in mixed-type muscles, Dransfield and Sosnicki (1999) reported that increasing growth rate leads to an increase in the percentage of glycolytic myofibres. This is the case in palmipeds such as geese (Klozowska *et al.*, 1993) and ducks (Pingel and Knust, 1993) as well as in quails (Fowler *et al.*, 1980), because fast-growing birds present more glycolytic breast muscle. In mixed thigh muscle of slow-growing (Kumamoto Cochin) chickens, Iwamoto *et al.* (1997) also found a higher

percentage of oxidative muscle fibres than in modern fast-growing broilers.

It has also been suggested that increasing muscularity in poultry could affect the morphology of the muscle fibres, especially in increasing the percentage of abnormal cells. This is not yet clear; for example, Swatland (1989) found that the sporadic occurrence of supercontracted or giant muscle fibres is more likely to be associated with the method of slaughter, because there was no evidence of ante-mortem abnormalities. Rémignon *et al.* (2000) made similar observations and concluded that the morphology of the muscle fibres can change during the onset of rigor mortis but in the same ways in fast-, medium- and slow-growing turkeys.

Other muscle abnormalities have been described in fast-growing turkeys, such as pale, soft and exudative meat (PSE), deep pectoral myopathy and inherited muscular dystrophy. The latter is under genetic control and is caused by a single point mutation (Bentley, 1999), while deep pectoral myopathy is known to be caused by an anatomical predisposition to ischaemia (Marthindale *et al.*, 1979). The PSE syndrome seems to be more complex because no clear evidence of genetic implication has been identified in turkeys or in chickens. PSE meat in poultry presents the same characteristics as those described in pigs and concerns colour (excessively pale), tenderness (too tough) and a poor water-holding capacity. In pigs, it has been shown (Otsu *et al.*, 1991) that a mutation in the ryanodine receptor gene on chromosome 6 is responsible for an excessive calcium release in the sarcoplasma. This high concentration of calcium in muscle fibres leads to hypercontraction and progressive irreversible muscle damage. At present, no direct evidence of the same single locus mutation has been found in turkeys or chickens. However, Wang *et al.* (1999) suggested that there are significant differences in proteins comprising the sarcoplasmic reticulum that could have resulted from genetic selection for increasing growth rate in turkeys. No direct effects of the growth rate in turkeys were reported to explain the PSE syndrome (Fernandez *et al.*, 2001). Currently, it is only known that any

factor that leads to an acceleration or prolongation of post-mortem glycolysis is likely to create PSE. Santé *et al.* (1991) reported that the rate of decline of post-mortem pH generally leads to a higher incidence of quality defects that characterizes PSE meat. McCurdy *et al.* (1996) and Barbut (1996) suggested that heat stress before slaughter seems to be the most important parameter to be avoided for limiting PSE occurrence in turkeys or chickens.

The evolution of the poultry market has been dictated by a rising demand for portioned and processed products (Barton, 1994; Morris, 1996). Breast muscle is the most valuable part of the chicken carcass and attention has been given to this trait, since it is known that the amount of meat has a high level of heritability ranging from 0.45 to 0.6 (Le Bihan-Duval *et al.*, 1998). For example, Berri *et al.* (2001) reported that broilers yield up to 19% breast meat compared with strains raised 30 years earlier, when the yields were only 11–12%. Despite this increase in breast meat yield, little is known about the consequences of such selection on the quality of the meat. Le Bihan-Duval *et al.* (1999) compared chickens selected for high breast meat yield and low abdominal fat content with unselected birds and reported few differences in pHu, a*(redness) and b*(yellowness) values, but drip losses were significantly lower in the selected birds.

Breeders have also selected to reduce fatness in poultry since the early 1980s. This selection was done because the fattening of a bird during the rearing period increases the final cost of the product. This is mainly due to the fact that fat birds generally have a higher weight gain:feed ratio and a higher total mortality as well as reduced carcass value at the processing plant. The other main reason is that poultry meat is one of the leanest meats and it is important to meet the consumer demand for low-fat meat. Different genetic methods have been used to try to reduce the fatness of poultry, especially chickens. Leclercq (1988) in France and Cahaner (1988) in Israel demonstrated that it is possible to obtain lines of chickens divergently selected on the quantity of abdominal

fat, which is well correlated to the total proportion of fat in the carcass. Pym and Nicholls (1979) in Australia, Leenstra (1988) in The Netherlands and Sørensen (1988) in Denmark also indirectly selected lean and fat chickens by maintaining distinct weight gain:feed ratios. The latter selection was less efficient than the former, but also led to chickens with a high or low quantity of fat in the carcasses. Ricard *et al.* (1983) compared the distribution of carcass fat and the meat quality characteristics of birds divergently selected for abdominal fatness. Their study showed that if there was a correlated decrease in the subcutaneous fat of the lean birds, the lipid content of the muscle itself did not differ between the two lines, probably due to different genetic controls for different fat depots. In a larger comparison of all the selected lines described above (from France, Israel, The Netherlands and Denmark), Ducro *et al.* (1995) did not find any clear influence of genotype on muscle enzyme activities, proving that selection for or against fat content does not greatly modify muscle metabolism.

Other work has compared different genotypes adapted to high ambient temperature. Growth rate, feed efficiency and meat yield decrease when broilers are reared at high temperature (Yalçin *et al.*, 1997). Broilers with modified feathering can be used because reduced feathering may help birds to dissipate internal heat more efficiently. Broilers with the naked neck (*Na*) gene have less feather coverage by reducing the number of feathers and restricting their distribution (Yunis and Cahaner, 1999). Transylvanian naked-neck poultry reared in intensive conditions have a lower meat-to-bone ratio and crude fat content but a higher final pH value (pHu) water-holding capacity (WHC) than standard broilers. In extensive conditions these differences were no more significant, indicating a clear interaction between genotype and rearing condition (Latif *et al.*, 1998).

Meat Qualities and Genetic Selection

Fletcher (1991) summarized the possible influences of genetics on defects affecting

the carcass or meat of poultry during production (Table 4.1). From Table 4.1, it is easy to see that genetics has (or is suspected to have) a great influence on the sensory and technological quality of poultry meat. Although the genetics of meat quality, including meat characteristics, has been used widely in pig selection, it is a more recent focus in the selection of poultry. Consequently, very little is actually known about the genetic variability of muscle fibre characteristics and of post-mortem muscle metabolism in relation to organoleptic and technological qualities of poultry meat.

Influence of selection for growth or fatness on sensory traits

No differences in sensory scores of the meat from different strains of chickens killed at the same age were reported by Ziauddin

Table 4.1. Summary of poultry carcass (C) and meat (M) defects and their suspected area of origin during production (Fletcher, 1991).

Defect	Carcass or meat	Cause
Bloody thigh	C, M	Management
Breast blisters	C	Management
Brown spots	C	Genetics
Bruises	C, M	Management, nutrition
Composition	C, M	Genetics, nutrition
Conformation	C	Genetics, nutrition
Fat stability	M	Nutrition
Feather colour	C	Genetics
Focal myopathy	M	Genetics
Haemorrhages	C, M	Nutrition
Leg problems	C	Management, nutrition
Meat colour	M	Genetics, management
Meat staining	M	Management, nutrition
Meat tenderness	M	Genetics, management
Muscular dystrophy	M	Genetics
Oily bird syndrome	C	Management, nutrition
Off flavours	M	Nutrition
Scabby hip	C	Nutrition
Skin colour	C, M	Genetics, management, nutrition
Yield	C, M	Genetics, management, nutrition

et al. (1996) or Gardzielewska et al. (1995a). Grey et al. (1986) compared lines of turkeys with different growth rates and concluded that some of the observed variations in tenderness might result from genetic differences among birds. Touraille et al. (1981) conducted a study in order to quantify the specific effect of selection for increased growth and age on the characteristics of chicken meat: they compared two experimental lines divergently selected for growth curve. Age was the most significant factor of variation of the sensory characteristics as, in both the thigh and breast muscles, tenderness and juiciness decreased between 9 and 16 weeks whereas the flavour intensity increased. These results agreed with the study of Chambers et al. (1989), who showed that, compared with experimental-strain broilers slaughtered at 47 days, birds slaughtered at the ages of 75 or 103 days had more intensely flavoured and less tender dark meat. As emphasized by Berri (2000), the effect of age on meat quality must partly explain the differences between genotypes with different growth rates. The effect of selection for growth per se on the sensory meat quality was not clearly demonstrated: while Chambers et al. (1989) observed a rather large variability of the sensory characteristics of the meat from broilers of modern and experimental strains slaughtered at 9 weeks, no significant variations of the sensory traits were observed by Touraille et al. (1981) between fast- and slow-growing genotypes at 16 weeks of age. If selection for growth has obviously modified the meat sensory characteristics by decreasing the age at slaughter, its impact on the overall acceptability of the product is not straightforward; indeed, preferences are being directly linked to the eating habits of consumers, which can lead to conflicting judgements for the same products (Berri, 2000). In the French study by Touraille et al. (1981), older birds had a higher overall acceptability mainly explained by their greater flavour. In contrast, Yamashita et al. (1976) reported that Japanese consumers preferred meat from young chickens because they were more tender and judged to have more flavour.

Sensory properties are also strongly influenced by the lipid content of the meat. If no significant differences between fat and lean birds could be found for cooking loss, juiciness and flavour of the meat, selection against fatness was associated with decreased tenderness. In their study on modern and experimental broiler lines, Chambers *et al*. (1989) found that carcass fatness had a significant and positive effect on flavour, juiciness and tenderness of dark meat. However, they estimated that the percentage of variation of these traits explained by carcass fatness did not exceed 7.8%. Ricard *et al*. (1983) concluded that breeding for a low abdominal fat depot would not significantly alter the sensory properties of the meat.

Variations in pH significantly affect the storage and the processing quality of the meat, by modifying its water-holding capacity and rheological properties (Kijowski and Niewiarowicz, 1978; Daum-Thunberg *et al*., 1992; Barbut, 1997a). In contrast to pigs, very little is known in poultry about the impact of genetics on muscle growth rate and extent of pH fall and subsequent meat quality. The existence of genetic variability was suggested by studies comparing the

meat quality of various chicken lines (Gardzielewska *et al*., 1995b). Xiong *et al*. (1993a) found that the pH values of breast or thigh muscle of eight strain crosses were within a narrow range averaging 5.98 ± 0.05 for the breast and 6.10 ± 0.04 for the thigh.

Berri *et al*. (2001) compared breast meat metabolism and meat quality of four broiler lines: an experimental and a commercial line selected for increased body weight and breast yield, and their respective unselected control lines. Birds from the experimental selected (ES) line exhibited similar body weight but higher breast yield (+21%) and lower abdominal fat percentage (−25%) than those of the experimental control (EC) line (Table 4.2). The breast and fat yields of the birds from the commercial selected (CS) line were higher (+61% and +18%, respectively) than those of the commercial control (CC) line. As expected, the glycolytic metabolism measured by the lactate dehydrogenase activity was preponderant in the pectoralis major of all the genotypes, whereas the enzyme activities associated with oxidative metabolism (citrate synthase and β-hydroxyacyl CoA dehydrogenase (HAD)) remained very low. There was no significant effect of the line on these enzyme activities,

Table 4.2. Body weight, body composition traits and meat quality indicators from the experimental selected line (ES) and its corresponding control line (EC) at 7 weeks of age, and the commercial selected line (CS) and its corresponding control line (CC) at 6 weeks of age (from Berri *et al*., 2001).

	Time post mortem	EC (n = 58)	ES (n = 58)	CC (n = 57)	CS (n = 57)	Line effect
Body weight (g)		$2237^b \pm 180$	$2223^b \pm 144$	$1306^c \pm 78$	$2966^a \pm 114$	***
Breast yield (%)		$12.5^c \pm 1.7$	$15.1^b \pm 1.9$	$11.5^d \pm 1.1$	$18.5^a \pm 1.2$	***
Abdominal fat yield (%)		$2.45^a \pm 0.76$	$1.84^c \pm 0.55$	$1.77^c \pm 0.5$	$2.09^b \pm 0.48$	***
pH	0.25 h	$6.31^c \pm 0.13$	$6.42^b \pm 0.12$	$6.12^d \pm 0.14$	$6.55^a \pm 0.12$	***
	1 h	$6.10^b \pm 0.17$	$6.27^a \pm 0.13$	$5.79^c \pm 0.11$	$6.31^a \pm 0.12$	***
	24 h	$5.84^c \pm 0.14$	$5.90^b \pm 0.14$	$5.75^d \pm 0.12$	$6.03^a \pm 0.14$	***
Lightness (L*)	1 day	$48.4^b \pm 1.6$	$49.8^a \pm 2.5$	$48.1^b \pm 2.3$	$49.7^a \pm 2.0$	***
	6 days	$52.0^b \pm 1.8$	$53.2^a \pm 2.4$	$49.3^c \pm 2.2$	$51.4^b \pm 1.6$	***
Redness (a*)	1 day	$0.64^a \pm 0.86$	$0.20^b \pm 0.69$	$0.27^b \pm 1.1$	$-0.96^c \pm 0.61$	***
	6 days	$1.49^b \pm 0.95$	$0.84^c \pm 0.59$	$1.94^a \pm 0.81$	$0.03^d \pm 0.77$	***
Yellowness (b*)	1 day	$10.86^a \pm 1.24$	$10.39^b \pm 1.18$	$9.38^c \pm 1.49$	$7.50^d \pm 0.80$	***
	6 days	$10.12^{ab} \pm 1.16$	$9.99^{ab} \pm 1.27$	$10.47^a \pm 1.39$	$9.77^b \pm 1.07$	***
Drip loss (%)	6 days	1.75 ± 0.45	1.62 ± 1.27	1.59 ± 0.42	1.64 ± 0.70	NS

a–dMeans with the same letter in the same row do not differ (P > 0.05).
NS, non-significant.
***$P \le 0.0001$.

except that the HAD activity appeared greater in the commercial lines, with the highest level in the CC line. A significant effect of the line was observed for the rate of pH fall after death in the muscle, which was delayed in the selected lines by comparison with their controls (Table 4.2). At the same time, the extent of the pH fall was less pronounced in the selected lines, which had a higher ultimate pH than their controls. This was consistent with the lower glycolytic potential they also exhibited. No effect of the selection was found on the drip loss of the meat, which remained rather low (no more than 1.75% after 5 days of storage). The colour of the meat appeared to be modified by the selection, as meat of the selected birds appeared paler and less red than those of the unselected birds. This loss in colour intensity was likely to be due to the decrease in the muscle haeminic pigment content that was observed in the selected birds.

In order to specify the possibilities of a selection on meat quality, Le Bihan-Duval *et al.* (1999, 2001) conducted a genetic study on several quality indicators measured on the breast muscle of experimental meat-type chickens. Measurements of pHu and colour variables (L*: lightness; a*: redness; b*: yellowness) were recorded for a total of 1076 birds. Rate of pH fall (pH at 15 min post mortem) and drip loss were also recorded for some animals (about 600). Restricted maximum likelihood (REML) estimates indicated that meat traits had very significant levels of heritabilities, ranging from 0.35 to 0.57 (Table 4.3).

Colour parameters were the most heritable traits, with estimates between 0.50 and 0.57. Although breast muscle contains only fast-twitch fibers (Rémignon *et al.*, 1996), a rather large variation in colour was observed, especially for meat paleness, which varied between hatches from 43.4 to 58.5. The estimated genetic correlation between $pH_{15 min}$ and the pHu was found to be equal to zero, showing that the rate and extent of the post-mortem pH fall were governed by different genes. The pHu of the meat is supposed to be mostly dependent on the initial glycogen reserves of the muscle at the time of slaughter, whereas the rate of the

pH fall is related to the glycolytic enzyme activities just after death (Bendall, 1973). This result was in agreement with the genetic results obtained in pigs, for which Larzul *et al.* (1999) showed that the *in vivo* glycolytic potential was genetically strongly related to pHu but not to the pH at 30 min post mortem. In this study, colour appeared to be mainly genetically related to the pHu of the meat, which was very strongly negatively correlated with paleness. Decreasing the genetic level for the pHu should result in higher drip loss, as a marked negative genetic correlation (−0.83) was found between both traits. This study thus confirmed at a genetic level the importance of the pHu for meat quality, which was already obvious from other phenotypic observations. For example, Barbut (1997b) observed that the relationships between the final meat pH and the colour or water-holding capacity were very significant, at −0.79 and +0.85, respectively. The measure of pH 15 min post mortem did not appear significantly genetically correlated with the other quality indicators, although this factor is usually considered as a major determinant factor of meat quality. However, in experimental conditions, the values of $pH_{15 min}$ remained high (between 6 and 6.75), above the threshold of 5.7 suggested by Kijowski and Niewiariwicz (1978) for characterizing PSE meat in broilers. The genetic correlations were estimated between meat quality traits and body weight as well as body composition traits. The pH levels, at either 15 min or 24 h post mortem, appeared poorly correlated with growth and muscle development of the birds. On the other hand, both characteristics exhibited poor to moderate negative genetic correlations with the redness and yellowness of the meat. These genetic parameters suggested that selection for growth and muscle development would not alter the pH of the meat but could slowly modify its colour by decreasing the redness and yellowness indicators. This was completely in agreement with the results obtained when comparing selected and unselected broiler lines (Berri *et al.*, 2001).

In turkeys, Fernandez *et al.* (2001) conducted a study on meat quality in a

Table 4.3. Heritabilities and genetic correlations with their approximate standard errors for meat quality and body composition traits[a] in broiler chickens (Le Bihan-Duval et al., 2001).

	$pH_{15\ min}$	pHu	L*	a*	b*	DL	BW	BRY	AFP
$pH_{15\ min}$	**0.49 ± 0.01**								
pHu	0.02 ± 0.04	**0.35 ± 0.03**							
L*	0.13 ± 0.03	−0.91 ± 0.02	**0.50 ± 0.03**						
a*	−0.23 ± 0.03	0.14 ± 0.06	−0.48 ± 0.05	**0.57 ± 0.02**					
b*	0.05 ± 0.04	−0.43 ± 0.04	0.20 ± 0.04	0.54 ± 0.04	**0.55 ± 0.04**				
DL	−0.29 ± 0.04	−0.83 ± 0.04	0.80 ± 0.03	−0.25 ± 0.07	0.16 ± 0.07	**0.39 ± 0.04**			
BW	−0.06 ± 0.02	0.07 ± 0.03	0.16 ± 0.01	−0.30 ± 0.04	−0.13 ± 0.05	−0.04 ± 0.05	**0.35 ± 0.02**		
BRY	0.12 ± 0.03	0.13 ± 0.03	−0.07 ± 0.03	−0.29 ± 0.04	−0.39 ± 0.04	−0.16 ± 0.04	0.17 ± 0.02	**0.55 ± 0.01**	
AFP	−0.04 ± 0.02	−0.54 ± 0.04	0.50 ± 0.05	−0.24 ± 0.03	−0.02 ± 0.04	0.29 ± 0.06	0.19 ± 0.03	−0.17 ± 0.02	**0.62 ± 0.03**

[a]$pH_{15\ min}$, pH 15 min post mortem; pHu, ultimate pH; L*, lightness; a*, redness; b*, yellowness; DL, drip loss; BW, body weight; BRY, breast yield; AFP, abdominal fat percentage.

grandparental female turkey line to achieve the effect of the rate of post-mortem pH decline on the processing ability of the meat and to estimate genetic variability of the latter trait in relation to the pHu and colour of the meat. The technological yield was significantly lower in the groups showing the lowest pH_{20} for either white meat (Fig. 4.2a) or thigh. Lower pH_{20} values were also associated with increased drip loss (Fig. 4.2b for the white meat) and a paler aspect (higher whiteness) of the meat.

These data confirmed the detrimental effect of excessive rate of pH fall on turkey meat quality. Such results could lead to practical recommendations, such as selecting the raw meat intended for processing on the basis of this criterion.

In order to determine the impact of the genetics on these meat quality traits, REML estimates of the genetic parameters for pH_{20},

pHu and colour indicators (L*; a*; b*) were calculated, on the total of the available 420 pedigree birds, slaughtered under industrial conditions (Table 4.4). These birds were the progeny of 30 sires and 118 dams. As reported, a moderate level of heritability was obtained for the rate of pH fall measured in the breast muscle ($h^2 = 0.22$) or in the thigh muscle ($h^2 = 0.20$). Other meat characteristics exhibited low to moderate heritabilities, ranging from 0.12 to 0.21, when measured in the breast muscle. By contrast, the impact of the genetics appeared extremely low for the same traits measured in thigh muscle, with heritabilities close to zero. There are no other results in poultry on the genetic parameters of meat quality of the thigh muscle, which has an intermediate metabolic type. However, in a study by Larzul (1997) on pigs, very low heritabilities for the L* and a* values were also obtained in a red
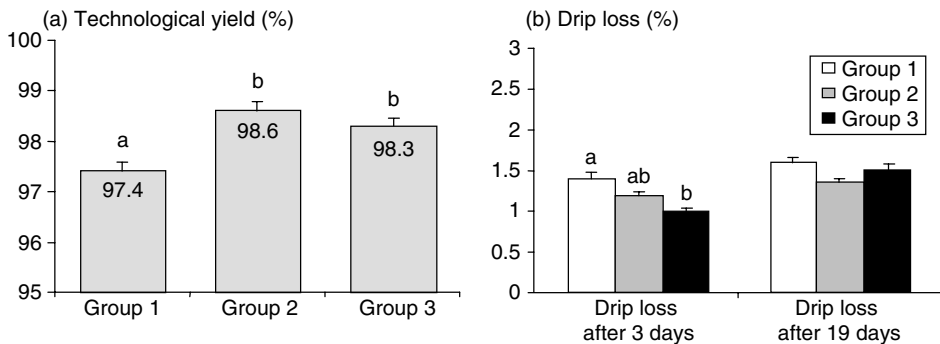
Fig. 4.2. Effect of pH group on (a) the technological yield during processing and (b) drip loss of pre-sliced and pre-packed processed products for the white meat. Group 1, low pH_{20} (5.66 < pH_{20} < 6.0); Group 2, medium pH_{20} (6.18 < pH_{20} < 6.3); Group 3, high pH_{20} (6.46 < pH_{20} < 6.9). Vertical bars show the standard error of the mean. [ab]Means lacking common letters differ significantly at α = 5%. (Fernandez et al., 2001.)

Table 4.4. Heritabilities and genetic correlations with their approximate standard errors for meat quality and growth traits[a] in a commercial turkey line (Le Bihan-Duval, 2001, unpublished results).

	BW	BRY	pH_{20}	pHu	L*	a*	b*
BW	**0.35 ± 0.05**	0.13 ± 0.10	0.55 ± 0.11	0.55 ± 0.11	−0.41 ± 0.19	0.15 ± 0.11	−0.38 ± 0.15
BRY		**0.32 ± 0.04**	0.61 ± 0.10	0.22 ± 0.14	−0.24 ± 0.17	−0.16 ± 0.12	−0.29 ± 0.13
pH_{20}			**0.22 ± 0.04**	0.60 ± 0.10	−0.79 ± 0.11	−0.25 ± 0.12	−0.20 ± 0.17
pHu				**0.16 ± 0.04**	−0.53 ± 0.18	0.08 ± 0.12	−0.01 ± 0.21
L*					**0.12 ± 0.04**	0.21 ± 0.13	0.47 ± 0.19
a*						**0.21 ± 0.05**	−0.05 ± 0.20
b*							**0.14 ± 0.04**

[a]BW, body weight; BRY, breast yield; pH_{20}, pH 20 min post mortem; pHu, ultimate pH; L*, lightness; a*, redness; b*, yellowness.

muscle, in contrast to the intermediate heritabilities estimated for some mixed or white muscles. Moreover, these results indicated a strong negative genetic correlation between the rate of pH decline and the paleness of breast meat. This was not surprising as both traits characterize the PSE syndrome, abnormally low pH at an early post-mortem time, when carcass temperature is still high, leading to protein denaturation responsible for alterations in colour (and water-binding capacity). Surprisingly, a significant positive genetic correlation was estimated between the rate and the extent of the pH fall in breast muscle. No explanation can be given at this time, as this was not in agreement with the first results in chickens or with the genetic results obtained in pigs.

These genetic results indicated that the lowest pH values, at either 20 min or 24 h post mortem, were associated with the lowest growth and muscle development performances, with very significant positive genetic correlations. These results, concordant with previous phenotypic observations in turkeys (Fernandez *et al.*, 2001) and chickens (Berri *et al.*, 2001), do not validate the hypothesis that birds intensively selected for growth and muscle development would be more susceptible to meat defects. This contrasts with pigs, for which a genetic antagonism between technological quality and growth or body composition traits is usually reported, the halothane susceptibility gene being the major factor responsible for this antagonism (Sellier and Monin, 1994).

It remains important to determine the changes in physical and chemical characteristics of muscles and of their constituents in different strains or crosses, as such characteristics can influence the quality of processed meat products. Xiong *et al.* (1993a) measured variations in muscle chemical composition, pH and protein extractability in male broilers of eight different genetic crosses of commercial strains. This study demonstrated the existence of different muscle chemical composition, and different water-binding properties (extractability) of protein (Table 4.5), which are associated with strain crosses.

The small differences in muscle chemical composition may slightly alter the nutritional value of the meat, while the small differences in muscle biochemical characteristics can have a significant impact on muscle functionality (emulsifying capacity, gel strength, water-holding capacity). In another study, Xiong *et al.* (1993b) showed that the strain cross influenced the cooking quality of broiler meat. Pre-cooking adjustments for meat pH and salt content significantly reduced the cooking loss. However, the effectiveness varied among different broiler strain crosses. These results indicate

Table 4.5. Body weight, proximate chemical analysis, pH and protein extractability of breast muscles from males of different broiler strain crosses at 8 weeks of age (Xiong *et al.*, 1993a).

Strain cross	Body weight (kg)	Moisture (%)	Protein (%)	Fat (%)	pH	Protein extractability (%)
1	2.43	75.9[a]	21.5[d]	1.3[bc]	6.00[ab]	75.9[a]
2	2.56	75.5[ab]	20.7[e]	1.4[bc]	5.99[ab]	74.3[ab]
3	2.79	75.1[ab]	22.2[c]	1.0[c]	5.97[abc]	66.2[c]
4	2.58	74.6[b]	22.3[c]	1.2[bc]	5.94[bc]	66.7[c]
5	2.70	75.0[ab]	22.8[bc]	1.5[bc]	6.04[a]	68.7[bc]
6	2.29	75.1[ab]	23.6[a]	2.0[a]	5.98[ab]	63.1[c]
7	2.60	75.4[ab]	23.0[ab]	1.7[ab]	5.88[c]	65.1[c]
8	2.63	74.8[ab]	23.6[a]	2.0[a]	6.03[ab]	64.2[c]
Pooled means		75.2	22.4	1.5	5.98	68.0
Pooled SEM		0.5	0.1	0.1	0.2	1.3

[a–e]Means within a column with no common superscripts differ significantly ($P \leq 0.0.5$). Each value represents the average for three samples; each sample was pooled from three birds per replicate and analysed in triplicate.

the importance of assessing optimum process variables to make adequate comparisons of the cooking quality of new varieties of broiler chickens.

Conclusion

As previously stated, meat quality is a large and complex notion. For a long time, it has only been considered from a sanitary or nutritional point of view. According to this, poultry meat has been more or less well thought of by the consumer, but always with the considerable advantage of remaining a good way of introducing large quantities of proteins in to a meal at a very low cost. This economic advantage of the poultry meat (when compared with beef or pork) is largely due to the efforts made by the poultry industry to remain competitive. Among all the participants in this challenge, breeders have a specific place. They have contributed to the production of birds that can be bought by most of the people because of the low cost of production (fast growth rate, better weight gain:feed ratio, low mortality, etc.). Today the consumer also wants a good product with specific organoleptic properties (depending on his or her cultural origin) and feels more concerned about the well-being of the animal, which explains the success of some extensive products such as the *Label Rouge* chickens produced in France. From the processing plant point of view, the demands have also changed slightly because most poultry carcasses are sold as portions or further processed products. As most of the breast fillets or thighs are cooked in large quantities, the industrial processing plants have expressed a higher exigency concerning the cutting yields and the appearance of the portions as well as some specific technological properties such as the colour or the water-holding capacity of the meat. According to the results on meat quality obtained until now, a very significant contribution of the breeders for resolving some existing problems (such as PSE meat in turkeys and chickens) could be

anticipated. However, the research effort to determine which physiological mechanisms and genes are involved in the variation of meat quality must be increased. This is a prerequisite for the establishment of breeding programmes that will ensure high-performance birds together with an acceptable meat quality for both the consumers and the processors.

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5 Behaviour Problems Associated with Selection for Increased Production

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Introduction

The initial domestication of poultry was possible because the wild ancestors of modern domesticated species exhibited characteristics favourable to this process. These characteristics include: a hierarchical group structure; sexual behaviour with males dominant to females and mating signals provided by posture; precocial young with early parent–offspring attachment; nonspecific dietary requirements; willingness to interact positively with humans; and adaptability to a wide range of environments (Hale, 1969). Some of these behaviours are no longer important in modern intensive agriculture (Siegel, 1975) because of major changes in housing and management, such as: keeping birds in large groups at high stocking densities; artificial insemination; artificial incubation and rearing of young chicks; the feeding of fully formulated mashed diets; and the use of environmentally controlled housing. Decades of strong selection for improved growth or egg production have not been associated with the major changes in behavioural traits needed to adapt the birds to these environments, and in many cases the consequence is maladaptive or abnormal behaviour.

Some management practices, such as beak trimming and very low light intensities, have masked (or have even been introduced to reduce) some of the behavioural problems caused by selection for higher production capacity. Because of animal welfare concerns and the growing markets for eggs and meat produced in alternative production systems (e.g. free-range systems), production practices are again changing rapidly and behavioural problems are reappearing. The more important of these behavioural problems in laying hens, as well as the major welfare issues associated with selection of meat-type birds for rapid growth (such as skeletal disorders and the severe feed restriction imposed on broiler breeders), will be the subject of this chapter.

Social Behaviour

Birds kept in social contexts that are very different from those that would be seen in the natural environment may experience difficulty in establishing appropriate social relations. This is likely to cause extreme aggression leading to injuries, which in turn can lead to higher mortality due to bacterial infections as well as exposure to cannibalistic pecking.

Domestic fowl are descendants of the Red Junglefowl, a species that lives in highly organized social groups in the wild (Mench

and Keeling, 2001). The basic social unit in feral fowl and Red Junglefowl consists of a dominant rooster and a harem of 4–12 females accompanied by subadult offspring. Before 1950, commercial chickens were also kept in small flocks, either outdoors or with outdoor access. To reduce housing and labour costs per bird, however, poultry flocks became larger and the use of indoor deep-litter systems became established. For layer-type strains, this changed gradually to housing on sloped wire floors (all slats), leading to extreme nervousness and hysteria (Hansen, 1976; Mills and Faure, 1988; Laycock and Ball, 1990). Battery cages were then adopted, first with a single hen per cage, but later with more hens and increasing stocking density. Today, most hens are kept in cages containing three to ten hens given 360–650 cm^2 of floor space each, while broiler chickens are still kept on litter but in flocks containing tens of thousands of birds. A gradual return to deep-litter systems for hens has been seen in the northern part of Europe, but in contrast to earlier times the typical flock size is now several thousand hens.

While these changes in housing and management were taking place, intense selection pressure was also being applied to increase egg production by increasing clutch size and accelerating onset of lay. This selection had correlated effects on social behaviour. Hens from lines subjected to selection for early maturity are more aggressive than unselected lines, and hens selected for either high production or early maturity are socially dominant to unselected hens (Lowry and Abplanalp, 1970; Craig *et al.*, 1975; Bhagwat and Craig, 1977). Conversely, selection for higher social dominance reduces the age at 50% lay, and increases egg production and the tendency to attack strangers (Craig, 1970). Modern layer strains, therefore, are supposed to have high levels of aggression. This is indeed often the case in medium-sized groups, but the number of agonistic interactions seems to be very low in high-density cages with small group sizes (Hughes and Wood-Gush, 1977). Interestingly, the same reduction of agonistic interactions is observed in very large groups

in modern floor systems (Hughes *et al.*, 1997) though this could also be due to recent genetic changes. The reason for these contrasting results remains obscure but the fact is that welfare or production problems due to dominance aggression are not the major issue in modern egg production systems. An exception is when hens are kept at high density with limited feeder space, in which case the subordinate birds may have difficulty accessing the feed and consequently go out of production (Cunningham and van Tienhoven, 1983).

Selection for growth characteristics appears to have effects on aggression that are opposite to those of selection for high egg production. Meat-type poultry show lower rates of pecking and threatening than laying-type birds (Mench, 1988) and aggressive interactions are rarely, if ever, observed in broiler flocks (Preston and Murphy, 1989; Estevez *et al.*, 1997).

Fear

Birds kept in environments where external stimulation is low, such as battery cages, may experience difficulty coping with subsequent environmental change. Indeed, they are likely to overreact to seemingly innocuous stimuli. Inappropriate fear responses can cause injury, pain or even the death of a bird or its companions. Fearfulness (the propensity to be easily frightened by a wide range of potentially alarming stimuli) is also negatively associated with productivity (Barnett *et al.*, 1992; Hemsworth and Coleman, 1998).

Increased docility has accompanied the domestication process even though many chickens, quail, turkeys and ducks still show pronounced fright reactions when they are exposed to unfamiliar objects, noises or people (Jones and Hocking, 1999). The potential scope for reducing fear through selection is illustrated by a range of results, from experiments using chickens and quail, that demonstrate that selection can be used to reduce tonic immobility, increase activity in a novel test arena and reduce the adrenocortical response to brief mechanical

restraint (Jones and Hocking, 1999). These selection programmes also appear to exert general (i.e. non-specific) effects on fear.

Locomotion and Activity

Proper use of the pen and free-range area is important in relation to both production and welfare. When birds spread over a wider area of the facilities provided to them, there is a more even use of resources (nests, feeders, drinkers, etc.) and this reduces stocking density. Reduced stocking density has been shown to alleviate problems of feather pecking and cannibalism in laying hens (see below). In larger groups of layers, some individuals tend to stay more in particular areas (Craig, 1982; Appleby *et al.*, 1985). There is a striking individual variation in patterns of pen usage and a large number of variables can influence movement and spacing in extensive environments, e.g. flock size, stocking density and breed (Mench, 1992). For example, the extent and pattern of use of the range area in flocks of 15 (Keeling and Duncan, 1991) or

35–40 hens (Kjaer and Isaksen, 1998) show large individual as well as breed differences (Fig. 5.1). The mechanisms responsible for these differences are not known, but differences in the intensity of social reinstatement behaviour or fearfulness could be involved.

Both fearfulness and social reinstatement behaviour may be amenable to genetic selection. Two commercial hybrids of laying hens were found to differ in sociality measured by a runway test and proximity in the home pen (Hocking *et al.*, 2001). Divergent selection for low or high levels of social reinstatement behaviour in Japanese quail results in significant line differences in just a few generations (Mills and Faure, 1991). Therefore, it might be possible to manipulate range behaviour in hens by genetic selection.

Meat-type birds have been selected over many generations for rapid growth, improved feed conversion and certain conformation traits (e.g. broad-breastedness). This selection has had correlated effects on behaviour. With the exception of feeding behaviour, market-age broiler chickens are
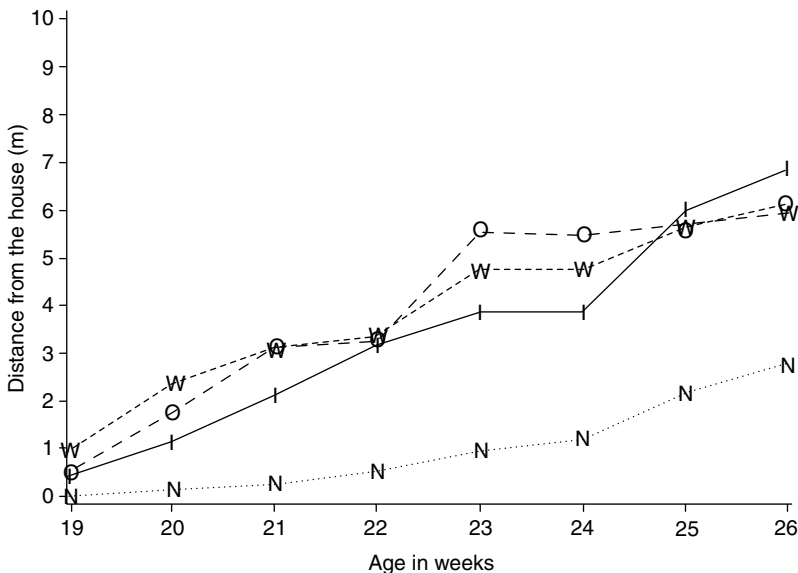

Fig. 5.1. The average distance from the house recorded at 19–26 weeks of age in four lines of laying hens: I, ISA Brown; N, New Hampshire; W, white Leghorn pure line; and O, cross between W and N (J.B. Kjaer, unpublished). There was a total of 24 groups with about 35 hens per group. First access to the range area was given at 16 weeks of age.

considerably less active than laying-strain chickens; for example, they may perform only limited dustbathing (Vestergaard and Sanotra, 1999) and locomotor behaviour. Faster-growing broiler strains are less active than slower-growing broiler strains, suggesting that there is a genetic basis for these differences in behaviour patterns (Bizeray et al., 2000).

Studies of activity patterns in broilers all indicate that the amount of locomotor activity declines with age while the amount of time spent lying increases (Newberry et al., 1988; Preston and Murphy, 1989; Lewis and Hurnik, 1990; Newberry and Hall, 1990; Gordon, 1994; Weeks et al., 1994; Estevez et al., 1997). The most likely reason for a reduction in movement and an increase in lying is an increase in the severity or incidence of skeletal problems with age. Broiler chickens are prone to a variety of infectious and non-infectious skeletal disorders (Whitehead et al., Chapter 3) including angular and torsional long bone deformities, femoral head necrosis, tibial dyschondroplasia (inadequate ossification of the bone growth plate) and perosis (crooked toes). Their bones are also porous and poorly mineralized (Williams et al., 2000). These skeletal abnormalities are associated with selection of broilers for rapid growth and can cause gait disorders. It is estimated that more than 30% of broilers have high-moderate to severe gait impairment (Sanotra et al., 2001). Broilers with mild to severe gait impairments

self-select feed containing an analgesic at rates significantly higher than those of broilers with no gait impairment (Danbury et al., 2000). Furthermore, birds with high lameness scores negotiate an obstacle course more quickly after administration of an analgesic (McGeown et al., 1999), which is evidence that pain is at least partly responsible for reduced mobility in broilers with leg problems.

Broiler chickens walk less and perform more behaviours (e.g. feeding, preening) while sitting rather than standing as they age, while at the same time gait scores worsen (Fig. 5.2) (Sørensen et al., 2000). Weeks et al. (1994) and Estevez et al. (1997) observed that broilers given access to a free-range area made little use of the area, which the former authors attributed to movement difficulties associated with gait impairment, although other factors in both studies could have affected outdoor use. Weeks et al. (2000) found a direct relationship between gait impairment and reduced activity. They recorded behaviours in 39–49-day-old broilers with different gait scores, and found that lamer birds spent more time lying and less time standing than birds with better walking ability, and also reduced the number of visits they made to the feeder. Reduced mobility has other health effects, since birds that spend more time lying down are also more prone to develop potentially painful problems such as breast blisters or footpad and hock burns (Kestin et al., 1999). Footpad burns in turn
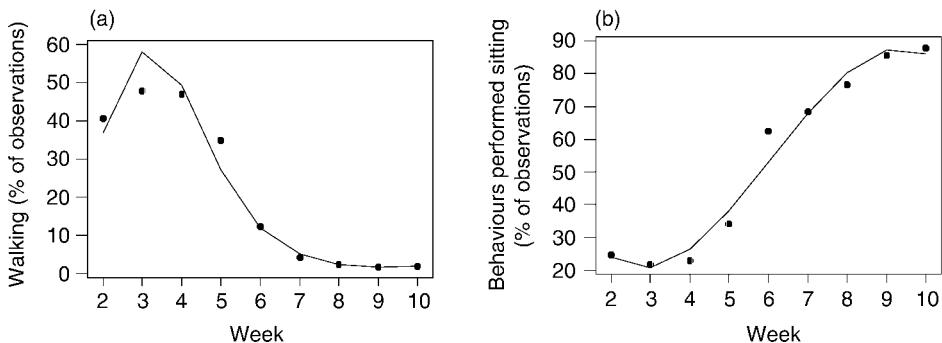

Fig. 5.2. Broiler chickens spend less time walking as they grow older, and also more time performing activities such as preening while sitting rather than standing (J.A. Mench, unpublished).

contribute to reduced gait performance (Sanotra, 1999; Su *et al.*, 1999).

Although skeletal problems may be the primary reason that activity is reduced in broilers, ironically that reduction in activity may cause skeletal problems to worsen. Physical loading in the form of mechanical stresses and strains is essential for normal bone formation (Thomas and Howard, 1964). Biomechanical forces on long bones cause tension and compression and influence the development of normal bone torsion or angulation (Lanyon and Baggott, 1975). Bone mass increases with bone use, and physical loading is critical for maintaining bone mass in poultry and other animals (Lanyon, 1993).

Exercise has been found to decrease skeletal problems in a number of agricultural species, including dairy cows (Gustafson, 1993) and pigs (Grondalen, 1974; Perrin and Bowland, 1977). Direct evidence associating lack of exercise with leg disorders in broilers was provided by Thorp and Duff (1988). They exercised birds on a treadmill daily, beginning when they were 8 days of age. At 33 days of age, 43% of unexercised birds had abnormalities of the physis and physeal vasculature as compared with only 20% of the exercised birds. Exercise thus promotes the blood flow and perfusion through skeletal tissue that is necessary for the maintenance of ossification. There have been several attempts to increase activity in order to decrease skeletal problems in broilers, but the results have been inconsistent (Haye and Simons, 1978; Newberry *et al.*, 1985, 1988; Weeks *et al.*, 1994). However, the lighting and feeding programmes currently used with some success by the industry to reduce leg problems may exert at least part of their effect by increasing bird activity (Riddell, 1983; Prayitno *et al.*, 1997). In a study by Mench *et al.* (2001), providing broilers with opportunities for increased activity in their pens in the form of ramps, dustbathing areas and perches led to an improvement in gait scores, although the birds used these features in quite complex ways that made it difficult to determine the exact relationship between exercise and leg problems.

Feeding Behaviour

The selection of broilers for increased growth rate has resulted in an increase in appetite (Siegel and Wisman, 1966) by modulating both central and peripheral mechanisms of hunger regulation (Lacy *et al.*, 1985; Denbow, 1989). The increased feed intake causes obesity, which must be controlled in broiler parent stock in order to maintain health and reproductive competence. This is accomplished by limiting the quantity of food provided. Feed allocations during rearing are 60–80% less than *ad libitum* consumption would be and are 25–50% less during the laying period (Yu *et al.*, 1992; Savory *et al.*, 1993b). This results in a reduction in the body weight of adults to approximately 45–50% that of birds fed *ad libitum* (Katanbaf *et al.*, 1989). The two most commonly used commercial restriction programmes are: skip-a-day, in which amounts of feed calculated to achieve desired body weights are fed on alternate days; and limited every day, in which half of the skip amount is fed daily. The skip-a-day programme is preferred for males, since it provides greater uniformity of body weight than limited every day feeding.

Despite its demonstrated positive influences on health and reproduction, there is mounting evidence that feed restriction also has negative effects on welfare. Fowl normally spend a considerable portion of their day in activities associated with foraging, and when given a choice prefer to work for at least part of their daily intake of food rather than having it provided for them (Duncan and Hughes, 1972). Feed-restricted broiler breeders consume their feed ration in a very brief period of time, less than 15 min (Kostal *et al.*, 1992; Savory *et al.*, 1993a), and show a number of behaviours indicative of boredom and feeding frustration (Duncan and Wood-Gush, 1971, 1972; Savory and Kostal, 1993). Restricted males are more aggressive during development than fully fed males (Mench, 1988; Shea *et al.*, 1990), while restricted hens and pullets are more active and also display high rates of stereotypical

oral behaviour (e.g. pecking at house structures and at the empty feeder) and pacing (van Niekerk *et al.*, 1988; Savory *et al.*, 1992). Their expression of these latter behaviours is positively correlated with the level of restriction imposed (Kostal *et al.*, 1992; Savory *et al.*, 1992; Savory and Maros, 1993; Hocking *et al.*, 1996) and is considered to be an indicator of poor welfare. Over-drinking (polydipsia) is also a common problem in broiler breeder flocks (Kostal *et al.*, 1992; Hocking *et al.*, 1996), resulting in the need to restrict water intake as well as feed intake in order to maintain litter quality.

There is also evidence that broiler breeders on restricted feed are chronically hungry. Savory *et al.* (1993b) restricted broiler breeder females according to commercial guidelines and then measured feeding motivation using operant conditioning. The restricted hens' level of motivation to consume food was approximately four times that of birds fed *ad libitum* and subjected to 72 h of feed withdrawal.

Genetic selection could be used to lessen the need for feed restriction of females while maintaining current levels of productivity in the progeny. Broilers can be produced by mating normal males with dwarf hens. These hens maintain reproductive competence even when less severely restricted than normal females (Proudfoot *et al.*, 1984; Whitehead *et al.*, 1985). It may also be possible to select genetically lean females that require less severe restriction (Hocking and Whitehead, 1990). As far as restriction of males is concerned, the adoption of artificial insemination technology could minimize the need for restriction, though the use of this technology would raise a number of other behavioural and welfare issues (Mench, 1995).

Another important aspect of feeding behaviour is residual feed consumption, which is the difference between the amount of feed actually consumed and the food consumption expected on the basis of weight gain, egg production and metabolic body weight. Selecting for lower residual feed consumption in laying hens leads to less aggression and less time spent feed-pecking,

walking, pacing and showing escape behaviour (Braastad and Katle, 1989). These last behaviour patterns are indicative of pre-laying frustration and indicate reduced welfare. The change in these behaviour patterns might be due to indirect selection against more energy-consuming behaviours in the high-efficiency line. Thus, selecting for lower residual feed consumption can have positive implications for welfare as well as production economy.

Reproductive Behaviour

Mating behaviour

Selection for production traits has had correlated effects on mating behaviour in meat-type birds. Because of their size and conformation, male turkeys can no longer mate and artificial insemination (AI) is used instead in turkey breeding programmes. Because of technical difficulties in implementing AI programmes for chickens, natural mating is still employed for layer and broiler breeder flocks. However, while fertility is high in commercial layer breeder flocks, maintaining good fertility and hatchability in broiler breeder flocks is becoming increasingly problematic. These difficulties can be traced directly to selection for production traits in the progeny, coupled with a lack of selection for traits relevant to mating behaviour.

In an early study on the effects of selection for rapid growth on fertility, Parker (1961) found that fertility was lower in Cornish cockerels mated to New Hampshire females than in New Hampshire cockerels. Although the fertilizing potential of the semen from the two breeds of males were the same, the Cornish males failed to inseminate 12% of the females in the flock. Broiler breeder males have difficulty completing mating and transferring semen, but it is unclear whether this is due to their size, conformation, the presence of musculo-skeletal lesions that cause leg weakness, or a combination of these factors (Hocking and Duff, 1989). Duncan *et al.* (1991) found that

adult male turkeys that received an injection of an anti-inflammatory drug (bethamethasone) had increased sexual motivation, suggesting that sexual behaviour was normally inhibited by pain associated with skeletal problems. However, even when libido is high, male broiler breeders have difficulty making cloacal contact, which is likely to be due to their size and conformation (Duncan *et al.*, 1990).

The industry has now begun to experience problems with broiler breeder males directing aggression towards females (Mench, 1993), a phenomenon that is normally rare in adult chickens (Wood-Gush, 1956) since males and females form separate social hierarchies and males dominate females passively (Guhl, 1949). As recent work shows, this problem results not from an increased general tendency toward aggressiveness among these males (Millman and Duncan, 2000a), but instead from deficiencies in male mating behaviour apparently correlated with selection for production traits.

Courtship in many domesticated bird species involves elaborate displays, including vocalizations, noises, postures and sometimes skin colour changes (Mench and Keeling, 2001). Roosters court females by vocalizing while performing feeding movements ('tidbitting'), wing-flapping, strutting, and dropping one wing while circling ('waltzing'). If the female is receptive she approaches the male and crouches; copulation then commences. This pattern of mating behaviour seems to have been disrupted in some way by selection for growth characteristics. Millman *et al.* (2000) found that broiler breeder males were much less likely to display courtship behaviour than Leghorns, and were also much more likely to chase females and force copulations on females, who often struggled when mated by these males (Millman and Duncan, 2000b). The pattern of injuries seen on broiler breeder females in commercial houses supports the data indicating that this problem is related to mating behaviour rather than social dominance aggression. Aggressive pecks are typically directed toward the head, but injured females in commercial houses have deep lacerations not only on the head but also along the torso, under the wings (Millman, 1999).

Selection for improved mating behaviour in broiler breeder males should be possible, since elements of mating behaviour in males have been shown to be heritable. In Leghorns, selection has been used to produce high-mating lines of males (Wood-Gush, 1960), and such males show more waltzing, rear approaching, mounting and treading than unselected males (Siegel, 1965; Bernon and Siegel, 1983). However, Leghorn males with high mating scores begin to produce semen later in life than lower-mating mates and also have some undesirable semen traits (lower sperm concentration and volume), so the effects of such selection would need to be balanced against improved behavioural competence. In addition, while problems with aggressive behaviour towards females might be decreased using such selection, fertility will not improve markedly unless male conformation traits are such that males can not only court females but also complete matings successfully.

Nesting behaviour

Nesting behaviour is a characteristic sequence of behaviours associated with nest site selection, nest building and oviposition. It starts with a period of restlessness followed by examination of potential nesting sites. When a nest site is finally selected, the hen performs rudimentary nest-building movements. Oviposition occurs following these behaviours. Incubation starts when all eggs of the clutch have been laid.

Incubation has almost completely disappeared in modern laying hens. This is due to direct selection against broodiness as well as indirect selection for high egg numbers, thereby increasing clutch size. Nevertheless, having access to some type of nesting area does appear to be very important to hens, since hens are prepared to work to gain access to a nest prior to laying (Cooper and

Appleby, 1996). If hens do not have access to nests, as is the case in battery cages, they still perform pre-laying behaviour, such as pacing (restlessness) and sitting. These behaviours are driven mainly by endogenous factors; they are considered to be indicators of frustration (Duncan, 1970) and appear to be under genetic control (Wood-Gush, 1981). Furthermore, it appears that pacing and sitting during the pre-laying period are inherited as separate characteristics. It has been suggested that pre-laying behaviour can be reduced by selection (Mills and Wood-Gush, 1982; Mills, 1987). The magnitude of additive genetic variation (heritability) has been found to be adequate for such selection. Heil *et al.* (1990) recorded the duration of restlessness, escape behaviour and stance (sitting or standing before oviposition) in hens from five Leghorn strains. Strains differed significantly for all three measures and heritabilities were estimated to be 0.12, 0.09 and 0.53, respectively. As mentioned earlier, indirect selection for pre-laying behaviour was obtained by selecting for increased feed efficiency (lower residual feed consumption) (Braastad and Katle, 1989). This led to less aggression and less time spent feed-pecking, walking, pacing and showing escape behaviour.

The use of nests by laying hens as well as broiler breeders is of importance for both economic and welfare reasons. Eggs laid outside the nests (floor eggs) easily become dirty or lost and a substantial amount of labour is needed to collect them (Ehlhardt *et al.*, 1989). The risk of cannibalistic pecking directed to the cloaca of floor-laying birds has been emphasized by Savory (1995), even though Gunnarsson *et al.* (1999) could not verify this in a cohort study of 59 commercial flocks. Nest-site selection and the use of nests for laying vary between strains (Appleby, 1984). In some cases these differences are due to better perching abilities in lighter strains, but factors other than this also seem to play a role. McGibbon (1976) selected for and against floor laying and found a response at one experimental site but not at another. Sørensen (1992) described a selection programme on egg number in which only eggs laid in trapnests were recorded. This was indirect selection for the use of nests; he concluded that there is a genetic variation in willingness to visit a nest when laying and that this willingness can be increased using a proper selection procedure.

Feather Pecking and Cannibalism

Feather pecking and cannibalism have been recorded in a range of poultry species, game fowl and ostriches. There has been a continuous increase in mortality caused by cannibalism in all brown layer strain crosses tested at the Random Sample Test stations in Germany since the late 1980s (Preisinger, 1997), suggesting that selection for higher egg production and lower body weight has led to higher levels of cannibalism.

A distinction is generally made between self-pecking and allopecking:

- If a bird pecks itself and the feathers, toes, etc. are damaged, this behaviour is referred to as self-pecking or self-mutilation.
- Pecking other birds is referred to as allopecking.
- Aggressive pecking is forceful allopecking, usually directed to the facial region (Kruijt, 1964). Feathers can be damaged, but it is generally acknowledged that aggressive pecking is not a major cause of feather loss (Kjaer *et al.*, 2001). In some cases, however, aggressive pecking can lead to damaged feathers or skin, which in turn leads to feather pecking or cannibalism (Fig. 5.3). A high level of feather pecking has been found to be correlated with a high mortality from cannibalism (Kjaer and Sørensen, 2002).
- Injurious pecking comprises aggressive and feather pecking as well as cannibalism.

The typical 'feather pecking act' has been described by Wennrich (1975). The hen about to perform feather pecking slowly approaches the target hen (recipient) from behind or from the side, focusing on the feathers of the recipient. The recipient in

most cases pays no attention to the performer at first but will often move away after having received some feather pecks, the response depending on the severity of the pecks.

In groups of young chickens, feather pecking is performed by most members of the group (Kjaer and Sørensen, 1997; Wechsler *et al.*, 1998). In pullets and adult hens, close to 50% are observed to feather peck (Bessei, 1984b; Kjaer and Sørensen, 1997). In layer chickens, feather pecking has been reported as early as 7 days of age (Allen and Perry, 1975) and in pheasants as early as 2 days of age (Kjaer, 2000b). One or more peaks in feather pecking can be seen from 3 weeks (Huber-Eicher, 1999) to 15 weeks of age (Hughes and Duncan, 1972; Blokhuis
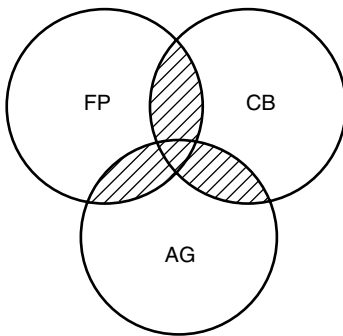
Fig. 5.3. Feather pecking (FP), cannibalism (CB) and aggression (AG) are supposed to have different motivational backgrounds but nevertheless some interactions occur, where one behaviour leads to another, indicated by the overlapping areas.

and Arkes, 1984; Martin, 1986). At the age of 15 weeks to sexual maturity, the level of feather pecking is low (Martin, 1986; Huber-Eicher, 1999). At this time growth of new feathers brings the plumage into perfect condition. Pecking rises at the onset of laying (Wechsler and Huber-Eicher, 1998) and this is the time when the risk of cannibalism is the greatest. This rise has been attributed to an increase in gonadal hormones (Hughes, 1973).

The diurnal rhythm of feather pecking seems to vary between strains. Kjaer (2000a) found a rise in feather-pecking activity in the last hours of the day in two 'brown shell' strains. Two White Leghorn strains, however, behaved significantly differently, with a more even distribution of feather pecking during the day and no rise in feather pecking activity just before dark (Fig. 5.4).

Often the occurrence of feather pecking and, in particular, the spread of cannibalistic pecking in a flock has an explosive character and the word 'outbreak' can be used (Allen and Perry, 1975). One major reason for this spread in a flock could be that feather pecking is elicited in a number of birds within a short period by some environmental factor or factors. Another reason is that damage to the plumage facilitates more pecking (Savory and Mann, 1997; Freire *et al.*, 1999; McAdie and Keeling, 2000). Whether one bird can learn from another by observing the other bird feather pecking is not known, but results from one experiment addressing this question indicated that this
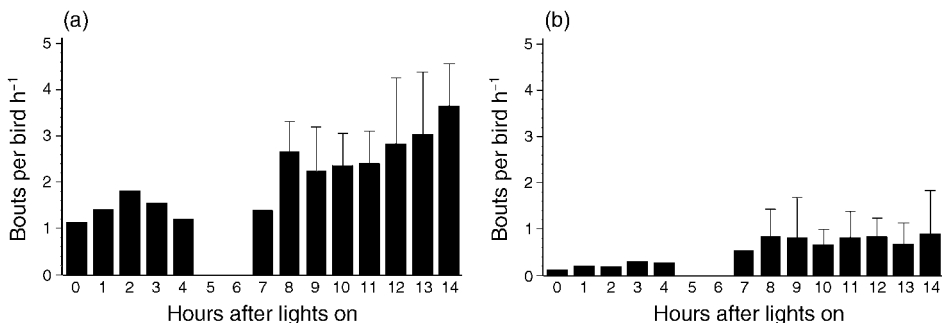
Fig. 5.4. Diurnal rhythm of feather pecking in brown shell vs. white shell hybrids. Brown shell hybrids had a significantly higher rise in level of feather pecking in the afternoon compared with White Leghorns (Kjaer, 2000a). (a) Feather pecking in brown shell (medium heavy) hybrids. (b) Feather pecking in White Leghorn hybrids.

kind of social learning is a possibility (Zeltner *et al.*, 2000).

Numerous authors agree that feather pecking is a redirection of behaviour related to foraging (e.g. Hoffmeyer, 1969; Wennrich, 1975; Martin, 1986; Blokhuis, 1989; Baum, 1992; Huber-Eicher, 1997). More precisely, a part of the exploratory pecking is redirected to the plumage of conspecifics, to a greater or lesser degree depending on the genetic tendency of the chicken, the environmental (including social) circumstances, and the birds' physiological state as far as stress is concerned. These factors may influence the birds from a very early age, before feather-pecking behaviour is seen, as well as at the moment of feather pecking.

Genetic aspects of feather pecking and cannibalism were neglected for a long time but differences between strains and family groups indicate that there is some kind of genetic background (Hughes and Duncan, 1972; Craig and Lee, 1990; Abrahamsson and Tauson, 1995; Craig and Muir, 1996; Ambrosen and Petersen, 1997). In addition to more feather pecking, one of four strains in a study by Sørensen and Kjaer (1999) also suffered higher mortality from cannibalism than the other strains. Other examples of such strain differences in mortality due to cannibalism are given in Robinson (1979), Craig and Lee (1990), Craig and Muir (1991, 1996), Lee and Craig (1991) and Ambrosen and Petersen (1997).

Moderate to high heritabilities (0.22 to 0.54) have been found for plumage condition (Damme and Pirchner, 1984; Grashorn and Flock, 1987; Craig and Muir, 1989). Plumage condition is only an indirect measure of feather-pecking behaviour and can be biased by abrasion. Direct observation of pecking behaviour is a more precise method for assessing an individual bird's tendency to perform feather pecking. Estimation of heritability based on individual performance of feather pecking has been estimated to be between 0.07 and 0.35 (Cuthbertson, 1980; Bessei, 1984a,b; Kjaer and Sørensen, 1997); the higher estimates were derived from observations of older birds.

Group selection has been very effective in reducing the incidence of beak-inflicted injuries in caged hens kept at high stocking densities and under conditions of high social stress and competition (Craig and Muir, 1993), and realized family heritability was estimated to be 0.65 ± 0.13. The term 'beak-inflicted injuries' includes feather pecking, aggressive pecking and cannibalistic pecking. Using a quite different trait for selection – individual rate of feather pecking in groups of 20 hens kept at a low stocking density on litter – high and low feather-pecking lines have been developed. After four generations of selection, significant differences in feather-pecking behaviour and plumage condition were found between the low- and the high-pecking lines (Kjaer, 2001; Kjaer *et al.*, 2001).

While feather pecking and cannibalism are behaviours that are hard to control in certain environments and under certain conditions (large group size, high stocking density, intact beaks, high light intensity) there is now accumulating evidence showing that reduction can be achieved by genetic selection. So far there seems to have been limited selection pressure applied to commercial lines. This could be due to the fact that most hens are still kept in cage systems, in which environmental factors can be manipulated in order to control these damaging behaviours.

Summary

The strong selection for improved growth or improved egg production has brought about changes that seem to reduce birds' adaptability. This, together with the introduction of specialized production systems, has resulted in a range of behavioural problems that reduce well-being. Meat-type birds suffer from reduced behavioural activity, skeletal disorders, hock burn and footpad necrosis causing pain and suffering. Severe feed restriction has to be imposed in order to alleviate reduced reproductive capacity due to obesity in broiler breeders, but the consequence is that the birds experience hunger and at least short-term stress. Using dwarf breeder hens could reduce the need for feed restriction. Selection for growth has

also led to problems with mating behaviour in broiler breeders, including extreme aggressiveness by males toward females and lowered fertility. Selection for improved mating behaviour in broiler breeders should be possible. In turkeys, however, natural mating is impossible due to the extreme conformation and heterogeneous size of the two sexes. In layer-type birds the major problem is injurious pecking behaviour. Selection for higher egg production combined with lower body weight has led to higher levels of feather pecking and cannibalism. There is accumulating evidence supporting the existence of additive genetic effects underlying feather-pecking behaviour, and with regard to cannibalistic pecking there is some indication of one or a few major genes having an influence. Selection lines differing in the propensity to perform feather pecking or cannibalistic pecking have been developed. There are several other behavioural problems shown by hens, including excessive fearfulness, floor laying and pre-laying behaviour in cages, that might be amenable to improvement by genetic selection. Overall, genetic selection is a realistic and effective instrument for improving the well-being of poultry in modern production systems.

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6 Genotype–Environment Interactions: Problems Associated with Selection for Increased Production

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Introduction

The phenomenon of genotype–environment interaction is reflected by the differential expression 'genotypes over environments'. As variability is a rule of nature, these interactions should be expected whenever more than one genotype or more than one environment is considered. However, the significance of these interactions may vary depending upon the magnitude of differences between the genotypic and environmental effects.

The genotypes can be breeds, strains, lines, specifically differentiated genotypes with respect to major genes or markers or individuals such as sires whose progeny have been raised in more than one environment. The environments can be nutrition, climate, housing, location, etc.

Genotype–environment interactions have been investigated in a number of studies in poultry as described in several extensive reviews (e.g. Tindell *et al.*, 1967; Pani and Lasley, 1972; Alihussain-Gadhia, 1983; Mathur, 1985; Merat, 1989; Cahaner, 1990; Hartmann, 1990; Sheridan, 1990).

In the presence of significant genotype–environment interactions, the relative advantages of genotypes may differ from one environment to the other. In some cases it is possible to adjust the environmental conditions to the requirements for the desired genotype. However, in many cases such adjustments are either not possible or not cost effective. Rather it becomes necessary and even useful to choose specific genotypes for specific environments. The choice of appropriate genotypes and selection for their further improvement depend upon the nature and magnitude of the interactions. Therefore, the genotype–environment interactions require additional considerations for selection and breeding programmes and offer several opportunities for production of breeding stock specifically suitable for the desired environmental conditions.

The Concept of Genotype–Environment Interactions

There are several ways to describe genotype–environment interactions. The various classifications that have been proposed in the literature differ greatly with respect to their basis, number of classes and terminologies, but they contribute to an understanding of the meaning of genotype–environment interactions. A classical approach was suggested by Haldane (1946), who tabulated six types of relations

depending upon the ranking order of the genotypes, considering two genotypes (A, B) and two environments (X, Y) as an example. The relative positions were assigned with the criterion that genotype A in environment X always had the highest rank. These relations were classified into four groups and graphically represented by McBride (1958). The comparative terms given by other researchers, based on these relations, are summarized in Fig. 6.1

(Mathur and Horst, 1994a). The six relations described by Haldane (1946) were later grouped into two by Lerner (1950) on the basis of additivity of the genotypic and environmental effects. Lerner (1950) suggested considering the interactions as linear when genotypic and environmental effects are additive and non-linear when they are not. Weber and Le Roy (1956) suggested classifying them on the basis of the statistical significance of interactions and considering

Relation	Ranks of genotypes	Additivity of G and E	Statistical significance	Relative magnitudes of G, E and I	Statistical significance and ranks
1(a)			No interactions	G > E > I	Type 1 No significant interaction
1(b)		Linear interactions		E > G > I	Type 3 Significant interaction
3				G > I > E	
2			Interactions	E > I > G	Type 2 No significant interaction
4(a)		Non-linear interactions		I > E > G	Type 4 Significant interaction
4(b)				I > G > E	
Source	Haldane (1946)	Lerner (1950)	Weber and Le Roy (1996)	Mather and Jones (1958)	Pani (1971)

Fig. 6.1. Classification and terminology for genotype–environment interactions. ◇ A, □ B are genotypes; X, Y are environments; G, E and I are the average effects of genotypes, environments and interactions, respectively. (Source: Mathur and Horst, 1994a.)

the relations 1(a) and 1(b) (Fig. 6.1) as cases of no interactions while the others could be cases of significant interactions.

The differences between these six relations are due to the relative magnitudes of genotypic (G), environmental (E) and interaction (I) effects. These effects can be expressed as absolute deviations from the overall mean, as suggested by Mather and Jones (1958). Here, G is estimated as the average effect of genotypes over both the environments, E as the average effect of environments over both genotypes, and the interaction effect, I, is estimated by $(AX + BY) – (AY + BX)$. In relations of type 1(a) and 1(b) (Fig. 6.1) the interaction effects are smaller than the genotypic or the environmental effects. In most such cases, the interactions are not significant. More common and statistically significant interactions are observed when they are larger than either the genotypic or the environmental effects, but not larger than both effects, as in relations 2 and 3 (Fig. 6.1). Sometimes the interaction effects can be greater than both genotypic and environmental effects, as in relations 4(a) and 4(b) (Fig. 6.1). It is common to observe that the environmental conditions that are unfavourable to one genotype are also unfavourable to the other. However, they might affect one genotype more than the other, leading to a change in the ranking of the genotypes from one environment to the other. It is very rare that the environmental conditions that are unfavourable to one genotype are favourable to others as in relations 3, 4(a) and 4(b) (Fig. 6.1).

Under practical circumstances in poultry breeding, a classification of interactions may not be used directly but it is very useful to know the changes in ranking order of genotypes from one environment to the other and the relative magnitudes of genotypic, environmental and interaction effects.

These classifications and terminology suggest that the three most important aspects while considering the nature of interactions are: (i) ranking order of the genotypes; (ii) relative magnitudes of genotypic, environmental and interaction effects; and (iii) statistical significance of interaction

effects. These three aspects can be illustrated with an experiment considering three different genetic groups tested in Germany (Mathur and Horst, 1994a). Each of the three genetic groups consisted of two genotypes (Table 6.1a) and there were two controlled environmental conditions (Table 6.1b). Figure 6.2 shows the interactions between these genotypes and the two environments with respect to three traits: body weight, shank length and egg production. These three traits have different heritabilities and show different kinds of interactions for the three genotypes. The magnitudes of genotypic, environmental and interaction effects are given in Table 6.2.

The interaction effects with respect to body weight show the relations of type 1(a) for the breeding groups and body size groups and of type 2 for feathering groups. It is important to note here that the main differences among the breeding groups, as well as among the body size groups, are due to the body weights. Therefore, the genotypic effects are substantially larger for these two groups while they are smaller for the feathering groups. The difference between the environmental effects is mainly due to high temperature in the warm stall. The heat stress has an adverse effect on body weight gain; therefore, the resulting body weight is substantially lower for both genotypes in the warm stall. However, the depression due to heat stress is higher on the heavier genotype than the lighter one, due to genotype–environment interactions. The interaction effects, although they are significant, are smaller than the genotypic as well as environmental effects. Therefore, the ranking order of the two genotypes has remained the same in the temperate stall as well as in the warm stall. There is a higher reduction in the body weight of genotype LM due to heat stress but it is still superior to genotype LL in the warm stall. If one were to choose a genotype for conditions similar to those in the warm stall, it would still be reasonable to select the same genotype (LM) as in the temperate stall. The interactions are statistically significant but have a limited impact on selection for increased

Table 6.1(a).   Genetic groups and their genotypes (Mathur and Horst, 1994a).

Genetic group	Genotype	Description
Breeding groups	LL	White Leghorn population lighter in body weight
	LM	Cross between LL and a Rhode Island Red medium heavy population (MM)
Body size groups	dw-	Group of layers with sex-linked recessive allele, smaller in body size
	Dw-	Group of layers with normal body size
Feathering groups	Nana	Group of layers with autosomal incompletely dominant naked-neck gene
	nana	Group of layers with normal feathering

Table 6.1(b).   Controlled environmental conditions.

Environment	Specific conditions	
	Code	Description
Temperate stall	CT	Controlled normal-temperature housing (temperature 18–22°C, relative humidity 70–80%), representing the environmental conditions prevailing in most temperate countries
Warm stall	CW	Controlled high-temperature housing (temperature 32°C, relative humidity 45%), to test the specific effect of high temperature and low humidity, the most important environmental stresses prevalent in most of the tropical countries

production under the given environmental conditions.

In the case of the feathering genotypes, the situation is very different. There are very small differences between body weights of the naked-neck and normal genotypes. The body weight of the naked-neck as well as normal genotypes is lower in the warm stall than in the temperate stall. However, the adverse effect of heat stress is higher on the normal genotypes than on the naked-neck genotypes due to genotype–environment interactions. The ranks of the genotypes in the warm stall are in the reverse order compared with the temperate stall. The interaction effects are statistically significant and are greater than the genotypic effects but smaller than the environmental effects. The normal genotypes seem to be more suitable for conditions similar to the temperate stall, but under conditions similar to the warm stall the naked-neck genotype seems to be more suitable.

Shank length (Fig. 6.2) represents body size and is a trait with very high heritability. This shows a classic situation where there are very limited environmental effects and almost no interactions. Egg production

represents an economically important trait with lower heritability. This shows the relations of type 2 for breeding and feathering groups and statistically and biologically significant interactions.

Genotype–Environment Interactions and Selection

One of the important problems in presence of interactions is that of selection. The question is whether the selection should be carried out under more favourable environmental conditions that allow maximum expression of the genotype or whether it should be carried out in the environment where the genotype is actually destined to live. There are arguments that suggest that progress can be maximized by selecting under conditions most favourable for the expression of the trait (Hammond, 1947), but there are reasons to believe that genetic superiority in one environment may not hold for other environmental conditions. Falconer (1952) suggested considering the phenotypic expression of the same
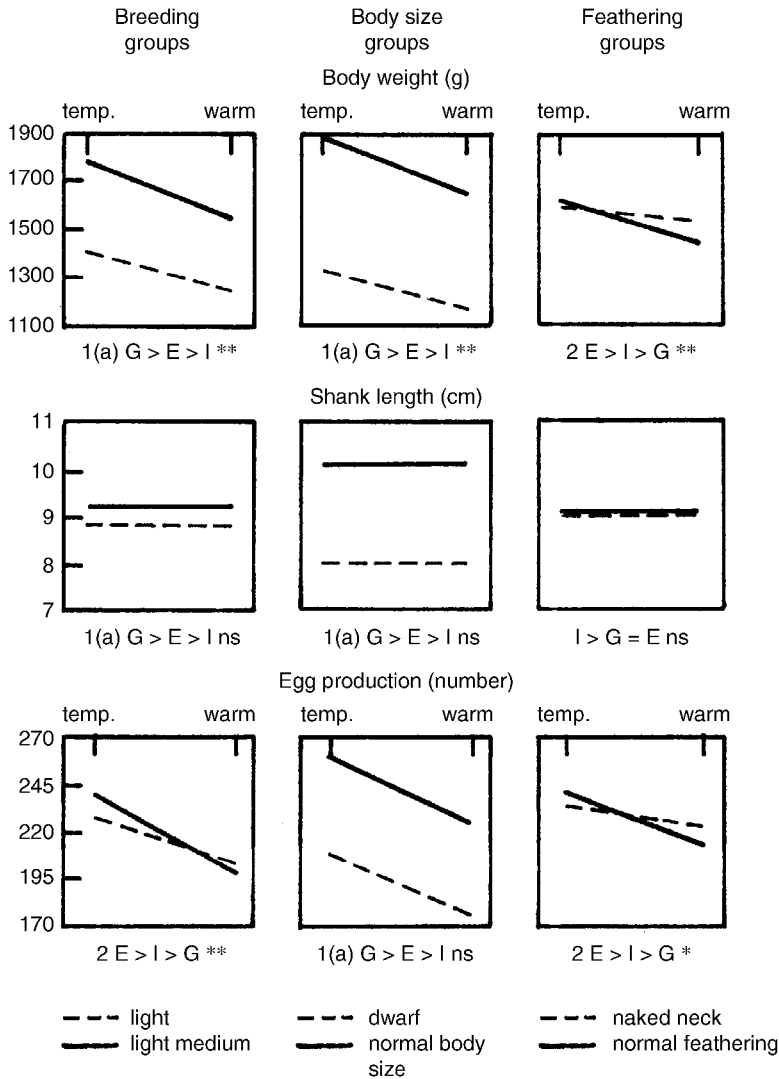
Fig. 6.2. Examples of classifications for genotype–environment interactions. $**P < 0.01$; $*P < 0.05$; ns, $P > 0.05$; 1(a), 1(b), 2, 3, 4(b) are the respective relations given by Haldane (1946); G, E and I are average genotypic, environmental and interaction effects, respectively, following Mather and Jones (1956). (Source: Mathur and Horst, 1994a.)

genotype in two environments as different traits. The concept allows the above question to be answered by estimating the relative progress that can be made from direct selection for performance in a given environment compared with that from indirect selection in a different environment. According to this concept, assume that the phenotypic expressions of a trait in environments 1 and 2 are P_1 and P_2, the direct response to selection for P_1 is ΔG_1, and the correlated response in P_1 based on selection in P_2 is $\Delta' G_1$. Assuming equal selection intensities in both the environments, the ratio of correlated response to the direct response can be obtained as follows (like any two correlated characters):

$$\Delta' G_1 / \Delta G_1 = h_2 r_g / h_1$$

Table 6.2.   Estimates of genotypic, environmental and interaction effects. (Source: Mathur and Horst, 1994a.)

	Effects	Breeding groups	Body size groups	Feathering groups
Body weight (g)	Genotypic (G)	176	262	11
	Environmental (E)	98	98	57
	Interaction (I)	17	20	25
Shank length (cm)	Genotypic (G)	1.8	1.1	0.01
	Environmental (E)	0.1	0.1	0.01
	Interaction (I)	0.0	0.0	0.02
Egg production (no.)	Genotypic (G)	1.6	24.9	1.4
	Environmental (E)	17.2	17.2	9.4
	Interaction (I)	3.7	0.2	3.8

where, h_1 and h_2 are the square roots of heritabilities in environments 1 and 2, respectively, and r_g is the genetic correlation between measures of the same trait in two environments. It can be noted here that, if the quantity $h_2 r_g$ exceeds h_1, indirect selection would be more efficient. However, genotype–environment interactions reduce r_g depending upon their own magnitude and may render the selection in one environment for performance in another rather useless. Thus the genetic correlations (r_g) and the differences between the heritability estimates within the environments are very important in recognizing the role of genotype–environment interactions in selection decisions.

Methods for Estimating Magnitude of Interactions as Genetic Correlation

The methods for estimating the genotype–environment interactions mainly depend upon the kinds of genotypes and environments studied in the biometrical sense. The environments can be a few fixed effects (e.g. location, poultry houses, feeds). The genotypes can be some fixed effects (e.g. breeds, lines, genetic group) or several random effects (e.g. sires, individuals). If there are only a few genotypes, the main interest is in changes in their average performance reactions to different environments revealed by genotypic means and interaction deviations. On the other hand, if there are several genotypes the variance among them

(genetic variance) and interaction variance become relevant and the magnitude of genotype–environment interactions can be estimated as a genetic correlation between the expression of the genotypes in different environments. This genetic correlation is expected to be 1 if there are no interactions. The greater the deviation from 1, the higher are the interactions. The methods for estimating the magnitude of genotype–environment interactions, as genetic correlations, have been described by Prabhakaran and Jain (1994) and Mathur and Horst (1994a).

This genetic correlation can either be estimated as an intraclass correlation or as a product moment correlation between part breeding values of the same individuals in different environments. The concept of intraclass correlation is only relevant if there are several randomly chosen genotypes. It cannot be used when there are only a few of them.

The following univariate factorial model is commonly used to describe the interactions:

$$Y_{ijk} = \mu + G_i + E_j + I_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is an observation of trait Y on the kth individual of the jth genotype, G_i is the effect of the ith genotype, E_j the effect of the jth environment, I_{ij} the interaction between the ith genotype and the jth environment, and ε_{ijk} the residual effect. The genotypes and the residual effects are assumed to be random, while the environments are either random or fixed effects.

In the case of few genotypes, the magnitude of interaction effects can be

estimated using least-squares procedure and the statistical significance of interaction effects can be tested through an analysis of variance followed by an F-test. If the genotypes are random effects, variance components can be computed by equating the mean squares to the expectations or by other direct procedures (maximum likelihood, restricted maximum likelihood, etc.). The interaction variance may be expressed as fraction of genetic variance, sum of genetic and interaction variance or total phenotypic variance to evaluate their relative significance.

The interaction sum of squares can be partitioned into two parts to determine the proportion of interaction that is due to heterogeneous variances and that due to imperfect correlations of rankings. Two methods for partitioning the interaction effects have been described by Muir *et al.* (1992). Their relative merits depend upon the nature of genotypes and environments, whether they are fixed or random and whether the interactions are mainly due to imperfect correlations between ranks or are due to heterogeneous variances.

The genetic correlation (r_g) between expressions of the same genotype in two or more environments can be estimated as intraclass correlation using the following formulae as suggested by Yamada (1962):

$$r_g = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_I^2 - V(\sigma_G)} \quad \text{(random model)}$$

$$r_g = \frac{\sigma_G^2 - \dfrac{1}{a}\sigma_I^2}{\sigma_G^2 + \dfrac{a-1}{a}\sigma_I^2 - V(\sigma_G)} \quad \text{(mixed model)}$$

where σ_G^2 and σ_I^2 are the variance component due to genotypes and genotype–environment interactions (σ_{GE}^2), a is the number of environments and the term $V(\sigma_G)$ is used to adjust for the bias when the variances are different. In the case of two environments:

$$V(\sigma_G) = \tfrac{1}{2}(\sigma_{G1} - \sigma_{G2})^2$$

A multivariate approach following the suggestion by Falconer (1952) considers the expression of a trait Y in two environments as variables, Y_1 in environment 1 and Y_2 in environment 2, affected by the same set of genotypes. A simplified form of the model for each of the variables can be given as:

$$Y_{ij1} = \mu_{1 +} G_{i1} + e_{ij1} \text{ and } Y_{ij2} = \mu_2 + G_{i2} + e_{ij2}$$

where Y_{ij1} is the observation of trait Y in environment 1, taken on the jth individual of the ith genotype and Y_{ij2} is the observation in environment 2 taken on a different individual j of the same genotype i; μ_1 and μ_2 are the respective means in environment 1 and 2, G_{i1} and G_{i2} the effects of genotype, and e_{ij1} and e_{ij2} residual effects. Genotypes and the residual effects are considered as random variables.

Following this approach, the genetic correlation between measures of the same trait in two environments can be estimated through variance and covariance analysis as in the case of any pair of correlated characters measured on different individuals (e.g. two sex-limited characters) as follows:

$$r_g = \frac{\sigma_{G\,12}}{\sigma_{G\,1} \cdot \sigma_{G2}}$$

where r_g is the genetic correlation using the multivariate approach, σ_{G12} is the covariance between the genotypic effects in the two environments and σ_{G1} and σ_{G1} are the genotypic standard deviations within environments 1 and 2, respectively.

However, since the observations of a genotype in different environments are taken in different individuals, they cannot be treated as paired observations and a simple covariance analysis cannot be used. A simpler method is estimating the breeding value of each sire in each of the two environments and calculating a product-moment correlation between them. This correlation between breeding values has been used in several studies. In addition to this, a bivariate distribution of these breeding values can be plotted to examine the pattern of relationship between them, as shown by Mathur and Horst (1994b). The sires can also be ranked on the basis of their breeding values within environments, and rank correlations as computed by Parekh and Pande (1982) can be used to make decisions about selection of superior individuals.

This multivariate approach is advantageous in various ways, but correlations between the estimated breeding values may also deviate from 1 because of reasons other than the genotype–environment interaction, i.e. expectations under the ideal situation of total absence of interaction may be lower than 1, depending upon sample size, heritability and accuracy of the estimates of breeding values. A test of significance under such circumstances is proposed by Simianer (1991). An alternative method is to use the expectation of this correlation determined by accuracy of the two progeny tests (Christensen, 1970). The magnitude of interaction effects should then be proportional to the deviation of the estimated genetic correlation from this computed expected value and not from the expected value of 1.

The two approaches – univariate and multivariate – are equivalent under only very restrictive conditions and using several assumptions (Fernando *et al.*, 1984; Yamada *et al.*, 1988; Itoh and Yamada, 1990; Mathur and Schlote, 1995). These assumptions may or may not be true under practical situations in poultry breeding. Consideration or omission of some of the assumptions may lead to inaccurate results. Hence, a univariate model may be used to test their significance (using an analysis of variance) and if they are significant, the genetic correlation should be estimated using a multivariate approach.

Examples of Genotype–Environment Interactions in Layers

Genotype–environment interactions can involve many environmental conditions. However, it is important to consider conditions that are not always under cost-effective control of the breeder or producer. Examples of such conditions include ambient temperature, humidity, geographical location, etc. They are especially important if some of the environmental conditions are unfavourable.

Poultry production in the unfavourable environmental conditions of the tropics mainly suffers from direct and indirect heat stress leading to a general depression in performance, which may not be uniform in all genotypes, revealing significant genotype–environment interactions. A series of experiments was conducted at the Institute of Animal Production, Berlin, over a period of about 20 years, to explore various aspects of such interactions in layers (e.g. Petersen and Horst, 1978; Mukherjee *et al.*, 1980; Alihussain-Gadhia, 1983; Horst, 1985; Mathur, 1985; Rauen, 1985; Mathur and Horst, 1988; Haaren-Kiso, 1991). The environmental conditions consisted of temperate (CT) and warm stall (CW) in Germany, as described earlier, as well as natural tropical open housing in Malaysia (TO; temperature 20–34°C; relative humidity 60–80%), representing humid tropics. These tests were later extended to several other countries (Horst *et al.*, 1995).

Three groups of genotypes were tested simultaneously under these environmental conditions: high-yielding layer lines; genetic groups carrying one or more major genes; and a number of sires whose progenies were distributed over the three environmental conditions. The interactions of each kind of genotype are discussed separately.

Line × environment interactions

Three lines were tested, the main differences among them being due to their body weights. These were: a White Leghorn type lightweight line (LL); a Rhode Island Red type brown medium heavy line (MM); and a cross between the two (LM). There were significantly larger interactions of these genotypes with the three environmental conditions (Mathur and Horst, 1989). In general, there was higher depression in the performance of the heavier genotype than the lighter ones, though magnitudes of the interactions were different for different traits and for different pairs of environments. The interactions were higher in production traits than in traits of growth and body size. This body-size-related expression of the performance reaction to unfavourable environments is supported by

the 'body size adaptability phenomenon', postulated by Horst (1981). This phenomenon reveals that body size is an important factor governing these interactions and the optimum level of body weight is lower for the tropics than for temperate environments (Horst, 1984).

Gene × environment interactions

Significant genotype–environment interactions may be caused not only by specifically differentiated genotypes, such as breeds or lines, but also by single major gene effects.

Several major genes, found in tropical local populations, have revealed significant genotype–environment interactions and the suitability of specific genotypes carrying these major genes for given environmental conditions.

The naked-neck gene (*Na*) responsible for general reduction of feathers over the body surface and total loss of feathers in the neck region has shown very favourable results under heat stress (Rauen, 1985; Merat, 1986). The heterozygous naked-neck layers (*Nana*) had significantly higher egg number, egg weight, egg mass and body weight under constant heat stress. The frizzle gene (*F*) also revealed its favourable effects on productivity but they were less pronounced than the naked-neck gene (Somes, 1988; Haaren-Kiso, 1991). The dwarf gene reduces body size and thereby causes a reduction in egg number and egg size but there is a significantly lower depression due to heat stress in dwarf layers than in the normal types. Thus the dwarf gene improves productive adaptability to heat stress. An especially favourable effect of this gene is improvement in feed efficiency (Merat, 1990).

These three major genes – naked-neck, frizzle and dwarf – were introduced into a commercial brown-egg layer line and were tested in Turkey, Egypt, Cuba, Burundi, Bolivia and Malaysia (Horst *et al.*, 1995). The performance of the normal type and deviation in performance of the major gene types

compared with the normal type are given in Table 6.3.

The effect of the naked-neck gene was mainly observed on egg number, egg weight, egg mass and productivity index, though there were large differences in different locations. Egg production of naked-neck layers was higher in the arid conditions of Egypt and Turkey but was lower in other locations. The gene also had a positive effect on egg weight, leading to substantial improvement in most locations. The frizzle gene showed favourable effect on egg production in Egypt and slightly in Malaysia. At the same time the egg production was significantly lower in Bolivia, Cuba and Burundi. The dwarf gene had a mainly depressing effect on body weight (ranging from 24 to 36%). This was also associated with reduction in egg number, egg weight and egg-mass production in most of the locations. However, considering the egg-mass production in relation to the metabolic body weight (productivity index), this gene seems to have very favourable effects.

In addition to the single major gene effects, interactions of their two-way and three-way combinations with environmental conditions were tested (Mathur and Horst, 1990), revealing distinct gene × gene and gene × gene × environment interactions. The magnitudes of these interactions are different for different gene combinations as well as for different environments. Comparison of combined effects of two genes with respective single effects reveals that the combined gene effects are associated with smaller but negative gene × gene interaction effects, reducing the superiority of the two-gene combination.

Specific major gene combinations, depending upon the locations and the traits considered, showed the most favourable effects in international tests (Horst *et al.*, 1995). In Turkey the effects of naked-neck or frizzle gene are not so favourable, but a combination of naked-neck and frizzle gene seems to be more suitable. In Egypt, the most favourable effects for egg-mass production were observed for naked-neck and frizzle genes. In general, combination of the three major genes yields lower egg mass but higher

Table 6.3. Performance of normal layers and deviations due to major gene effects (%) in different tropical locations. (Source: Horst *et al.*, 1995.)

Genotype[a] and traits	Ankara, Turkey	Cairo, Egypt	Havana, Cuba	Bujumbura, Burundi	Cochabamba, Bolivia	Kuala Lumpur, Malaysia
Normal (*nana ff Dw-*)						
Body weight (at 20 weeks) (kg)	1.69	1.73	1.75	1.46	1.53	1.64
Egg number (at 64 weeks)	216.70	228.80	221.80	178.80	157.00	183.20
Egg weight (kg)	65.40	59.20	60.17	53.92	60.75	56.17
Egg mass (g)	14.71	13.98	13.32	9.55	9.54	10.26
Productivity index	46.43	49.72	41.47	33.97	35.34	38.05
Naked-neck (*Nana ff Dw-*)						
Body weight	−1.78	−1.16	−0.57	2.74	−2.60	0.61
Egg number	1.85	13.14	−4.97	−3.37	−5.09	−0.55
Egg weight	−1.07	3.38	3.65	1.11	2.47	−0.71
Egg mass	−4.08	12.86	−1.50	−2.11	−2.11	−0.97
Productivity index	−3.45	7.24	−1.21	−4.42	−17.83	7.10
Frizzle (*nana Ff Dw-*)						
Body weight	−2.96	−1.73	−1.71	−1.37	0.65	−3.05
Egg number	−3.24	14.98	−12.20	−10.10	−12.71	2.73
Egg weight	1.68	2.87	3.16	−0.74	0.99	−0.53
Egg mass	1.36	13.57	−9.77	−10.53	−11.58	1.94
Productivity index	3.66	6.03	−8.92	−9.71	−14.15	−2.10
Dwarf (*nana ff dw-*)						
Body weight	−35.50	−24.28	−26.86	−36.30	−29.22	−28.05
Egg number	5.09	9.20	−26.66	−24.68	0.64	−1.64
Egg weight	−11.16	−3.04	−5.65	−7.79	−6.25	−4.45
Egg mass	−19.05	2.14	−30.83	−30.53	−5.26	−5.83
Productivity index	19.18	11.47	−12.54	−2.36	16.98	8.94

[a]Genotypes: *Nana*, naked-neck; *nana*, normal feathering; *Ff*, frizzle; *ff*, normal feathers; *dw-*, dwarf; *Dw-*, normal body size.

productivity index. The genes did not show any favourable effects in Cuba and Burundi, since there was a very low degree of heat stress. In Burundi, in particular, the stress is due to high altitude rather than to heat. The frizzle–dwarf combination had a very favourable effect in Bolivia, while no other gene or gene combination had such a prominent effect. In Malaysia only the frizzle gene had some favourable effects.

Sire × environment interactions

Having identified a particular genotype (breed, line, strain) for a specific environment, an important decision needs to be taken regarding the environment (e.g. temperate or tropical) in which selection for further improvement should be conducted.

This decision can be based on estimates of genotype–environment interactions as genetic correlation, considering each individual or sire family as a genotype. Such correlations between sire breeding values for each pair of the three environments were estimated by Mathur and Horst (1994b) and are given in Table 6.4. The estimates were high for body weight and egg weight but lower for egg number, egg weight, egg mass, feed intake and egg mass/metabolic body weight. These results suggest higher interactions for the traits of reproduction and production efficiency and also that traits with lower heritability display higher genotype–environment interactions (Mathur and Horst, 1989; Cahaner, 1990).

The lower genetic correlations were also associated with distinct changes in ranking orders of sires from one environment to the other. Thus the relative superiority of

Table 6.4. Correlations between breeding values of sires in different environments. (Source: Mathur and Horst, 1994b.)

Traits	CT–CW			CT–TO			CW–TO		
	Observed correlation	Expected correlation	Difference (%)	Observed correlation	Expected correlation	Difference (%)	Observed correlation	Expected correlation	Difference (%)
Body weight	0.78	0.82	5.1	0.73	0.81	9.9	0.70	0.81	13.6
Egg number	0.31	0.49	36.7	0.28	0.50	45.1	0.19	0.49	61.2
Egg weight	0.72	0.75	4.0	0.73	0.78	7.6	0.72	0.77	6.5
Egg mass	0.33	0.52	36.5	0.29	0.54	46.3	0.19	0.54	64.8
Feed intake	0.55	0.62	17.7	0.22	0.65	66.2	0.51	0.60	15.0
Egg mass/metabolic BW	0.39	0.57	31.6	0.34	0.52	34.6	0.31	0.50	38.0

CT, temperate stall; CW, warm stall; TO, tropical open housing; BW, body weight.

individuals in temperate environments can hardly be maintained in the tropics and indirect selection under temperate conditions seems to be less effective than direct selection in the tropics. It was also observed that the experiments in climatic chambers provide useful information pertaining to specific stressors but cannot completely replace field tests.

There was higher depression in the performance of the heaviest genotype than the lighter ones, and the intermediate type showed optimum performance. This pattern was more clearly observed in the warm stall but was less pronounced in natural humid tropical conditions in Malaysia. A plot of the breeding values of sires with respect to body weight and egg-mass production showed a non-linear relationship. The sires with lowest body weights had the lowest productivity but those with highest body weight also had lower egg-mass production (Mukherjee *et al.*, 1980; Mathur and Horst, 1994b). The body weight for optimum production under heat stress was much lower than that for normal temperatures. These results revealed that body size is an important determinant of productivity in the tropics. There should be a specific range of optimum body weight for specific locations that needs to be considered while identifying a breed or line for the given conditions in layers as well as in broilers. The genotype–environment interactions are important not only when considering the temperate and tropical environments but also for different locations, levels of temperature, humidity and other environmental factors.

Genotype–Environment Interactions in Broilers

Broilers are marketed at an early age compared with layers; therefore, they have a shorter period of exposure to the environment and less chance of genotype–environment interactions. However, there is evidence of significant genotype–environment interactions in broilers, especially with respect to environmental conditions such as heat stress and nutrition.

Genotype–environment interactions in broilers with respect to heat stress have been investigated in a series of experiments at the Hebrew University of Jerusalem, Rehovot, Israel. These investigations reveal significant interactions of the naked-neck, frizzle and dwarf genes with ambient temperature (Cahaner, 1990; Cahaner *et al.* 1993; Deeb and Cahaner, 1999, 2001; Petek *et al.*, 1999; Yunis and Cahaner, 1999).

Deeb and Cahaner (2001) studied the effects of normal (25°C) and high (30°C) ambient temperature on broiler progeny of hens from a sire line and two dam lines, differing in growth rate and meat yield, carrying the naked-neck (*Na*) gene. The advantage of the *Nana* genotype was much more pronounced at high ambient temperature in broilers, with genetically higher growth rate and breast meat yield.

Petek *et al.* (1999) studied the effect of genotype–environment interactions on the performance of commercial broilers in western Turkey. The genotypes were 29 sires and natural climatic conditions in spring and summer were considered as environments. The interaction was evaluated as correlations between sire breeding values in summer with those estimated from their spring offspring. The genotypes that gained more weight in the spring gained less weight under the hot conditions of summer. The correlation between the two seasons for weight gain from 0 to 4 weeks of age was 0.26, i.e. significantly lower than 1. It was even negative, though not significantly lower than 0, for weight gain from 4 to 7 weeks of age and body weight at 7 weeks of age. The analysis of variance revealed highly significant genotype–season interaction effects on all traits. They also observed that this variation was somewhat related to growth potential.

Interactions between broiler genotypes and heat stress have also been investigated in several tropical locations. Singh *et al.* (1998) compared the performance of naked-neck (*Nana*) and normal (*nana*) broilers in winter and summer in India. The naked-neck broilers were superior to normal broilers in terms of growth rate, feed efficiency, dressing percentage and liveability

in both seasons, but the difference between the two genotypes was higher in summer than in winter. The results show that the naked-neck genotypes are more suitable for the tropical climatic conditions and their superiority is greater with increasing heat stress. The results observed in layers also apply to the broiler populations, though the magnitude of the interactions again depends upon the differences between the environmental conditions.

Ali *et al.* (2000) studied the importance of genotype–environment interactions in broilers and layers in Tanzania. The genotypes were four commercial broilers: White Plymouth Rock, Tanzania Local fowls and crosses between White Plymouth Rock and Tanzania Local fowls. The environments were two rearing systems: intensive and extensive management systems from 10 to 18 weeks of age. The effect of interactions was evaluated on live weight, body weight gain, feed intake, carcass weight and gastrointestinal traits. The broilers had the highest live weight and fastest gain on both systems, but performed much better on intensive than on extensive management. Tanzanian Local fowls had the lowest weight and slowest gain. The live weight of the crosses was higher than the average of their parents. The intensively reared fowls gained about four times more weight than those on extensive rearing. Feed intake, carcass weight and intestinal length followed the same trends as live weight. The genetic groups ranked similarly in the two rearing systems, suggesting that genotype–environment interactions were of little practical importance.

An experiment to investigate the effect of heat stress on haematological parameters was conducted by Pingel *et al.* (1995) on divergently selected lines from White Rock fowls for plasma corticosterone concentration for three generations, and an unselected control line, after heat stress (2 h at 40°C). Heat stress decreased the leucocyte count in all groups. Antibody production against sheep erythrocytes was similar in fowls of the three lines kept at normal and high temperatures. However, the genotype–environmental temperature interactions were not significant.

Genotype–nutrition interactions in broilers were reviewed by Leenstra (1989). This review considered the effects of genotype × dietary composition (protein and fat) and genotype (strains and sex) × temperature interactions on broiler performance. The interaction effects are especially important when the genotypes differ in protein metabolism, body composition and protein efficiency. There is a need for diets of specific composition for optimal performance of the desired genotype.

Marks (1990) compared the performance of commercial broiler stock and the Athens-Canadian random-bred population (controls) on high-protein or high-energy diets. Broilers were heavier and consumed more feed and water. They had better feed efficiency, higher abdominal fat levels and poorer feather scores than controls. Body weights of broilers fed the high-energy diet were, in general, higher than those of contemporaries fed the high-protein diet, but the body weights of controls did not respond in a similar manner. Feed intake patterns were similar to those for body weight. Water intake and water:feed ratio values were consistently greater in controls and broilers fed the high-protein diet. The broilers had higher percentages of abdominal fat than control birds, and broilers fed the high-energy diet had higher abdominal fat (89%) than those fed the high-protein diet; the difference was only 33% in the controls. The results suggest significant genotype–nutrition interactions.

The behaviour of 2400 chickens from four commercial strains – Lohmann Selected Leghorn (LSL), Norbrid 41 (NB), Lohmann Brown (LB) and ISA Brown (ISA) – was compared in aviary systems by Kjaer (2000) with respect to rise in feather-pecking activity between 8 and 14 h of daylight. Feather-pecking behaviour was recorded by time sampling from video recordings at 38 weeks of age. Feather-pecking activity was highest in the medium heavy strains, with ISA doing the most pecking followed by LB. The pecking activity was considerably lower in White Leghorn strains, NB and LSL. The activity increased with an increase in exposure to daylight. This increase was significantly

higher in the medium heavy strains than in the White Leghorns, suggesting significant genotype–environment interactions.

Role of Genotype–Environment Interactions in Breeding and Selection Programmes

Genotype–environment interactions are usually evaluated as the relative change in the performance of two or more genotypes in two or more environments. In many cases it is not sufficient to detect presence or absence of interactions. There is a need to estimate the magnitude of the interactions to evaluate their biological significance and role in selection programmes. Statistically significant interactions may or may not be biologically relevant, e.g. if they do not affect the ranking order of specific breeds or lines from one environment to the other. However, they can be of significant value if superior individuals such as sires in one environment cannot maintain superiority in a different environment.

Experiments considering different lines, major genes and their combinations as well as individual sires have revealed significant genotype–environment interactions in layers and broilers. The magnitudes of these interactions are different for different traits, genotypes and environments. In layers, significant interactions are observed with respect to temperate and tropical conditions. In broilers, the magnitude of such interactions is usually lower, due to limited exposure to the environmental factors during the short growth period, but there is evidence of significant genotype–location and genotype–nutrition interactions.

The interactions are usually higher for traits with lower heritabilities, such as reproduction and feed efficiency, but lower for traits with higher heritabilities, such as growth and body size.

The unfavourable effects due to genotype–environment interactions on the desired genotype can be partially overcome by adjusting management conditions to those required for the optimum performance. However, in many cases such adjustments are either not possible or not cost effective. In many parts of the world, it is hard to simulate the optimum environmental conditions for high-yielding genotypes, since it requires substantial capital investments – for example, to control the climatic conditions and to provide specific types of cages, a disease-free environment, or highly concentrated or balanced diets. In many places, it is also possible that these suboptimal environments may prevail for many years. Under such circumstances it is more appropriate to select specific genotypes for specific environmental conditions.

There are three important considerations concerning genotype–environment interactions: the choice of a suitable breed or line; selection for further improvement within the selected breed or line; and further improvements in the selected genotype through specific biological effects such as body size or the use of major genes.

The phenomenon of genotype–environment interactions requires additional efforts in the choice of breeding stock with a general adaptability to more than one environmental condition. At the same time it offers several opportunities for high production levels under unfavourable environmental conditions. Appropriate use of this phenomenon can prove very useful for production of genotypes that are well adapted and genetically superior for the given environmental conditions.

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7   Breeding Objectives and Selection Strategies for Layer Production

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Introduction

Livestock production is a means of producing human food, other products for human consumption, and intermediates to be used as inputs for plant production. Livestock production also serves human interest in other ways, such as banking and social status. In general, livestock production is a means of creating human welfare and well-being by converting resources (production factors: labour, land and capital) to products of higher value.

Genetic improvement is a technological development (Cochrane, 1958). The essence of technological development is to save on the input of production factors by setting up a new production function. Through an alternative use, the saved production factors get a (market) price or an opportunity cost. Depending on many factors (e.g. the state of development of the country), key issues in the use of saved production factors are to increase food security or food quality, to enhance (economic and social) well-being of producer, consumer and citizen, or to increase the sustainability of the system. The definition of breeding goals involves deciding on the direction of a technological change: genetic improvement for the traits that will lead to the specifically desired saving of production factors.

Genetic improvement schemes arise through the initiatives of governments, industrial investors or farmers. From each perspective, investments (in recording, housing potential breeding stock and evaluation) anticipate future benefits such as sustainable food security, continued farm profits or appropriate returns on investment. Those who have control over decisions in a genetic improvement scheme will try to optimize benefits, given their specific perspective. Optimizing benefits involves appropriate identification and use of superior stock. However, optimization of genetic improvement schemes is not only technical, determined by animal breeders; it is also a socio-economic optimization, reflecting the broader wishes and demands of those who initiated and are involved in the scheme. For example, the choice for crossbreeding schemes is a result of the industry's wish to control return on investments rather than a consequence of technical superiority of crossbred breeding stock. Other examples are the preference of an individual farmer for a specific breed, or public concern about animal welfare and biodiversity.

This chapter describes general aspects on the definition of breeding goals with an example for the layer sector. Emphasis will be on the effect of the perspective taken in decision making with respect to genetic improvement schemes.

An Aggregate Genotype

The breeding goal looks at traits that are subject to genetic improvement and gives each trait a value. In this way a weighted summation of traits is made – an aggregate genotype to be improved (Hazel, 1943). An important implicit effect of defining the breeding goal is the quantification of the (true) genetic variance among animals subject to selection (σ^2_H).

Ideally, the breeding goal should include all animal traits for which genetic improvement will lead to saving production factors. In practice, the choice of traits is also based on the potential for genetic improvement of the trait (e.g. genetic variability) and cost (in labour, facilities and time) of accurate prediction of the breeding value for the trait (Harris, 1970).

The values used for weighting traits are generally called 'economic values' or 'economic weights'. In this chapter, they will be called 'goal values'. The goal value includes a current market economic component (economic value; Hazel, 1943), a component that reflects the benefit of using an animal with a superior genotype (cumulative discounted expression; McClintock and Cunningham, 1974), and also a non-current market component (social value; Olesen *et al.*, 2000). In matrix notation:

$$H = \mathbf{a'g} \qquad \text{US\$ per animal}$$
$$\mathbf{a} = \mathbf{c'}\,(\mathbf{ev} + \mathbf{sv}) \quad \text{US\$ per animal per unit}$$

where $\mathbf{g}$ is a (m*1) vector with genetic superiorities of m aggregate genotype traits (unit), $\mathbf{a}$ is a (m*1) vector with goal values of m aggregate genotype traits (US\$ per animal per unit), $\mathbf{c}$ is a (m*m) diagonal matrix with cumulative discounted expressions of m aggregate genotype traits (animal.year per animal), $\mathbf{ev}$ is a (m*1) vector with economic values of m aggregate genotype traits (US\$ per animal per year per unit), and $\mathbf{sv}$ is a (m*1) vector with social values of m aggregate genotype traits (US\$ per animal per year per unit). (NB: animal.year is the unit of counting the number of expressions of the trait.)

Generally, economic and social values are specified for specific production circumstances, and cumulative discounted expressions are specified per selection path included in the genetic improvement scheme. Relative levels of goal values of different traits are important for an accurate definition of the breeding goal, giving optimum levels of genetic improvement, i.e. a technological change optimally addressing predicted future societal needs. To obtain an accurate calculation of revenues of the genetic improvement scheme (in order to optimize its structure), primarily the absolute levels of goal values are important.

Selection of animals to be parents of the next generation is not directly on the individual aggregate genotype, as (true) genetic values of individuals are unknown. Therefore, current genetic improvement strategies in livestock have widely adopted the so-called selection index theory (Hazel, 1943), or its advanced version in best linear unbiased prediction (BLUP) of breeding values (Henderson, 1973). The tool used in deciding which males and females will become parents of the next generation is the selection index or predicted breeding value. The predicted breeding value is a summary of observations, information on measurements and scores, weighted so as to account for: (i) the genetic possibilities of improvement (by considering the genetic and phenotypic (co)variances in the population); (ii) the number of observations on the animal and its relatives; and (iii) the relative importance of traits (given by goal values) in the aggregate genotype (Hazel, 1943).

Initially the selection index was a single-step procedure with a fully multi-trait approach. In practice, a two-step procedure is generally applied: (i) a (single trait) prediction of breeding values per aggregate genotype trait; and (ii) weighting predicted breeding values to an overall index. The error of this simplification is dependent on the accuracy of (single trait) predicted breeding values and the correlation structure among aggregate genotype traits.

The extent to which the (true) genetic variance (σ^2_H) can indeed be exploited in selection depends on the accuracy of the breeding value prediction (r_{IH}, i.e. the

correlation between the index and aggregate genotype: $\sigma^2_I = r^2_{IH} \sigma^2_H$).

As mentioned, the breeding goal determines the variance subject to selection, σ^2_H, and breeding value prediction determines the accuracy of selection, r_{IH}. Selection and mating strategy determine the intensity of selection (i). The revenue of the genetic improvement scheme in terms of genetic gain per year is calculated by the equation $\delta g = \Sigma(i_l \cdot r_{IH,l} \cdot \sigma_H)$, where summation is over l selection paths. It should be remembered that, in the definitions used in this chapter, the cumulative discounted expression (cde) is in the variance of the aggregate genotype. In a steady-state genetic improvement strategy (in other words, an infinite time horizon applied in gene flow) and an interest rate of zero (in other words, ignoring discounting), the cde for all traits in all selection paths is $1/\Sigma L$ (L is the generation interval). In this situation, the calculation of genetic gain per year equals the formula of Rendel and Robertson (1950).

Usually economic goal values are assumed to be linear, independent of the population means of the traits considered. However, traits might have a non-linear economic value: egg weight (Kempthorne and Nordskog, 1959) is a classic example. A quadratic component for **g** might be, where goal values depend on the level of the trait itself and/or on the level of other traits (Wilton *et al.*, 1968):

$$H_q = \mathbf{a}'(\boldsymbol{\mu} + \mathbf{g}) + (\boldsymbol{\mu} + \mathbf{g})'\mathbf{A}(\boldsymbol{\mu} + \mathbf{g}) \text{ US\$ per animal}$$

where **a** is a vector with goal values for linear terms of genotype traits, **μ** is a vector with population averages, and **A** is a matrix with goal values for quadratic terms of genotype traits. Specific procedures to select for non-linear profit are quadratic indices (Wilton *et al.*, 1968), mate selection (Allaire, 1977), group selection (Bulmer, 1980) and maximizing response over a multiple generation response curve (Goddard, 1983; Dekkers *et al.*, 1995). However, if goal values are non-linear, use of the first-order linear approximated goal value will give very close to optimum economic selection responses (Goddard, 1983; Gibson and Kennedy, 1990), especially when goal values are updated regularly (Groen *et al.*, 1994).

Goal values can be assigned non-objectively or derived objectively. Non-objective methods assign goal values in order to achieve a desired or restricted amount of genetic gain for some traits (Kempthorne and Nordskog, 1959; Brascamp, 1984). Certainly, these methods may be useful in examining the borders of the possible solution area for genetic improvement. Schultz (1986) suggested that non-objective methods may be useful in commercial breeding. In defining breeding goals, it is usually assumed that a below-average performance for one trait can be compensated by an above-average performance for another. Breeding companies often consider this not to be the case. They experience problems in marketing animals that are too far behind for some traits even if they excel in others. Therefore, they tend to put more emphasis on poor traits and less on superior ones (Schultz, 1986). The purpose of this strategy is to avoid decreases in sales, although obviously it leads to lower savings in production factors than would be achieved applying normative economic. De Vries (1988) presented a method for objectively assigning goal values incorporating the competitive position. The method modifies the goal value based on the performance for each trait for the stock concerned, the average performance for all traits of all competitors, a compensation factor expressing to what extent a low level for one trait may be compensated by a high level for another traits, and the minimum trait level required for acceptance by farmers. Although it is a problem to acquire these parameters, all of them are assumed when adopting the approach of desired gains. The advantage of the De Vries method is that the different considerations are made more visible.

Methodology for Deriving Economic Values

The general principle in deriving economic values is illustrated in Fig. 7.1. An equation,
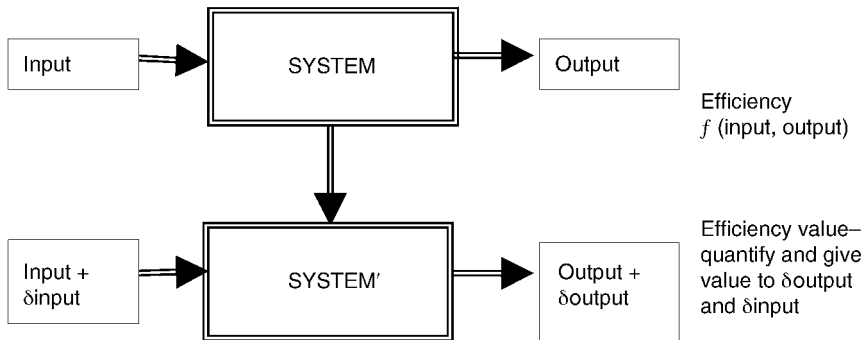
Fig. 7.1. The general principle in deriving economic values. The ' denotes a marginal change in genetic merit of one aggregate genotype trait.

or a set of equations (a model), represents the behaviour of a system. The equations identify modelling elements and their relationships (e.g. regression coefficients or class effects). Genetic merit for animal traits is defined unambiguously by certain modelling parameters.

Economic efficiency of production is a function of costs and revenues of the production system. Costs can be defined as the total value of production factors (input) required for production within the system; revenues can be defined as the total value of products (output) resulting from production within the system. The economic value of a trait expresses to what extent economic efficiency of production is improved at the moment of expression of one unit of genetic superiority for that trait. Derivation of economic values, therefore, involves: (i) quantifying changes in physical amounts (and qualities) of each production factor required and product produced; and (ii) giving a value to the changes in production factors required and product produced.

Dickerson (1970) advocated the improvement of a biological efficiency rather than economic efficiency as it seems unlikely that breeding for biologically less efficient livestock in the long term is sustainable. Differences between biological and economic efficiency are restricted to differences in defining costs and revenues. In the biological definition, costs and revenues are expressed in terms of energy and/or protein; in the economic definition this is in terms of money. The major problem arising with the

biological definition is that not all costs and revenues can be expressed in terms of energy and/or protein. The economic definition largely deals with this problem but a disadvantage of the economic expression is weakness in stability in time and place of monetary units (Schlote, 1977). Notwithstanding imperfectness, money is 'the standard for measuring value' (Stonier and Hague, 1964) and, therefore, efficiency of production is usually considered to be economic efficiency. Nevertheless it is very relevant to check whether genetic improvement strategies based on economic valuation of production factors and products indeed also lead to improvement of biological efficiency.

In the literature, a difference is often made between the use of profit functions and bio-economic modelling. However, there is no principal difference. A profit function is a single-equation model; regarding the strict definition of profit as output minus input, the more general term 'efficiency function' better represents this type of modelling. A multi-equation simulation model is referred to as a bio-economic model (e.g. Groen *et al.*, 1998). Using simulation models, economic values are derived by partial budgeting; with efficiency functions, this can also be performed by partial differentiation. Bio-economic modelling offers opportunities for considering large numbers of elements and their relationships. Thus, bio-economic modelling allows for the implementation of mathematical programming techniques to optimize production systems. Mathematical

programming allows for finding (given farm characteristics) the best use of saved production factors, in other words the highest opportunity cost (Steverink *et al.*, 1994).

Deriving economic values requires specific choices on perspective to be taken (Groen, 1989): (i) allowed changes (in terms of δ input and δ output, where δ is marginal change) of the system by the level (animal, farm, national, etc.) and planning term (operational, tactical or strategic); (ii) defined efficiency of the system in terms of an interest of selection: maximize profit (output minus input), minimize cost price (input/output) or maximize revenues on investment (output/input); and (iii) assumed size of the system in terms of a base of evaluation: fixed number of animals, fixed input of a production factor or fixed output of a product.

The theoretically appropriate level to be used in deriving goal values in animal breeding is the one for which limited resources and prices of products and production factors are influenced by an improvement of a trait (Fewson, 1982). A good example is given in a dairy industry with a milk quota system limiting the amount of product at farm level. Improvement of genetic merit for milk production per cow will have to result in a reduction in the number of cows at a farm. To include the effects of a reduction in the number of cows (reduced costs of housing, feeding, labour and so on), derivation of economic values should be performed at farm or higher level. For practical purposes, however, modelling is generally limited to animal or farm level.

In fact, the system level and planning term together determine for which saved production factors an alternative use is feasible in the time frame considered. Fixed costs are not subject to changes, while variable costs are. In other words, input of production factors that are considered fixed does not have an alternative use, while input of production factors that are considered variable does have an alternative use.

Groen (1989) presented the concepts of economic production theory regarding different combinations of interests of selection and bases of evaluation in deriving economic values. For example, with a fixed number of animals and profit maximization, the economic value is positive when the marginal revenues of increased output per animal exceed the marginal costs of increased output per animal (see also Melton *et al.*, 1993). In this case, breeding for increased output per animal will be beneficial.

The essence of improvement of efficiency of a production system is saving inputs of production factors per unit product and/or a change towards use of cheaper production factors. Saved production factors can either be used in the system from which they are saved (and thus extend product output of that system) or be transferred to another system (via the market) (Willer, 1967). Likewise, additionally required production factors may be drawn either from the market or from an alternative use in the system. Obtained differences in concepts of production theory originate directly from differences in assumed use of saved production factors. For the 'profit, fixed number' perspective, saved production factors are sold at the market. This means that differences in concepts between perspectives will indeed lead to differences in economic values when the values of (saved) production factors differ between alternative uses. However, assuming markets of products and production factors to be purely competitive markets and assuming industry and all individual firms to be in equilibrium, market prices will equal average total costs of production (Stonier and Hague, 1964). This is the approach considered by Brascamp *et al.* (1985) in proposing to set profit to zero. In terms of Table 7.1, for example, economic values on the basis of fixed numbers of animals are equivalent when derived within profit and cost-price interests. On the basis of fixed output, economic values within a profit interest are equivalent to economic values within a cost-price interest. These economic values will also be equivalent to economic value 'fixed number, cost price' when all costs of the farm are considered to be variable per unit product. This equivalence was pointed out by Smith *et al.* (1986), who proposed to express fixed costs per

Table 7.1. Economic values expressed in concepts of economic production theory (after Groen, 1989; see also Groen *et al.*, 1997; third column on maximization of revenue over cost not previously published).

Base of evaluation	Selection interest		
	Profit maximization	Cost price minimization	Maximization of revenues over costs
Fixed number of animals	Marginal revenues[a] – marginal costs[b]	Average total costs[a] – marginal costs[b]	Marginal revenues over marginal costs – average revenues over costs
Fixed input	Marginal revenues[a] – average (revenues – fixed costs per animal)[c]	Average total costs[a] – average fixed costs farm[c]	Marginal revenues – average (revenues over variable costs)
Fixed output	Average variable costs[a] – marginal costs[b]	Average variable costs[a] – marginal costs[b]	Marginal revenues – average (revenues over all costs)

[a]Per δy units of product.
[b]Per δy units of product, corresponding to δx_v units production factor.
[c]Per δx_v units production factor.

animal or per farm as variable costs per unit of output.

However, in agricultural industries, products and production factors are commonly heterogeneous and not fully divisible. Heterogeneity of products and production factors leads to division of markets (Dahl and Hammond, 1977) and causes the average costs of production to be different for individual firms. Given (equilibrium) market prices, some firms will have a lot of profit; other firms will be just efficient enough to continue production (Stonier and Hague, 1964). As an important result, the equivalence of perspectives may hold under certain conditions for the sector as a whole but will not be valid from an individual producer's point of view. In defining breeding goals, the definition of efficiency function has to correspond to the individual livestock producer's selection interests; the producer's primary reason for buying a certain stock at a certain price, will be based upon his assessment of how animals will perform.

The point of assuming fixed costs per animal or per farm to be variable per unit product is subject to debate. Costs may be fixed (constant or discontinuously variable) with respect to the size of the farm (Horring, 1948). Considering these fixed costs to be variable per unit of product requires an assumption on the (continuously optimum)

size of the farm. Smith *et al.* (1986) proposed to express all fixed costs per animal or per farm as per unit of output, thereby assuming a given optimum farm structure or size, with efficient use of resources. The condition of fixed cost to be constant per unit of product is arithmetically correct when assuming: (i) that all farms are of the same size; and (ii) that changes in output and input are accomplished by a change in the number of farms. However, structural developments in industry are detached from improvements in the efficiency of production, which is not correct considering long-term effects of the implementation of new techniques (Zeddies *et al.*, 1981; Amer and Fox, 1992).

A similar discussion is on the system subdivision: are economic values different for different levels of the production system (e.g. nucleus breeder, hatchery, multiplier and commercial grower)? In fact, the normal profit theory by Brascamp *et al.* (1985) also holds here. Jiang *et al.* (1998), for a broiler example, illustrated that in a situation where trading between system levels is based on cost prices (e.g. in a fully integrated enterprise), breeding goals are the same from each system level perspective. However, when assumed market prices substantially differ from cost prices (i.e. one system level is making an 'excess' of profit), breeding goals start to differentiate between system levels.

Methodology for Deriving Cumulative Discounted Expressions

Identification of genetically superior animals is only useful when the identified animals can subsequently be used to produce progeny. In other words, the value of identified genetic superiority depends on the time and frequency of expression of the superiority in offspring. Time and frequency of expression differ between traits and therefore it is important to include cumulative discounted expressions in goal values. McClintock and Cunningham (1974) proposed the use of a 'standard discounted expression' of an individual's genotype in the progeny in the year in which the mating took place. To deal with more complex designs of mating systems, the discounted gene flow method was proposed by Elsen and Mocquot (1974) and Hill (1974). The discounted gene flow is expressed as a number of cde, as a consequence of one mating; 'cumulative' refers to an accumulation of expressions over generations or years; 'discounted' implies that future return is discounted to today's values by a discounting factor (Brascamp, 1978).

Let $m_t(i)$ be the proportion of genes of interest in age class i at time t. There are two processes that give a transfer of genes in time from one age class to another age class: reproduction and ageing. Two matrices can describe these processes: $\mathbf{R}$ for reproduction and $\mathbf{Q}$ for ageing. One might be interested in gene flow as transmitted to the first generation of offspring through one single selection path, while transmission to following generations is by all paths. To do this, a matrix $\mathbf{R}_l$ is defined, containing zeros except for the one row describing the transmission of genes through path l. If an $\mathbf{R}_l$ is defined for all paths and $\mathbf{P}$ is a transmission matrix, then

$$\mathbf{P} = \sum_l \mathbf{R}_l + \mathbf{Q}$$

The transmission of genes can be described as two processes:

$$\mathbf{n}_{l,t} = \mathbf{Q}^t \mathbf{n}_{l,0}$$
$$\mathbf{m}_t = \mathbf{P}\mathbf{m}_{t-1} + \mathbf{R}_l \mathbf{n}_{l,t-1}$$

The vector $\mathbf{n}_{l,0}$ generally will contain zeros but a 1 in that class for which the fate of genes has to be followed. The first process describes ageing and allows genes to arrive in those classes of $\mathbf{n}_{l,t}$ that contribute to reproduction. The second term of the second process describes how these genes are transmitted by reproduction to the whole population, to $\mathbf{m}_{l,t}$. Vector $\mathbf{m}_{l,t}$ contains zeros initially ($\mathbf{m}_{l,0} = 0$), but as soon as genes have arrived in $\mathbf{m}_{l,t}$, further transmission to following generations is described by $\mathbf{P}\mathbf{m}_{l,t-1}$. After the considered genes reached the oldest age class, $\mathbf{n}_{l,t}$, gets 0 (the initial genes die).

So far, theory gives us derivation of $\mathbf{m}_{l,t}$: the response to selection in different age classes in year t as a result of gene transmission and one cycle of selection. The interest, however, is in computing the contribution of a basic set of genes to future expression of traits. To obtain the total response in terms of expression of genetic superiority in improved performance in different age classes in different years, it is necessary to account for: (i) which age classes express genetic superiority together with their frequencies (at any time); this is specified in the incidence vector $\mathbf{h}$; and (ii) discounting of future revenue to a base year ($t = 0$); this is accomplished by regressing revenue in year t by a factor $\delta^t = [1/(1 + q)]^t$ where q is the interest rate in real terms.

Summarizing, the cumulative discounted expression up to time horizon t_h from one cycle of selection for a single trait in a breeding structure is:

$$c_{l,t_h} = \sum_{i=0}^{t_h} h' m_{l,t} \delta^i$$

Methodology for Deriving Social Values

Economic values generally relate to direct food production uses of livestock and relative price ratios assumed generally reflect current market validation procedures. However, especially in lower-input production environments, livestock serves many more roles than only food production, such as fibre, power, fertilizer, fuel, transportation,

insurance, social status and banking, and these roles should also be considered when defining breeding goals. The benefit of part of these roles can be modelled normatively. For example, Bosman *et al.* (1997) developed a concept for estimating the insurance value of keeping livestock based on flock values during the year. When defining breeding goals, effects of changes in genetic merit on benefits of non-food production roles can be included in social values or assumed to be an integral part of economic values.

Olesen *et al.* (2000) also considered non-food production roles of livestock to be included in defining breeding goals for sustainable production systems, but they emphasized that current market validation procedures do not account for important cultural/social aspects, such as concerns about animal welfare and loss of historical breeds. According to Olesen *et al.* (2000), these so-called non-current market values of animal traits (e.g. ethical values of improved animal welfare through less suffering from diseases or stress, and a higher quality of life) should be quantified. There is a clear link to valuation of, for example, natural resources and the environment. A total environmental value includes both use (consuming or non-consuming) and non-use values such as insurance premiums, existence, intrinsic or bequest values (e.g. Smith, 1993). Olesen *et al.* (1999) pointed out that methodology used for obtaining empirical estimates of environmental values can also be used to derive social values for defining breeding goals. For example, contingent valuation can be applied to assess people's willingness to pay for animal welfare (Bennett, 1996) and meat quality (Von Rohr *et al.*, 1999).

The contingent valuation method includes setting up a hypothetical market where products are specified. Then individual people are asked to state their maximum willingness to pay for a certain improvement (e.g. animal welfare) or minimum willingness to accept compensation for a certain reduction. Willingness can be obtained using, say, a bidding game, or from choice experiments.

Breeding Goal Definition in Layers

Here an attempt is made to define an ideal situation of breeding goal traits; for these traits a layer breeder might want to know predicted breeding values for his selection candidates. The listing in Table 7.2 is based on several literature sources, including Nordskog (1986).

Nordskog (1986) identified four primary trait groups in an egg-type layer breeding enterprise: (i) value of saleable baby chicks per breeder hen; (ii) value of saleable market eggs per hen; (iii) carcass value of a spent hen; and (iv) cost of feed consumed per hen. Some early studies on goal values assumed only some of these trait groups. Hogsett and Nordskog (1958) derived goal values for egg production rate, body weight and egg weight. Nordskog (1960) defined the value of improving egg production, hen-day production, mortality, egg size and body weight. An important feature of the work by Nordskog (1960) is that it showed the dependency of 'goal values' on production level and layer type. Arboleda *et al.* (1976) considered only the last three trait groups and determined the relative importance from average market prices for three representative traits: body weight of cull hens, egg mass and layer feed consumption. Other studies giving goal values for laying hens are Yamada *et al.* (1975), Hagger and Abplanalp (1978), Luiting (1991) and Katle (1992). A review of relative importance of trait groups is not given here: applied methodology and parameter assumptions differ too much to draw any consistent conclusion.

For broiler chickens the same tendency towards considering more trait groups is seen. Considering average income over feed costs, Moav and Moav (1966) expressed a simple selection objective for a broiler operation with a profit function that included egg production of parents and growth rate of their progeny. Work by Akbar *et al.* (1986) and Jiang *et al.* (1998) included a broad range of dam traits, broiler traits and carcass traits.

No comprehensive study using goal values for the layer sector has been

Table 7.2. Traits for breeding goals.

Main trait group	Traits
Egg production level	Age first egg
	Hen-day egg production
	Persistency of production (rate of lay)
	Pauses (broodiness)
	Egg size/weight
Product quality	Outer: Egg deformation
	Shell thickness, colour, texture, porosity and shape
	Inner: Albumen quality
	Blood spots
	Taste
Production efficiency	Mature body weight
	Feed consumption (level and type of feed)
Reproductive performance (breeder stock)	Female and male fertility
	Hatchability of eggs
Meat production characteristics	Growth rate/body weight
	Body conformation (carcass, breast meat, wing and leg yields)
Functional traits (breeder and commercial stock)	Heat tolerance/adaptability
	Disease resistance
	Leg strength
	Survival
	Feeding behaviour
	Maternal care
	Cannibalism
	Temperament (e.g. flightiness)
Others (e.g. type)	Plumage colour standard for the breed

published. The literature on economic values generally includes only some of the important traits and is usually based on simplified profit functions. There is no application of cumulative discounted expressions to layers in the literature; there is one application to broiler production that includes cumulative discounted expressions for female reproductive traits (Jiang *et al.*, 1999). Derivations of social values in layers are not known.

The ideal situation, as presented in this chapter, is partly a redefinition of the listing by Nordskog (1986), partly a refinement (by underlying traits), but also partly an elaboration towards new traits. The emphasis on product quality and functional traits especially should be mentioned. This illustrates the increasing importance of consumer behaviour and public attitudes towards livestock production as a whole, and more specifically the need for a shift in breeding. This change in direction of technological development requires renewed definition of

breeding goals. Specialized layer and broiler chickens and also dual-purpose chickens play an important role in modern western livestock systems such as organic farming and also in lower-production environments in developing countries. Defining breeding goals according to specific environments is an important issue, to optimize production but also to preserve biodiversity.

Discussion

Genetic improvement does not aim at an optimum; it dynamically searches for improvements. Given animal genetic variation (within or between breeds), there is always a means of improvement. In fact, this approach by farmers originates from the historical and continuing natural process of re-establishing genetic variation (i.e. mutations). This approach is also an incentive for conserving genetic variation during the process of selection. Directing genetic

improvement is an important area of decision making; genetic change is lasting and therefore valuable. The state of the next generation depends partly on the state of the present generation, and so on.

Society itself is diverse and dynamic. Therefore, the breeding goal for a population needs to be defined in relation to the predicted ecological, social and economic circumstances under which the future generation of animals is to produce. Keeping track of societal changes is very important for animal breeders, because changes in (predicted) future circumstances require the updating of breeding goals. Also technological changes in, for example, biotechnology and information technology will have their influence on optimal design of breeding value prediction and selection and mating of parents. Breeding goals ought not to change according to seasonal or cyclical variations of circumstances, but should be adjusted as soon as structural and lasting changes are observed, in order to avoid losses. Definition of breeding goals should be robust, but also continuously require reconsideration of economic and social developments.

There is no 'worldwide standard' or even 'country standard' breeding goal. Differences in economic, social and ecological production environments give rise to different approaches and wishes in terms of human welfare and well-being. These differences give rise to different development objectives. Diversification of breeding goals is important to serve farmers facing different local situations. Diversification of breeding goals according to local production environments will support genetic improvement for locally adapted breeds, and thus help to conserve genetic resources. Future studies should include aspects of developing customized indices (for specific individual farming situations) and robustness of indices (e.g. Amer and Hofer, 1994). Although breeding in the layer sector is to a large extent by private breeding organizations, there is a clear need for public research on definition of breeding goals in layers to give appropriate direction towards technological change in layer production systems.

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8 Breeding Objectives and Selection Strategies for Broiler Production

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Introduction

Success in the poultry breeding business is dependent on the integration of a number of activities. Primary breeders must have efficient pedigree programmes that are structured and operated in a manner to maximize annual genetic gains. They must have efficient schemes for the multiplication of commercial products to provide quality products in sufficient quantities to meet market demand. Breeders are expected to provide technical recommendations and support to ensure that customers achieve the genetic potential of the product produced. However, there are examples of breeders who were successful in all these areas but are no longer in business as a result of pursuit of the wrong goal. The breeding goal provides the compass by which a breeding programme is directed and identification of the breeding goal is an essential starting point for any successful breeding programme.

The process of establishing breeding objectives involves a blend of economics, science and consumer psychology. The poultry industry is a very competitive business and, consequently, breed decisions are economically driven. The sciences of genetics and physiology provide the basis for efficient genetic change and, therefore, these disciplines are a key component in

developing breeding goals. The output of the breeding process is a product that must be marketed to the broiler industry. While breed decisions are economically driven, there is a significant emotional component to these decisions as well. Thus, breeding companies must create a balance between technical and psychological considerations. The technical issues can be defined through empirical analysis but psychological factors defy such approaches and can only be appreciated through direct interaction with the production sector.

The industrial nature of poultry breeding has a large influence on the process of development of breeding plans. The poultry breeding industry is highly concentrated and is a high-risk, high-reward business. Consequently, breeders must be concerned with short-term sales as well as long-term viability. An idealized approach that focuses on theoretical optima is not suitable if it sacrifices current sales or if it does not create perceived value for customers. Primary breeding is an international business. Grandparent franchises and partnerships play a key role in the distribution of genetic products and the impact of a given breeding scheme on multiplication costs is an important consideration. Consequently, practical breeding objectives often differ from objectives that would be established for a fully integrated process encompassing

everything from pure line development through broiler production and processing.

Geneticists commonly focus on factors influencing genetic change as a result of within-line selection. Multiple line crosses improve profitability when there is non-additive variation for one or more traits contributing to the profit function (Moav, 1966) and, consequently, commercial broilers are the result of three- and four-way strain crosses. As a result, combining ability and between-line selection are important tools for industry geneticists. This shift in focus from pure lines to commercial products creates an additional level of complexity that complicates the development of breeding goals.

As there is a relatively long timeline from selection of pedigree stock to recognition of results in the field, market signals that serve to drive genetic development are often delayed and diffuse. In addition, products must ultimately satisfy multiple production sectors as well as the consumer. This often results in mixed signals that frequently defy logic and empirical analysis. In addition, the human factor cannot be ignored, as there is an emotional element to most purchase decisions. Customer feedback provides an essential component of the process, as there is no point in developing and producing products for which there is no market demand even when they are more cost effective than existing products.

Establishment of breeding objectives is a process and breeding goals must be regularly reviewed and re-evaluated. Just as the poultry industry has evolved since the 1950s, the poultry breeding business has increased in complexity. The evolution of the poultry industry has provided new demands and a greater focus on economic drivers. While there is a danger in modifying successful programmes, there is inherent risk in continuing in an inappropriate direction. The discussion that follows is intended to provide an overview of some of the considerations that influence practical breeding objectives and to provide some insight regarding the process that occurs in primary breeding organizations.

Establishing Breeding Objectives

Market sectors

Primary breeding is an international business with a customer base spread across the globe. There is almost unlimited diversity of markets that must be served, ranging from live bird markets to highly industrialized markets. It would be possible to develop products specifically designed for many of these markets based on local breeding programmes and improved indigenous populations (Notter, 1999). This approach may be appropriate in some very specialized situations in developing countries (Mukherjee, 1990) but inevitably such programmes cannot keep pace with more sophisticated breeding programmes. Even developing markets benefit from the structured research and development efforts of the international primary breeders (Narrod and Fuglie, 2000). There is an association between market size and the adoption of advanced genetic technology which has contributed to the integration and technical development of the poultry industry (Johnson and Ruttan, 1997).

Market size is one of the key determinants of private investment in agricultural research (Narrod and Fuglie, 2000). Thus, primary breeders must identify market clusters that can be served by a single product in order to justify significant research and development effort. These clusters can then be viewed as market segments. The challenge is to develop products that satisfy the broad needs of the majority of the cluster, though it is sometimes possible to produce several alternative products for grandparent markets. For example, most breeders produce both feather-sexable and fast-feathering products, some with similar characteristics, for their product range.

The majority of the world broiler industry can be divided into three broad sectors. There is a significant sector, primarily in developing markets, that requires high reproductive performance balanced with rapid broiler growth to relatively low market weight (< 2 kg). The second sector is

primarily focused on broiler performance, with secondary interest in processing characteristics and lower standards for reproduction. The third sector represents further processing, which is primarily driven by processing yield with secondary emphasis on broiler performance. This generalized view provides a suitable model to explain the major broiler-producing areas of the world. However, it should be noted that there is considerable variation in product requirements within each sector and overlap between these sectors.

Despite this generalization, many niche markets exist. While existing products designed for larger market sectors are sometimes suitable for these niches, there are situations where special products are required. In many cases, these markets are supplied by specialized breeding groups that focus their efforts on these unique sectors. However, these segments are of increasing interest to the large multinationals, as they may represent developing trends rather than long-term niche markets. The *Label Rouge* market in France and the developing 'drug-free' market in North America represent niche markets that might eventually have a significant impact on production practice in the broader markets.

Economic factors

The economics of production are the primary consideration in the establishment of any breeding goal. As economic considerations are the primary driver in any breed decision, so must economics be a primary consideration in product development. This would appear to be a straightforward process based on the economics of poultry production. However, there are many variable market requirements, production systems and market conditions employed in the poultry industry. Economic models provide a tool for examining the impact of these variables on production costs, product output and profitability (Groen *et al.*, 1998). Model assumptions and market definitions have an impact on the outcome of any economic evaluation. Unfortunately, there are often no definitive parameters for many of these inputs. This is particularly true when one considers the large variation observed both within and between various market sectors and individual companies. In addition, analysis of relative trait values involves independent assessment of the impact of individual traits on profitability, holding all other factors constant. The obvious shortcoming is that performance traits do not change independently, due to the multiple genetic relationships between most breeder and broiler characters. Thus, these modelling exercises typically provide general direction rather than definitive solutions.

The structure of a market has a large impact on the economics of production. Costs and returns accrue in integrated systems and economic value is based on the total cost or net value of product produced. It has been suggested that, even in non-integrated markets, there is transfer of costs from one sector to the next and so even non-integrated markets should be evaluated based on total profitability to the system, particularly for the purpose of long-term improvement through genetic selection (Moav and Moav, 1966). However, in some markets there are distinct sectors under separate ownership and economic evaluation becomes more difficult. When this is the case it is critical to identify the balance of influence of each sector on genetic decisions. Inevitably, one production sector will dominate breed decisions and product goals must focus on the requirements of this sector. Economic analysis can be extremely difficult for undeveloped markets where there are many small producers and unsophisticated systems for the marketing of poultry products. Distributors and breeder and hatchery operators tend to dominate these markets; consequently, breeder performance and chick cost are the primary economic criteria. In any case, it is essential to identify the primary measure for breed selection in order to conduct a suitable economic evaluation.

Different sectors of the industry compare economic performance based on

different scale factors, depending on the manner in which they derive income. In non-integrated markets, hatchery productivity is evaluated based on total costs per viable chick. The broiler sector is generally paid based on the number of kilograms produced and so this sector evaluates performance based on the total cost per live kilogram. Processing cost is directly related to the capital cost of a plant and plant capacity; plant capacity is generally fixed based on line speed and, thus, processing efficiency is most appropriately evaluated based on margin per bird processed. Most integrated producers base economic decisions on profitability per shackle, since investment in processing facilities represents a high percentage of total capital investment and processing efficiency is the most significant factor influencing return on capital investment. An evaluation of the relative impact of a 1% improvement in performance based on these evaluation criteria is presented in Table 8.1. Under the assumptions of this analysis, it is apparent that reproductive traits, feed conversion and meat yield have the greatest impact on evaluation criteria for the hatchery, broiler and processing sectors, respectively. However, when evaluated on an integrated basis, yield has a larger impact than any other single factor.

Another factor that has a large impact on production economics is the manner in which poultry products are marketed to the consumer. In general, the impact of processing yield and efficiency increases with greater degrees of further processing. Live weight and general health and appearance are most important for live bird markets. Fast-food and cut-up markets place premiums on carcass yield, grade and uniformity. The economic impact of white meat yield increases rapidly as the percentage of deboning increases (Fig. 8.1). Although the relative value of processed parts and white meat vs. dark meat has some influence on the impact of yield on production economics, yield remains a dominant economic driver even with low product prices or equity in the relative value of white meat and dark meat, due to the significant biological variation that exists.

The incremental value for trait improvements also varies significantly with market weight. As market weight increases, the relative contribution of chick cost to total production cost declines. For example, chick cost represents approximately 20% of live production cost at 1.8 kg market weight but only 12.5% of live production cost at a market weight of 3 kg, while the relative contribution of feed cost to production cost is relatively constant over this same weight range. Consequently, for one particular scenario, four eggs are equal in economic value to one point of feed conversion at 1.8 kg while 13.5 eggs are required to offset a single point of feed conversion at 3 kg. Thus, trait equivalency is not static across market weights.

Market assumptions such as the cost of labour and feed ingredients and the relative market value of various products obviously impact on any economic evaluation. Short-term fluctuations seldom have significant

Table 8.1. Influence of improvement in trait performance on profitability of individual production sectors.[a]

Trait	Sector			
	Hatchery	Broiler	Processing	Integrated
Egg production	0.56	0.11	0	0.23
Hatchability	0.76	0.15	0	0.46
Broiler weight	0	0.21	0	0.32
Broiler mortality	0	0.19	0	0.42
Feed conversion	0	0.60	0	1.31
Breast meat %	0	0	0.70	3.10

[a]Expressed as the percentage improvement in profit criteria resulting from a 1% improvement in trait performance.
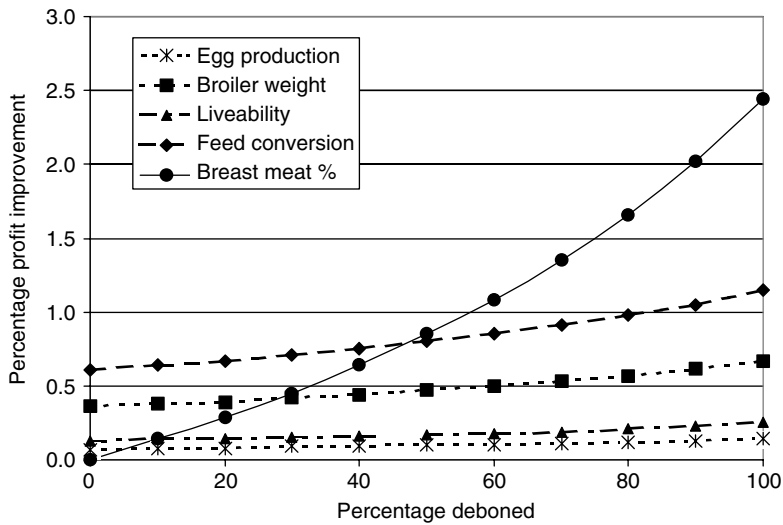
Fig. 8.1.   Percentage improvement in profit in a fully integrated operation for varying product mix.

impact on breed decisions since the purchase of breeding stock represents a long-term investment. Parent stock is generally ordered 6–18 months before placement and will remain in production for 65 weeks. The time horizons extend even further in the case of grandparent purchases. Thus, it would be short-sighted to base genetic decisions on short-term market fluctuations. However, intrinsic differences in the price of inputs or outputs in markets can influence product requirements (Jiang *et al.*, 1998). For example, consumers in many markets prefer dark meat as compared to the preference for white meat in the United States and Western Europe. White meat sells for a premium in most western markets, which increases the impact of white meat yield. However, the impact of intrinsic market factors remains relatively small since biological conversions and production relationships are largely conserved except when there is excessive variation in market price relationships. International trading of poultry products has greatly stabilized market price variation as well.

Competitive position

Due to the competitive nature of primary breeding, it is often desirable to adjust

breeding objectives in order to overcome product shortcomings (Emsley, 1993). Product performance is generally evaluated in relative terms, with the competitor serving as a benchmark. For some highly visible traits, a competitive disadvantage will negatively affect sales even though the overall economic value of the package is superior. Thus, it is necessary to put greater selection emphasis on these traits than would be justified by any objective analysis. Likewise, a competitive advantage is often viewed as a sacred position that must be maintained even when not justified by market conditions. There is also the potential to capture market share by creating a point of difference for a novel trait where no breed variation currently exists.

Customer perceptions

Historical perceptions are sometimes very difficult to overcome even when a competitive disadvantage has been eliminated. Thus, it may be important for performance to be superior to the competition for such a trait. For traits that are not primary drivers but are of historical importance, it may be necessary to be competitive but not necessarily superior, while the market may also

punish a product that is last in the field for the same traits.

Recognition limits

While the poultry industry is a competitive market-driven industry, there are practical limits to its ability to measure the impact of individual management inputs on profitability. As a result, factors that can be readily measured often receive undue emphasis. For example, it is currently possible to measure the impact of a given breed on broiler performance and the cost of live production as a part of ongoing operations but, under field conditions, it is very difficult to measure yield performance directly in an objective and technically sound way, due to the large scale of operations. While most companies recognize the impact of meat yield on production costs, managers have great difficulty measuring the impact of a breed change on yield and there is reluctance to depend on information from research trials rather than actual production data. Thus, there are limits to the ability of the production sector to measure advances in genetic performance despite a general recognition of the benefit of these advances. These limits have a profound influence on breeding objectives in a competitive marketplace.

Threshold traits

There are many traits that the marketplace treats as threshold traits, where performance below some arbitrary level constitutes product failure. This is most common for highly visible traits that carry an emotional component. For example, late mortality is sometimes viewed as a threshold trait for growers who must remove and dispose of mortality on a daily basis. This may hold true even when economic performance is acceptable. Similarly, producers have traditionally placed a minimum threshold on egg production that must not be crossed even though advantages for

broiler performance offset higher egg cost. The requirement for additional capital expenditure to achieve the same level of production can present a business and psychological constraint as well.

Biological considerations

Genetic factors play a key role not only in the proper design of a breeding programme but also in the development of a breeding goal. All else being equal, it is more effective to select for traits with high heritabilities and economically positive genetic correlations. Indeed, it can be more effective to select for a trait with a relatively low economic value but a high heritability than a trait with higher economic value but a lower heritability. It is also beneficial to select economically important traits that are positively correlated as compared with traits of similar economic value that are genetically independent of other traits under selection. Economically important traits that are genetically antagonistic represent the worst scenario. In such cases it is generally necessary to strike a balance that is acceptable to the market.

There is a tendency to rely on a genetic solution to most poultry production issues. While it is true that genetic progress is possible for any trait that demonstrates genetic variation, there will always be a question of relative benefit that must be considered. Some production problems can be addressed more cost effectively through proper management, nutrition or environmental control than through genetic selection. Likewise, some issues can be improved more effectively on a small scale through genetic selection of elite breeding populations than on a large scale in the production sector. Thus, it is important to determine the proper balance between genetic and non-genetic solutions and to establish the proper priorities on various traits.

There are also situations where there may be economic advantage in genetically improving a trait for which there is limited biological variation. For example, it would

be economically desirable to select for an increased percentage of wing for some markets. However, biological variation for the percentage of wing is limited, presumably because this is a fitness trait that has been conserved through natural selection. In contrast, breast meat percentage demonstrates significant biological and genetic variation and, consequently, is very responsive to genetic selection.

Social and political pressures

There is increased public and consumer recognition of issues related to food safety, product quality, environmental pollution, animal welfare (Douglas and Buddiger, 2002) and genetic diversity (Notter, 1999), particularly in affluent markets. Primary breeders have adopted improved production and biosecurity practices and have taken a leadership position on food safety issues. Improvement of animal productivity improves the efficiency of conversion of feed to food and indirectly reduces the production of animal waste per unit, although additional methods of reducing nitrogen and phosphorus waste will ultimately be required. Welfare concerns have resulted in greater emphasis on skeletal and metabolic disorders, disease resistance (McKay *et al.*, 2000) and behavioural genetics in commercial breeding programmes (Hartmann, 1989). Although it is difficult to assign an economic value to these considerations, primary breeders will be expected to develop novel approaches to help provide sustainable solutions to these problems.

Selection Strategies

Establishing a proper breeding objective is only the first step in developing a viable breeding programme. The breeding objective provides the intended balance between individual trait goals and quantitative information regarding the annual genetic progress that can be expected. However, there are many nuances that cannot be captured in any numerical analysis but that are essential in achieving the market objective. Thus, the actual outcome of selection is highly dependent on the selection strategies employed. These less tangible design factors affect physiological and product characteristics and influence the market suitability of the resulting products.

Line differentiation

A breeding objective defines the relative performance and directional improvement for important traits within the breeding matrix for a specific product type. However, commercial breeding schemes rely on line crossing for the protection of genetic assets and to capitalize on non-additive genetic variation for lowly heritable traits. Thus, it is important to distinguish between product goals and breeding objectives for individual component lines. Commercial meat-type chickens and turkeys are the product of three- and four-way crosses of closed populations (Hunton, 1990). Theoretically, a scheme where all component lines in a commercial cross are selected according to a similar breeding goal would provide the most efficient approach. This strategy assumes that the selection objective is clearly defined and not subject to rapid change. This strategy also has the potential to produce a more uniform final product, since variability resulting from recombination should have a small impact on product variation. However, diversity of market sectors and risk exposure limit the commercial viability of this approach.

Moav and Moav (1966) demonstrated the economic value of specialized sire and dam lines when male and female parents provide unequal contributions to reproductive success. Thus, in the case of meat-type poultry, male and female lines are highly differentiated with male lines being approximately 20–30% heavier at a given age. A similar strategy can be extended to differentiation of female lines to optimize reproduction within the multiplication scheme and to provide greater flexibility of

final product characteristics. For example, it would be possible to produce three or more distinct products utilizing a common female-line female (line D) mated to males from highly differentiated female lines (line C). In this way three products could be produced from a base of four pure lines, compared with a total of six lines required to produce the same range of products using undifferentiated pure lines. This provides a more streamlined and cost-efficient strategy to produce distinct products for different market sectors and it also provides insurance should market requirements shift more rapidly than can be compensated for through within-line selection.

Body weight and growth

While body weight is one of the easiest and most repeatable traits to measure, selection for growth is not straightforward. Growth is a dynamic process that involves the coordinated development of many tissues and physiological systems. Negative consequences of genetic selection for growth rate have been well documented (Siegel and Dunnington, 1987). In some cases, the magnitude of these complications may be influenced by the selection strategy employed (Barbato, 1991).

The intensity, timing and method of selection for growth rate can have a significant effect on the actual outcome of selection. Anthony *et al.* (1991) hypothesized that the timing of selection relative to physiological maturity can influence the shape of the growth curve in addition to changing the magnitude of growth. Divergent selection for juvenile or adult body weight, alone or in combination, has been demonstrated to influence growth in a manner consistent with this hypothesis (Mignon-Grasteau, 1999). Similar results have been reported for commercial strains with similar growth potential (Lilburn and Emmerson, 1993). Research reports regarding the influence of single-trait selection on growth curve parameters are consistent with commercial observations involving less differentiated

genotypes. In general, selection at an early age appears to change the shape of the growth curve, resulting in an earlier point of inflection and a greater slope at the point of inflection. In contrast, selection at a later age increases the magnitude of growth without significantly changing the form of growth.

There are several important physiological implications related to growth curve modification. A growth pattern with relatively slower early growth and more rapid late growth is more energetically efficient. In addition, this growth trajectory should support better cardiovascular health and skeletal development, as development of critical supply organs is established prior to the rapid growth of demand organs. Thus, theoretically, there are biological benefits to selection for this type of growth pattern. In practical breeding programmes this approach also gives ample time for the expression of leg disorders and other physical defects, allowing more effective selection against these conditions. This selection strategy has been applied in the development of roaster stocks and Large White turkeys for deboning markets.

In contrast, selection for rapid early growth influences allometric growth relationships and accelerates the rate of developmental maturity. Selection for early growth rate not only results in an earlier point of inflection of the growth curve but also leads to earlier achievement of mature body size (age and weight at asymptote). Thus, at any chronological age, a line developed under this strategy will have greater physiological (developmental) maturity. Advanced maturity is reflected in carcass composition and results in greater meat yield and carcass fatness. There is greater maternal influence on early growth, which results in correlated responses for increased egg weight and reduced egg number. This approach also potentially has less impact on gender dimorphism as selection is occurring prior to the development of significant sexual dimorphism, when male weight and female weight are genetically more similar (Mignon-Grasteau, 1999). This selection strategy is analogous to common practice in the development of broiler stocks.

The scenarios outlined above represent two extreme strategies; in practice, there are unlimited combinations between these extremes. It is important to note that there are negative consequences associated with each approach. These consequences cannot be viewed in a void without regard to selection practised for other traits. Indeed, selection for other traits provides a method of ameliorating some of these consequences and still benefiting from the positive aspects of the strategy. For example, many of the negative aspects of selection at a very early age can be overcome through simultaneous selection for improved efficiency and reduced mortality due to metabolic disease.

The primary breeding industry typically follows one of three basic approaches in selecting for growth rate that can lead to quite different physiological and chronological selection points, depending on the line, gender and species (Fig. 8.2). Historically, selection at a commercial age has been the most common approach. Pure lines are selected at an age that corresponds with market age under this system. This strategy is still common in turkey breeding, where the commercial industry has a goal of weight for age and processing weights are not targeted as precisely. This approach has the additional advantage of not being complicated by the weight differential between male and female lines.

Selection at a commercial weight is generally a more appropriate strategy when market requirements demand narrow or precise weight specifications for end use. Pure lines are selected at a weight that matches market weight and the age at selection becomes progressively earlier as growth potential increases. This approach ensures that selection is focused on improving all traits at an appropriate target weight. This also takes into account the fact that male lines are 30–40% heavier than female lines and that growth rates are significantly greater in a structured pedigree programme than would be observed under field conditions. Thus, primary selection might occur at a much younger age than typical market age but at a weight and developmental maturity that is consistent with the ultimate breeding objective. Selection at a commercial weight is the most common strategy employed in industrial broiler breeding.

Multi-stage selection represents a practical compromise between selection at a commercial age and selection at a commercial weight. Multi-stage selection is appropriate for development of multi-purpose products that must perform over a relatively large range of market weights or where a secondary breeding objective exists. In turkey production, market weights for males and females are developmentally incompatible, as females are significantly less mature at market age than their male counterparts
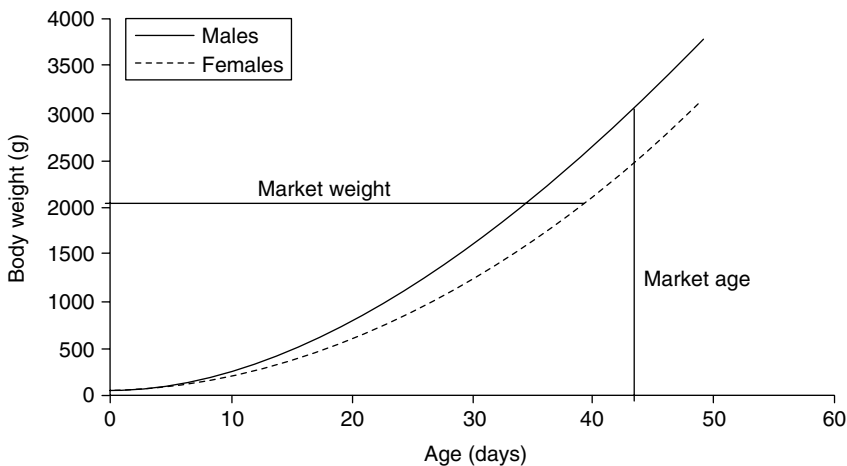

Fig. 8.2. Potential selection strategies for improvement of body weight.

(Fig. 8.3). Indeed, male and female body weights at a common age represent genetically different traits in turkeys, as evidenced by the relatively low genetic correlation between male and female live weight.

Conformation and meat yield

Selection for conformation and meat yield will be discussed together since the two subjects are closely related, though it should not be assumed that the two are synonymous. While conformation and yield are genetically related, from a market standpoint conformation is currently more of a secondary trait in breeding plans than in the past, due to greater consumer interest in deboned and value-added products. This shift in emphasis has changed the way in which the breeding industry selects for yield traits.

Traditional selection for yield involved heavy emphasis on phenotypic evaluation and the use of subjective conformation scores. The original intent of conformation selection was to improve the visual appeal and consumer acceptability of whole carcasses. This method has also been very effective in improving yield, due to the high genetic correlation between conformation scores and white meat yield. The degree of fleshing is the primary factor in any conformation scoring system. However,

selection based on conformation has several less obvious side effects. Anecdotal evidence suggests that intense selection for conformation produces correlated changes in skeletal structure resulting in the development of a shorter and less prominent keel structure, greater spread to the ribs and shorter legs and shanks. Nestor *et al.* (2001) observed significant differences in the length and weight of leg bones and in the dimensions of the keel and the body cavity between two commercial sire lines as compared with a line selected exclusively for body weight, which supports this general hypothesis. These characteristics have negative production implications relating to breeder fertility as well as leg strength in both breeders and broilers. Since conformation *per se* is not a primary commercial issue, breeders have sought strategies to increase breast meat yield through more direct and less subjective means.

Ultrasound technology represents one potential strategy for the improvement of yield through objective means. It can be used to measure muscle thickness in the live animal and muscle thickness is highly correlated with total meat yield. When properly applied, ultrasound measurements are more highly correlated with meat yield than subjective conformation scores. This strategy utilizes direct selection for muscle thickness as a means of producing a correlated response for percentage yield. Fillet
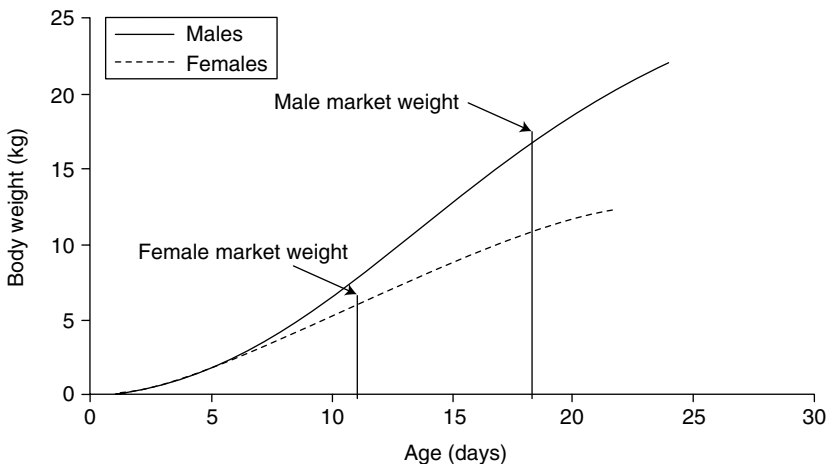

Fig. 8.3. Typical market weight for male and female turkeys in relation to standard commercial growth.

thickness has a higher phenotypic correlation with white meat yield than fillet length or width and has greater variability (Lubritz, 1997). Consequently, selection based on ultrasound potentially has a greater impact on fillet thickness than other methods of selection for yield, which can present a problem for the processing sector. There are currently two major sectors within the deboning market segment. One sector uses deboned meat for products that involve significant further processing and the resulting product does not resemble the original form; in this case deboned meat is simply a raw material. The other sector uses deboned fillet meat in its original form, following minimal processing; consumer preference in this sector generally requires uniformity of portion size with tight specifications for fillet dimensions, including muscle thickness.

The most direct method in selecting for yield is to utilize yield information from pedigree sibs to identify families with superior yield potential. While this approach does not account for variation in individual performance, it is very effective because yield is a highly heritable trait. With its potential for rapid genetic progress, this has become a common selection approach. It also has the advantage of being free of the incidental correlations with frame size and fillet dimensions that result from indirect measurements. However, biologically driven negative correlations resulting from pleotropic effects of genes associated with yield still remain.

Genotype × environment interactions

Concentration within the poultry breeding industry has greatly reduced the number of sites devoted to the genetic improvement of poultry. As a result, selection is focused on a relatively narrow range of environmental conditions that can be quite divergent from commercial conditions (Douglas and Buddiger, 2002). In addition, breeders generally operate in quality facilities that are biosecure (low disease challenge) and

offer a high degree of management control. Theoretically, genetic progress is maximized through optimization of biological performance (reproduction, liveability) and elimination of environmental variation. Under these conditions, heritabilities are optimized and phenotypic variation becomes more representative of genotypic variation. Thus, sophisticated housing with environmental controls, precise management and optimal nutrition will support rapid genetic progress, which is the primary objective of breeding companies.

However, poultry production systems typically do not achieve the same level of performance under field conditions, as a result of environmental variables. Environmental variation arises from diversity of climatic conditions, production and housing systems, feed ingredients and disease challenges present throughout the world. Additional variation exists due to variable management expertise, production philosophies (low live cost, low meat cost, optimal performance), market conditions and welfare standards. Genetic stocks, which have been developed in a limited number of locations in relatively ideal environments, are expected to perform consistently under variable environmental conditions. This apparent conflict is of particular concern to production in developing markets (Hartmann, 1989).

This creates a fundamental dilemma for poultry breeders. The market requires products that are disease and pathogen free but resistant to disease under field conditions. Products must be robust and resistant to physiological challenges associated with rapid growth, such as ascites, sudden death syndrome and tibial dyschrondroplasia, but must also be rustic and able to withstand stress presented by low feed quality, poor environmental control, high environmental temperatures and local and varied disease challenges. Products must have high genetic potential and must perform equally well in both optimal and challenging environments.

Genotype-by-environment (G × E) interactions have historically been ignored in commercial breeding programmes (Siegel, 1988). This has occurred because it is

difficult to establish their existence clearly under controlled conditions within the range of commercially relevant genotypes and environments (Emmerson, 2000). Although empirical evidence is generally limited, anecdotal evidence from commercial conditions suggest that $G \times E$ may be widespread. This apparent discrepancy is generally poorly accepted since, by definition, it cannot be explained scientifically. Variation within and between poultry operations often exceeds variation attributable to $G \times E$ interactions (Douglas and Buddiger, 2002). Controlled research regarding $G \times E$ interactions requires a high degree of replication to successfully detect statistically significant interactions and such research must generally be focused on single sources of environmental variation. However, under field conditions, many different sources of environmental variation overlap and interact to create a more rigorous standard (Emmerson, 2000). Multiple challenges limit animal resources and make it more difficult to mount effective responses. Thus, resource allocation theory increases the likelihood of $G \times E$ interactions under field conditions.

The simplest genetic strategy to deal with $G \times E$ interactions would be to select pedigree populations in a defined commercial environment, but there is no single set of commercial conditions that would be representative of the world poultry industry. Even the relatively developed markets in Western Europe and North America differ greatly in their production practices, nutritional programmes and climatic conditions. Although genotypes selected in one environment are broadly successful in the other, there are many limitations to the viability of this approach. For example, broiler and turkey genotypes selected in North American systems typically have excessive mortality from metabolic disease when grown in European management systems, while European genotypes do not generally perform at their genetic potential under North American feeding programmes.

Another potential strategy is to select in a 'blended' environment that includes critical environmental factors from several different markets. To an extent, this strategy is employed by all breeding organizations since it is not practical to utilize many conditions encountered in the field. For example, it is possible to feed commercial diets and use common commercial densities but breeders must maintain a higher degree of biosecurity to minimize the risk of disease exposure. This general approach could be extended in a more overt way to develop a selection environment that combines critical environmental factors from a number of sectors. This strategy would be likely to produce products that are generally adaptable to many environments but they might not represent the best genotype in any single environment. Success with this approach would depend on the identification and implementation of the proper collection of environmental conditions.

Some companies have established satellite pedigree breeding programmes. Although these operations are often primarily initiated for business reasons, such as import or quarantine restrictions, they have the potential to produce products that are more adapted to the local environment. However, due to the great cost associated with operating a pedigree breeding programme, this is only practical for markets that are sufficiently large and unique to sustain this scale of operation.

Breeders of chicken stocks for table-egg production have adopted the most progressive approach for dealing with $G \times E$ interactions. Although the issues are quite different, breeders of egg-type stocks have adopted systems for the evaluation of crossbred performance in commercial environments as part of recurrent testing programmes (Ansah, 2000). This provides the opportunity to evaluate pedigree performance in systems that are more commercially oriented without sacrificing biosecurity in the pedigree programme. Although these programmes are very costly to operate, they allow breeders to select pedigree populations under more challenging conditions than would normally be encountered in a controlled pedigree programme. This strategy is only feasible when the genetic correlation between

pedigree performance and field performance is low to moderate (Douglas and Buddiger, 2002). A similar approach could be used in broiler or turkey breeding to overcome G × E interactions and effectively select for adaptation to challenging environments. Such an approach may become necessary as developed and emerging markets become more diverse and commercial genotypes develop to greater physiological extremes.

Conclusions

The initial step in any successful breeding project is the establishment of an appropriate breeding goal. Successful programmes must incorporate both quantitative and biological considerations and be closely aligned with market requirements. The industrial nature of poultry breeding has a large impact on genetic development. Poultry breeding stock represent genetic products that must be marketed and sold and, hence, a product must have perceived value that translates into customer demand.

While economics plays a central role in establishing breeding goals, a number of assumptions go into economic analysis and there are many intangible factors that cannot be accounted for in any economic model. In addition, many variable market requirements, production systems and market conditions are employed in the poultry industry and these variables cannot be accounted for in any static model. Empirical models are essential tools that provide general directions to the development of breeding plans but many intangible factors must also be considered.

Whereas breeding goals provide quantitative targets for genetic progress, selection strategies influence the manner in which those goals are met. Selection strategies incorporate biological considerations and influence the production and quality characteristics and market acceptability of genetic products. These strategies must continue to evolve as additional market challenges appear and as more diverse genotypes are developed.

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9 Use of Mixed Model Methodology in Breeding Strategies for Layers

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Features of Layer Breeding Programmes

Layer breeding programmes present three main features which are worth recalling before discussing statistical approaches used by geneticists.

1. Breeding programmes use large populations that are placed in homogeneous environments. Layers are housed in farms where temperature, ventilation, lighting, etc. are very well controlled, and receive the same treatment for nutrition and disease control.
2. Birds are managed in separate cohorts or contemporary groups; future breeding stocks, either roosters or hens, are selected from a group of birds of the same age. This separation helps to maintain a good sanitary status in the management of the flocks.
3. Poultry breeding schemes are characterized by a hierarchical family structure: a male is mated to many females (between five and ten) whereas a female is seldom mated to more than one male. Full-sib families may consist of more than ten daughters but only two or three of the sons are retained.

These features explain, at least partly, the success of classical methods of genetic evaluation based on the selection index theory, and estimation of genetic parameters based on Professor C.R. Henderson's methods. These classical methods have interesting properties but they also have some theoretical and practical drawbacks that explain the increasing interest of poultry breeders in a new generation of methods, limited to large domestic species until the early 1990s. These include mixed model approaches such as best linear unbiased prediction (BLUP) for breeding value prediction and restricted maximum likelihood (REML) for estimation of genetic parameters, both applied to animal models.

Estimation of Genetic Parameters and Genetic Values

Classical approaches

Estimation of genetic parameters by analysis of variance

Estimation of variance components leads to the computation of the elements of genetic and residual variance–covariance matrices, which are key elements in quantitative genetics. Their estimation is necessary to quantify the determinism of the traits of interest and predict the corresponding breeding values.

The use of large populations with structures close to well-balanced designs on the one hand, and within-generation selection on the other hand, allowed the estimation of genetic parameters using simple methods.

The most frequently used are Henderson's methods, applied (because of the hierarchical family structure) to the following nested model:

$$y_{ijkl} = \mu + h_i + s_j + d_{jk} + e_{ijkl} \qquad (1)$$

where y_{ijkl} is the observed performance, μ is the population's mean, h_i is the ith hatch (fixed) effect, s_j is the effect of the sire j, considered as random, d_{jk} is the effect of the dam k mated to the sire j, considered as random as well, and e_{ijkl} is the residual effect associated with observation l.

Two situations are generally described in the literature. In the first the sire, the dam nested within the sire and the residual components are estimated from the data adjusted for the fixed effects (Pirchner and von Krosigk, 1973; Flock, 1977), i.e. using standardized deviations, $y^*_{ijkl} = y_{ijkl} - \mu$, reducing model (1) to a purely random model. Consequently, Henderson's method I can be used to obtain genetic parameters. The second situation is more frequent and comprises estimating the variance components based on the full model using Henderson's method III. Most of the results compiled by Fairfull and Gowe (1990) are based on this method.

In both situations, the estimates are unique and unbiased. However, with these quadratic methods, the probability of getting estimates outside the parameter space (negative variances, correlation >1 or <−1, heritability >1) is not null (Searle, 1989).

Considering the long history of selection that layers have gone through, accounting for this selection is a major problem facing the geneticist when estimating the variance components. These estimators may be biased due to selection on correlated traits, which explains, at least partly, the shift to maximum likelihood estimators.

Genetic evaluation by the selection index theory

Historically formalized by Smith (1936) in plant genetics, Hazel (1943) in animal genetics and Osborne (1957) in relation to poultry, the selection index theory is a statistical method for prediction, by multiple linear regression, of additive genetic value of candidates to selection. This prediction is based on observations adjusted for environmental effects that are supposed to be well known (Henderson, 1973). The use of homogeneous and very well controlled environments has encouraged poultry geneticists to consider this hypothesis to be fulfilled, i.e. adjusted performances are as precise as raw data, and consequently to use the index:

$$I_x = b_1 y_f + b_2 (y_x - y_f) \qquad (2)$$

where y_x is the adjusted performance of the candidate and y_f is the mean performance (including its own) of the candidate's family. The coefficients b_1 and b_2 maximize the correlation r_{IA} between the index and the breeding value. They depend on the heritability, family size and coefficients of relationship. Such an expression can easily be extended to multivariate cases. An adjustment usually considered is to express the observations in standardized deviations sometimes called probit (probability unit). The index I_x in model (2), which includes information on the candidate and on the family, is called a combined index (Falconer, 1981; Minvielle, 1990). It is still widely used in poultry breeding and its contribution to the tremendous genetic trend realized in these species is unquestionable.

If the assumption of a priori knowledge of the expectation and variances of the observations is correct, prediction based on the selection index theory maximizes the correlation between estimated and true breeding values, is unbiased, and maximizes the probability of pairwise correct ranking of estimated breeding values (Henderson, 1963). Furthermore, it maximizes the expectation of the genetic value of the n best candidates (Goffinet and Elsen, 1984), for any value of n.

For laying hens, hatches do not necessarily have the same genetic level. Different levels exist when the proportion of young vs. old breeders varies from one hatch to another. In such a case, the above assumption is not correct and the comparison between indices of animals from different

hatches may be biased. The selection index theory presents other theoretical and practical drawbacks: it does not account for changes in additive genetic variance due to selection, inbreeding and preferential mating. It quickly becomes cumbersome as the coefficients of the index have to be recalculated each time the amount of information on the candidate changes. This has pushed geneticists to limit the information to close parentage (e.g. parents, full-sibs, half-sibs) while information from more distant relatives is ignored.

Mixed model-based methods

Infinitesimal model

Mixed model methodology has been introduced in poultry breeding more recently than for other farm animal species. The first applications of REML and BLUP were reported in the early 1990s (Besbes *et al.*, 1992; Wei and van der Werf, 1993).

For most traits of interest, little (if anything) is known about the true genetic background, e.g. the number of genes involved or their respective position and effects. Fisher (1918) suggested a model with a large (strictly speaking, infinite) number of independent loci, each locus with a relatively small effect on the trait of interest. This infinitesimal model continues to dominate the world of quantitative genetics. This is not because the above assumption is always true (loci with large effects exist but less frequently than loci with small effects) but because it is mathematically tractable, has proved to be reasonably robust and leads to some simple solutions of genetic values (Hill, 1994). It is a formal requirement for most desirable properties of BLUP, as well as the selection index theory, in livestock improvement.

Under this infinitesimal model, additive effects can be added across loci. Asymptotically, these effects can be treated as normally distributed random variables (Simianer, 1994), leading to the basic mixed linear model, which can be written in matrix notation as follows:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{a} + \mathbf{e} \qquad (3)$$

where $\mathbf{y}$ is the vector of observations, $\boldsymbol{\beta}$ is the vector of fixed environmental effects (e.g. contemporary groups or hatches), $\mathbf{a}$ is the vector of random additive genetic effects of animals with observations and their relatives, $\mathbf{e}$ is the vector of residuals, and $\mathbf{X}$ and $\mathbf{Z}$ are incidence matrices associated with $\boldsymbol{\beta}$ and $\mathbf{a}$.

In this model, which most animal breeders identify as the animal model, numerous assumptions are implicitly made – such as the independence of additive genetic and residual terms, i.e. no genotype-by-environment $(G \times E)$ interaction. But does it really matter if it is wrong? To this question, Hill (1999) answered: 'with directional truncation selection, the prediction equations from the infinitesimal model work very well, even when selection is at high intensity. It works not so much because all terms are small, but because some cancel out.'

Restricted maximum likelihood estimation

REML is a general method for estimating variance components in the case of unbalanced designs. It is applicable to all types of models (mixed or random, classified or nested, with or without interaction) and presents well-defined properties (Harville, 1977), i.e. REML estimates are asymptotically unbiased, invariant and within the parameter space.

Under normality, this method accounts for the effect of selection on estimated parameters, provided that all the information related to selection is included in the analysis (Im *et al.*, 1989). Harville (1977) and Banks *et al.* (1985) showed that REML estimators are robust even when this hypothesis is not satisfied. Besbes *et al.* (1993), analysing egg production data, confirmed this robustness but only for slight departure from normality.

Best linear unbiased prediction

The principle of BLUP is as follows:

1. Estimate the fixed effects $\boldsymbol{\beta}$ by generalized least-squares.

2. Apply selection index theory to data corrected for $\hat{\beta}$.

The resulting solutions are 'best linear unbiased estimates' (BLUE) of β and 'best linear unbiased predictions' (BLUP) of a. Unfortunately, this approach needs the inversion of the variance–covariance matrix between observations, which is impossible with field data sets. In fact, Henderson (1949) realized that the solutions of β and a could be obtained by solving what became known as Henderson's mixed model equations:

$$\begin{bmatrix} \mathbf{X'R^{-1}X} & \mathbf{X'R^{-1}Z} \\ \mathbf{Z'R^{-1}X} & \mathbf{Z'R^{-1}Z+G^{-1}} \end{bmatrix} \begin{bmatrix} \beta \\ a \end{bmatrix} \begin{bmatrix} \mathbf{X'R^{-1}y} \\ \mathbf{Z'R^{-1}y} \end{bmatrix} (4)$$

The genetic $\mathbf{G}$ and residual $\mathbf{R}$ variance–covariance matrices are positive definite and generally diagonal, bloc diagonal or of a particular (easy to invert) structure. When the data are ordered by trait within animal, $\mathbf{G} = \mathbf{A} \otimes \mathbf{G_0}$, where $\mathbf{A}$ is the relationship matrix, $\mathbf{G_0}$ is the genetic variance–covariance between traits and $\otimes$ indicates the direct (Kronecker) product. The above system became widely used only three decades later, when Henderson (1976) proposed his simple rules to calculate $\mathbf{A^{-1}}$ directly without constructing and inverting $\mathbf{A}$, and the computing requirements of the method could be satisfied by modern computers. The fact that $\mathbf{A^{-1}}$ is a sparse matrix also helped in developing efficient solving strategies.

BLUP is therefore considered as a selection index that uses corrected data based on the best possible estimation (in the sense of generalized least-squares) of environmental fixed effects. If these effects were exactly known, BLUP would be equal to the selection index. Consequently, it has many properties of the latter but under less restrictive hypotheses. When applied to an animal model, BLUP takes into account the evolution of the genetic variance due to selection, genetic drift and inbreeding (Kennedy *et al.*, 1988). Moreover, if the complete pedigree traces back to the base population of non-selected, non-related and non-inbred animals, and all data used for previous selection steps are included in the analysis, BLUP is unbiased by selection (Im *et al.*, 1989).

The number of traits incorporated in breeding programmes for layers is constantly increasing, but not all traits are selected in the same way. One group of traits may be selected through an aggregate index based on genetic values, while others are selected with independent culling levels based upon genetic or phenotypic values. Exclusion of certain traits from multiple-trait evaluation is due either to computational cost or sometimes to the difficulty in obtaining reliable estimates of the genetic and environmental (co)variances for these traits. Gowe *et al.* (1993) added two other situations where independent culling level selection may be preferable. These are for traits approaching a biological limit (e.g. fertility and hatchability) and compound traits (e.g. mortality).

As a selection objective includes more than one trait, combining single-trait predictions of breeding values $\hat{a}$ into an aggregate genotype $\mathbf{b'\hat{a}}$ is not an easy task. The weight of vector $\mathbf{b}$ for each trait depends on its economic value and the accuracy of $\hat{a}$, as well as the genetic correlation with other traits. With multiple-trait BLUP evaluations, this combination becomes straightforward and the optimal $\mathbf{b}$ values are simply the economic weight for each trait (van der Werf *et al.*, 1992).

Uncertainty on the level of genetic correlations is put forward as an argument to use single-trait methods instead of multiple-trait methods for the prediction of breeding values. It should be remembered, however, that single-trait analyses implicitly assume null correlations between traits and, therefore, are not free of selection bias themselves. This is the case, for example, when animals are recorded for two correlated traits and only the first one is selected for. Univariate prediction based on the second trait is biased, except in the special case when the regression coefficients of the residual and genetic parts of the second trait on the first one are equal (Pollak *et al.*, 1984).

Further benefits from the joint consideration of several traits in genetic evaluations essentially come from the following:

● Direct information on traits of interest (e.g. carcass and meat quality

measurements or sex-limited egg production traits) is sometimes not available on the selection candidates themselves. Consideration of these traits in breeding programmes may require the use of indirect indicators obtained from these selection candidates (e.g. conformation or breast angle) or of direct information available on related animals (e.g. slaughtered sibs).

- Whether direct information is available or not, the use of indirect information on other correlated traits often increases the accuracy of the genetic evaluation, depending on the genetic and environmental correlations.
- Whether this accuracy is improved or not, systematic biases in the evaluations can be reduced by accounting for the selection on correlated traits.

A typical situation is the meat-type poultry breeding scheme, where dam-line birds are sequentially measured, evaluated and culled on two negatively correlated traits: growth and reproduction. The latter is measured on best (selected for growth) birds only. Therefore, their expected value for growth is not equal to zero but varies depending on their ranking for growth. If expected values for reproduction are all considered to be equal to zero, as in univariate analyses, the evaluation will be biased.

Indeed, the knowledge of the change in average genetic merit on the selected trait in the population is necessary to specify the expected value of any correlated trait of interest. However, sometimes broilers are culled on the farm based on body weight or leg problems with no data recording. The magnitude of resulting bias will depend then on the culling intensity and (co)variances between traits (Pollak *et al.*, 1984).

In addition, multivariate animal models are able to cope with $G \times E$ interactions by considering performance data measured in different environments as different traits. Data measured on crossbred animals might be incorporated in the genetic evaluation in a similar way (Ducrocq, 1994; Wei and van der Werf, 1995).

Despite the increasing power of modern computers, a BLUP evaluation based on a multiple-trait animal model may become an onerous task when the population size is large and the number of traits is high (sometimes more than 20 for layers). To reduce computational costs, some authors suggest the use of equivalent models either to reduce the size of the coefficient matrices of the mixed model equations or to transform the variance–covariance matrices in order to manipulate them more easily. For example, since the parents represent fewer than 10% of the total number of animals in poultry, the reduced animal model (RAM) of Quaas and Pollak (1980) can be used (Besbes *et al.*, 1992). In this model, the equations of the non-parents are absorbed into those of the parents, reducing the number of equations to the number of parents. Linear transformation of the original data was also considered, using, for example, the canonical transformation (Thompson, 1977). The new 'canonical' variables are genetically and phenotypically independent. This transformation is not directly applicable in poultry, due to the presence of missing data. Ducrocq and Besbes (1993) circumvented this problem by recurrently replacing the missing data with their estimates. Their approach was successfully applied to layers.

BLUP vs. family index or phenotype-based selection

Because of the features of their breeding programmes, poultry breeders and many poultry scientists are reluctant to stop using index selection theory. Adoption of mixed model methods by poultry geneticists requires the demonstration of clear and quantifiable benefits in order to justify the (apparent) cost and complexity of the implementation.

Many simulation studies have compared selection responses based on BLUP with those based on either phenotype or family index. For example, in two simulation studies of a pig population, phenotypic selection led to response rates of 64 and 91% of those for BLUP for heritabilities of 0.1 and 0.6, respectively (Belonsky and Kennedy,

1988; Sørensen, 1988), whereas index selection responses ranged from 75 to 85% and from 81 to 96% of those for BLUP when the heritabilities were 0.1 and 0.5, respectively. These results indicate that the relative advantage of BLUP over phenotypic or family index selection will depend on both the heritability of the trait of interest and the structure of the data.

The stochastic simulation of various two-stage two-trait culling strategies by Mehrabani-Yeganeh *et al.* (1999) showed that culling based on phenotypic evaluation and not carrying information to the second stage reduced rates of response by 9–17% and led to inbreeding levels higher than or close to that of BLUP selection. They concluded that the failure to capture all first-stage data for later use in genetic evaluation was the primary cause of lower response with first-stage culling on phenotype. Similar results were obtained by Morris and Pollott (1997) using records from a commercial broiler breeding programme where selection was on juvenile body weight (JW), percentage breast meat yield (BM) and hen-day rate of egg production (EP). Estimated genetic response obtained with phenotypic selection for JW was 77.7% of the one obtained with BLUP, and estimated genetic responses with selection index were 90.7, 66.9 and 88.4% relative to BLUP for JW, EP and BM, respectively.

The superiority of BLUP was lower in a study by Jeyaruban *et al.* (1995), who simulated a balanced breeding structure typical of layer poultry breeding operations. The relative selection response with the selection indices compared with the BLUP estimates were from 94.5 to 99.4% of BLUP. Increases in inbreeding rates were considerably higher in the BLUP selected populations, but neither selection response nor inbreeding gave a powerful argument for or against use of BLUP. It should be recalled, however, that some advantages of BLUP are generally not modelled in the simulations, such as accounting for evolution of the variances through generations of selection, and the ability to compare environmental and genetic effects in time and space.

Recording of phenotypic data is still the major driving force for genetic progress for egg production traits. To minimize recording errors, considerable efforts and resources are allocated by breeders. It is then 'common sense' to make similar effort to improve the accuracy of breeders' evaluations. Decisions on implementation of BLUP may well have non-measurable benefits by forcing more rigorous analysis, automatically generating estimation of genetic trend and inbreeding, and allowing easy monitoring of programme performance.

Animal models used in poultry breeding

The 'animal' model is a general way of modelling the polygenic additive component in the context of BLUP of breeding values. Simianer (1994) reported that the concept was implicitly used by Henderson (1973) as 'evaluation using all records', but the term 'animal model' was introduced by Quaas and Pollak (1980), who defined it as a model in which 'the equation for a record contains a term for the breeding value of the animal making the record'.

The success of the animal model in practical applications can be attributed to three factors: (i) the development of efficient algorithms to set up directly the inverse of the numerator relationship matrix (Henderson, 1976; Quaas, 1976); (ii) the huge and continuous increase in computing power since the early 1980s; and (iii) the development of efficient solving strategies for very large linear equation systems (Schaeffer and Kennedy, 1986). However, this is sometimes done at the expense of a reduction of the number of parameters in the model by 'deliberately' omitting some effects (Ollivier, 1999). Ignoring some random effects in genetic evaluation may or may not yield biased predictions, often with increased prediction error variance, and therefore lower responses to selection (Henderson, 1975). In the multivariate case, using an incorrect model to estimate (co)variance components in selected populations results in incorrect parameters, which do not allow proper accounting for selection bias (Ducrocq, 1994).

Most of the poultry results published in the early 1990s were based on a multiple-trait model including only the additive genetic effect (Besbes *et al.*, 1992; Wei and van der Werf, 1993; Wang *et al.*, 1994). Misztal and Besbes (2000) clearly showed that this model is not appropriate for analysing traits related to egg production. Other effects should be included to account for the additional sources of full-sib (or daughter–dam) covariance. The relevance of this point becomes more evident if one remembers the frequent differences between sire and dam components in variance analyses in nested designs, the dam component being sometimes substantially higher than the sire component.

FULL-SIB EFFECT. The inclusion of a full-sib effect in the animal model accounts for the full-sib covariances caused by factors other than additive genetic effects. The corresponding variance component is usually small but its exclusion from the model has a detrimental impact on inferences about heritability and response to selection.

In poultry, where the mating structure is mainly hierarchical, the full-sib variance can be partitioned into dominance and non-dominance variances, where the latter may also include some maternal, common environmental and epistatic variances (Falconer, 1981). Brade and Groeneveld (1996) claimed that low estimates of variance for the full-sib effect indicated low dominance variance in the population.

DOMINANCE EFFECT. In the past, estimation of dominance variance for real data has been restricted to small populations for which the dominance relationship matrix could be inverted. However, for a similar accuracy, the estimation of the dominance variance requires much more data than the estimation of the additive variance (Chang, 1988).

Smith and Mäki-Tanila (1990) were the first to propose an algorithm to build up the inverse of the complete variance–covariance matrix for models allowing dominance and inbreeding. They were followed by Höschele and VanRaden (1991), who developed rules for directly creating the inverse of the dominance relationship matrix. Their methodology for estimating the variance components uses sire and sire–dam models only (VanRaden *et al.*, 1992). Misztal (1997) extended it to a full animal model using either REML or method R (Reverter *et al.*, 1994). The latter is an algorithm for estimating variance components based on the linear regression of genetic predictions obtained with full data on those based on partial data.

Wei and van der Werf (1993) used an animal model to estimate dominance variance for egg production traits in three White Leghorn purebred lines. The estimates of the dominance variance to total variance were somewhat higher for egg numbers than for egg weight and specific gravity. More recently, Besbes and Gibson (1999) estimated variances of five traits in two lines of brown egg layers using tilde-hat (an approximation method to REML estimation) and method R methodologies. They obtained similar results. In fact, estimates of d^2, which is the ratio of dominance variance to phenotypic variance (σ_d^2 / σ_t^2), are not direct measurements of the degree of dominance gene action, since the dominance variance also depends on the allele frequencies and the number of loci affecting the trait. Simulation results suggest that higher ratios are probably due to loci with large partial dominance or over-dominance (Uimari and Gibson, 1998).

Hill (1999) postulated that published estimates of dominance variance may include full-sib common environmental effects and thus are inflated. Mizstal and Besbes (2000) re-estimated the variance components for five traits (Besbes and Gibson, 1999) by REML using several models. The full model included hatch group effect, additive genetic, full-sib, parental dominance and inbreeding depression. In the absence of the full-sib common environmental effect in the model of analysis, its variance mostly inflates the parental dominance effect, and vice versa. With the full model, the estimates of full-sib variance were generally higher for egg production traits and lower for egg characteristics than those of the parental dominance variance. This model probably partially failed in separating these two

components. Joint estimation of parental dominance and full-sib common environmental effects is possible only if the full-sib group sizes are large, i.e. exceeding 100 individuals (Rye and Mao, 1998).

As full-sib hens were randomly distributed across cages and the traits are expressed late in life, maternal and common environmental effects can be ignored for egg production traits (Fairfull and Gowe, 1986; Wei and van der Werf, 1993). Therefore, the full-sib variation in the complete model could be due to epistasis. Indeed, epistasis has been found to have a significant effect on egg production in line-crossing experiments (Sheridan and Randall, 1977; Fairfull and Gowe, 1986). The underlying genetic mechanism determining heterosis seems to vary among traits; it is mainly due to epistasis for egg production and to dominance for egg characteristics (Misztal and Besbes, 2000). These results agree to some extent with those of Fairfull *et al.* (1987) who reported, in White Leghorn, that heterosis for sexual maturity and egg weight is determined mainly by dominance, with a small but significant epistatic component, while heterosis for egg production has a major epistatic component.

MATERNAL EFFECT. A great amount of work has been carried out on the estimation of maternal effects among domestic livestock, in particular for mammals (Willham, 1963, 1980; Robinson, 1994, 1996). In poultry, where maternal effects on juvenile broiler body weight, viability or disease resistance are important (Fairfull and Gowe, 1986; Chambers, 1990; Fairfull, 1990), few attempts have been made to partition this maternal variance into genetic and environmental components.

Koerhuis (1994) and Koerhuis and Thompson (1997) performed REML estimations for juvenile body weight of broilers under an animal model for two large broiler data sets. Different animal models were fitted, ranging from a simple model with animals as the only random effect to the most comprehensive model allowing for both genetic and environmental maternal effects and genetic covariance between direct and maternal effects. The latter model resulted in the best fit, but gave a highly negative direct maternal genetic correlation ρ_{AM}. Negative estimates of the correlation between direct and maternal genetic effects are quite common, but often without simple or convincing explanations. In the case of broilers, parental flocks of different ages and farms have offspring in a given hatch week. A more detailed fixed-effects model, accounting for the age difference, reduced estimates of ρ_{AM} but, as mentioned by Hill (1999), 'this is not necessarily the correct or whole explanation either, but it does point to the need to consider alternative models'.

The choice of the fixed-effects part in the model appears to be paramount for detailed maternal-effect models. Differences in the results of studies with the same data set illustrate the sensitivity of maternal effects to different fixed-effects models. Such a conclusion has already been drawn in dairy cattle by Robinson (1994), who showed that additional variation (e.g. sire × year or dam–offspring covariances) unaccounted for in the model affected estimates of maternal effect and of the correlation between direct and maternal effects.

The main difficulties associated with the use of increasingly complex models are lack of robustness and computational problems. More complex models often involve more dispersion parameters, which are usually less accurately estimated for a given amount of data. For instance, standard errors of heritabilities can be three to five times larger with a maternal-effect model as compared with a model involving only direct effects (Thompson, 1976).

Estimation of Selection Response

Inferences about response to selection can be based on least-squares or via mixed model methods. Use of least-squares estimators requires the use of control lines in order to disentangle genetic and non-genetic changes with time. Assuming no interaction between non-genetic effects and lines, no antagonistic natural selection

peculiar to the control and discrete generations, deviations between selected and control lines reflect genetic changes. In practice, most commercial breeders have abandoned the use of control lines and maintain limited, but active, test programmes to measure the effectiveness of their programme, compare their stocks with those of their competitors and search for new crosses.

Methods based on animal models include two approaches. The first is a two-stage procedure: in the first stage, variances are estimated using the data at hand; in the second stage, the computed variances are used instead of the true parameters to solve the mixed model equations. In this approach, inferences about selection response ignore the uncertainty associated with estimated variances. Using analytical derivation, Ollivier (1999) showed how dependent the animal model estimators are upon the heritability assumed in the model. Low sensitivity is observed either for traits with high heritability or for very large family sizes. The sensitivity of a design may be evaluated a posteriori, by estimating responses with increasing values of the prior h^2. In most cases responses have been shown to increase markedly when h^2 increases (Blair and Pollak, 1984; Perez-Enciso and Toro, 1992). A posteriori evaluations of responses with varying values of prior h^2 should be tested in the case of field data.

Selection induces linkage disequilibrium tending to reduce the additive genetic variance (Bulmer, 1971). In selected lines of limited size, an additional factor reducing the response is the decrease of genetic variance due to genetic drift. The animal model takes into account the two phenomena of variance reduction due to drift and the Bulmer effect (Sørensen and Kennedy, 1983, 1984). This model, when applied to long-term selection experiments, yields unbiased estimates of selection responses over successive generations when BLUP is used (Thompson, 1979). However, different studies (Blair and Pollak, 1984; Thompson, 1986; Sørensen and Johansson, 1992) have shown that the estimates of selection response obtained by this approach are dependent on the prior values of the genetic parameters.

The second approach makes use of the Bayesian philosophy: all the parameters of the model (environmental effects, additive genetic values and variance components) are estimated simultaneously. Inferences about response to selection are based on the marginal posterior distribution of response (Sørensen *et al.*, 1994) and therefore account for the estimation of all other parameters in the model. This marginalization usually requires the computation of highly multi-dimensional integrals, which is now possible with the use of techniques such as the Gibbs sampler.

The animal model provides trends in breeding value predictions based on a specific genetic model. The consequences of using a wrong genetic model for evaluating responses over several generations are expected to be different from the consequences on the breeding value predictions and selection efficiency. In breeding value predictions, precision is more important than bias, as pointed out by Johansson *et al.* (1994). When responses are evaluated, the errors may be cumulative over generations, and may create a sizeable bias (Ollivier, 1999).

Short-term vs Long-term Response

An overall breeding objective usually tries to balance genetic change and risk. The primary components of risk are variance of response and inbreeding. Increased genetic gain in the short term is usually associated with increased inbreeding, which leads to decreased genetic gain in the long term, due to declines in fitness and genetic variance.

Compared with mass selection, use of family information in BLUP (and to a lesser extent selection index) increases the correlation between predicted breeding values of relatives and therefore the probability of co-selection of close relatives, which in turn leads to a reduction in the effective population size and an increase in the variance of selection response and inbreeding

(Robertson, 1961). As short-term response depends mainly on the accuracy of selection, and long-term response depends on both accuracy of selection and effective population size, BLUP-based selection is expected to give larger short-term but smaller long-term responses than mass selection (Woolliams, 1989; Wray and Hill, 1989; Verrier *et al.*, 1993). Quinton *et al.* (1992) showed that, at equal levels of inbreeding, the increase in response induced by increased accuracy of BLUP–based selection can be offset in small populations.

Various studies have investigated selection methods to reduce inbreeding while maintaining high rates of genetic gain. All proposed methods are based, in one way or another, on the same principle: that is, to reduce the average relationship between selected individuals. This can be done by restricting the number of close relatives selected (Nicholas and Smith, 1983), by increasing the number of parents selected (Quinton and Smith, 1994), or by reducing the weight given to family information in the genetic evaluation (Grundy and Hill, 1993), i.e. using an estimate of h^2 higher than its actual value.

A more direct method is maximization of the selection objective combining breeding values and coancestries of individuals (Meuwissen, 1997; Grundy *et al.*, 1998; Sonesson and Meuwissen, 2000). Jeyaruban and Gibson (1994) investigated the effects of number of females mated to each male and of the population size on response and inbreeding when selection was on an index involving egg production, egg weight and deformation. They found that an intermediate population size of around 3000 females per generation and a male:female mating ratio in the range of 1:6 to 1:8 would provide an appropriate balance between response and inbreeding.

Mating schemes proposed to control inbreeding also include matings among the least related animals (Toro and Perez-Enciso, 1990), and mating individuals from families in which many are selected to others from families in which few are selected, known as compensatory mating (Caballero *et al.*, 1996). The mating systems reduce the cumulative effects of selection on drift over generations while the use of upward-biased h^2 mainly reduces the variance of family size.

Different Strategies for Different Traits

Commercial breeders of layers work with a complete array of traits. It includes, for example, egg numbers (sexual maturity and persistency of lay), feed efficiency, egg size, eggshell strength and colour, interior egg quality criteria such as proportion of yolk and albumen and their solid contents, fertility and hatchability, etc. Because of their international scope, breeding companies aim at providing birds that are able to cope with a wide variety of field conditions. This implies selection for liveability and adaptability too.

Egg production traits

As seen above, mixed model methods are based on the infinitesimal model and its inherent assumptions that genetic and environmental effect are additive and that experimental errors are independent, have equal variances and are normally distributed. The latter assumptions do not hold for egg production traits, which show markedly skewed distribution; hence genetic parameters estimates are biased if REML is used, which in turn reduces the efficiency of BLUP.

Cochran (1947) suggested either the omission of certain or all outlier observations or that these data should be transformed on a scale on which the above assumptions are better satisfied. Different transformation methods have been used for egg production data such as probit (Cochez and Péro, 1954), logarithmic (Brah *et al.*, 1982) or arcsin $\sqrt{\%}$ transformation (Boukila *et al.*, 1987).

Box and Cox (1964) suggested transformation of dependent variables through a family of power transformations that became known as the Box–Cox family. The

transformation depends on a single parameter t and has the following form:

$$y^{(t)} = g_t(x) = \begin{cases} \dfrac{x^t - 1}{t} \text{ if } t \neq 0 \\ \log(x) \text{ if } t = 0 \end{cases}$$

Their general idea was to restrict attention to transformations indexed by unknown parameters t and estimate t, and then estimate the other parameters of the model for a given t. Gianola *et al.* (1990), using the Bayesian approach, estimated t jointly with the parameters of interest in the case of mixed model situations.

Box–Cox transformation was successfully applied to egg numbers in laying hens by Ibe and Hill (1988) and Besbes *et al.* (1993), and in turkeys by Chapuis *et al.* (1996). These authors chose the optimal t value empirically in such a way that several criteria were simultaneously satisfied: minimum residual variance of the model describing the transformed records, linearity of the relationship between records of related animals (which can be assessed graphically or using the coefficient of determination R^2 of the regression of (groups of) half-sib records on (groups of) individuals), the Kolmogorov–Smirnov normality test (which should be minimum) and the symmetry assessed by the coefficient of skewness (which should be close to zero).

In general, the further from normality the distribution of the trait is before transformation, the larger (in absolute value) is the optimum value of t. For left (positively) skewed data, such as age at first egg, t values are negative (Ibe and Hill, 1988). For such a trait, a logarithmic transformation is sometimes suitable (Lehman, 1970, cited by Savas *et al.*, 1998). Ibe and Hill (1988) pointed out that t-transformed data lead to more normally distributed variables, but not necessarily to homogeneous error variances.

The Box–Cox transformation of egg production traits often results in an increase in their heritabilities without drastically modifying the genetic and residual correlations between transformed traits. The genetic values estimated from original and transformed data are highly correlated (> 0.9) but the percentage of hens selected with both evaluations may be low when the original distribution is far from the normal situation (Besbes *et al.*, 1993). Similar results were reported by Savas *et al.* (1998) who found that selection differentials for egg production are higher for laying hens ranked on breeding values estimated from transformed data.

Working on the new scale causes some problems to the geneticist who wants to express the estimated breeding values or the genetic trend in egg numbers. Also, it makes the calculation of new economical weights for these transformed traits more difficult when building a global selection index.

Categorical traits

Some egg quality traits, such as egg size, shell colour intensity or yolk and albumen solid content, are continuous. Others are binary (all or none) traits, such as presence or absence of dark blood spots in the yolk, or ordered categorical traits, such as shell texture or smoothness. Average score or frequencies are generally used to analyse these discrete traits using linear models.

Fertility and hatchability are binary traits in nature: the egg is either fertile or not and similarly the embryo either hatches or does not (Sewalem and Johansson, 2000). However, they are generally considered as traits of the parents, expressed in percentage of fertile or hatched eggs and analysed with linear models. This approach ignores the interaction between male and female gametes to produce a viable zygote and the potential of the latter to develop.

From a statistical point of view, the distribution of the frequencies are skewed. To better satisfy the normality criteria, some authors used Box–Cox (Beaumont, 1992) and arcsin transformations (Chaudhaury *et al.*, 1994). The arcsin transformation was also applied for percentage of dead chicks at hatch without a reduction of departure from normality (Sewalem and Wilhelmson, 1999).

Mixed linear models may be used to analyse these discrete traits using Snell scores, but they do not adequately handle the heterogeneity of incidence rate and of variance among fixed-effect subclasses (Gianola, 1982). To account for these, discrete traits should be analysed with generalized mixed linear models (McCullagh and Nelder, 1983).

Binary or ordered categorical traits are often analysed by means of the threshold-liability model, first used by Wright (1934) in studies of the number of digits in guinea pigs. In the threshold model, it is postulated that there exists a latent or underlying non-observable variable (called liability), whose value y_i for record i is described using a mixed linear model:

$$y_i = \mathbf{w}'_i\boldsymbol{\theta} = \mathbf{x}'_i\boldsymbol{\beta} + \mathbf{z}'_i\boldsymbol{a} + e_i$$

where $\boldsymbol{\theta} = (\boldsymbol{\beta}', \boldsymbol{a}')'$ and $\mathbf{w}'_i = (\mathbf{x}'_i, \mathbf{z}'_i)$ is the ith row of $\mathbf{W} = (\mathbf{X}, \mathbf{Z})$. The usual assumptions about polygenic inheritance are considered to hold for this liability variable. In particular, $\mathbf{a} \sim N(\mathbf{0}, \mathbf{A}\sigma_a^2)$ with $\mathbf{A}$ being the relationship matrix. The e_i's are independent normally distributed residuals with mean 0 and variance 1, hence defining, without loss of generality, a unit of measurement.

A response Y_i in a given category j ($j = 1,2,\ldots,J$) is observed if the actual value of liability y_i falls between the thresholds defining the appropriate category ($\tau_j \leq y_i < \tau_{j+1}$). The probability of this response is:

$$\Pr(Y_i = j | \boldsymbol{\theta}) = \Phi(\tau_{j+1} - \mathbf{w}'_i\boldsymbol{\theta}) - \Phi(\tau_j - \mathbf{w}'_j\boldsymbol{\theta})$$

where Φ is the normal cumulative distribution function.

Algorithms for estimating genetic parameters and for predicting genetic merit based on a univariate threshold model were described in the early 1980s by Gianola and Foulley (1983) and Harville and Mee (1984). Many methodological developments under a Bayesian framework allowed for generalization of the threshold concept to multiple dichotomous responses (Foulley *et al.*, 1987) and to joint consideration of discrete and continuous traits (Foulley *et al.*, 1983; Janss and Foulley, 1993; Jensen, 1994; Wang *et al.*, 1997).

Meijering and Gianola (1985) compared BLUP with threshold model-based selections for one generation of selection of progeny-tested sires. The threshold model resulted in significantly higher genetic responses only when the binary trait had a low incidence (< 25%) of the undesirable phenotype and when heritabilities in the underlying scale were moderate (0.20) or high (0.50).

Application of threshold models in laying hens is quite recent. For example, Johansson *et al.* (1998) estimated the heritabilities and relationship between shell crack (a threshold trait) and resistivity (a continuous trait) using an animal threshold model via Gibbs sampling, under a Bayesian setting. Similarly, the (co)variance components of direct and maternal genetic effects of fertility or hatchability (categorical traits) and the direct genetic effect of egg weight (continuous) have been estimated (Sewalem and Johansson, 2000). Genetic maternal effects are assessed with a sire, maternal grandsire and dam within a maternal grandsire model, which is not possible with a simple sire model. Furthermore, the possibility of estimating variance components for categorical traits based on an animal model is questionable even with Gibbs sampling.

Fertility and hatchability are at very high levels in most improved lines such that they can be viewed as approaching a limit. To maintain them at a high level, Gowe *et al.* (1993) recommended using a culling scheme, which has little effect on the selection pressure available for other traits. Knowing the heritabilities of these traits, a culling strategy based on underlying breeding value instead of the phenotypic value would, however, lead to higher genetic progress as a consequence of greater accuracy and more efficient selection.

From a statistical point of view, the threshold model can be considered as a generalized linear model with probit link function Φ^{-1} (Gianola and Foulley, 1983). However, it is not applicable when the response categories are not ordered, because the relationship (in particular the ranking) between the categories is unknown. This is

the case with, for example, leg disorders in broilers when they are expressed as different pathologies ('valgus' and 'varus' angulations) and scored as disjoint categories. A multivariate logit transformation (Cox, 1970) provides a relevant link function between continuous latent variables and expected occurrences (Le Bihan-Duval *et al.*, 1996).

Longevity versus mortality traits

In poultry, mortality rates are generally low. Under controlled conditions, average mortality is usually below 5% per year of egg production. This rate tends to increase. Selection for lower mortality has been practised directly or indirectly for many years, but it has not been very effective. Heritability estimates for mortality of pure-line hens in single cages are typically near zero, because the level of mortality is too low to express significant family differences (Flock, 1996).

Mortality (or its opposite, liveability) is a compound trait, where each cause of mortality is a different trait (usually with low incidence) and is highly dependent on environmental conditions. Causes of mortality on the breeding farm and under commercial conditions are different. Under the controlled conditions of breeding farms, mortality is mainly due to particular pathogens. Caging of birds in commercial multiple bird cages is stressful and can result in injuries through aggression, flightiness and cannibalism, leading to high mortality. A group selection (Graig and Muir, 1996; Muir, 1996) based on mortality and other indirect traits such as injury occurrence (analysed as either a binary or an ordered categorical trait) and feather cover score can be performed, provided that these traits are heritable and correlated to mortality.

In most cases mortality is analysed as a 0/1 trait, depending on whether the hen is still alive at the end of the test or not. With this definition no distinction is made between a hen dying just after housing and a hen dying just before the end of the test.

Therefore, longevity or number of days of life is a more precise measurement.

Longevity measures describe the width of the interval between an origin point and an end point. The origin corresponds to date of birth or date of housing, and the end point corresponds to death or removal. Longevity has often been considered as a continuous trait and analysed with a linear model. However, this measure has a special feature requiring special statistical tools: the end point (death) is not observed for > 95% of the hens, which are still alive at the end of the test. Records of removed live birds are considered as 'censored'. Survival analysis is the statistical art of combining 'censored' and 'uncensored' observations in a single analysis (Kalbfleisch and Prentice, 1980). It is becoming a standard technique for genetic analysis of length of productive life in dairy cattle, for example.

The main characteristic of survival analysis is that it uses all the information available, from dead animals as well as from animals still alive when the analysis is performed. The model describes at what rate animals are dying over time. Ducrocq *et al.* (2000) analysed survival in the rearing and production periods of eight generations of a pure line of laying hens. The overall mortality rates were very low: 2.2% in the rearing period and 5.9% in the production period. Heritabilities were equal to 0.48 and 0.19, respectively, and non-additive genetic effects could not be estimated properly because of the structure of the data and the low incidence of mortality.

Crossbred traits

Crossbreeding is a standard practice in poultry breeding programmes. However, debate continues among geneticists on the most effective way of maximizing the genetic response in crossbred animals (Bell, 1982; Wei and van der Steen, 1991). A selection strategy based on purebred and crossbred data is referred to as combined crossbred purebred selection (CCPS), whereas selections based only on

information from the pure line or the cross are referred to as pure line selection (PLS) and crossbred selection (CS), respectively. In the absence of direct experimental comparison, estimates of genetic parameters related to the crossbred and purebred populations might give some indication as to which method of selection will be most effective in maximizing the genetic response in the cross. The genetic parameters of interest are the heritabilities in both populations (h_p^2, h_c^2) and the correlation between crossbred and purebred performances (r_{pc}) considered as two different traits.

Because purebred hens are often caged singly in well-controlled environments, whereas crossbred hens are kept in multiple-bird cages under commercial conditions, the estimates of r_{pc} are confounded with a possible $G \times E$ interaction. Considering crossbred and purebred performances as two traits with a genetic correlation between them does not require distinction between these effects as long as crossbred performance is measured in the same environment for which the breeding goal has been defined (Wei and van der Werf, 1994, 1995).

Wei *et al.* (1991) considered r_{pc} to be the most reliable indicator of the relative emphasis to be given to purebred vs. crossbred information when selecting for crossbred performance. This parameter depends on the degree of dominance and the difference in allele frequencies in the parental population; its value decreases with increasing dominance effect and/or the allele frequency difference.

Besbes and Gibson (1999) reported high correlation between purebred and crossbred performance (> 0.80 and even > 0.90) for egg numbers, egg weight and shell strength, with different patterns between the sire and dam lines. For the sire line, r_{pc} was fairly well estimated because of the large number of progeny per sire; but estimates were much less accurate for the dam line, being based on dam's sibs' crossbred progeny only. In their study, crossbred animals were kept in individual bird cages.

Wei and van der Werf (1995) analysed egg production records of purebred and crossbred laying hens. These birds were produced by a crossing scheme in which two sire lines were crossed to a dam line, each sire producing on average 45 purebred and 105 crossbred daughters. Purebred data were recorded individually whereas crossbred data were cage means recorded for three to five half-sib hens per cage. The multivariate model containing purebred and crossbred performance was as follows:

$$
\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{X}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_2 \end{bmatrix} \begin{bmatrix} \boldsymbol{\beta}_1 \\ \boldsymbol{\beta}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_2 \end{bmatrix} \begin{bmatrix} \mathbf{a}_1 \\ \mathbf{a}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix}
$$

where $\mathbf{y}_1$ and $\mathbf{y}_2$ are vectors of purebred and crossbred observations, respectively; $\boldsymbol{\beta}_1$, $\mathbf{a}_1$, $\mathbf{e}_1$ and $\mathbf{e}_2$ are unknown vectors of fixed effects, additive sire effects and residuals in purebreds and crossbreds, respectively; $\mathbf{X}_1$ and $\mathbf{Z}_1$ are known incidence matrices relating observations to fixed effects and sire effects. The variances of additive sire and residual effects are:

$$
\text{var}\begin{bmatrix} \mathbf{a}_1 \\ \mathbf{a}_2 \end{bmatrix} = \mathbf{G}_0 \otimes \mathbf{A}; \quad \text{var}\begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{I}\sigma_{e1}^2 & 0 \\ 0 & \mathbf{D}\sigma_{e2}^2 \end{bmatrix}
$$

where $\mathbf{A}$ is the relationship matrix, $\mathbf{I}$ is an identity matrix, $\mathbf{G}_0$ is a sire genetic (co)variance and σ_{e1}^2 and σ_{e2}^2 are the residual variances for records in purebreds and crossbreds. $\mathbf{D}$ is a diagonal matrix with diagonals equal to $1/n_i$, where n_i refers to the number of hens per cage.

Wei and van der Werf (1995) reported estimates of 0.04 to 0.51 for heritabilities of crossbred traits recorded among sib groups and equal to or less than 0.73 for the genetic correlations. Crossbred heritabilities were generally lower than purebred ones for all traits and much lower for egg production traits. These authors attributed this low heritability in crossbreds to: (i) the stressful condition under which the traits were measured, as illustrated by the large environmental variation in the crossbreds compared with the purebreds; and (ii) the bias induced by considering a fraction of full-sibs within a cage as half-sibs. This bias could probably be avoided if an animal model were used. The above results can also be explained by a loss of information induced by working with cage mean.

Analysis of egg production data from multiple bird cages presents three difficulties that should be addressed.

- The records should be adjusted for mortality occurring within the cage and affecting the cage performance.
- The heterogeneity of residual variances of cage performances due to different cage group sizes should be accounted for.
- The distinction between cages of full-sibs and cages of half-sibs is recommended. In fact, full-sib groups are better because they allow the use of a sire–dam model for the analyses.

The first difficulty can be solved by considering an 'equivalent' cage group size, e.g. number of hens per cage weighted by total number of days present. The solution for the other difficulties is more complex and needs specific developments.

Conclusion

Layer geneticists were late in adopting mixed model methods for the analysis of data. The features of layer breeding programmes on the one hand, and the exaggeration of the cost and complexity of the implementation of these methods on the other hand, may explain this delay. Today, these methods have become the methods of reference in poultry breeding and are used by most major breeding companies. Two main reasons explain such a move: the continuous increase in computing power; and the development of general, user-friendly and easily accessible software.

It took more than one decade to see mixed model methods applied widely for the analysis of classical continuous egg production, egg quality and feed efficiency traits. Hopefully, it will not take such a long-time to analyse, 'properly', important discrete traits such as shell texture, fertility and hatchability, or censored traits such as longevity. Currently, these traits are evaluated using univariate analyses and are selected for using independent culling levels. Further studies are needed to build up an aggregate index that includes all traits.

Another aspect briefly considered here concerns the study of multiple-bird cage performances. Whether they are measured on crossbred or purebred hens, these data are very useful because they are measured under commercial conditions. However, some statistical problems remain to be solved before they can be used efficiently in breeding programmes by layer geneticists.

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10 Application of Mixed Model Methodology in Breeding Strategies for Meat-type Birds

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Introduction

Methods aimed at the genetic improvement of farm animals, including poultry, were employed many centuries before the Mendelian laws of inheritance were discovered and before Sir Roland Fisher (1918) devised his model describing the effects of genes on quantitative traits. It can thus be assumed that the genetic evolution of animals started along with the process of their domestication.

Thanks to the pioneering works of Fisher and S. Wright, selection methods based on population genetics and quantitative genetics theory were first employed in the 1930s and further developed by J.L. Lush in the 1950s. During the same period, Lerner (1950) established the foundations of modern poultry selection based on population genetics. According to Bell (1982), the practical application of quantitative and genetic variance with regard to polygenic traits is confined to the additive effect of genes. The last 30 years (i.e. since 1970) have seen the wide-ranging generalization of selection theory. The use of efficient and rapid selection methods is expensive, especially for large farm animals. On the other hand, economic conditions are changing so quickly that it is difficult to determine the profitability of a given genetic improvement method. It can

be assumed that current knowledge of population genetics is based on the achievements of science and practice over the last 60 years of the 20th century.

In many countries, conventional breeding strategies for meat-type poultry are based on a pyramidal structure involving pedigree, reproductive (grandparental and parental) and commercial stocks (Wężyk, 1978). Selection based on breeding value has been used in pedigree stocks where the birds are kept in closed populations as strains or lines. The breeding goal is achieved in commercial stocks, which are produced from crosses of strains at grandparental or parental levels.

With regard to the genetic improvement of poultry the main concern is the performance of individuals under selection in the following year or their progeny in the next generation. The attention of breeders of egg-laying and meat-type hens and ducks is focused on progeny performance. Only in the case of geese and turkeys, where the generation interval is more than 1 year, is the breeder also interested in statistical models used to estimate predicted progeny performance. Birds in a flock can be arranged according to their breeding value from the highest to the lowest, taking into account both the individual breeding value and the values of full and half-sibs. In the simplest

case, the genetic progress can be expressed using the Dempfle (1989) formula as:

$$\Delta G = i \mathrm{R_{HI}} \sigma_H$$

where i means the intensity of selection, which depends largely on the number of individuals selected from the flock under evaluation; σ_H reflects genetic variance, which is most often beyond the breeder's control; and $\mathrm{R_{HI}}$ reflects the correlation between actual and predicted breeding value. The correlation coefficient value will depend on the statistical procedure used.

Pedigree breeders of poultry usually have at their disposal pedigrees reaching back several generations and more than one result of bird performance (at least five traits), which distort the information available about various individuals. Moreover, birds (males and females) reared and kept in the same environmental conditions originate from different hatches and the appropriate statistical procedures should be employed to balance the results obtained.

As has already been mentioned, the genetic improvement of pedigree poultry stocks is usually carried out within closed populations, while the breeding aim is determined according to the places (positions) they are assigned in the crossbreeding scheme as male and female strains and as paternal and maternal strains. Identical housing conditions for birds restrict the differentiating effect of environmental conditions that largely complicate the evaluation of breeding value for other species of livestock, such as cattle. In addition, as has already been mentioned, poultry are characterized by a relatively short generation interval (less than 1 year), making the breeding effects noticeable in a relatively short period of time (Jiang *et al.*, 1999). A number of desirable poultry characteristics are closely related to their biology, contributing substantially to the intensification of breeding and production. They include an enormous reproductive ability, efficient artificial hatching techniques, high fertility rates of properly stored hatching eggs, the possibility of stimulating sexual maturity, the identification of sex at hatching, sensitivity to light stimulation during the laying period, and a high adaptability to changes in feeding and housing conditions (Hartmann, 1992). Moreover, non-overlapping generations and the straightforward measurement of individual performance traits facilitate the prediction of breeding value. The large size of groups of full and especially half-sibs should also be stressed.

Unlike other species of animal (notably those used in artificial insemination), poultry are characterized by smaller differences between the genetic effect of both parents on their progeny despite the use of polygamous mating systems (one cock to ten hens; one gander or drake to five geese or ducks; and one stag or tom to 10–40 artificially inseminated female turkeys).

Modern meat-type poultry breeding programmes generally have a sequential structure in which selection for reproductive traits is preceded by selection for growth traits. Multiple-trait genetic evaluation makes it possible to overcome selection bias and prevents the depression of genetic progress in the population (Pollak *et al.*, 1984; Koerhuis and van der Werf, 1994). In genetic evaluation, estimating the variance matrices requires the use of ANOVA-type procedures, which may yield heavily biased results (Rothshild *et al.*, 1979; Meyer and Thompson, 1984). According to Patterson and Thomson (1971), when an analysis includes all the necessary data for making selection decisions, the restricted maximum likelihood (REML) method applied in the individual animal model (IAM) can alleviate an undesirable selection bias. This fact may complicate computational procedures but rapid advances in computer technique and mathematical procedures for the algorithms used have made the IAM–REML method very useful in solving some of the above problems, involving large numbers of birds (Koerhuis and McKay, 1996).

Statistical Procedures for Estimating Parameters of Meat-type Poultry Breeding Value

In estimating genetic parameters of juvenile body weight (JBW) in meat-type hens

and the reproductive traits, Koerhuis and McKay (1996) used the IAM–REML method according to the following linear model:

$$Y_{ijklm} = \mu + SEX_i + HW_j + AGE_k + a_l + p_m + e_{ijklm} \tag{1}$$

where Y_{ijklm} is the observed phenotypic value of the animal; μ is the mean for the population; SEX_i is the fixed effect of sex; HW_j is the fixed effect of the jth hatch week ($j = 1,2, \ldots, 304$); AGE_k is the fixed effect of the kth class of age of dam ($k = 1,2, \ldots, 7$); a_l is the random additive genetic effect of the lth animal; p_m is the random environmental effect of the mth dam; and e_{ijklm} is the random error.

With the exception of the sex effect, the model applied to the reproductive traits was the same, while the number of hatch weeks was smaller ($j_{MAX} = 284$).

The following analysis procedures can be used for further calculations. Equation (1) takes a general matrix notation (2), with body weight and egg production being the traits:

$$\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{X}_1 & 0 \\ 0 & \mathbf{X}_2 \end{bmatrix} \begin{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{bmatrix} +$$

$$\begin{bmatrix} \mathbf{Z}_{a1} & 0 & \mathbf{Z}_{p1} & 0 \\ 0 & \mathbf{Z}_{a2} & 0 & \end{bmatrix} \begin{bmatrix} \mathbf{a}_1 \\ \mathbf{a}_2 \\ \mathbf{p}_1 \\ \mathbf{p}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix} \tag{2}$$

where for trait i ($i = 1,2$), $\mathbf{y}_i$ is a vector of observations, $\mathbf{b}_i$ is a vector of fixed effects, $\mathbf{a}_i$

is a vector with random additive genetic effects, $\mathbf{p}_i$ is a vector with random maternal environmental effects; $\mathbf{e}_i$ is a vector with random errors, and $\mathbf{X}_i$, $\mathbf{Z}_{ai}$, $\mathbf{Z}_{pi}$ are incidence matrices for respective fixed and random effects.

The assumed variance structure was (see equations at bottom of page) where σ^2_{ai}, σ^2_{pi}, σ^2_{ei} are the additive genetic variance, the maternal environmental variance and the error variance for trait i; σ^2_{a12}, σ^2_{p12}, σ^2_{e12} are the corresponding covariances between the traits 1 and 2; $\mathbf{A}$ is the relationship matrix; $\mathbf{I}$ is an identity matrix; $\mathbf{B}$ is an incidence relating progeny records of body weight and egg production to the dam; $\mathbf{C}$ is an incidence matrix relating body weights to egg production records; and $\otimes$ denotes tensor product.

REML estimation of these variances and covariances yields a nine-dimensional maximization problem. To make the computation process simpler, Thompson $et\ al.$ (1995) proposed scaling and transformation models. The two correlated error effects in matrix $\mathbf{R}$ were reparameterized into two independent effects (σ^{*2}_{e1} and σ^{*2}_{e2}) by introducing an additional effect (σ^2_b) common to both traits:

$$\sigma^2_{e1} = \sigma^{*2}_{e1} + a^2\,\sigma^2_b$$

$$\sigma^2_{e12} = ab\,\sigma^2_b$$

$$\sigma^2_{e2} = \sigma^2_{e2} + \sigma^2_b$$

$$\mathbf{V}\begin{bmatrix} \mathbf{u} \\ \mathbf{e} \end{bmatrix} \equiv \mathbf{V} \begin{bmatrix} \mathbf{a}_1 \\ \mathbf{a}_2 \\ \mathbf{p}_1 \\ \mathbf{p}_2 \\ - \\ \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{A}\sigma^2_{a1} & \mathbf{A}\sigma_{a12} & 0 & 0 & | & 0 & 0 \\ \mathbf{A}\sigma^2_{a12} & \mathbf{A}\sigma^2_{a2} & 0 & 0 & | & 0 & 0 \\ 0 & 0 & \mathbf{I}_1\sigma^2_{p1} & \mathbf{B}\sigma^2_{p1} & | & 0 & 0 \\ 0 & 0 & \mathbf{B}_a\sigma_{p12} & \mathbf{I}_2\sigma_{p12} & | & 0 & 0 \\ - & - & - & - & | & - & - \\ 0 & 0 & 0 & 0 & | & \mathbf{I}_1\sigma^2_{a1} & \mathbf{C}\sigma_{12} \\ 0 & 0 & 0 & 0 & | & \mathbf{C}\sigma_{a12} & \mathbf{I}_2\sigma^2_{e2} \end{bmatrix} \equiv$$

$$\begin{bmatrix} \mathbf{A} \otimes \mathbf{G} & 0 & |0 \\ 0 & \mathbf{P} & |0 \\ - & - & - \\ 0 & \mathbf{0} & |\mathbf{R} \end{bmatrix} \equiv \begin{bmatrix} \mathbf{T} & 0 \\ 0 & \mathbf{R} \end{bmatrix}$$

$$\mathbf{V}\left(\mathbf{y} = \begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{bmatrix} \right) = \begin{bmatrix} \mathbf{Z}_{a1} & 0 & \mathbf{Z}_{p1} & 0 \\ 0 & \mathbf{Z}_{a2} & 0 & \mathbf{Z}_{p2} \end{bmatrix} \mathbf{T} \begin{bmatrix} \mathbf{Z}_{a1} & 0 & \mathbf{Z}_{p1} & 0 \\ 0 & \mathbf{Z}_{a1} & 0 & \mathbf{Z}_{p2} \end{bmatrix}' + \mathbf{R} \equiv \mathbf{ZTZ}' + \mathbf{R}$$

where $a(= \sigma^{*2}_{e1})$ and $b(= \sigma^{*2}_{e2})$ are scaling factors. Univariate algorithm is then used to evaluate the likelihood (Meyer, 1989).

After scaling $\mathbf{G}$ and $\mathbf{P}$ into $\mathbf{G}_s$ and $\mathbf{P}_s$ with $\mathbf{G}_s = s\mathbf{G}s$ and $\mathbf{P}_s = s\mathbf{P}s$ where

$$s = \begin{bmatrix} 1/\sigma^{*2}_{e1} & 0 \\ 0 & 1/\sigma^{*}_{e2} \end{bmatrix}$$

Koerhuis and McKay (1996) obtained estimations σ^{*2}_{e1} and σ^{*2}_{e2} from partial derivatives of the log-likelihood (log L), and using $\mathbf{G}_s$, $\mathbf{P}_s$ and σ^2_b they reduced the problem from nine to seven dimensions (for details see Thompson *et al.*, 1995). The log L values were found directly as partial derivatives (Meyer, 1989).

The coefficient matrix for equations in the mixed model matrix (MMM) and the right side vector were rearranged using a multiple minimum degree reordering (George and Liu, 1980), before Gaussian elimination was performed on MMM. The maximum log L was determined by the SIMPLEX method (Downhill Simplex). A threshold factor was used for initial runs (Thompson *et al.*, 1995) with 10^{-3} being the operational zero for off-diagonal elements in MMM, and convergence was assumed when the variance of log L values was less than 10^{-2}. In this way, the multidimensional likelihood surface was roughly 'scanned', with time savings of over 60% for computations.

The parameters obtained from the maximum of the approximate run were used in the final run, where the operational zero was set to 10^{-8}, and a convergence criterion of 10^{-5} was assumed.

This two-step procedure is robust and very time efficient, particularly when the initial choice of priors was poor. Koerhuis and McKay (1996) based the estimation of heritability errors and environmental and genetic variances on Taylor's series expansion about the maximum log L (Smith and Graser, 1986). Sampling errors of genetic and environmental correlations were approximated according to Robertson (1959). The bivariate analysis of JBW and the individual reproductive traits in terms of hen housed production, daily rate of lay, sexual maturity and egg weight resulted in estimates of variance components and

parameters for JBW, which were presented as an arithmetic average.

When presenting the evaluation based on the multivariate model, Koerhuis and McKay (1996), to illustrate equation (2) and variance–covariance structure, assumed equal incidence matrices (i.e. $\mathbf{X}_1 = \mathbf{X}_2$, $\mathbf{Z}_{a1} = \mathbf{Z}_{a2}$, $\mathbf{Z}_{p1} = \mathbf{Z}_{p2}$) for traits y_1 and y_2. In order to reduce computations only to estimation of variance components, Thompson and Hill (1990) suggested a sequence of univariate analysis based on the transformation:

$$\mathbf{z} = \mathbf{H}_0 \mathbf{y} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 1 & 1 \end{bmatrix} \begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} y_1 \\ y_2 \\ y_1 + y_2 \end{bmatrix}$$

to determine $\mathbf{G}$ and $\mathbf{P}$ (see above) and the covariance matrix for error $\mathbf{E}$. Covariance terms in matrices $\mathbf{G}$, $\mathbf{P}$ and $\mathbf{E}$ were estimated using the formula:

$$\text{cov}\,(y_1, y_2) = 0{,}5\,[\text{var}\,(y_1 + y_2) - \text{var}\,(y_1) - \text{var}\,(y_2)]$$

The authors also used canonical transformation $\mathbf{T}$ reducing $\mathbf{G}$, $\mathbf{P}$ and $\mathbf{E}$ to diagonal and identity form.

Back transformation of the canonical variances to the original scale was repeated until $\mathbf{T}$ gave approximately uncorrelated canonical variates. This algorithm was applied to the reproductive traits (i.e. hen housed production, rate of lay, sexual maturity and egg weight) simultaneously in a quadrivariate analysis that involved ten separate univariate analyses. A convergence criterion of 10^{-6} was assumed on the canonical and original scale, locating the maximum log l as described above.

The iteration procedure was ceased when the absolute values of correlations on the canonical scale were less than 10^{-4}, or after 15 iteration rounds. Test runs showed that differences in variance and covariance estimates were minimal when the iteration procedure went beyond this point. Koerhuis and McKay (1996) also performed univariate variance estimation for JBW and the reproductive traits to compare the bivariate and multivariate estimates. They obtained the maximum log L in the same manner, assuming convergence at 10^{-8}.

In addition to the above advantages, the estimation of poultry breeding value involves some difficulties such as the occurrence of additional relationships (other than parent–progeny) and the non-additive effects. Neither of these elements can be identified in the classical sire–dam model and for this reason they can be a source of biased estimates (Wei and van der Werf, 1993).

Over and above genetic variability, selection intensity and generation interval, breeding progress is determined by the accuracy of evaluation of the genetic or breeding value of individuals (Rendel and Robertson, 1950; Falconer, 1981; Wężyk and Szewczyk, 1993). This calls for the greater use of as many sources of information as possible, mainly in order to increase the accuracy of estimating breeding value (additive effect) for individual birds in a flock. Genetic variability is conditioned by the genetic structure of a population and is practically independent of the breeder. Selection intensity and generation interval are determined by the reproductive ability of individuals. In this case, too, the direct impact of the breeder is limited. The only stimulator of genetic progress within the breeder's control is the accuracy of the evaluation of breeding value, corresponding to the sum of all average gene effects (Falconer, 1981).

It is assumed that measurable quantitative traits of poultry, such as the number of eggs laid, the number of progeny, the growth rate, feed conversion and slaughter yield, are determined by unknown genes localized in particular loci and by the effect of the environment. The summed effect of genetic and environmental factors can be illustrated using the simple formula:

$$P = G + E$$

where P is the phenotypic value, G is the genetic value and E is the environmental value. Variance P is expressed by the formula:

$$\sigma^2_P = \sigma^2_G + \sigma^2_E + 2\mathrm{cov}(G,E)$$

Assuming that $\mathrm{cov}(G,E) = 0$,

$$\sigma^2_P = \sigma^2_G = \sigma^2_E$$

The summing effect of both factors will not be so simple when the genotype × environment interaction occurs. For quantitative traits, only phenotypes are calculated. According to Fisher's model, the genes localized in loci sum their individual (additive) effects with the interaction between loci (domination). Additive and dominant effects and the effects of interaction between loci (epistasis) are summed in every locus. This makes it possible to estimate the transmission of additive effects from one generation to the next.

Selection based on additive effects involves determination of the bird's breeding value, as measured by the double phenotypic deviation from the average for the progeny sired by one sire and from the population average. Since it is practically impossible to compare the progeny mean with the mean for the whole population, the problem of estimating the additive genetic (breeding) value should be solved based on phenotypes.

The general formula for breeding value estimation can be written as:

$$G_x = P + b_{G/P} (P_x - P)$$

where G_x is the genotype value of the individual estimated, P is the mean phenotypic value of trait in a flock, P_x is the phenotype of the individual estimated, and $b_{G/P}$ is the coefficient of genotype's regression into phenotype (in this case h^2).

The above arguments suggest the need for methodological adjustments to ensure more effective use of available sources of information, and above all to increase accuracy when evaluating breeding value (additive effects) for individual birds. The evolution of breeding methods, several decades in the making, towards the early and accurate evaluation of animals has resulted in statistical procedures which ensure that good results are achieved. However, their implementation faces temporary problems due to the limited operational memory capacity of computers. In addition, the accuracy of the results obtained depends on whether the accepted model corresponds with real situations (real sources of trait variation) and on the accuracy of individual performance evaluation.

In the second half of the 20th century, conventional poultry breeding programmes came to apply selection indices which used only one source of information about the animal's genotype, e.g. its own performance. This is exemplified by the selection index developed by Hazel (1943) and Hazel and Lush (1943):

$$I = P_1 h^2_1 w_1 + P_2 h^2_2 w_2 + \ldots + P_2 h^2_n w_n$$

where I is the selection index, P_i is the phenotype of individual traits, h^2 is the heritability coefficients of the traits, and w_i is the economic weight of trait i.

It can be generally assumed that the index is a surplus of the bird's estimated genotype over the flock's mean total genotype. The phenotypic values of traits used in the selection index should be presented in comparable units, e.g. standard deviation units. When creating a selection index, the most important thing is the correct estimation of individual coefficients of regression β_i, which are estimated based on the correlation between individual sources of information and total genotype according to the formula:

$$b_i = \beta_i \frac{\sigma_{GT}}{\sigma_I}$$

Correct estimation of coefficients β_i requires the knowledge of the matrices of correlation coefficients between individual sources of information and the numerical values of the vector of multiple regression coefficients (b_i) between individual sources of information and total genotype.

The following data need to be estimated to elaborate the selection index.

- phenotypic variances of individual traits: σ^2_{Px}, σ^2_{Py}, σ^2_{Pz}
- genetic variances of individual traits: σ^2_{Gx}, σ^2_{Gy}, σ^2_{Gz}
- genetic covariances between traits: σ^2_{GxGy}, σ^2_{GxGz}, σ^2_{GyGz}
- coefficients of heritability: h^2_x, h^2_y, h^2_z
- coefficients of genetic correlations between traits: r_{GxGy}, r_{GxGz}, r_{GyGz}
- coefficients of genetic correlations between full-sibs: t_{xx}, t_{yy}, t_{zz}
- coefficients of phenotypic correlations between half-sibs: T_{xx}, T_{yy}, T_{zz}

- coefficients of phenotypic correlations between traits under selection
- determination of economic weights for the traits

Selection index theory has evolved with such modifications as 'classical', 'optimum' and 'reduced' selection indices (Hazel, 1943; Wężyk, 1978; Jeyaruban *et al.*, 1995). The result has been considerable genetic and productive progress for most of the traits being improved. For example, the rearing period has been reduced for broiler chickens from 56 to 35 days. During this period, the birds achieve over 2 kg of body weight with feed conversion of 1.80 kg feed kg^{-1} body weight gain. During the period 1970–1999, body weights of commercial male and female turkeys (at a given age) increased by 6.7 and 2.7 kg, and feed conversion improved by 0.49 and 0.71 kg kg^{-1} body weight gain, respectively. In geese, the number of hatching eggs produced increased significantly (from 32 to 64) with accelerated rate of growth.

By contrast, and as a consequence of different family structures and reproductive abilities, the improvement of large farm livestock, particularly cattle and pigs, has developed rather differently since the 1960s or 1970s.

The development of statistical methods and computational computer techniques since the 1980s has resulted in new prospects for evaluating the breeding value of farm animals. Most attention has been focused on the best linear unbiased prediction (BLUP) method (Henderson, 1975; Mao, 1982), particularly the animal model (AM) as developed by Quaas and Pollak (1980), and other models based on the greatest likelihood principle.

Best Linear Unbiased Prediction under an Animal Model (BLUP–AM)

General considerations

The animal model makes it possible to use records on the performance of an evaluated individual, its ancestors and collateral

relatives (sibs, half-sibs). Another asset is the possibility of applying the breeding value evaluation to individuals included in the calculations and to those not observed. This method enables several environmental and genetic factors that affect the variability of trait(s) to be included in the model. The BLUP animal model has been increasingly used to evaluate the breeding value of dairy cattle (Mäntyssari and Straden, 1991) and other livestock species such as pigs (Wood *et al.*, 1991; Duniec and Różycki, 1993). This method has so far seen little application in poultry breeding. The application of the animal model to poultry breeding was studied by Pang *et al.* (1989) and Hagger (1991b). According to Hartmann (1992), the diminished interest of poultry breeders in this method results from high computation costs, relatively low environmental variability, and the specific nature of poultry genetic improvement programmes that are mainly designed to obtain so-called crossing effects (heterosis). With the limited environmental variability of selected poultry compared with other livestock species, the effect of the hatching date is the environmental factor most frequently included in the evaluation. Generation can be another fixed effect in this model.

The BLUP theory was first proposed by Henderson in 1949 to describe the environmental and genetic trend estimates in cattle and was subsequently developed (Henderson, 1973, 1975). The term 'animal model' was introduced by Quaas and Pollak (1980). In general, the applicability of this method is determined by the interest of the breeders and computer limitations (capacity of operational memory). However, in recent times increased computer power has enabled huge amounts of data to be gathered, processed and analysed (e.g. animal models involving several genetic effects, unequal incidence matrices).

The main advantages of the BLUP animal model (BLUP–AM) are the high level of accuracy of the breeding (or genetic) evaluations and the versatility of its application when compared with the so-called classical selection index, which includes phenotypic value data on only some known relatives and

produces less precise estimates of environmental fixed effects. According to Buddiger and Albers (2000), the accuracy of turkey selection depends largely on the statistical technique used to estimate the breeding value. While the accuracy of physical (hands-on) selection is 10% for egg production improvement, 30% for selection index, 60% for BLUP–AM and 100% for biotechnological methods such as marker assisted selection (MAS), the accuracy of selection for body weights of turkeys is 45, 60, 70 and 100%, respectively.

Application of BLUP–AM to the evaluation of poultry breeding value

The evaluation of breeding value under an animal model is based on the following mixed linear model:

$$\underline{y} = X\underline{b} + Z\underline{a} + \underline{e}$$

where $\underline{y}$ is the vector of observations $n*1$ (n is the number of observed individuals, assuming that 1 individual = 1 observation), $\underline{b}$ is the unknown vector of fixed effects $p*1$ (p is the number of levels of all fixed effects included), $\underline{a}$ is the unknown vector of additive genetic effects $q*1$ (q is the number of individuals evaluated), $\underline{e}$ is the unknown vector of random errors $n*1$, X is the incidence matrix $n*p$, and Z is the incidence matrix $n*q$. The assumptions of the model are:

$$E\begin{bmatrix} \underline{y} \\ \underline{a} \\ \underline{e} \end{bmatrix} = \begin{bmatrix} X\underline{b} \\ 0 \\ 0 \end{bmatrix}$$

$$V\begin{bmatrix} \underline{y} \\ \underline{a} \\ \underline{e} \end{bmatrix} = \begin{bmatrix} ZAZ'\sigma^2_\alpha + R & ZA\sigma^2_\alpha & R \\ AZ'\sigma^2_\alpha & A\sigma^2_\alpha & 0 \\ R' & 0 & R \end{bmatrix}$$

$$R = \sigma^2_e I$$

A is the matrix of additive relationships between the individuals. Principles for the construction of this matrix were given by Kennedy (1989). Values σ^2_α and σ^2_e are components of additive genetic variance and error variance. These components can also be obtained based on the animal model (Meyer, 1989). Methodological similarity

of the way variance components, fixed estimators and predictors of additive genetic effect are obtained enables some stages of both operations to be carried out once.

Solving the following system of mixed equations (Kennedy, 1989) can yield predictors of additive genetic effects:

$$\begin{bmatrix} \mathbf{X'R}-1\mathbf{R} & \mathbf{X'R}-1\mathbf{Z} \\ \mathbf{Z'R^{-1}X} & \mathbf{Z'R^{-1}Z}+\mathbf{A^{-1}}\lambda \end{bmatrix} \begin{bmatrix} \underline{\mathbf{b}} \\ \underline{\mathbf{a}} \end{bmatrix}$$

$$= \begin{bmatrix} \mathbf{XR^{-1}}\underline{\mathbf{y}} \\ \mathbf{Z'\,R^{-1}}\underline{\mathbf{y}} \end{bmatrix}$$

where $\lambda = \sigma^2_e / \sigma^2_a$.

With a large number of individuals evaluated, the conventional solution of this system of equations is faced with serious numerical barriers, one of them being the computation of the inverse of the additive relationship matrix. Henderson (1976) and Quaas (1976) suggested a solution to this problem, which evades computational complications related to the inversion of large relationship matrices. The elements of the inverse matrix are then computed directly. Until recently, the evaluation of components of vectors $\underline{\mathbf{b}}$ and $\underline{\mathbf{a}}$ also presented a serious challenge.

Currently there are many iteration procedures (Misztal *et al.*, 1987) enabling a system of equations to be solved without the need for inverting 'left-hand' matrices. It should be stressed, however, that accuracy of the final results and computation time depend on the iteration parameters assumed and the applied iteration approach (Misztal *et al.*, 1987; Reents and Swalve, 1991). One factor that limits the scale of numerical difficulties is the absorption of those parts of equations related to fixed effects, for in practice the estimators of some fixed effects are not always important. Absorption leads to a decrease in the size of 'left-hand' matrices.

One way of reducing the size of a mixed equation matrix is to replace the observations of individual birds with means for full-sib groups. Although this conceals the information about individual phenotypic variation, Simianer and Gjerde (1991) regard these 'losses' as very small. In addition to decreasing the matrix size and the computation time, the method suggested makes it possible to evaluate groups of individuals, especially where individual measurement of traits is not possible. The argument for this procedure is the fact that family selection is often carried out in poultry.

The above procedures can be used to make single-trait analysis, i.e. to evaluate the breeding value of individuals based on one trait. The above method can also be used to evaluate individuals based on a larger number of traits. In single-trait evaluation based on an animal model, the knowledge of additive genetic covariances and covariances of random errors between traits is also necessary (Da and Grossman, 1991; Graser and Tier, 1991).

Comparison of the above theoretical solutions for the problem of breeding evaluation with relatively limited computer capacities of poultry farms and companies shows that the applicability of the latest achievements to practical breeding will be fairly limited. This conclusion leads one to make efforts aimed at optimizing the computational algorithms and adapting them to local conditions. These efforts must also include estimation of genetic parameters as integral elements of the breeding evaluation. Comparative studies by Beaumont (1991) on the estimation of heritability coefficients based on Henderson's method I (Henderson, 1953) and the REML method (Patterson and Thompson, 1971) showed great differences in the results obtained by both methods. Brodacki *et al.* (1996) obtained similar values of h^2 coefficients for Sussex hens that were estimated with variance analysis according to Henderson's methods I and III, but they were considerably different from REML estimates. The results obtained by these authors failed to confirm the thesis of Wężyk and Szewczyk (1993) that the higher the heritability, the greater is the conformation of its value estimated by different methods. Differences in h^2 estimations obtained by analysis of full-sib groups often results from the type of variance component (sire or dam) on which the evaluation is based (Kasznica *et al.*, 1988; Malik *et al.*, 1991; Mou, 1991; Wężyk and Szewczyk, 1993; Koerhuis *et al.*, 1997). In the cited papers,

heritability coefficients of hen performance traits obtained from the sire component were much lower than those from the dam component. These differences result from estimators' bias, which, as already mentioned, is the result of both methodological faults and the interaction with gene pairs (dominance) and between gene pairs (epistasis), and can also be the result of specific dam effects (Bosch, 1990; Barbato and Visilatos-Youchen, 1991; Koerhuis *et al.*, 1997). The use of animal models helps considerably to eliminate the above biases, because analysis includes the genotypes of sire, dam and other relatives. This method also makes it possible to identify individual components of genetic variance. As shown by the studies of Szwaczkowski (1992), differences in the results of evaluation of genetic parameters are largely affected by the number of available pedigree records.

The efforts to reorient the model of poultry performance testing that focus mainly on those traits which are the final product, such as the number of eggs produced, average egg weight, juvenile body weight, are regarded as justified. The findings obtained by many authors for meat-type hens (Hagger, 1990, 1991a; Luiting, 1991; Malik *et al.*, 1991; Nordskog *et al.*, 1991; Sabri *et al.*, 1991; Morris and Pollott, 1997; Su and Sørensen, 2000), turkeys (Havenstein *et al.*, 1988), geese (Rosiński, 2000) and ducks attest to the possibility of carrying out meat-type poultry selection for such traits as feed intake, feed digestibility, intensity of metabolic changes and indicators of feed costs. Resistance to disease is an economically important group of traits. The inclusion of the above traits in the breeding evaluation will lend it a more economic dimension and this should increase the interest of the breeders in the method presented.

The use of the BLUP animal model boils down not only to the prediction of genetic additive effects and possible fixed effects. The extent of application for this method is much broader and also includes prediction of the above-mentioned genetic effects, which are not exactly additive effects but mainly dominance and epistasis effects. Studies with other populations of hens

(Hagger, 1986; Flock *et al.*, 1991) and turkeys (Emmerson *et al.*, 1991) showed that dominance or epistasis effects of genes may be an important source of performance trait variation. Non-additive gene effects, identified in practice with heterosis, occur most often in the crosses of various breeds or genetically distant lines.

Prediction of genetic effects is based on the following linear model (Kennedy, 1989):

$$\underline{y} = X\underline{b} + Z\underline{a} + Z\underline{d} + Z\underline{q}_1 + Z\underline{q}_2 + \ldots + Z\underline{q}_j + \underline{e}$$

where: $\underline{y}$ is vector $n*1$; X and Z are known incidence matrices with fixed dimensions $n*p$ and $n*q$, respectively; $\underline{b}$ is the unknown vector of fixed effects $p*1$; $\underline{a}$ is the unknown vector of genetic additive effects $p*q$; $\underline{d}$ is the unknown vector of genetic dominance effects $p*q$; $\underline{q}_j$ is the unknown vectors of genetic epistasis effects $q*1$ ($j = 1, 2 \ldots, t$), with each vector corresponding to the epistasis effect of one combination of interactions between pairs of genes (additive* additive, additive*dominance, dominance* dominance, etc.); and $\underline{e}$ is the unknown vector of random errors $n*1$.

The possibilities of using the animal model in poultry breeding go far beyond the issues presented here. This especially concerns the estimation of genetic effects related to the introduction of such techniques as cloning, mapping or gene transfer into animal breeding, including poultry breeding (Kennedy, 1989; Hartmann, 1992; Brodacki and Wężyk, 1993). These issues, however, still have only limited practical application and the breeding work is mainly based on quantitative genetics theory.

Modelling and data transformation

The adequacy of the linear model to describe the variability of traits is one of the important factors determining the precision of the estimation and prediction. In general, the modelling of fixed effects in meat-type poultry linear models is similar to that for other livestock species. In the case of

one generation, the fixed effect of hatch is usually included. When evaluation concerns individuals from more than one generation, the year effect is often also specified.

One of the main assumptions of the method of parameter estimation and prediction in linear models is the normal distribution of the residual vector effect. The normality of distribution is often connected with other assumptions of this method, i.e. homogeneity of group (subclass) variances, additivity of the model, linearity of genotypic regression and heritability (Carroll, 1980; Ibe and Hill, 1988). Unfortunately, these assumptions do not usually hold for egg production traits (Clayton, 1975; Besbes *et al.*, 1993; Koerhuis, 1996; Koerhuis and McKay, 1996), sexual maturity and body weight of hens (Szwaczkowski *et al.*, 1997). These traits most often exhibit skewed distributions, leading to misestimation of genetic parameters (variance error estimates are increased, which in turn reduces the efficiency of BLUP–AM). Thus, the Box–Cox transformation (Box and Cox, 1964) is recommended in order to obtain variables that satisfy the assumptions of the additive linear model with normal errors and homogeneous error variances (Szwaczkowski and Wężyk, 1994, 1995).

Variance component estimation

There are many methods (and algorithms) available to estimate variance components under an animal model. Most of them were developed for unbalanced data in general linear models in the 1970s. These include first of all the REML approach (Patterson and Thompson, 1971) and MINQUE (minimum norm quadratic unbiased estimation) and MIVQUE (minimum variance quadratic unbiased estimation) (Rao, 1971a,b; La Motte, 1973). The widespread use of these methods is conditioned by a large computational base. As a consequence there was a tendency for the use of other statistical methods for the estimates, such as translation invariance, unbiasedness, minimum

mean squared error and consistency. Easier numerical algorithms were gradually developed. Several methods (REML) to reduce the computational demands appeared in the literature, such as Henderson's method IV (Henderson, 1980), the pseudo-expectation procedure (Shaeffer, 1986) and the tilde-hat procedure (Van Raden and Jung, 1988). Moreover, many alternative numerical algorithms based on the REML were described, such as Henderson's algorithm (Henderson, 1973), the expectation–maximization (EM) algorithm (Dempster *et al.*, 1977) and the derivative-free algorithm (DFREML) presented by Graser *et al.* (1987). These new approaches to estimating variance components facilitated increasingly accurate analysis and could be applied to sophisticated animal models. More details concerning types of animal models are given by Henderson (1988) and Kennedy (1989). Currently, the DFREML algorithm is frequently employed to analyse large sets of livestock data (Meyer, 1989; Wężyk and Szwaczkowski, 1997).

It should be stressed that the application of REML (and in consequence BLUP) to an animal model was not computationally feasible until a method for computing the inverse of the additive relationship matrix ($\mathbf{A}^{-1}$) was found. The first procedures to obtain the inverses of large relationship matrices were described by Henderson (1976) and Quaas (1976). As already mentioned, using the new REML algorithms with animal models allowed a more precise description of particular components of the genetic variability in poultry performance traits. Investigations conducted by many authors (Fairfull *et al.*, 1987; Graml and Pirchner, 1993; Wei and Van Der Werf, 1993) indicated the importance of dominance and epistasis in egg production trait variation. Non-additive genetic variances are estimated according to Wei and Van der Werf (1993) in three cases: (i) as an unbiased estimation of heritability in the narrow sense; (ii) for more precise prediction of additive effects; and (iii) in the use of dominance and epistasis effects in crossbreeding or a special mating strategy. Both the estimation of the non-additive genetic variance components and

the prediction of respective genetic effects require the inverses of non-additive relationship matrices. Currently, such procedures are known (Hoeschele and Van Raden, 1991; Van Raden and Hoeschele, 1991).

Many authors have reported a relatively large influence of the dam on productive traits in egg-laying and meat-type hens (Wężyk, 1970; Barbato *et al.*, 1983; Leenstra, 1986; Liu *et al.*, 1993; Koerhuis and McKay, 1996; Koerhuis *et al.*, 1997; Su and Sørensen, 2000), ducks (Rouvier *et al.*, 1994; Mignon-Grasteau *et al.*, 1998) and turkeys (Havenstein *et al.*, 1988).

Prediction of genetic effects

Predictors of random genetic effects and estimates of fixed effects are obtained by solving the mixed model equations (MME) presented by Henderson (1973). Solution of the equations entails overcoming a number of numerical barriers such as obtaining the inverse of the relationship matrix (one or more as mentioned above) and, from a theoretical point of view, the inverse of the 'left-hand' matrix in the MME. Questions concerned with inverses of numerator relationship matrices are presented above. The dimensions of the 'left-hand' matrix depend on the number of individuals in the analysis, number of covariables and the number of levels of fixed effects. Finding the inverse of the 'left-hand' matrix is usually impossible. Hence the elements of the vector of unknown fixed parameters and unknown random (genetic) effects are, in the case of livestock data, obtained by the use of iteration procedures (Misztal *et al.*, 1987) to solve the MME. Moreover, the size of this matrix may be reduced by absorbing some of the effects of fixed factors on many levels (when solutions for these effects are not important from a practical point of view). It should be stressed that accuracy of the final results and computation time depend on the assumed iteration parameters and the applied iteration approach (Misztal *et al.*, 1987).

As already mentioned, in poultry analysis the number of fixed effects is limited compared with for instance, cattle data. Hence, as given above, the size of the 'left-hand' matrices is mainly determined by the number of recorded and base individuals and the number of predicted genetic effect types (direct additive, direct dominance, maternal additive, etc.). Current methodology may be employed in both single-trait and multi-trait analysis. Backgrounds of multi-trait prediction under an animal model have been described by Thompson and Meyer (1986), among others. To predict the effects in multi-trait models, the estimates of respective genetic and residual covariances also have to be known. This obviously leads to additional large computational requirements in estimating the genetic parameters. Multi-trait genetic evaluation may be required to overcome selection bias and a possible depression of genetic progress in populations selected (Pollak *et al.*, 1984). It should be stressed that multi-trait BLUP analysis leads to greater accuracy of the breeding value of correlated traits when information on a trait is limited, or even non-existent, and when different traits are recorded in different environments. Multi-trait evaluation with BLUP–AM is also applied in poultry breeding (Hagger, 1992; Wężyk and Szewczyk, 1993; Szwaczkowski and Wężyk, 1994; Brodacki *et al.*, 1996; Koerhuis and McKay, 1996; Szwaczkowski *et al.*, 1997).

Available computer package programs

A considerable amount of animal breeding software is available, which uses mixed model methodology. The software can be used successfully in animal breeding (Wężyk and Szwaczkowski, 1997). Most of these programs are species oriented (mainly for dairy and beef cattle) and all support animal models with fixed and random hierarchical and cross-classified effects and co-variables. Basically, all may be employed to solve mixed model

equations for fixed effect estimates and additive genetic predictors as well as to estimate genetic parameters by REML. In the case of more sophisticated problems, the programs differ in many features (size of data, testing hypothesis in mixed model equations). Several packages are currently used in animal breeding, e.g. DFREML (Meyer, 1993), MTDFREML (Boldman et al., 1993), PEST (for prediction and estimation of the effects) and VCE (for variance component estimation) (Groeneveld, 1993), ABTK, JAA/MTC (Misztal, 1992) and DMU (Jensen and Madsen, 1995). It should be noted that all of them (except MTDFREML, which is written for PC/DOS) require the UNIX system. In practice, the above-mentioned computer packages may be used for the breeding evaluation of all species and utility types of poultry.

Discussion

Generally, the advantages of BLUP under an animal model over different classical methods for breeding evaluation of livestock have been confirmed by both simulation and empirical studies. Hudson and Schaeffer (1984), on the basis of computer simulation (cattle population with selection and random mating), concluded that breeding evaluation from an animal model was about 65% more accurate compared with evaluation from a sire model.

Morris and Pollot (1997), who compared the results of selection with three methods of breeding evaluation – i.e. BLUP, selection index (SI) and phenotype (SP) including three traits: juvenile body weight (JW), percentage of breast muscle (BM) and egg production (EP) – stated that the application of BLUP in commercial breeding can increase selection response compared with SI, particularly for low heritability traits and when the number of performance-tested birds is low. Breeding values estimated with BLUP on the basis of individual phenotypic records were correlated to a much lower degree with breeding values estimated with the selection index. The highest correlations obtained between SI and BLUP indicate

an increase in accuracy resulting from the inclusion of additional information on relatives.

Similarly, Danbaro et al. (1995) in their studies on the application of REML to estimation of meat-type hen genetic parameters found that the use of BLUP–AM estimated breeding value in selection was justified when heritabilities of body-weight traits of birds were low or average.

Mielenz et al. (1994) showed that the degree of advantage of BLUP over the traditional selection index is determined by the structure of the breeding programme and the mating strategy. A cumulative response to selection and the average inbreeding coefficient in a simulated poultry population were taken as criteria to compare these methods. Results obtained by Wężyk and Szewczyk (1993) indicated a high concordance in rankings from the animal model and selection index in laying hens. Similar results were obtained by Szwaczkowski et al. (1997). However, when analysis was restricted to the group of best families (formed on BLUP evaluations) the rank correlation coefficient ranged from +0.41 to +0.50. Differences in rankings are often associated with the size of the heritability coefficient. Sørensen (1988), on the basis of a simulation study, reported that an increase of trait heritability resulted in a higher concordance between the BLUP–AM and selection index rankings. As the heritability rises, less emphasis is placed on the performance of relatives and more on the individual's own record; thus, increasing agreement between BLUP and the selection index is to be expected. It was also shown that, for traits with low heritability (for instance, fertility and hatchability), employing the BLUP induced about 5% extra response to selection and about 15% extra inbreeding over a family selection index procedure. In the case of highly heritable traits such as egg weight, BLUP increases selection response by about 2% and inbreeding by about 9%. An increase in the inbreeding level may lead to a loss in reproductive and productive trait performance. However, many authors report that inbreeding depression in poultry is not particularly severe, due to the short period of

reproductive performance (Szwaczkowski, 1995) and the specific hierarchical structure of breeding (Hartmann, 1992).

The expected benefits of using an animal model compared with a selection index obviously depend in part on the costs of implementing a new evaluation methodology and especially on the computer, software and computation costs. It must be stressed that these computational requirements may be reduced by application of the so-called reduced animal model (Quaas and Pollak, 1980) or, in the case of estimation of variance components, the procedure is simplified by including full-sib group (or full-sib × fixed-effect class) means in the computation. All these arguments are important when deciding on the introduction of the animal model to poultry breeding programmes.

As already mentioned, the genetic evaluation of poultry is connected to the estimation of genetic parameters, a subject investigated by many authors (Beaumont, 1991; Spilke and Mielenz, 1992; Wężyk and Szewczyk, 1993; Mielenz et al., 1994; Brodacki et al., 1996). Breeding evaluation of poultry most often employs a selection index developed by Henderson (1953) with later modifications, whereas BLUP evaluation as the linear model is used together with REML to estimate variance components. REML is superior to Henderson's methods, particularly when heritability of traits is low. It should be stressed that the bias of estimates obtained by the conventional approach may result from selection (Beaumont, 1991) and from ignoring relationships (ancestors and collateral relatives). These disadvantages are eliminated when REML under an animal model is employed.

Dempfle and Grundl (1988) concluded that one of the main differences between the more traditional breeding of the past and the scientifically influenced breeding in major livestock species carried out today lies in the understanding of what is best (an individual with the highest phenotypic value or genetic or breeding value). The evaluation of an individual using the classical selection index is based on phenotypic values, assuming a typical polygenic inheritance model of traits (a very large number of loci with small and equal and independent effects contributes to the genetic variance with no linkage in segregation). According to the current status of knowledge concerning the genetic background of poultry traits, this assumption seems questionable. Investigations conducted on dairy and beef cattle and sheep indicate the significance of cytoplasmic influence on the phenotypic variability of performance traits (Schulz et al., 1994).

The identification of genes with large effects resulted in a revision of the polygenic inheritance model and, in consequence, influenced the realized breeding strategies. When the difference between alternative homozygotes (for genotypes with large effects) is no less than 1 standard deviation, the gene with large effects is called a major gene. An evaluation of the genetic value of an individual consists of two elements: the effect of a major gene (treated as fixed in the linear model) and the polygenic effect (treated as random). Such a model of genetic determination of a trait is defined as a mixed (or combined) model. Merat (1993) reviewed a considerable number of examples of identified genes with large effects. Several studies showed pleiotropic and linkage action of the genes (Merat, 1993), which increases the number of traits in a combined inheritance model. Thus, an improvement in the population requires identification of genetic markers to detect the major genotypes (and their frequencies) of individuals as well as estimates of major gene effects and predictors of polygenic effects.

The possible application to poultry breeding of such developments as gene transfer and chimeras will require improved methods of genetic evaluation. Unquestionably, the use of new techniques will complicate the process of determining genetic worth. The animal model, after some modification, is likely to be the most effective means of individual bird evaluation (Kennedy, 1989; Wężyk and Szwaczkowski, 1997).

The considerations presented here do not concern the dilemma of choosing between the BLUP animal model or selection index because best linear unbiased predictors are usually used to construct

a selection index, particularly in livestock species and also in laying and meat-type hens, turkeys, ducks and geese (Pribil and Pribilova, 1991). The main differences between the more or less traditional types of selection index (optimum, classical, reduced) concern the base files that are included in the computations (individual, full-sibs and half-sibs, phenotypic values or genetic merits). Comparisons of breeding evaluations from the traditional selection index and BLUP–AM do not always lead to the same conclusions. It seems that development of new reproductive techniques and genetic engineering will enhance the application of BLUP under an animal model to breeding strategies of various species and utility types of poultry (Merat, 1993).

Summary

Evaluation of the breeding value of poultry includes the results of performance and progeny testing. In pedigree flocks of egg-laying and meat-type hens and ducks, the breeder's attention is focused on the performance of progeny, parents, grandparents and collateral relatives (full- and half-sibs). In the case of geese and turkeys, where birds of different age are kept in the same flock, the evaluation also includes reproductive performance in the first, second and third seasons of reproductive utilization. Much attention has been devoted to statistical models for estimation of progeny value.

Modern meat-type poultry breeding programmes generally have a sequential structure in which evaluation of growth traits and slaughter value in the first stage is followed by evaluation of reproductive traits after the birds have attained sexual maturity. Multiple-trait genetic evaluation makes it possible to overcome selection bias and prevents the depression of the genetic progress in the population. In genetic evaluation, estimating variance matrices requires the use of ANOVA-type procedures, which may yield heavily biased results. When an analysis includes all the necessary data for making selection decisions, the restricted maximum likelihood method applied in the individual animal model can alleviate an undesirable selection bias. This method is very useful when the computations involve large numbers of birds.

The chapter has discussed: basic statistical procedures used for estimating the breeding value of meat-type poultry, with special regard to the BLUP–AM model; the ways of estimating genetic variance components and of predicting genetic effects; and the advantages and disadvantages of the methods for estimating the breeding value of poultry.

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11 Use of Mixed Model Methodology in Poultry Breeding: Estimation of Genetic Parameters

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Introduction

Although a number of single loci have been identified, genetic backgrounds of a majority of poultry performance traits are still explained by the probability approach. Generally, three main genetic models are employed for the description of trait inheritance: monogenic, polygenic and mixed models.

To begin, consider a monogenic model: here, a trait is determined by a single locus without environmental effects (e.g. some plumage variants). For more than one locus (say, several), the model is outlined as an oligogenic one (e.g. comb variants). Thus, a statistical characterization of the variability of the trait studied (if genotypes are identifiable) is relatively simple. It should be noted that the monogenic model, based on Mendelian theory, was chronologically the first to be described.

Unfortunately, the approach did not prove sufficient to explain the genetic backgrounds of numerous productive poultry traits. However, probability rules are an antidote to ignorance. Hence, the polygenic model based on an infinite number of loci with equal and small effects has been adopted. The approach resulting from probability circumstance allows for the application of a statistical framework (e.g. under assumption of normality). As already mentioned, many single loci (the so-called quantitative trait loci – QTL) have been discovered and mapped since the early 1990s. The genetic variability of traits determined both by polygenes and by single loci with large effects is described by the mixed (combined) inheritance model. More details concerning the model are given in later chapters of this book.

This chapter considers only the polygenic model. It should be noted that a majority of poultry performance traits (e.g. egg production, weight gain) are created in a relatively long and complex physiological process. Thus, such traits may be determined by many loci and environmental effects as well as genotype-by-environment interaction (see Fig. 11.1). A genotype-by-environment interaction ($G \times E$) can be defined as a change in the relative performance of two or more genotypes measured in two or more environments (Merks, 1988). Other definitions of $G \times E$ can also be found in the literature (Hartmann, 1990; Mathur and Horst, 1994). Basically, interactions may involve changes in rank order for genotypes between environments and magnitude of variance between environments. It should be stressed that, in poultry, genotypes are
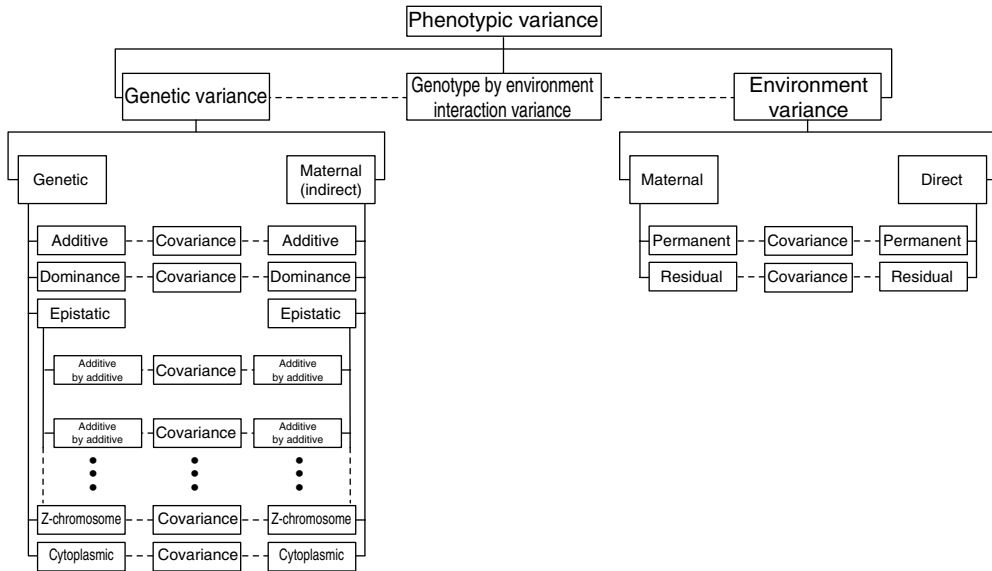
Fig. 11.1. Phenotypic variance components in birds.

usually represented by genetic groups (strains, lines, breeds), whereas environments are represented by, for instance, the housing system, management system or sex.

In an infinite number of loci a genetic variability (variability of genetic effects) can be divided into direct and indirect maternal components. Direct genetic variability is classified into the following:

- Additive genetic variability (resulting from no interaction of intralocus and interloci effects). Thus, the aggregated effect of genotype is the sum of single allele effects. It is noted that one parent (say, sire) transmits to progeny half of its additive genetic effect.

- Dominance genetic variability. Dominance effects result from interactions of pairs of alleles at the same locus (joint effect of single locus does not equal the sum of two allele effects). Only one allele from each pair is transmitted to given progeny, so an individual cannot transmit its dominance effect directly to its offspring. Transfer of dominance effects is traced through pairs of parents.

- Epistatic genetic variability. There are many types of interlocus interactions

for two or more loci. For two loci, three types of epistasis can be considered: additive by additive (for no interaction in both loci), additive by dominance (for no interaction in one locus only) or dominance by dominance (for interaction in both loci). Similarly, these interactions may also be studied for three loci (e.g. additive by additive by additive) and more. Transmission of epistatic effects from parent to progeny depends on the type of non-allelic interaction.

- Cytoplasmic genetic variability. The presence of cytoplasmic genetic effects has been hypothesized since mitochondria contain their own DNA (so-called mtDNA). It is known that ovum cytoplasm has about 100,000 copies of mtDNA whereas the spermatozoon has only about 100 copies of mtDNA. Therefore, mitochondria are passed from dam to offspring (with no gene segregation). Why does mitochondrial variability exist? Two main reasons are reported in the literature: (i) mtDNA has evolved more rapidly than nuclear DNA; and (ii) interactions of extra-nuclear by intranuclear DNA have been registered. Both factors contribute to a

high polymorphism of some mitochondrial chicken genes (Li *et al.*, 1998).

● Z-chromosome variability. Sex determination in birds operates through a ZZ/ZW sex chromosome system, in which the female is heterogametic. Unlike mammals, male birds have two identical sex chromosomes. Pigozzi (1999) reported that in most bird species the W chromosome is largely heterochromatic. The chicken W constitutes less than 2% of the genome and contains only a few coding sequences (Nanda *et al.*, 2000). Hence, much interest has focused on the genetic effects of the Z chromosome. In contrast to mammals (where one of the two X chromosomes in female somatic cells is randomly inactivated to ensure comparable dosages for most X-linked genes in individuals of both sexes), no Z chromosome is inactivated. The 'surplus' of gene actions may be treated as specific paternal genetic effects (see, e.g. Ledur *et al.*, 2000).

It should be stressed that, for a number of mammal and plant species, gametic imprinting phenomena are also considered to be a source of genetic variability (Ruvinsky, 1999). To our knowledge, no gametic imprinting has been registered in birds.

Other components of phenotypic variability of traits determined by many loci are specific maternal variabilities. Generally, these maternal effects have been defined as any influence from a dam on its progeny, excluding the effects of directly transmitted genes (see, e.g. Albuquerque *et al.*, 1998). Although the maternal effects (and in consequence maternal variability) are more exhibited in mammals, it is known that the development of a chicken embryo depends on egg environment during incubation, which is the vehicle for maternal effects of the fertility of egg and development of the embryo as well as other traits, e.g. body weight. Koerhuis *et al.* (1997) reported that a difference in egg weight of 1 g is reflected in about 10 g gain of body weight of a juvenile broiler. The so-called egg environment is determined by both dam genotype and the external environment (maternal environmental effects). From the standpoint of the mother, maternal effects on progeny performance result from maternal traits controlled by her genotype as well as associated environmental factors. The maternal (indirect) genetic variance may be classified into the same components as mentioned above (additive, dominance, additive by additive, etc.). On the other hand, from the standpoint of the offspring, indirect maternal effects are considered as environmental. Therefore, by analogy to the interaction of genotype by environment, attention should be drawn to the dependence between direct and maternal effects (see Fig. 11.1). If a negative dependence exists between a direct and maternal effect, the dam either gives her offspring a plus set of genes for the direct effect and a poor maternal effect, or the other way around. Similarly to genetic variability, an environmental variability can also be divided into direct and maternal (indirect) with dependencies (covariances) between permanent and temporary (residual) components (see Fig. 11.1).

This chapter presents some methodological aspects of genetic parameter estimation focused on restricted maximum likelihood (Patterson and Thompson, 1971) under an animal model. Other statistical procedures are also reported. Results involving the genetic parameters of egg production, growth, meat production and related traits are discussed.

Preliminaries

The main applied measure of trait variability is a variance denoted as σ^2 (as unknown population parameter). Thus, estimate of variance is expressed as $\hat{\sigma}^2$. For simplicity, in subsequent considerations, the following symbols concerning the above described variances will be employed:

Symbol	Variance
σ^2_P	Phenotypic
σ^2_G	Genetic
$\sigma^2_{G(d)}$	Direct genetic
$\sigma^2_{G(d)A}$	additive
$\sigma^2_{G(d)D}$	dominance
$\sigma^2_{G(d)epist}$	epistatic
$\sigma^2_{G(d)AA}$	additive by additive
$\sigma^2_{G(d)AD}$	additive by dominance
$\sigma^2_{G(d)DD}$	dominance by dominance
$\sigma^2_{G(d)Z}$	associated with Z-chromosome effects
$\sigma^2_{G(d)C}$	cytoplasmic
$\sigma^2_{G(m)}$	Maternal (indirect) genetic
$\sigma^2_{G(m)...}$	Respective maternal genetic (in order given above)
$\sigma_{G(dm)A}$	Covariances between respective direct and maternal effects
$\sigma_{G(dm)D}$	
$\sigma_{G(dm)AA}$	
$\sigma_{G(dm)AD}$	
σ^2_E	Environmental
$\sigma^2_{E(d)}$	direct
$\sigma^2_{E(d)p}$	permanent direct
$\sigma^2_{E(d)t}$	residual direct
$\sigma^2_{E(m)}$	Maternal environmental
$\sigma^2_{E(m)p}$	permanent
$\sigma^2_{E(m)t}$	temporary
$\sigma^2_{E(dm)p}$	Covariance between direct and maternal permanent environmental
$\sigma^2_{E(dm)t}$	Covariance between direct and maternal temporary environmental
σ^2_{GE}	Genotype-by-environment interaction

Genetic parameters of a single trait are any function of variance components resulting from practical implications. Generally, the ratio of some genetic variance to total variance is called the heritability coefficient and is denoted as h^2, previously classified into heritability in a narrow sense (defined as $\sigma^2_{G(d)A} / \sigma^2_P$) and heritability in a broad sense (defined as σ^2_G / σ^2_P).

In the past, heritability in the broad sense was usually treated as less useful than heritability in the narrow sense. From a practical point of view heritability in the narrow sense was more useful in realized breeding strategies. However, the current status of knowledge of phenotypic variance components makes it possible to estimate other heritabilities, which cover various sources of genetic variability. So, the ratios of particular genetic variance components to total variance can be estimated. For instance, the participation of additive direct variance in total variance is named additive direct heritability ($h^2_a = \sigma^2_{G(d)A} / \sigma^2_P$). Other single direct and maternal heritabilities can be defined in similar ways. It should be stressed that nowadays only some heritabilities and respective other functions of the variance components are reported in the literature and they will be presented here. Apart from the already mentioned additive direct heritability, the following heritabilities have been estimated: direct dominance ($h^2_d = \sigma^2_{G(d)D} / \sigma^2_P$), direct additive by additive ($h^2_{aa} = \sigma^2_{G(d)AA} / \sigma^2_P$) and maternal additive ($h^2_m = \sigma^2_{G(m)A} / \sigma^2_P$).

As already noted, genetic direct and maternal effects are correlated. Therefore, the respective covariance ($d_{am} = \sigma_{G(dm)A} / \sigma^2_P$) between these effects as proportional to total variance is also estimated. For easier interpretation, the dependence between direct and maternal effects can be expressed as a linear correlation ($r_{am} = \sigma_{G(dm)A} / \sqrt{\sigma^2_{G(d)A}\sigma^2_{G(m)A}}$). The literature supplies estimates of maternal permanent environmental variance and direct permanent environmental variance (when repeated measures per animal are included) as proportions of the total, denoted usually as c^2 and p, respectively. When genetic variances of uncorrelated effects are considered, the so-called total heritability (h^2_T) is defined as the sum of respective components to phenotypic variance. In the case of correlated effects (e.g. when direct and maternal additive variances are studied), the total heritability is calculated according to Willham (1972):

$$h^2_T = (\sigma^2_{G(d)A} + 0.5\sigma^2_{G(m)A} + 1.5\sigma_{G(dm)A}) / \sigma^2_P$$

The equation is equivalent to

$$h_T^2 = h_a^2 + \tfrac{1}{2}\,h_m^2 + \tfrac{3}{2}\,r_{am}h_a h_m,$$

where h_a and h_m are square roots of direct and maternal additive heritability coefficients, respectively.

Another reported genetic parameter for a single trait (for repeated observations) is the repeatability coefficient (r). Generally, repeatability may be expressed as follows: $r = (\sigma_G^2 + \sigma_{E(d)p}^2 + \sigma_{E(m)p}^2)/\sigma_P^2$. It must be noted that repeatability is usually considered in a simpler genetic model. Thus, it is expressed as the sum of genetic and permanent environmental variance to total variance.

Why are not all functions of variance components evaluated? First, some estimates of the variance components are negligible and their accurate estimation is rather difficult. Secondly, the variances may be confounded with other genetic or environmental effects. Thirdly, simultaneous estimation of the number of variance estimates is still computationally very demanding. Fourthly, estimation of the number of variance components requires a sufficient size of population with respective pedigree structure (relationships between individuals). Misztal *et al.* (1995) reported that evaluation of the dominance variance (for a level of significance equal to 0.05) requires about 20 times more data than estimation of the additive variance. Thus, for estimation of dominance direct variance with 3% accuracy, about 65,000 individuals are needed; and to estimate three genetic direct variances (additive, dominance, and additive by additive) about 380 times more data (compared with a simple additive genetic model) is necessary.

The next parameter covering joint genetic variability of two traits is genetic correlation. From a theoretical perspective, the correlation coefficient may be estimated for any genetic effects of two traits (direct additive, direct dominance, direct additive by additive, etc.). In practice, only the correlation between genetic direct additive effects (the so-called breeding value) is evaluated. Thus, the correlation coefficient (r_G) is defined as follows: $r_G = \sigma_{G(d)A,XY}\,/\,\sqrt{\sigma_{G(d)A,X}^2\,\sigma_{G(d)A,Y}^2}$, where $\sigma_{G(d)A,XY}$ is the genetic direct additive covariance between traits X and Y. The correlations are determined by pleiotropy phenomena and linkage between quantitative loci. The magnitude of the correlation coefficient can also be influenced by factors disturbing a Hardy–Weinberg equilibrium (e.g. selection, gene migration).

Motivation

Poultry breeding programmes are aimed at improving the genetic potential for economic traits through selection and cross-breeding plans. Initially, genetic parameter estimates were used in the prediction of selection responses. Currently, an application of mixed model methodology (leading to the best linear unbiased prediction of genetic effects) requires knowledge of variance components (direct additive genetic variance and residual variance for the simplest animal model). Additionally, use of multiple-trait genetic evaluation is connected with estimation of respective variances and covariances between them. However, when the direct additive genetic model is insufficient for describing the genetic variability of a trait, other genetic effects should be included in the model.

Generally, there are three main reasons for estimating non-additive and maternal genetic variances (e.g. Wei and Van der Werf, 1993): (i) an unbiased estimation of heritability in a narrow sense (ignoring these effects will lead, first of all, to overestimation of error variance); (ii) more accurate prediction of breeding values; and (iii) usage of these genetic effects in crossbreeding. As already mentioned, crossbreeding has become a standard practice in poultry breeding programmes. On the other hand, it may be noted that non-additive effects (especially dominance) were the first hypotheses of heterosis phenomena (Bruce, 1910). Mating of individuals from different breeds (genetic groups) may lead to a large expression of interaction within and between loci as well as specific genetic effects.

Methods of Estimation –
from a Historical Perspective

Basically, three main approaches to the estimation of genetic parameters are described in the literature. The first, called realized heritability, is defined as ratios of selection responses to selection differences under long-term selection over many generations. Hence, the method is not preferred for the estimation of genetic parameters from both field-collected data (with number of source of trait variability) and crossbreeding data. Additionally, the objective of this approach is to examine the intensity of the selection performed. More details concerning the estimation of realized heritability are given by Dickerson and Grimes (1947).

The second approach to the estimation of the genetic parameters is based on the parent–progeny resemblance (in the case of heritability and genetic correlation) and similarity of earlier and later measurements of the character (for repeatability). Resemblance for single traits is evaluated by the use of linear regression. Thus, the heritability (or repeatability) estimator is expressed by the linear regression coefficient (Kempthorne and Tandon, 1953). Offspring–parent genotypic regression is linear if the quantitative trait is additive. Unfortunately, this genotypic regression is influenced by non-genetic effects (Gimelfarb, 1986) as well as environmental factors. Moreover, from a practical point of view the method has strong assumptions. It should be noted that Hazel (1943) introduced an analogous method, based on parent–progeny resemblance, for the estimation of genetic correlation. The regression methods were not widely applicable in poultry and livestock populations.

The third approach is derived from intra-class correlation formulae. Thus, the variance and covariance components were estimated for half-sib and/or full-sib groups. Breeding flocks of domestic fowl are characterized by a typical hierarchical structure (when 10–20 females mate to one male). Hence, a given poultry trait can be described by following equation (linear model):

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z_1 s} + \mathbf{Z_2 f} + \mathbf{e}$$

where $\mathbf{y}$ is the $n \times 1$ observation vector (n is the number of observations, recorded individuals), $\mathbf{\beta}$ is the $p \times 1$ vector of fixed effects (e.g. hatch period), $\mathbf{s}$ is the $N \times 1$ vector of random effects of sires (N is the number of sires), $\mathbf{f}$ is the m $\times$ 1 vector of random effects of dams, $\mathbf{e}$ is the $n \times 1$ vector of residual effects, and $\mathbf{X}$, $\mathbf{Z_1}$ and $\mathbf{Z_2}$ are the $n \times p$, $n \times s$ and $n \times m$ incidence matrices associated with these effects, respectively. The analysis of the model is based on the following assumptions:

$$E\begin{bmatrix} \mathbf{s} \\ \mathbf{f} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \end{bmatrix} \text{ and } V\begin{bmatrix} \mathbf{s} \\ \mathbf{f} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{I}\sigma_s^2 & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{I}\sigma_f^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_e^2 \end{bmatrix}$$

where s_s^2, s_f^2, s_e^2 are the sire, dam and residual variances, respectively, $E[.]$ is the expected values, $V[.]$ is the (co)variance and $\mathbf{I}$ is the identity matrix. It implies that $\mathbf{y} \sim N(\mathbf{X\beta}, \mathbf{Z_1 Z_1'}\sigma_s^2 + \mathbf{Z_2 Z_2'}\sigma_f^2 + \mathbf{I}\sigma_e^2)$. So, it assumed that both sires and dams are unrelated. Moreover, the distribution of traits should be normal. Several statistical tools are available to estimate these variance components. The ANOVA (analysis of variance) estimation from balanced data – the same number of observations in each of the subclasses (here: full-sib groups) – has several important properties, such as translation invariance (estimators are not affected by changes in the fixed effects), unbiasedness (when the expected value of the parameter estimator is equal to the parameter), etc. However, the approach does not guarantee unbiasedness from the selection data and non-negative variance component estimates. Unfortunately, as already mentioned, the sizes of full-sib families are usually unbalanced, which leads to biased estimates of variance components. Hence, new methods for unbalanced data have been developed. Henderson (1953) introduced three methods (known in the literature as Henderson's methods) that are adaptations of the ANOVA. Henderson's method I covers a random model, Henderson's method II corrects data for fixed effects while Henderson's method III is applicable to all

general mixed models and has some advantages over methods I and II. Subsequent approaches to the estimation of variance components MINQUE (minimum norm quadratic unbiased estimation) and MIVQUE (minimum variance quadratic unbiased estimation) have been developed independently by Rao (1971) and La Motte (1973). These methods have considerable properties, but still do not guarantee two desirable features: (i) unbiasedness influenced by selection; and (ii) non-negativity of variance component estimates (error variance estimates are always positive, but other variance estimators can yield a negative value). For this reason, these approaches, and similarly the maximum likelihood method (downwardly biased because of the loss of degree of freedom due to leaving fixed effects out of consideration; for more details see, e.g. Hofer, 1998), are not widely applicable in livestock and poultry breeding. Searle (1989) reported that when a negative estimate of variance (which by definition is positive) is obtained, the following four courses of action are possible: (i) use of null instead of negative estimator; (ii) re-estimation of the variance components after a new modelling of data; (iii) collection of more data and re-estimation; and (iv) use of another method to estimate variances. Thus, the situation inspired a search for new methods, mainly restricted maximum likelihood (REML) (Patterson and Thompson, 1971) and related algorithms. These will be presented in more detail later.

Computations performed via these methods are usually based on the classical sire–dam model. The heritability estimator may be obtained simultaneously from the following three functions of variance components: sire component $\hat{h}_s^2 = 4\hat{\sigma}_s^2 / (\hat{\sigma}_s^2 + \hat{\sigma}_f^2 + \hat{\sigma}_e^2)$, dam component $\hat{h}_f^2 = 4\hat{\sigma}_f^2 / (\hat{\sigma}_s^2 + \hat{\sigma}_f^2 + \hat{\sigma}_e^2)$ and joint sire and dam components $\hat{h}_{(s+f)}^2 = 2(\hat{\sigma}_s^2 + \hat{\sigma}_f^2) / (\hat{\sigma}_s^2 + \hat{\sigma}_f^2 + \hat{\sigma}_e^2)$. Thus, the estimates are frequently different, because in conventional methods of statistical analysis it is not possible to estimate direct additive genetic variance free from the influence of other genetic effects. The different formulae for heritability estimates

complicate the next steps of genetic evaluations. On the other hand, the procedure may supply approximate information about some non-additive and maternal genetic variances. When the direct dominance and/or maternal effects are considerable, the dam variance is overestimated. On the contrary, the occurrence of additive by additive effects leads to an increase in the sire variance estimator. The structure of sire and dam variances under a hierarchical two-way model is as follows (Tempelman and Burnside, 1990):

Sire/dam component	Causal components
σ_s^2	$\dfrac{1}{4}\sigma_a^2 + \dfrac{1}{16}\sigma_{aa}^2$
σ_f^2	$\dfrac{1}{4}\sigma_a^2 + \dfrac{1}{4}\sigma_d^2 + \dfrac{3}{16}\sigma_{aa}^2 + \dfrac{1}{8}\sigma_{ad}^2 +$
	$\dfrac{1}{16}\sigma_{dd}^2 + \sigma_m^2 + \sigma_c^2 + \sigma_{ec}$

where σ_a^2 is direct additive variance, σ_{aa}^2 is additive by additive variance, σ_d^2 is direct dominance variance, σ_{ad}^2 is direct additive by dominance variance, σ_{dd}^2 is direct dominance by dominance variance, σ_m^2 is maternal variance, σ_c^2 is cytoplasmic variance, and σ_{ec} is covariance between direct and maternal permanent environmental effects.

Additionally, some authors (Wei and Van Der Werf, 1993) have suggested that sire variance estimates may also be influenced by sex-linked effects.

Some non-additive and maternal heritabilities can be estimated from sire and dam variance components but an approximate evaluation is performed under strong assumptions. The dominance heritability (Tempelman and Burnside, 1990) is estimated as follows:

$$\hat{h}_d^2 = 4(\hat{\sigma}_f^2 - \hat{\sigma}_s^2) / (\hat{\sigma}_s^2 + \hat{\sigma}_f^2 + \hat{\sigma}_e^2)$$

However, the other causal components are assumed to be non-significant. By contrast, to estimate the maternal heritability, dominance variance and other causal components are taken as null. Thus, the maternal heritability estimator (Tempelman and Burnside, 1990) can be expressed as:

$$\hat{h}_m^2 = (\hat{\sigma}_f^2 - \hat{\sigma}_s^2) / (\hat{\sigma}_s^2 + \hat{\sigma}_f^2 + \hat{\sigma}_e^2)$$

Before the 1990s the sire–dam model was usually applied to estimate genetic parameters of poultry performance traits. Hence, single generations of the recorded individuals were separately included in the analysis. However, Dong *et al.* (1988) reported that full relationships from ancestors of about two generations resulted in slighty larger estimates of heritability as compared with relationships to one generation. Application of the animal model seems to be necessary to estimate the genetic parameters.

To recapitulate: over many decades the conventional methods based on a sire–dam model were sufficient for poultry breeding programmes. First of all, this was due to the fact that some bird characteristics, related to their biology, facilitate the intensification of breeding and production (Hartmann, 1989). On the other hand, the approach does not allow estimation of particular genetic and environment variance components, especially non-additive and maternal variances. Moreover, the methodology may lead to biased estimators of variance components by selection. Additionally, these variance estimates may be negative and, in consequence, complicate the use of parameters in further genetic evaluation.

REML Estimation Under an Animal Model

Some aspects of theoretical backgrounds and related problems

In recent years, restricted (residual) maximum likelihood (REML) under an animal model has emerged as the main method of choice in livestock and poultry breeding for variance components. As already mentioned, the more sophisticated methods such as MINQUE, MIVQUE and ML estimations in mixed models have several disadvantages. As REML is a modification of ML, it removes bias from ignoring the loss in degrees of freedom due to fitting of fixed effects by maximizing only the part of the likelihood independent of the fixed effects

(Patterson and Thompson, 1971). The natural logarithms of the likelihood function of $\mathbf{K'y}$ (the so-called error contrasts) are expressed as:

$$\ln(\boldsymbol{\theta}; \mathbf{K'y}) = -0.5[(N - r(\mathbf{X}))\ln(2\pi) + \ln|\mathbf{K'VK}| + \mathbf{y'K}(\mathbf{K'VK})^{-1}\mathbf{K'y}]$$

or

$$\ln(\boldsymbol{\theta}; \mathbf{K'y}) = \mathbf{C} - 0.5\ln|\mathbf{V}| - 0.5\ln|\mathbf{X}^{*\prime}\mathbf{V}^{-1}\mathbf{X}^*| - 0.5(\mathbf{y} - \mathbf{X}\tilde{\boldsymbol{\beta}})'\mathbf{V}^{-1}(\mathbf{y} - \mathbf{X}\tilde{\boldsymbol{\beta}}) \quad (1)$$

where $\boldsymbol{\theta}$ is the vector of unknown variance components; $\mathbf{K}$ is the matrix that contains any $r(\mathbf{X})$ linearly independent rows of $\mathbf{I} - \mathbf{X}(\mathbf{X'X})^{-1}\mathbf{X'}$; $\mathbf{X}$ is the incidence matrix for fixed effects; $\mathbf{y}$ is the observation vector; $\mathbf{C}$ is the constant; $\mathbf{X}^*$ is the full column rank submatrix of $\mathbf{X}$; $\mathbf{V}$ is the (co)variance matrix of $\mathbf{y}$; $\tilde{\boldsymbol{\beta}}$ is the vector of estimators of fixed effects found according to the formula: $(\mathbf{X'V}^{-1}\mathbf{X})^{-1}\mathbf{X'V}^{-1}\mathbf{y}$; and $r(.)$ is a rank of matrix.

The REML methodology has been described by many authors (e.g. Meyer, 1985). Generally, the approach can be applied to several types of models, including the sire–dam model, and this is the model for which the backgrounds of REML will be presented here. Thus, for three estimated variance components ($\sigma_s^2, \sigma_f^2, \sigma_e^2$) the REML equations to be solved (Meyer, 1985) are then given by:

$$\mathbf{y'P}\frac{\partial \mathbf{V}}{\partial \sigma_i^2}\mathbf{Py} = \sum_{n=1}^{3} tr\left(\mathbf{P}\frac{\partial \mathbf{V}}{\partial \sigma_i^2}\mathbf{P}\frac{\partial \mathbf{V}}{\partial \sigma_j^2}\right)\sigma_j^2 \quad (2)$$

where $\mathbf{P}$ is the projection matrix outlined as: $\mathbf{P} = \mathbf{V}^{-1} - \mathbf{V}^{-1}\mathbf{X}(\mathbf{X'V}^{-1}\mathbf{X})^{-1}\mathbf{X'V}^{-1}$. In our case: $\mathbf{V} = \mathbf{Z_1Z_1'}\sigma_s^2 + \mathbf{Z_2Z_2'}\sigma_f^2 + \mathbf{I}\sigma_e^2$, $\boldsymbol{\theta}' = [\sigma_s^2, \sigma_f^2, \sigma_e^2]$ and $tr(.)$ is a matrix trace (a sum of diagonal elements). The equations can be summarized as:

$$\mathbf{B}\boldsymbol{\theta} = \mathbf{w} \quad (3)$$

where $\mathbf{B}$ here is the 3×3 matrix with $b_{ij} = tr(\mathbf{PV}_i\mathbf{PV}_j)$, $i, j = 1, 2, 3$. Matrices $\mathbf{V}_i$ and $\mathbf{V}_j$ are defined as follows: $\mathbf{V_1} = \mathbf{Z_1Z_1'}$, $\mathbf{V_2} = \mathbf{Z_2Z_2'}$, $\mathbf{V_3} = \mathbf{I}$. The ith element of vector $\mathbf{w}$ is described as: $\mathbf{w}_i = \mathbf{y'PV}_i\mathbf{Py}$. Hence, $\mathbf{V}$ can be rewritten as:

$$\mathbf{V} = \sum_{i=1}^{3} \mathbf{V}_i\sigma_i^2$$

Solution of equation (3) by iterations leads to restricted maximum likelihood estimates of variance components. However, the results from first iteration are identical to those of MIVQUE. It should be noted that the non-negativity requirement for variance estimates is guaranteed by the use of the iteration process. The REML estimation removes two main disadvantages of earlier approaches: bias from selection and negativity of variance estimates.

Although computer power doubles every 18 months (Misztal, 1999), the direct solving of equation (3) is computationally very demanding. From a practical point of view the algorithm can be used to estimate the variance components from only a small data set under a simple linear model. Unfortunately, poultry data sets usually have many thousands of records. Moreover, large data are necessary for accurate estimation of some genetic variances. Implementation of an animal model for genetic evaluation is connected with more complex structures of random effect variances; for example, the inverse of relationship matrices are needed (more details are given below). Computing requirements also increase when a multiple-trait model is analysed. Hence, easier numerical algorithms (REML or approximated REML) were gradually developed.

The following main approaches to obtaining REML estimates (e.g. Hofer, 1998) have been developed on the basis of: (i) first-order partial derivatives; (ii) no derivatives (so-called derivative-free); and (iii) first- and second-order partial derivatives. The first of these approaches is based on computation of derivatives of the REML function. It can be derived in mixed model equations mainly via the expectation–maximization algorithm introduced by Dempster *et al.* (1977) or the Newton–Rapshon algorithm described by Press *et al.* (1989). Since the rate of convergence of the algorithms is relatively slow, the algorithms may be used for relatively simple models. In the case of more complex genetic models, computations of the derivatives are difficult. More details concerning the numerical algorithm were given by Henderson (1984).

Recently, the popular algorithm in estimating genetic parameters has been the derivative-free (DF) algorithm introduced by Graser *et al.* (1987). DF requires the calculation of the determinant in each iteration and uses search procedures to locate the maximum of restricted likelihood function, e.g. the simplex algorithm of Nelder and Mead (1965). The simplex procedure has proved to be easy and robust for use with numerous genetic models including different random effects with various covariance structures (Meyer, 1989, 1991). In contrast to the derivative algorithm the DF procedure is strongly dependent on the number of traits as well as the type of algorithm (Misztal, 1994). For example, Campos *et al.* (1994) obtained very different heritability estimates of yield and reproductive traits (in dairy cattle) from single- and two-trait models via DF. Investigations carried out by Misztal (1994) suggested that for a three-trait model, the Powell algorithm of DF is already twice as fast as the simplex procedure. It should be noted that DF convergence depends also on the priors. In general, this algorithm has relative computational feasibility but is likely to have insufficient numerical properties, especially when more variance components are to be estimated.

In the third approach, the so-called average information restricted maximum likelihood (AIREML) has been described by Johnson and Thompson (1995). The method is based on average and observed information. Sparse matrix techniques are employed to derive the coefficient matrix required to calculate the first partial derivatives of the likelihood function. Averages of observed and expected values are used to compute second derivatives of the likelihood. The use of the AIREML procedure for multiple-trait analysis (with one random effect) resulted in the shortest computing time compared with the two approaches mentioned above (Ashida and Iwaisaki, 1998). However, Johnson and Thompson (1995) pointed out that the use of AIREML is correct when the parents are not selected, but discrepancy between the average observed data increases if a selected population is analysed. Thus, the number of iterations may also increase.

These three numerical approaches can be employed to analyse data under an animal model (see below). However, the literature also supplies several numerical procedures, called approximative REML, to reduce the computational demands; these include Henderson's method IV (Henderson, 1980), the pseudo-expectation procedure (Schaeffer, 1986), the tilde-hat procedure (Van Raden and Jung, 1988) and the modified pseudo-expectation method (Iwaisaki, 1992). It must be stressed that a majority of them have several desirable features (e.g. applicability to very large data sets), but can also work with relatively simple linear models (excluding animal models).

Alternative approaches to REML as well as statistical models to estimate variance components are still being developed. They will be characterized briefly in the next sections.

Some single-trait genetic animal models

Knowledge of genetic variances is necessary for the use of the BLUP of considerable genetic effects. These two stages of analysis must be based on the same statistical model. As already mentioned, certain classes of models (the so-called animal models) due to their versatility, incorporating known relationships between individuals into variance–covariance matrices of random effects, have been widely used in livestock and poultry programmes. The term 'animal model' originated from a paper by Quaas and Pollak (1980) on reduced animal models. Although poultry populations have a typical hierarchical structure, advantages of the animal model to the description of variability of genetic effects have been reported by many authors (e.g. Wężyk and Szwaczkowski, 1997). Currently, numerous types of animal model are known. The following are the main ones, which seem to be important in poultry genetic improvement.

Additive genetic linear model

The model can be expressed as:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Za} + \mathbf{e}$$

where $\mathbf{y}$ is the $n \times 1$ vector of observation (n is the number of single records of individuals), $\mathbf{\beta}$ is the $p \times 1$ vector of fixed effects, $\mathbf{a}$ is the $q \times 1$ vector of random additive direct genetic effects (q is the number of evaluated individuals), $\mathbf{e}$ is the $n \times 1$ vector of residual effects, and $\mathbf{X}$ and $\mathbf{Z}$ are the $n \times p$ and $n \times q$ known incidence matrices associated with respective effects.

The model assumes that $\mathbf{a} \sim N(\mathbf{0}, \mathbf{A}\sigma_a^2)$ and $\mathbf{e} \sim N(\mathbf{0}, \mathbf{I}\sigma_e^2)$ as well as $\text{cov}(\mathbf{a}, \mathbf{e}) = \mathbf{0}$. Thus $\mathbf{y} \sim N(\mathbf{X\beta}, \mathbf{ZAZ'}\sigma_a^2 + \mathbf{I}\sigma_e^2)$. Here $\mathbf{A}$ is the $q \times q$ additive relationship matrix (e.g. Quaas, 1976) and $\mathbf{I}$ is the $n \times n$ identifying matrix.

It implies that both the genetic and phenotypic distributions of the trait studied have to be normal. In others words, this trait should be determined by many loci (polygenic model) with numerous phenotypes. Moreover, assumptions concerning other negligible genetic effects are necessary. The model is widely used in many poultry reports for various performance traits.

More complex genetic models to analyse some trait are now strongly recommended (Koerhuis and Thompson, 1997; Sewalem, 1998; Misztal and Besbes, 2000; Anang *et al.*, 2001).

Additive genetic threshold model

Some poultry traits (e.g. hatchability, fertility, disease resistance) are measured on a discrete scale and determined by many loci (polygenic). It is assumed that response (phenotype) is related to an underlying normal variable (genetic), usually called liability. Threshold concepts of trait analysis were introduced by Dempster and Lerner (1950). However, theoretical backgrounds of analysis of the discrete data were given by Gianola and Foulley (1983). On the basis of the theory, discrete phenotypic observations are arranged into a contingency table with s rows representing combinations of subpopulations (variants of studied effects) and m columns representing classes of phenotypes.

Liability (l_{ij}) is described by the following equation (Matos et al., 1997):

$$l_{jk} = \eta_j + e_{jk}$$

where $j = 1, 2, \ldots, s$ and $k = 1, 2, \ldots, n_j$; η_j is the location parameter of jth combination of included effects (explanatory variables); and e_{jk} is the random error. It is assumed that $e_{jk} \sim N(0, 1)$.

The above equation can be rewritten (corresponding to the linear model) as:

$$\mathbf{l} = \mathbf{W\Theta} + \mathbf{e}$$

where $\mathbf{W}$ is the blockmatrix as follows: $\mathbf{W} = [\mathbf{X}, \mathbf{Z}]$, $\mathbf{\Theta}' = [\mathbf{\beta}', \mathbf{a}']$. Thus (Matos et al., 1997):

$$\eta_j = \mathbf{v} + \mathbf{w}'_j \mathbf{\Theta}$$

where $\mathbf{v}$ is defined as the overall mean to all subpopulations (rows) in the contingency table; $\mathbf{w}'_j$ is the known incidence row vector; and $\mathbf{\Theta}$ is the vector of unknown location parameters.

Such specification of the model is necessary to construct a likelihood function. The variance components are usually estimated by using the expectation–maximization type algorithm based on the mixed model equations (under assumed stopped criteria for the iteration process).

Animal linear models for repeated records

The additive model is applied when the recorded individual has one measure of trait (e.g. slaughter body weight, age at first egg) as well as when the cumulative and/or test measure of a character is analysed (e.g. body weight, number of eggs). However, the longitudinal traits can be more frequently registered. For a joint analysis of repeated records various models can be used, but the most popular approach is the so-called repeatability model:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z_1 a} + \mathbf{Z_2 p_e} + \mathbf{e}$$

where $\mathbf{p_e}$ is the $q \times 1$ vector of random permanent environmental effects; $\mathbf{X}$, $\mathbf{Z_1}$ and $\mathbf{Z_2}$ are the $n \times p$, $n \times q$ and $n \times q$ incidence matrices for fixed, additive genetic and permanent environmental effects, respectively; and $\mathbf{y}$, $\mathbf{\beta}$, $\mathbf{a}$ and $\mathbf{e}$ are as above.

The (co)variance structure of random effects for the model is as follows:

$$V\begin{bmatrix} \mathbf{a} \\ \mathbf{P_e} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{A}\sigma_a^2 & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{I}\sigma_{pe}^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_e^2 \end{bmatrix}$$

where σ_{pe}^2 is the permanent environmental variance.

Recently, other models previously described for test day analysis in dairy cattle have also been implemented for poultry. For instance, Anang et al. (2001) reported results of comparative studies covering three approaches to the genetic evaluation of monthly egg production. They concluded that a random regression model could be the most favourable for the data. More details concerning the models (fixed and random regressions) are given by, among others, Jamrozik and Schaeffer (2000). In general, the genetic evaluation based on partial records is better than the cumulative productive approach. Thus, genetic parameters are estimated for particular period measures of the trait studied. Sometimes, a typical multiple-trait model is applied to analyse repeated records. Thus, partial observations are treated as single traits. In consequence, the covariances between partial records can be estimated.

Non-additive genetic linear models

When direct dominance genetic effects are important, the additive model described above (for single records) can be extended as follows:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Za} + \mathbf{Zd} + \mathbf{e}$$

where $\mathbf{d}$ is the $q \times 1$ vector of random dominance direct genetic effects; $\mathbf{y}$, $\mathbf{\beta}$, $\mathbf{a}$, $\mathbf{e}$, $\mathbf{X}$ and $\mathbf{Z}$ are as above.

The expected values for random effects are assumed to be zeros. Dispersion matrices are as follows:

$$V\begin{bmatrix} \mathbf{a} \\ \mathbf{d} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{A}\sigma_a^2 & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{D}\sigma_d^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_e^2 \end{bmatrix}$$

where σ_d^2 is the direct dominance genetic variance, and $\mathbf{D}$ is the dominance genetic

relationship matrix (see, e.g. Hoeschele and Van Raden, 1991).

Some authors (Wei and Van der Werf, 1993; Misztal and Besbes, 2000) suggested that dominance effects should be included in the model to analyse traits related to egg production. As already mentioned, in poultry the mating structure is hierarchical. The dominance variance estimates are mostly derived from full-sib covariances, but also common environmental and other specific variances. Thus, full-sib effects should be included in the model for a more accurate extraction of the dominance effects (Varona *et al.*, 1998; Misztal and Besbes, 2000).

It must be noted that estimation of dominance variances requires the inverse of dominance relationships matrices because, for large data sets, direct computing of the matrix inversion is still impossible. The algorithm to create the inverse of dominance relationship matrix directly from pedigree information for non-inbred populations has been described by Hoeschele and Van Raden (1991).

Estimation of dominance variance will also be biased if epistatic effects exist. Some authors (Sheridan and Randall, 1977; Fairfull and Gowe, 1987) have reported that epistatic effects are significant for egg production in line-crossing experiments. From a practical point of view an estimation of total epistatic variance from field-collected data is impossible. As already mentioned, the total epistatic variance can be divided into many components depending on the type of extra-allelic interaction. Moreover, when several epistatic effects are included in the model, estimation of these variance components could be very demanding. So, to estimate particular epistatic variances, the respective inverses of relationship matrices are necessary. Van Raden and Hoeschele (1991) discovered an algorithm to obtain the inverse of the additive by additive relationship matrix. In practice, however, only additive by additive variances are estimated (Szwaczkowski, 1999). The model to estimate additive by additive variance can be expressed in matrix notation as follows:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Za} + \mathbf{Zd} + \mathbf{Za:a} + \mathbf{e}$$

where **a:a** is the $q \times 1$ vector of random additive by additive direct genetic effects; **y**, **β**, **a**, **d**, **e**, **X** and **Z** are as above.

Thus, the following assumptions are made:

$$E\begin{bmatrix} \mathbf{a} \\ \mathbf{d} \\ \mathbf{a:a} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \end{bmatrix} \text{ and}$$

$$V\begin{bmatrix} \mathbf{a} \\ \mathbf{d} \\ \mathbf{a:a} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{A}\sigma_a^2 & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{D}\sigma_d^2 & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{A\#A}\sigma_{a:a}^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_e^2 \end{bmatrix}$$

In consequence, $\mathbf{y} \sim N(\mathbf{X\beta}, \mathbf{V})$, where $\mathbf{V} = \mathbf{ZAZ'}\sigma_a^2 + \mathbf{ZDZ'}\sigma_d^2 + \mathbf{ZA\#AZ'}\sigma_{a:a}^2 + \mathbf{I}\sigma_e^2$, $\sigma_{a:a}^2$ is the additive by additive variance; and $\mathbf{A\#A}$ is the additive by additive genetic relationship matrix (# denotes a Hadammard product of these additive relationship matrices).

Maternal linear models

In contrast to dominance and epistatic effects, maternal effects are not important for egg production. As noted in the previous section, they may be significant for early growth rate, viability and disease resistance as well as hatchability. These maternal influences are usually reported in the literature as maternal genetic additive (thus the covariance between direct and maternal effects is also considered) and maternal permanent environmental. A simple maternal model including genetic additive and permanent environmental effects can be written as:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Za} + \mathbf{Z_m m} + \mathbf{Z_c c} + \mathbf{e}$$

where **m** is the $q \times 1$ vector of random maternal additive genetic effects; **c** is the $m \times 1$ vector of random maternal permanent environmental effects; $\mathbf{Z_m}$ and $\mathbf{Z_c}$ are the $n \times q$ and $n \times m$ incidence matrices corresponding to respective effects; **y**, **β**, **a**, **e**, **X** and **Z** are as above; m is the number of dams.

The (co)variance matrix for the random effects of the model is:

$$V \begin{bmatrix} \mathbf{a} \\ \mathbf{m} \\ \mathbf{c} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{A}\sigma_a^2 & \mathbf{A}\sigma_{am} & \mathbf{0} & \mathbf{0} \\ \mathbf{A}\sigma_{am} & \mathbf{A}\sigma_m^2 & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_c^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_e^2 \end{bmatrix}$$

where σ_m^2 is the maternal additive genetic variance, σ_c^2 is the maternal permanent environmental variance, and σ_{am} is the covariance between direct and maternal additive genetic effects. Thus, the variance matrix of observation vector (**V**) is as follows:

$$\mathbf{V} = \mathbf{ZAZ'}\sigma_a^2 + \mathbf{Z_m AZ'_m}\sigma_m^2 + (\mathbf{ZAZ'_m} + \mathbf{Z_m AZ'})\sigma_{am} + \mathbf{Z_c Z'_c}\sigma_c^2 + \mathbf{I}\sigma_e^2$$

Recently, algorithms to estimate genetic maternal dominance variances have also been developed (Duangjinda *et al.*, 2001). However, evaluation of these effects is seriously limited by size of sample and number of estimated variance components. The modelling of these effects is similar to those presented above for additive genetic ones.

Maternal effects are also transmitted by the mitochondrial genome. To estimate a cytoplasmic variance, the maternal lineages should be determined by tracing female paths to the last identified female ancestor. Nowadays the cytoplasmic groups may be formed on the basis of the mitochondrial DNA polymorphism. A majority of such studies have been performed in cattle. It should be noted that, generally, two statistical approaches have been adopted when estimating the effects of maternal lineages, considered as random or fixed effects. When the effects are included in the model as fixed, *F* tests can be used to evaluate their significance.

Multiple-trait linear models

Generally, multiple-trait analysis is most advantageous (Mao, 1982) when: (i) some traits are measured on limited numbers of individuals and estimation accuracy may be attained by analysing these traits jointly with other traits, which were measured on more representative individuals of the population; (ii) the correlations between traits are high; and (iii) some individuals may

lack records on some traits as a result of selection on other traits.

A simple additive genetic model for *t* traits when each individual is recorded for each trait is:

$$\gamma = \omega\theta + \phi\alpha + \varepsilon$$

where γ is the $N \times 1$ vector of observations of *t* traits for *n* individuals (thus: $N = tn$), θ is the $P \times 1$ vector of fixed effects ($P = pt$, *p* is the number of all fixed parameters for each trait); α is the $Q \times 1$ vector of random direct additive genetic effects ($Q = qt$, *q* is the number of these effects for a single trait); ε is the $N \times 1$ vector of unknown random effects of residuals; ω and ϕ are the $N \times P$ and $N \times Q$ incidence matrices, respectively.

The observations are arranged so that vector γ has the following form: $\gamma = (\mathbf{y'_1}, \mathbf{y'_2}, \ldots, \mathbf{y'_t})'$. Thus, matrices ω and ϕ as well as vectors θ, α and ε have the following forms: $\omega = (\mathbf{I_t} \otimes \mathbf{X})$, $\phi = (\mathbf{I_t} \otimes \mathbf{Z})$, $\theta = (\mathbf{\beta'_1}, \mathbf{\beta'_2}, \ldots, \mathbf{\beta'_t})'$, $\alpha = (\mathbf{a'_1}, \mathbf{a'_2}, \ldots, \mathbf{a'_t})'$ and $\varepsilon = (\mathbf{e'_1}, \mathbf{e'_2}, \ldots, \mathbf{e'_t})'$. Here **X** and **Z** are the incidence matrices (equal) for single traits, respectively, and $\otimes$ denotes a Kronecker product. When these incidence matrices are unequal for the traits studied, some modifications in the formulae are needed.

For this model it is assumed that:

$$E\begin{bmatrix} \alpha \\ \varepsilon \end{bmatrix} = \begin{bmatrix} \mathbf{0} \\ \mathbf{0} \end{bmatrix} \text{ and } D\begin{bmatrix} \alpha \\ \varepsilon \end{bmatrix} = \begin{bmatrix} \mathbf{G} & \mathbf{0} \\ \mathbf{0} & \mathbf{R} \end{bmatrix}$$

Thus $\gamma \sim MVN(\omega\theta, \phi\mathbf{G}\phi' + \mathbf{R})$ with $\mathbf{G} = \mathbf{G_0} \otimes \mathbf{A}$ and $\mathbf{R} = \mathbf{R_0} \otimes \mathbf{I}$, where $\mathbf{G_0}$ is a $t \times t$ additive (direct) genetic variance–covariance matrix, $\mathbf{R_0}$ is a $t \times t$ residual variance–covariance matrix, **A** is the $q \times q$ additive relationship matrix (see above), and *MVN* is a multivariate normal distribution.

Generally, all methods and algorithms summarized previously for univariate analysis are applicable to multiple-trait estimation, but these are computationally very demanding. Although the number of equations is proportional to the number of traits studied, a computational requirement for *t*-trait analysis is more than *t* times that of univariate analysis. Additionally, application of certain algorithms (e.g. DFREML) is limited (see above section). Hence, some

simplifications of the multiple-trait analysis have been developed.

The first is based on the following formulae (Searle and Rousville, 1974):

$$\sigma^2_{(x+y)} = \sigma^2_x + \sigma^2_y + 2\sigma_{xy}$$

thus:

$$\sigma_{xy} = 0.5(\sigma^2_{(x+y)} - \sigma^2_x - \sigma^2_x)$$

where σ_{xy} is the covariance between traits x and y, $\sigma^2_{(x+y)}$ is the variance of sum of two traits, and σ^2_x and σ^2_y are these variances of traits x and y, respectively. This procedure is easy to use but some limitations exist. It cannot be employed for unpaired records or for models with covariance between random effects (e.g. including direct and maternal effects).

Secondly, the so-called canonical transformation of initial correlated traits to uncorrelated canonical traits can also be applied (Jensen and Mao, 1988). It leads to greatly reduced computational requirements. Several extensions have been developed, allowing, among others, an inclusion of more than one random effect and/or analysis of missing data (Gengler and Misztal, 1996).

Accuracy of estimates

Accuracy of obtained variance component estimates can be considered from two points of view: (i) goodness of fit of the studied model compared with other models; and (ii) standard deviation of the estimate.

Adequacy of model

Various criteria to compare a goodness of fit of the models have been reported in the literature. One of the simplest is mean squared error (MSE) and the model with smallest MSE is considered to be the best. Unfortunately, this inference is usually too broad and sometimes it may lead to wrong conclusions – for instance, when a fixed effect (model I) is included in model II as a random effect. As a consequence, the error variance estimate is smaller for model II though this model is not necessarily better.

In the case of sire and sire–dam models, statistical inferences about goodness of fit and accuracy of estimates can be performed by the use of classical approaches described for balanced and unbalanced experimental designs. The methodology includes numerous formulae for standard deviations, confidence intervals for genetic parameters and hypothesis testing (Ofversten, 1993).

These statistical methods cannot apply to REML under an animal model. As already noted, magnitude of residual variance estimator may be a criterion for choosing the best model. However, the complexity of random genetic effects summarized in the relationship matrix does not allow for the employment of other classical statistical tools.

The testing of a hypothesis about the significance of a particular genetic effect (in other words, the adequacy of two models) is usually performed using the likelihood ratio (LR) test. Thus, the test statistics are expressed as follows (Dobson, 1990):

$$\chi^2 = -2\ln\frac{L_I}{L_{II}}$$

to be equivalent to:

$$\chi^2 = -2(\ln L_I - \ln L_{II}) \sim \chi^2_{\alpha,r}$$

where $\ln L_I$ is the logarithm of a maximum likelihood function for a reduced model (without checked genetic effect), $\ln L_{II}$ is the logarithm of a maximum likelihood function for a full model (with checked genetic effect), α is the significance level and r is the degree of freedom, defined as the difference in the number of effects in a full model and a reduced one. Occasionally, the LR test does not give satisfactory results for statistical inferences, especially for a negligible magnitude of checked effects. Hence, other criteria have been developed.

One of the more popular approaches to ranking models according to their power to fit the animal data is Akaike's information criterion (AIC) presented by Akaike (1977). The criterion is not a statistical test but is very useful for finding the true genetic model (Thaller *et al.*, 1996). For the ith model, it is defined as:

$$\text{AIC}_i = -2\ln L_i + 2t_i$$

where $\ln L_i$ denotes a natural logarithm of an obtained maximum of likelihood function for the ith model and t_i is the number of independently estimated parameters on the basis of the ith model. The model reaching the smallest AIC fits the data best.

Standard deviation of estimates of variance component function

In general, the size of the sample and the magnitude of the true parameter determine the precision of estimates. Robertson (1959) proposed formulae to approximations of standard deviations of heritability, repeatability and genetic correlation estimates obtained from balanced data under a sire and/or sire–dam model. Unfortunately, the formulae cannot be applied to an animal model with typical data structure. The accuracy of estimates is also affected by numerical procedures.

As already mentioned, one of the most popular approaches in estimating variance components in the 1990s was the derivative-free restricted maximum likelihood (DFREML). To describe formulae for standard deviation of estimates, a simple genetic model including direct additive and residual effects will be considered. Thus, the logarithm of likelihood is approximated on the following second polynomial function:

$$a_0 + a_1\gamma + a_2\gamma^2 = \ln L(\gamma \mid \mathbf{y})$$

where $\gamma = \sigma_a^2/\sigma_e^2$. Thus $\text{var}(\hat{\gamma}) = (-2a_2)^{-1}$, hence standard deviation (SD) of estimate $\hat{\gamma}$ is expressed as $\text{SD}(\hat{\gamma}) = (-2a_2)^{-0.5}$. Since $h^2 = \sigma_a^2/(\sigma_a^2 + \sigma_e^2)$ and $\gamma = \sigma_a^2/\sigma_e^2$ the variance of heritability estimate can be described as follows:

$$\text{var}(\hat{h}^2) = \text{var}(\hat{\gamma})/(1 + \text{var}(\hat{\gamma}))$$

In consequence, an approximated standard deviation of heritability estimate is:

$$\text{SD}(\hat{h}^2) = \text{SD}(\hat{\gamma})/(1 + \text{SD}(\hat{\gamma}))^{0.5}$$

The approximation of standard deviations of estimates of genetic parameters can also be derived from the third polynomial function under various animal models. A simulation study conducted by Szwaczkowski *et al.* (1997) indicated that the obtained standard deviation estimates based on second and third polynomials were very similar. More details on sampling variances and confidence intervals for estimates of REML variance components are given by Meyer and Hill (1991).

Other Methods of Estimating (Co)variance Components Under an Animal Model

Method R

Although REML algorithms are very popular in estimation of (co)variance components in livestock and poultry data, sometimes these approaches are less attractive. In some situations, when very large data sets are analysed, application of the REML algorithms may be difficult. These procedures have been prohibitively expensive from a computational standpoint for routine application to field-collected data with unequal sizes of subclasses, even with assumed homogeneity of genetic and residual variances (Reverter *et al.*, 1994b).

Generally, the main idea of the method described by Reverter *et al.* (1994a) is based on a procedure to investigate whether changes in genetic predictions such as accuracy values increase over time, given that the expected value of the regression of recent on previous genetic prediction equals unity. Parameter estimation by method R, recommended for large data sets, is based on repeated solutions of mixed model equations. In the case of a simple genetic model, the estimate of h^2 is obtained by iterative computation of the following ratio (Reverter *et al.*, 1994a):

$$R = \frac{\hat{\mathbf{a}}'_{\mathbf{T}}\mathbf{A}^{-1}\hat{\mathbf{a}}_{\mathbf{p}}}{\hat{\mathbf{a}}'_{\mathbf{p}}\mathbf{A}^{-1}\hat{\mathbf{a}}_{\mathbf{p}}}$$

where $\hat{\mathbf{a}}'_{\mathbf{T}}$ is the vector of predictors of additive direct genetic effects of all individuals included in the analysis; $\hat{\mathbf{a}}_{\mathbf{p}}$ is the vector of predictors of additive direct genetic effects of some of the individuals (random subsets

of the complete data set) included in the analysis; and **A** is the additive genetic relationship matrix.

All values of R will be 1 for the parameters (e.g. heritability) appropriate for the population studied. Heritability coefficient is overestimated for $R < 1$, in contrast to $R > 1$ when h^2 is underestimated. Sampling procedures have been described by, among others, Cantet *et al.* (2000). It can be noted that estimation of variance components via method R is based on standard approaches outlined in the past for solution of mixed model equations. Some of the theoretical properties of estimates of R are not known. Cantet *et al.* (2000) concluded that estimates of additive genetic variances via method R are unbiased for both selected and unselected data. Van Tassell *et al.* (2000) reported that the difficulty of this method, when applied to multiple random effects, is that constrained optimalization is needed to obtain parameters that result in all regressions of 1.

Currently, the method is applied to dairy cattle data to estimate direct additive and dominance variances (Misztal *et al.*, 1997). Misztal and Besbes (2000) also employed the method to analyse laying hen data.

Standard errors of variance component estimators can be approximated by repeating R analyses many times and computing the standard deviations of obtained estimates. Details of approximation of standard deviation and confidence intervals for heritability estimated by method R are given by Mallinckrodt *et al.* (1997).

Cantet *et al.* (2000) concluded that estimates of h^2 by method R are more variable than REML estimates, either for unselected data or for data undergoing selection, with a complete or an incomplete relationship matrix. Additionally, the difficulty of this approach when used to multiply random effects is that constrained optimization is needed to obtain parameters that result in all regressions of 1.

Gibbs sampling

It should be recalled that all of the above methods and algorithms give point estimates, which, in practice, is very convenient. However, from the Bayesian perspective, a single sample is only one of numerous realizations. Although many Bayesian methods (Gilks *et al.*, 1997) are currently available, the Gibbs sampling approach (Geman and Geman, 1984) is widely used in livestock and poultry breeding. Gibbs sampling has several advantages over REML algorithms. First of all, it can be used to estimate the whole posterior densities of variance component estimates instead of only their maximum points. Moreover, confidence intervals can be derived for the estimates of variances with skewed distributions. Thus, simple tests are applied to determine significance of estimates. It should be noted that the use of Gibbs sampling often reduces the demand for computer memory and very large data sets may be analysed. However, the application of Gibbs sampling for complex genetic models may be more complicated.

Gibbs sampling is a Monte Carlo method of numerical integration and has been used to marginalize densities in various procedures. It allows inferences to be made about joint and marginal distributions. In the Bayesian framework all analysed parameters (genetic and environmental effects, 'traditional' fixed effects, regression coefficients and variance components) are considered as random. This leads to assumptions about distributions of these parameters. More recently, this idea has been realized for both linear models (Su *et al.*, 1997) and threshold models (Sewalem *et al.*, 1998).

The algorithm is applied by generating a random value for each parameter in turn, assuming other parameters are known (thus treated as known). For a simple genetic model (including fixed, direct additive genetic and residual effects) the following full conditional densities are required: $f(\mathbf{a} \mid \boldsymbol{\beta}, \mathbf{y})$ and $f(\boldsymbol{\beta} \mid \mathbf{a}, \mathbf{y})$ to estimate $f(\mathbf{a} \mid \mathbf{y})$, $f(\boldsymbol{\beta} \mid \mathbf{y})$ and $f(\mathbf{a}, \boldsymbol{\beta} \mid \mathbf{y})$.

To initiate the sampling process, starting values are necessary. The distribution of all the parameters is as follows:

$$p(\mathbf{y}, \mathbf{a}, \boldsymbol{\beta}, \sigma_a^2, \sigma_e^2) = p(\mathbf{y} \mid \mathbf{a}, \boldsymbol{\beta}, \sigma_e^2) p(\mathbf{a} \mid \sigma_a^2)$$
$$p(\boldsymbol{\beta}) p(\sigma_a^2, \sigma_e^2)$$

Since no knowledge about fixed effects is available, it is assumed that the prior distribution of $\boldsymbol{\beta}$ is the distribution with the density function proportional to a constant (thus: $f(\boldsymbol{\beta}) \propto$ constant).

As already noted, the genetic effects are assumed to be normally distributed. Thus:

$$p(\mathbf{a}|\sigma_a^2) \sim N(\mathbf{0}, \mathbf{A}\sigma_a^2) \text{ (it can also be noted}$$
$$\text{as: } \mathbf{a}|\sigma_a^2 \sim N(\mathbf{0}, \mathbf{A}\sigma_a^2))$$

Variance components are assigned scaled inverted chi-squared distributions:

$$p(\sigma_a^2, \sigma_e^2) \propto \left(\frac{1}{\sigma_a^2}\right)^{\frac{\nu_a}{2}+1} e^{\frac{-\nu_a S_a^2}{2\sigma_a^2}}$$
$$\left(\frac{1}{\sigma_e^2}\right)^{\frac{\nu_e}{2}+1} e^{\frac{-\nu_e S_e^2}{2\sigma_e^2}}$$

where ν_a and ν_e can be interpreted as degrees of freedom parameters, and S_a^2 and S_e^2 are prior values for σ_a^2 and σ_e^2, respectively.

So, under independent normally distributed errors, $\mathbf{y}$ has the following density distribution:

$$p(\mathbf{y}|\boldsymbol{\beta}, \mathbf{a}, \sigma_a^2) \sim N(\mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{a}, \mathbf{I}\sigma_e^2)$$

Series of values for each parameter are generated over many rounds. Since the consecutive samples are positively correlated, to obtain independent samples (needed for posterior density estimation) only some of them (for instance each 500th) are stored. The number of rounds between uncorrelated samples is called a thinning interval. The initial period of sampling (called the burn-in period) is also rejected. A diagnosis of the convergence of the process is necessary. Details concerning convergence criteria are given by, among others, Gelman and Rubin (1992) and Raftery and Lewis (1992).

In contrast to traditional point estimation, the result of this sampling process is a posterior distribution of each parameter. For fairly symmetrical one-mode distribution, the inference about any parameter is relatively easy. Thus, estimates based on posterior means and modes have quite similar properties. However, distributions of posterior densities are frequently skewed. Thus, the mean (average) or mode of the distribution is considered as the 'true' (point) estimate.

Simulation investigation carried out by Van Tassell *et al.* (1995) showed that genetic additive variance estimates derived from the mean of the distribution have smaller mean squared errors than those derived from the mode. They concluded that Gibbs sampling mode estimates may be biased when variance components are small and the posterior distribution is skewed. It should be stressed that a mode inference is preferred in some situations – for instance, in analysis of mixed inheritance models when a locus with large effects is detected.

Some comparisons of results from Gibbs sampling and other approaches are also reported in the literature. Wright *et al.* (2000) concluded that classical approaches (BLUP using REML estimates of variance components) and Bayesian analysis (BLUP using posterior mean estimates) lead to different predictions of direct additive genetic effects. Van Tassell *et al.* (1995), however, pointed out a similarity between Gibbs sampling mean and REML estimates of variance components, especially for highly heritable traits. Moreover, the authors showed that the mean squared error of genetic variance estimates is smaller for Gibbs sampling mean (for various types of population structures) than for REML, MIVQUE and Gibbs sampling mode estimates. It seems that further comparative studies covering Bayesian and classical point estimation should be performed.

Available Computer Package Programs

Public animal breeding software is available that uses mixed model methodology based on linear and threshold animal models as well as Bayesian methods (Gibbs sampling). Most of these packages are applicable due to different models, methods and species orientations. Basically, all may be employed to obtain estimates of genetic parameters, fixed effects and regression coefficients for covariables as well as predictions of random effects. Otherwise, in the case of more

sophisticated problems, the programs differ in some features (number of simultaneously studied traits, testing hypothesis in mixed model equations). The following packages are usually used in animal breeding: ASREML (Gilmour *et al.*, 1998), DFREML (Meyer, 1993), DMU (Jensen and Madsen, 1995), MTDFREML (Boldman *et al.*, 1993), MTGSAM (Van Tassell and Van Vleck, 1995), PEST/VCE (Groeneveld, 1998) and REMLF90 (Misztal, 1998). In practice, these computer packages may be used for the estimation of genetic parameters in poultry.

Overview of Genetic Parameter Estimates Reported in the Literature

Numerous estimates of genetic parameters for performance traits in birds can be found in the literature. These reported estimates vary widely across authors, years, methods and genetic groups (lines, strains) for the same traits. Although a majority of the studied estimates concern chickens, several species of poultry are considered. Hence, to clarify this review, genetic parameters are divided as follows: egg production, egg quality, body weight and meat production, reproduction, feed efficiency, disease resistance and conformation, and behaviour. The review presents estimates from recent years only.

Egg production traits

Since about 1950, egg production by laying hens has increased markedly. Albers (1998) reported that the average number of eggs per hen per year increased from 190 in 1950 to 309 in 1998. The total (phenotypic) progress was achieved through a combination of environmental changes and genetic improvement programmes, especially selection and crossbreeding. On the other hand, several traits (e.g. body weight, sexual maturity) were included in layer selection. Hence, there are questions about how the improvement schemes influenced the heritability estimates of egg production

traits and their relationship with other controlled characters.

Table 11.1 gives some estimated heritabilities of egg production traits. These estimates vary from 0.11 (Szydłowski and Szwaczkowski, 2001) to 0.53 (Wei and Van der Werf, 1993). Although these heritabilities were obtained for various populations in different periods (both times and lengths) of examination of the number of eggs, many authors reported that the genetic parameters are strongly influenced by the methodological approach.

Generally, the review shows that several new aspects of genetic parameter estimations for egg production were developed in the 1990s. However, it should be noted that classical analysis of variance under both sire and sire–dam models is still applied. According to current knowledge the estimates obtained via the classical models are biased for several reasons, including the ignoring of relationships other than parents–progeny as well as genetic dominance effects. This corresponds with the results obtained by Wei and Van der Werf (1993), Besbes and Gibson (1999) and Misztal and Besbes (2000). Wei and van der Werf (1993) also concluded that an additive model including all relationships leads to over-estimated heritability (in the narrow sense) because it ignores dominance effects. Misztal and Besbes (2000) suggested that, in the case of a complex pedigree structure (several generations included) of the full-sib, permanent environmental effects and inbreeding coefficients of recorded individuals (as covariable) should also be incorporated into the dominance effects. Some authors (Savas *et al.*, 1999; Misztal and Besbes, 2000) reported moderate estimates of inbreeding depression on egg production. It is known that the evaluation of inbreeding effects increases when more pedigree information is available. Hence, fewer negative inbreeding effects are detected due to a smaller number of generations studied (Misztal and Besbes, 2000). As has already been mentioned, egg production is also influenced by epistatic gene action and some authors (Sheridan and Randall, 1977; Fairfull and Gowe, 1987) have shown in

Table 11.1. Heritability estimates for egg production.

Trait	Species/population	Period	h_a^2	h_d^2	Method/model	Reference
Egg number	Pure/cross RIR × WPR	60 weeks	0.31		DFREML/AM	Hagger (1992)
	Two layer pure lines	19–26 weeks	0.25/0.27		EM-REML/AM	Besbes et al. (1992)
		26–38 weeks	0.09/0.13			
		26–54 weeks	0.18/0.16			
	Commercial laying stocks	26–32 weeks	0.10/0.26		DFREML/AM	Engström et al. (1992)
		33–39 weeks	0.10/0.26			
		40–46 weeks	0.04/0.28			
	Three lines WL	18–25 weeks	0.48–0.53	0.10	DFREML/AM	Wei and Van Der Werf (1993)
		26–65 weeks	0.28–0.37	0.20		
		18–65 weeks	0.35–0.40	0.18		
	WL	273 days	0.19		ANOVA/SD	Poggenpoel et al. (1996)
		479 days	0.17			
		274–497 days	0.18			
	Three local breeds	39 weeks	0.20–0.33		DFREML/AM	Francesch et al. (1997)
	RIR	20–60 weeks	0.16[a]/0.17[b]		ANOVA/SD/REML/AM	Preisinger and Savas (1997)
	WR	20–60 weeks	0.14[a]/0.20[b]			
	Two lines WL	42–63 weeks	0.21/0.34		EM-REML/AM	Sewalem et al. (1998)
	Two layer lines (RIR, WR)	24 weeks	0.30/0.37		REML/AM	Savas et al. (1999)
		32 weeks	0.14/0.12			
		44 weeks	0.25/0.24			
	WL	1–12 months	0.20–0.48		EM-REML/AM	Anang et al. (2000)
	RIR	19–25 weeks	0.32/0.38[c]	0.13	EM-REML/AM	Misztal and Besbes (2000)
		26–38 weeks	0.19/0.29[c]	0.14		
		26–54 weeks	0.19/0.24[c]	0.07		
	WL	31 weeks	0.23		Gibbs/AM	Szydłowski and Szwaczkowski (2001)
	NH	31 weeks	0.11			

Table 11.1. *Continued.*

Trait	Species/population	Period	h_a^2	h_d^2	Method/model	Reference
Egg weight	Pure/cross RIR × WPR		0.75		DFREML/AM	Hagger (1992)
	Two layer pure lines		0.47/0.48		EM-REML/AM	Besbes et al. (1992)
	Three lines	30–35 weeks	0.41–0.47	0.07	DFREML/AM	Wei and Van Der Werf (1993)
	WL	40–45 weeks	0.52–0.53	0.02		
	WPR	34 weeks	0.56–0.67		DFREML/AM	Danbaro et al. (1995)
	WC	34 weeks	0.46–0.68			
	WL	1st 10 eggs (average weight)	0.47		ANOVA/SD	Poggenpoel et al. (1996)
		37th week	0.44			
	Three local breeds	39 weeks	0.48–0.59		DFREML/AM	Francesch et al. (1997)
	RIR, WR	20–60 weeks	0.72[a]/0.59[b]		ANOVA/SD/REML/AM	Preisinger and Savas (1997)
			0.66[a]/0.50[b]			
	Two lines WL	42–63 weeks	0.61/0.70		EM-REML/AM	Sewalem et al. (1998)
	Two layer lines (RIR, WR)	20–60 weeks	0.36/0.60		REML/AM	Savas et al. (1999)
	WL	29 weeks	0.34		REML/AM	Hartmann et al. (2000)
	RIR	38–54 weeks	0.65/0.67[c]	0.08	EM-REML/AM	Misztal and Besbes (2000)
	WL	33–35 weeks	0.08		GS/AM	Szydłowski and Szwaczkowski (2001)
	NH	33–35 weeks	0.38			
Mean oviposition time	Three layer lines		0.19–0.81		REML/SD	Lilpers and Wilhelmson (1993)
Mean within-sequence oviposition interval			0.42–0.55		REML/SD	Lilpers and Wilhelmson (1993)
Clutch number	RIW		0.11–0.22		ANOVA/SD	Bednarczyk et al. (2000)
Average clutch size	RIW		0.09–0.16		ANOVA/SD	Bednarczyk et al. (2000)

Heritability: h_a^2, direct additive; h_d^2, direct dominance.

Breeds: NH, New Hampshire; RIR, Rhode Island Red; RIW, Rhode Island White; WL, White Leghorn; WPR, White Plymouth Rock.

Methods: ANOVA, analysis of variance; DFREML, derivative-free restricted maximum likelihood; EM-REML, expectation–maximization type REML; GS, Gibbs sampling.

Models: AM, animal model; SD, sire–dam model.

[a]Estimates obtained from Henderson method III (sire–dam model).

[b]REML estimates (animal model).

line-crossing experiments that epistasis has a significant effect on the number of eggs. An evaluation of non-additive genetic effects is relatively simpler in crossbreeding experiments than in purebred populations, due to the magnitude of these effects and to the methodology applied. Therefore, the epistatic variance components from field-collected data are rarely reported in the literature. On the basis of a genetic model including additive and additive by additive effects, Szwaczkowski (1999) estimated negligible epistatic variance components.

Table 11.1 also presents various methods and models used. Comparative studies carried out by several authors (Mielenz *et al.*, 1994; Brodacki *et al.*, 1996) indicate considerable 'methodological' effects on heritability estimates. Many methods (briefly described in earlier section) have been employed to estimate variance components. Generally, both from the empirical and theoretical points of view, it was concluded that REML (under an animal model) has many advantages for poultry analysis (see Wężyk and Szwaczkowski, 1997). On the other hand, the Bayesian approach has been intensively developed in recent years. A majority of investigations (in poultry) conducted via Gibbs sampling concerned an analysis of mixed inheritance models, when the single locus variance is also estimated. Therefore, the comparisons of variance estimates from polygenic and combined genetic models are difficult. However, some studies with mammals have shown higher heritability estimates from the Bayesian polygenic analysis than from the REML method (Miyake *et al.*, 1999).

As previously noted, one of the main assumptions of many estimation methods (including REML) is a normal distribution of residuals (in consequence, as it is also shown, observations). Unfortunately, egg production has usually been shown to exhibit considerably non-normal distribution (e.g. Besbes *et al.*, 1993). This leads to overestimated error variance and so heritability is usually underestimated. The power transformation technique, the so-called Box–Cox transformation, is recommended for obtaining variables intended to satisfy an additive linear model with normal errors. Details of the method were given by Box and Cox (1964) and by Ibe and Hill (1988). In contrast to heritability estimates, a high correlation of breeding values between transformed and untransformed data was found by Savas *et al.* (1998). Other aspects of data transformation referring to egg production traits have been reported by Besbes *et al.* (1993) and Koerhuis (1996).

Egg production in poultry is denoted by egg production curves, usually summarized on a weekly or monthly basis. From this perspective, 'new' traits describing egg production can be formed. For example, the ovulatory cycle of the domestic hen is thought to be controlled by a circadian rhythm, entrained by the daily light–dark cycle, that governs the timing of the pre-ovulatory surge of luteinizing hormone and by the growth and maturation of the follicles (Lippers and Wilhelmson, 1993). The cyclic process is the basis for the formation of a clutch. Hence the clutch number, clutch size and maximum clutch size (Sheldon and Yoo, 1993; Bednarczyk *et al.*, 2000) can be analysed. Generally, the heritability estimates (obtained from a sire–dam model) reported in the literature are moderate (see Table 11.1). Bednarczyk *et al.* (2000) concluded that heritability estimates for the 64th week of age are higher than for the 38th week. Incidentally, it should be noted that the differences between heritability from sire and from dam components can be indicated by considerable maternal and/or non-additive genetic effects.

Published heritability and genetic correlation estimates indicate a relatively large variability in individual egg production. Generally, low genetic correlations between monthly egg production (excluding dependencies between early periods) are shown in the literature (Preisinger and Savas, 1997; Anang *et al.*, 2000). Some genetic correlations for different periods are listed in Table 11.2. Analogous relations were also registered for heritability of partial egg production (see Table 11.1). Preisinger and Savas (1997) and Anang *et al.* (2000) concluded that there is high heritability in the first month of the laying period. However, they

Table 11.2. Estimates of genetic correlations (r_G) between egg production traits.

Trait	Species/population[a]	Period	r_G	Method/model[a]	Reference
Egg number	Two layer pure lines	19/26–26/38 weeks	0.27/0.36	EM-REML/AM	Besbes *et al.* (1992)
		19/26–26/54 weeks	0.06/0.21		
		26/38–26/54 weeks	0.66/0.78		
	WL	1–2 months	0.76	EM-REML/AM	Anang *et al.* (2000)
		1–6 months	0.08		
		1–12 months	0.17		
		6–12 months	0.29		
		11–12 months	0.98		
Egg number/ egg weight	Pure/cross RIR × WPR		−0.27	DFREML/AM	Hagger (1994)
	Two lines WL		−0.25/−0.39	EM-REML/AM	Sewalem *et al.* (1998)
Initial egg production/ egg weight	RIR		−0.12	GS/AM	Szwaczkowski *et al.* (2001)
	RIW		0.10		

[a]Breed names and models as in Table 11.1.

result from the variation in age at first egg. It should be stressed that selection based on first-month egg production is not very effective, because of the relatively low correlation between first-month and later egg production. The optimum testing period has been studied in a number of papers (e.g. Flock, 1977).

Another perspective for analysing the egg production curve is the possibility of applying test day models (TDMs) to genetic evaluation in poultry. This approach has been widely used in dairy cattle and may also be applied to laying hens because, in general, partial (weekly or monthly) and cumulative records of egg production are relatively similar to tests for milk yield. One of the main advantages of TDMs over traditional approaches is that they enable the evaluation of both environmental and genetic day effects. Thus, genetic egg production curves for single individuals can be outlined. In consequence, the heritability coefficients can be derived from respective variability of genetic and environmental effects for each 'day in egg'. Many TDMs (and the so-called submodels), including both fixed and random regression for genetic evaluation in dairy cattle, have been presented (Szyda and Liu, 1999). The methodology to fit monthly records for genetic evaluation in laying hens was introduced by

Anang *et al.* (2001). Many applied breeding methods for dairy cattle have later been implemented for other livestock species and poultry.

Although the main effort in layer improvement genetic strategy is focused on the number of eggs, egg weight is also a factor in selection schemes. The objective of commercial breeding programmes is to obtain lines characterized by moderate egg weight. In general, the heritability estimates of egg weight shown in Table 11.1 are relatively high. Egg weight is strongly correlated with layer age and, in consequence, with body weight (Di Masso *et al.*, 1998). So, it leads to individual egg weight curves. Therefore, the magnitude of genetic parameter estimates of the trait is also determined by measurement period. Poggenpoel *et al.* (1996) reported higher heritability of weight of the first ten eggs compared with egg weight in the 37th week. The same authors, on the basis of a sire–dam model, also reported higher heritability estimates from the sire component than from the dam component or from sire–dam components jointly. Larger estimates for the sire than for the dam component may suggest additional sire influences, e.g. due to sex-linked effects. This corresponds with results obtained by, for example, Poggenpoel and Duckitt (1988) and Mou (1991). Unfortunately, from the literature

review an alternative genetic model may also be created. It is known that egg weight is positively correlated with body weight and research carried out by Di Masso *et al.* (1998) confirmed a partial pleiotropic basis for genetic correlation between these traits. Hence, maternal effects may also be considered in modelling egg weight. Koerhuis and McKay (1996) reported estimated maternal permanent environmental variance proportional to the phenotypic variance equal to 0.02 (in broiler chickens). On the other

hand, as also for the number of eggs, some authors (Wei and Van der Werf, 1993; Misztal and Besbes, 2000) suggest including dominance effects in the linear model. The genetic modelling of egg weight needs further study.

Examples of genetic relationships between the number of eggs and egg weight are listed in Table 11.2. As expected, a majority of the correlations are negative, which is analogous to many earlier reports (e.g. Fairfull and Gowe, 1993).

Table 11.3. Estimates of genetic correlations (r_G) between egg production traits and some other performance traits.

	Traits				
Egg production	Other performance	Species/ population	r_G	Method/ model	Reference
Egg number	Body weight	Two layer pure lines	−0.09/−0.12	EM-REML/AM	Besbes *et al.* (1992)
	Body weight	Commercial line of laying hens	−0.26	ANOVA/S	Müller and Mielenz (1993)
	Body weight	Pure/cross		DFREML/AM	Hagger (1994)
	Female	RIR × WPR	−0.04		
	Male		−0.16		
	Income feed cost	Pure/cross RIR × WPR	0.49	DFREML/AM	Hagger (1992)
	Feed intake	Pure/cross RIR × WPR	0.28	DFREML/AM	Hagger (1994)
	Residual feed consumption	RIR		DFREML/AM	Tixer-Boichard *et al.* (1995)
	Male		−0.16		
	Female		0.11		
	Age at first egg	RIR	0.78	GS/AM	Szwaczkowski *et al.* (2001)
		RIW	0.21		
		WL	−0.41	ANOVA/SD	Poggenpoel *et al.* (1996)
	PF	WL	−0.06	EM-REML/AM	Sewalem *et al.* (1998)
	PHT	WL	0.46		
Egg weight	Body weight	Commercial line of laying hens	0.21	ANOVA/S	Müller and Mielenz (1993)
	Body weight	Pure/cross		DFREML/AM	Hagger (1994)
	Female	RIR × WPR	0.29		
	Male		0.34		
	Income feed cost	Pure/cross RIR × WPR	0.53	DFREML/AM	Hagger (1992)
	Feed intake	Pure/cross RIR × WPR	0.39	DFREML/AM	Hagger (1994)
	Age at first egg	RIR	0.09	GS/AM	Szwaczkowski *et al.* (2001)
		RIW	−0.02		

Breeds: See Table 11.1.
Methods: See Table 11.1.
Models: AM, animal model; S, sire model; SD, sire–dam model.
Traits: PF, per cent fertile eggs; PHT, per cent hatched total set eggs.

A literature review of estimates of genetic correlations between the above egg production traits and other performance traits is shown in Table 11.3. In spite of their variability, these estimates are in agreement with those formerly reported. It is well known that egg number is negatively correlated with body weight. Currently, the final effect results from joint efforts by geneticists and breeders to reduce maintenance requirements in layers. Fortunately, egg production is often positively correlated with feed efficiency. A brief review of the genetic relationships between egg production and reproductive traits is also shown in Table 11.3. Generally, the estimates are strongly influenced by population as well as the method applied, e.g. the dependence on age at first egg.

Egg quality traits

In contrast to typical egg production, egg quality traits are not usually subject to routine control in commercial farms. Hence, the number of literature reports is relatively small. Numerous egg quality characters can be checked, such as egg density, yolk proportion, eggshell colour and frequency of cracked eggs. Heritability estimates of some traits reported in the literature are listed in Table 11.4. Generally, the estimates are

relatively high. It should be stressed that the majority of these estimates are obtained via the additive genetic model. Investigations carried out by Besbes and Gibson (1999) and by Misztal and Besbes (2000) indicated a considerable participation of dominance variance in the total variance (about 0.14, whereas the direct additive heritability is 0.23–0.24) for shell strength. Eggshell strength can be expressed in various ways and, in consequence, studied as several single traits (Johansson *et al.*, 1996). Heritability estimates of some traits obtained by these authors are very high, e.g. 0.70 for resistivity and 0.68 for specific breaking strength. This indicates the possibility of achieving satisfactory genetic progress. However, both genetic and environmental correlations between the frequency of cracked eggs and a majority of other egg quality traits are negative (Johansson *et al.*, 1996). From the breeder's perspective the relations between egg quality and quantity seem to be important. Nirasawa *et al.* (1998) concluded that selection for lower non-destructive deformation can increase shell strength but reduces egg number. Besbes *et al.* (1992) studied relationships between egg density and some egg production traits; in general, the correlation estimates exhibited negligible dependencies between them.

Another trait group is egg composition, which mainly concerns yolk. Hartmann and

Table 11.4. Estimates of egg quality traits.

Trait	Species/population	h^2	Method/model	Reference
Egg density	Two layer pure lines	0.33/0.34	EM-REML/AM	Besbes *et al.* (1992)
Egg specific gravity	Three lines WL	0.34/0.38	DFREML/AM	Wei and Van Der Werf (1993)
Eggshell colour	Three local breeds	0.27–0.53	DFREML/AM	Francesch *et al.* (1997)
Attachment strength	WL	0.41	DFREML/AM	Johansson *et al.*
Breaking strength		0.48		(1996)
Membrane thickness		0.30		
Shell thickness		0.33		
Frequency of cracked eggs		0.58		
Shell strength	Two lines RIR	0.23/0.27	EM-REML/AM	Besbes and Gibson (1999)
Yolk proportion	WL	0.38	REML/AM	Hartmann *et al.* (2000)
Yolk weight		0.22		
Albumen weight		0.41		

Breeds, methods and models as in Tables 11.1 and 11.2.

Wilhelmson (2001) reported that heritability estimates of yolk weight ranged from 0.22 to 0.57. Similar genetic variation in terms of heritability were found for other yolk traits (e.g. yolk dry matter concentration, percentage content of yolk). Although it is a well-established fact that egg weight is positively correlated with yolk weight, genetic antagonism is shown for the relationship between egg weight and yolk percentage (Hartmann *et al.*, 2000). The proportion of yolk is also negatively correlated with other egg production traits. More details concerning genetic backgrounds and

relationships were shown in a review by Hartmann and Wilhelmson (2001). For a long time, studies on the genetic aspects of egg yolk cholesterol content have been conducted for various species of birds. The lowest level of percentage of egg yolk cholesterol was found in fowl, quails and pheasants (Baumgartner, 1995). Generally, the heritability estimates varied between 0.11 to 0.28. Additionally, it should be noted that the associations of yolk cholesterol content with egg production traits and hatchability are almost negligible.

Table 11.5. Heritability estimates of body weight and meat production traits.

Trait	Species/population	h_a^2	h_m^2	Method/model	Reference
Body weight	Two layer pure lines	0.51/0.51		EM-REML/AM	Besbes *et al.* (1992)
	Three lines of WL	0.51–0.68		EM-REML/AM	Tufvesson *et al.* (1999)
	WL	0.32		GS/AM	Szydłowski and
	NH	0.25			Szwaczkowski (2001)
7 weeks	Broiler hen lines	0.10–0.33		REML/AM	Danbaro *et al.* (1995)
30 weeks		0.14–0.34			
Juvenile	Commercial broilers	0.28		DFREML/AM	Koerhuis and McKay (1996)
Body weight	Two lines of hen	0.24/0.37		EM-REML/	Le Bihan-Duval *et al.*
Body weight	broilers	0.61/0.63		MGS MGS-D	(1997)
Breast angle		0.47/0.45			
Breast meat yield		0.18/0.41			
Body weight (male)					
8 weeks	Meat type chickens	0.39	0.24	REML/AM	Mignon-Grasteau *et al.*
36 weeks		0.61	0.11		(1999)
Body weight (female)					
8 weeks		0.45	0.21		
36 weeks		0.64	0.08		
Meat quality parameters					
pHu	Hen broilers	0.49		REML/AM	Le Bihal-Duval *et al.*
Lightness		0.75			(1999)
Redness		0.81			
Yellowness		0.64			
Body weight (female)	Muscovy ducks	0.51/0.67		EM-REML/AM	Mignon-Grasteau *et al.*
6/12 weeks					(1998)
Body weight (male)		0.40/0.33		EM-REML/AM	Mignon-Grasteau *et al.*
6/12 weeks					(1998)
Body weight (female)	Turkey	0.23		ANOVA/SD-S	Havenstein *et al.*
Body weight (male)		0.60		ANOVA/SD-S	(1988)
Shank width (female)	Turkey	0.55		ANOVA/SD-S	
Shank width (male)		0.47		ANOVA/SD-S	

Note on symbols: MGS, maternal grandsire model; MGS-D, heritability estimated from dam component on basis of maternal grandsire model; pHu, ultimate pH; other symbols, as above.

Body weight and some meat production traits

Excellent and comprehensive reviews of genetic parameters of growth and meat production in chickens, turkeys, waterfowl and other galliforms were given by Crawford (1993); hence, only new aspects (especially methodological) are presented here. Generally, as shown in Table 11.5, heritability estimates of body weight and meat production traits are varied.

Although layer breeding strategies focus on decrease in body weight, most studies on body weight and its changes over time are conducted in broiler chickens. Body weight is influenced by, among others, genetic and environment maternal effects, as confirmed by the results of special crossbreeding experiments (Liu *et al.*, 1993). Koerhuis and Thompson (1997) analysed two commercial lines: by comparing various models to estimate maternal effects for juvenile body weight in broiler chickens, they concluded that maternal heritability was relatively low (0.02–0.13), whereas correlation between direct and maternal genetic effects was negative (−0.11 to −0.92). Hence, the selection on maternal breeding values for the trait may not be effective. The authors also reported relatively low maternal environmental variance as a proportion of phenotypic variance. However, higher maternal effects (see Table 11.5) were shown by Mignon-Grasteau *et al.* (1999). It seems that studies on maternal effects will continue.

Body weight and its changes (growth) may be checked for different ages and, basically, heritability increases proportionally with age. Genetic correlations between body weights registered at different ages are rather varied (Mignon-Grasteau *et al.*, 1999). Different heritabilities over time in body weight have been reported in Muscovy ducks (Mignon-Grasteau *et al.*, 1998). Considerable variability in individual phenotypic and genetic growth curves is demonstrated via evaluated genetic correlation coefficients. These correlations are also highly varied. One important source of body weight variability and its changes is sexual dimorphism, reported by Mignon-Grasteau *et al.*

(1998, 1999), who concluded that selection on growth curve parameters could modify the difference in body gain between sexes. Moreover, the growth curve parameters were shown to be highly heritable. By analogy with other livestock species (mainly dairy cattle) and laying hens, it seems that more modern methodologies will be employed to estimate the genetic parameters of body weight in broilers.

Selection of meat-type birds focuses not only on increasing growth performance, but also on the improvement of carcass quality, especially a better body composition with higher yields of breast meat and lower abdominal fat. Le Bihan-Duval *et al.* (1999) reported that body composition can be significantly improved by selection, as shown by the relatively high heritability estimates given in Table 11.5. Generally, these results are in agreement with those presented in other papers (Le Bihan-Duval *et al.*, 1998) and a similar tendency has been registered in turkeys (Benkova and Jankowski, 1995), geese (Rouvier, 1995) and ducks (Książkiewicz, 1995).

Reproductive traits

There are a number of reproductive traits in poultry. The most important of them include fertility, hatchability and complex embryo development traits, semen characters and age at first egg. These reproductive traits are known to be usually of low heritability, with the exception of sexual maturity. This corresponds with the results of many investigations carried out in different species of livestock and poultry (Beaumont, 1992; Poivey *et al.*, 2001).

It should be noted that poultry are characterized by some features related to their biology that facilitate the intensification of breeding and production (Hartmann, 1989), such as immense natural fecundity, mechanized incubation and brooding, and persistent fertility of properly stored hatching eggs. Therefore, a breeder's interest is focused, first of all, on the improvement of

environmental conditions. From the genetic point of view, fertility and hatchability in laying hens are still probably the least understood aspects of poultry breeding. In fact, fertility is generally considered a common trait of both parents, being a consequence of the interaction of a male and a female gamete to produce a viable zygote. In practice, fertility is checked by candling on the 5th to 8th day of incubation. This leads to an overestimated percentage of fertility due to early embryonic mortality (eggs with embryos that died in the first days of incubation are classified as infertile).

Recently, new approaches of quantitative genetics and statistical tools have been employed to analyse some reproductive traits. Fertility is usually analysed on a percentage basis and exhibits departure from the normal distribution. Thus, classical analysis of the data is troublesome. Two approaches are recommended in the literature: one is based on data transformation – arcus sinus of the original observation

(e.g. Foerster, 1993); the other (preferred) approach applies a threshold model, because fertility is a phenotypic discrete trait determined by many loci (Sewalem, 1998). Additionally, the heritability estimates of reproductive traits may be significantly modified by the method and model used (Beaumont, 1991; Beaumont *et al.*, 1997).

Hatchability is determined by embryonic development and modified by additional maternal effects; therefore, maternal effects should be included in the model (Szwaczkowski *et al.*, 2000). A brief literature review of direct and maternal heritability estimates is given in Table 11.6. Unfortunately, estimates of covariance between genetic direct and maternal effects are sometimes negative. This can imply a reduction of genetic gain in this trait. The heritability estimates for hatchability are higher than those for fertlity. Some aspects of the genetic relationships of these traits with egg production have already been

Table 11.6. Heritability estimates for reproductive traits.

Trait	Species/population	h_a^2	h_m^2	Method/model	Reference
Age at first egg	Commercial broiler type	0.34	0.02[a]	DFREML/AM	Koerhuis and MacKay (1996)
	Four lines WL	0.27–0.44		EM-REML/AM	Sewalem (1998)
	RIR	0.08		Gibbs/AM	Szwaczkowski *et al.* (2001)
	RIW	0.15			
Fertility[b]	Four lines WL	0.03–0.15		EM-REML/AM	Sewalem (1998)
	NH	0.14/0.01[c]	0.08	DFREML/AM	Szwaczkowski *et al.* (2000)
	WL	0.10/0.05[c]	0.05		
Male fertility	Chicken	0.08		REML/AM	Barbato (1999)
Sperm number		0.26		REML/AM	Barbato (1999)
Hatchability	Layers	0.05		LLF	Beaumont *et al.* (1997)
	WL	0.24	0.18	GS/TEM	Sewalem (1998)
	NH	0.21/0.20[c]	0.11	DFREML/AM	Szwaczkowski *et al.* (2000)
	WL	0.14/0.11[c]	0.02		
TEM	Two lines of WL	0.09–0.24[d]		ANOVA/SD	Brah *et al.* (1999)
		0.001–0.54[e]			
		0.05–0.33[f]			
NEF	Two lines of Brown	0.30/0.26		REML/AM	Poivey *et al.* (2001)
NED	Tsaiya ducks	0.06/0.09			
NEH		0.18/0.19			

[a]Maternal environmental permanent variance as proportion to total variance; [b]per cent fertile eggs; [c]estimates from model included genetic maternal additive effect; [d]heritability estimates obtained from sire component; [e]heritability estimates obtained from dam component; [f]heritability estimates obtained from sire and dam component; LLF, logistic link function; TEM, total embryo mortality; NEF, number of fertile eggs at candling; NED, total number of dead embryos; NEH, number of hatched male ducklings.

discussed briefly. Generally, some results show that selection for production traits does not lead to a decrease of reproduction traits (Szwaczkowski *et al.*, 2000).

Another important trait, especially in laying hens, is age at first egg (AFE). The trait is easy to measure and many reports are available. Some of the heritability estimates are shown in Table 11.6. Bednarczyk *et al.* (2000) obtained higher heritability estimates from the dam component than from the sire component, which might indicate maternal and/or dominance effects.

Danbaro *et al.* (1995) reported various genetic correlations of AFE with body weight of two lines of broiler chicken ranging from −0.15 to 0.75 at 7 weeks and from −0.19 to 0.89 at 30 weeks. Similar (various) relations are also reported for egg production traits (Szwaczkowski *et al.*, 2001). Other heritability estimates for reproductive traits are listed in Table 11.6.

Feed consumption and conversion

Feed comprises the major portion of total costs of egg and meat production. The literature reports a number of traits to measure the efficiency of feed conversion and intake.

One of the first traits to be studied was food conversion efficiency (FCE) expressed as a ratio of egg mass to food intake (Pirchner, 1985). The inverse relation is known as the food conversion ratio (FCR). Unfortunately, especially for laying hens, the measurements focused on production level and thus maintenance requirements were omitted. Therefore a new trait, residual feed consumption (RFC), has been proposed, derived from multiple regression including feed intake, body weight and its changes as well as production level (Tixier-Boichard *et al.*, 1995). Other traits combining feed intake and economic aspects have also been studied, e.g. income minus feed cost (Hagger, 1992). As shown in Table 11.7, the heritability estimates of feed efficiency are varied. However, these traits are satisfactorily inherited; hence, feed efficiency traits have been significantly improved by both indirect and direct selection (Flock, 1998).

As expected, feed intake (FI) is positively correlated with performance traits. Hagger (1994) found very high positive genetic correlation between body weight and FI at 0.79 for females and 0.65 for males. In the case of egg production, the correlation was 0.28. Tixier-Boichard *et al.* (1995) studied relationships between RFC and

Table 11.7. Estimates of feed efficiency traits.

Trait	Species/population	h^2	Method/model	Reference
IFC	Pure/cross RIR × WPR	0.38	DFREML/AM	Hagger (1992)
FI	Pure/cross RIR × WPR	0.68	DFREML/AM	Hagger (1994)
RFC	WL	0.20	ANOVA/SD	Mielenz *et al.* (1994)
RFC	WL	0.22	REML/AM	Mielenz *et al.* (1994)
FI			DFREML/AM	Tixier-Boichard *et al.* (1995)
Male	RIR	0.33		
Female	RIR	0.43		
RFC			DFREML/AM	Tixier-Boichard *et al.* (1995)
Male	RIR	0.33		
Female	RIR	0.27		
FI	RIR	0.33/0.38	ANOVA/SD/REML/AM	Preisinger and Savas (1997)
	WR	0.38/0.40		
FI	RIR	0.18	REML/AM	Savas *et al.* (1999)
	WR	0.19		
FC	RIR	0.26	REML/AM	Savas *et al.* (1999)
	WR	0.31		

Note on symbols: FC, feed consumption; other symbols, as above.

some productive traits in laying hens. They obtained low genetic correlations between RFC (for males and females) and egg number, and the female RFC was not correlated with egg weight. This suggests that selection took place on a set of independent traits.

Hagger (1992), analysing layer data from two generations under selection, found a positive genetic correlation between income minus feed cost and both egg number and egg weight (0.49 and 0.53, respectively). Respective correlations with body weight and feed conversion were negative (–0.16 and –0.80, respectively).

Resistance to disease and conformation defects

Diseases play a major role in the poultry industry because they lead to losses in commercial production. Hence, the efforts of many breeders and geneticists have focused on challenging methods for genetic improvement of disease resistance and rejection of genetic defects. The biological aspects of immune responses and related problems are widely discussed in other chapters of this book. Genetic parameter estimates of resistance to pathogens, some skeletal defects and other more important diseases are presented briefly in Table 11.8.

One of the main topics is the genetic background of antibody response to *Escherichia coli*. It should be noted that such studies require special experiments conducted with young birds; therefore the number of reports on genetic parameter estimation is relatively small. Yonash *et al.* (1996) reported that phenotypic variation in the immune response of young chicks could reflect incomplete development of their immune system, partial expression or regulation of its genetic control, and possible variation among lines in the maternal effect. Heritability may be derived from each of these sources of variation. Leitner *et al.* (1992) estimated heritability within the range of 0.15 to 0.43 for antibody titre 10 days after immunization with *E. coli* vaccine at 10 days of age. Heritability estimates (from a sire–dam model) for different vaccination age, lines (high and low antibody) and time for antibody development obtained by Yonash *et al.* (1996) were varied.

Table 11.8. Heritability estimates of disease resistance and conformation defects.

Trait	Species/population	h^2	Method/model	Reference
Antibody response	Meat-type			
to *E. Coli* ($8d_w$)	chickens (HC)	$0.03^a/0.58^b$	ANOVA/S/SD	Yonash *et al.* (1996)
($10d_w$)		$0.10^a/0.78^b$		
($8d_w$)	(LC)	$0.04^a/0.39^b$		
($10d_w$)		$< 0.0^a/0.88^b$		
CCSSE	Chickens	0.06	ANOVA/TSD	Berthelot *et al.* (1998)
PCTT	Broiler chickens	0.19	REML/AM	Bautista-Ortega *et al.* (1999)
SRT	Broiler chickens		REML/TAM	Janss and Bolder (2000)
Mortality (%)		0.12		
Survival time		0.06		
Cecal carriage		0.09		
SRBC	Chickens	0.18	GS/AM	Bovenhuis *et al.* (2002)
TD (4 weeks)	Broiler chickens	0.37	DFREML/AM	Kuhlers and McDaniel (1996)
(7 weeks)		0.42		
Embryonal scoliosis	RIR	0.67	GS/AM	Pryszcz *et al.* (1997)

[a]Obtained from sire component (sire–dam model). [b]Obtained from dam component (sire–dam model).
Note on symbols: CCSSE, cecal carrier state of *Salmonella entertidis*; PCTT, plasma cardiac troponin T; SRT, salmonella resistance traits; HC, high antibody line; LC, low antibody line; SRBC, antibody response of chickens to sheep red blood cells; TSD, threshold sire–dam model; d_w, days for antibody development; TD, tibial dyschondroplasia.

Comparison of three types of heritability estimates (from sire, dam and combined sire–dam components) suggests maternal effects. From the methodological point of view, it should be noted that some heritability estimates from some sire variance components were negative. Heritability estimated from the combined sire and dam components ranged from 0.25 to 0.44 in the high antibody line and from 0.21 to 0.42 in the low antibody line of broiler chicks.

A second major topic for genetic study is salmonella resistance. Berthelot et al. (1998) obtained heritability for resistance to the caecal carrier state of *Salmonella enteritidis* at 0.08 from the sire component and 0.29 from the dam component. These differences between the two types of heritability estimate may be influenced by maternal and dominance effects. More sophisticated methodology to estimate genetic parameters was employed by Janss and Bolder (2000), who applied both threshold and linear animal models. This also resulted in low heritability estimates of three salmonella resistance traits (see Table 11.8).

Another health problem in broiler chicken is the ascites syndrome, the so-called heart-API. Moghadam et al. (2001) found heritability estimates for heart-API that ranged from 0.12 to 0.41. The genetic association of this syndrome with body weight is not clear. For instance, Greef et al. (1998) reported negative correlation, which implies a favourable genetic relation, but Moghadam et al. (2001) reported less optimistic results.

Tibial dyschondroplasia (TD) is a genetic leg defect in chicken and turkey broilers described by Leach and Nesheim (1965). TD is related to a rapid growth rate early in life and incidence of TD increases in heavier lines (Wong-Valle et al., 1993). The results obtained by Kuhlers and McDaniel (1996) indicate a possibility of effective selection (see Table 11.8). Broadly similar heritability estimates were found by Le Bihan-Duval et al. (1997). The heritabilities of valgus angulation at 6 weeks for two lines of broiler chickens ranged from 0.15 to 0.39 and for varus angulation from 0.21 to 0.30. In one line, the magnitude of estimates

was considerably influenced by statistical methods. Estimation of heritability on the basis of sire/maternal-grandsire component (sporadically applied in poultry) leads to lower estimates.

Osteoporosis in laying hens is an important condition that involves the progressive loss of structural bone during the laying period (Whitehead, 1999). Whitehead and Wilson (1992) showed a relatively high heritability of some properties of layer bones. Four analysed traits (keel radiographic density, humeral strength, tibial strength and bone index) were moderately to highly inherited (0.27–0.59). Thus, selection for improved bone characteristics should be a successful strategy for combating osteoporosis.

Behavioural traits

Intensive egg and meat production leads to changes in bird behaviour. Behaviour is defined as the physical activity of a living organism that can be recorded by an observer (Gerken, 1995). To our knowledge, most behavioural traits are determined by many loci and environmental effects. There are many reasons for being interested in genetic backgrounds to behaviour. From a practical point of view, several traits are associated with production level: for example, broodiness resulted in a decrease in the number of eggs, while food consumption (as a behavioural trait) positively influenced growth rate. Moreover, there are international and national regulations to protect the welfare of poultry and livestock. Various aspects of animal well-being are widely discussed in others part of this book: here, just a short review of heritability and genetic correlation is given. In contrast to most performance traits, the measurement of behavioural characters is more complex. Generally, the reported heritability estimates for these traits tend to be lower compared with those for many production traits. Dunnington and Siegel (1983) estimated the heritability for mating behaviour at 0.18, whereas h^2 of tonic immobility in

Japanese quails ranged from 0.09 to 0.23. It should be noted that tonic immobility is negatively correlated with mortality (Craig and Muir, 1989).

One of the more important problems in layer husbandry is feather pecking, characterized as non-aggressive pecking directed to the plumage of other individuals. It has been shown to be the main reason for deterioration of the plumage of laying hens, whether housed or in a range production system (Kjaer, 1999). Heritability estimates of feather pecking are varied. As already mentioned, it can be strongly influenced by methodological approach. Craig and Muir (1993) estimated that realized heritability (two generations under selection) is 0.65. A summary of many experiments indicates that the magnitude of heritability in feather pecking increases with age in layers.

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12 Use of Mixed Model Methodology in Poultry Breeding: Assumptions, Limitations and Concerns of BLUP-based Selection Programmes

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Introduction

The poultry breeding industry has made substantial genetic improvements in commercial birds, despite the fact that the industry was not developed to any extent until the last half of the 20th century (Hunton, 1990). Large-scale breeders in particular continue to make rapid advances, due to more effective use of genetic selection strategies. Improved management techniques have also allowed these breeders to use more sophisticated prediction methods, which rely on accurate data recording and pedigree records.

Artificial genetic selection is based on the principle of controlling the reproductive rate of birds within a particular flock, so that those predicted to produce inferior offspring do not reproduce. Traits that are determined by a very small number of genes, such as colour, can be predicted using Mendelian genetics, but most economically important traits are quantitative, i.e. determined by a large number of genes, together with many random environmental effects, including measurement error. Without extensive molecular information, the only sources of information on these traits are the phenotypes, pedigrees and management groups of

the birds involved. For these traits, the offspring performance of each individual, known as the breeding value, is predicted from its own phenotype, and/or those of its relatives, and is relative to others in the group. Information from relatives is, of course, the only source of information for many reproductive or meat quality traits. Quantitative traits may be either continuous, covering a range of many possible values (e.g. body weight), or discrete, with only a few possible values (e.g. presence vs. absence of disease). Discrete traits are more difficult to predict, and prediction methods for continuous traits are usually applied to traits with several possible ordered values, such as annual egg production and body condition score.

Most selection programmes for continuous traits are conducted using one of three techniques for predicting breeding values: individual phenotypes or selection index (Hazel, 1943), or best linear unbiased predictor (BLUP) (Henderson, 1984). There are no essential differences in the philosophy underlying the use of these techniques as the basis for selection. Breeding values, for all three methods, are predicted based on a regression model that was derived from Galton's original approach and formed the

basis of all regression analysis. Selection on individual phenotype is based on the idea that offspring performance is linearly related to that of the parent, and is extended in the selection index method, which uses a multiple regression model to predict offspring performance, based on the phenotype of the individual and/or those of any of its known relatives. The regression coefficients are calculated based on the trait heritability and the expected covariance of each information source with the individual's breeding value, and the phenotypes are pre-adjusted for any systematic (management) effects.

Henderson (1984) extended the selection index method, by using phenotypes from all known relatives, rather than only particular ones, for the prediction, and by estimating systematic effects simultaneously, using weighted least-squares, rather than pre-adjusting the phenotypes. Estimates of systematic effects are produced, as well as predicted breeding values, and these can provide invaluable management information.

In fact, one of the biggest reasons the method has been so successful is that it is more effective at disentangling systematic and genetic effects. BLUP-based selection programmes are used extensively in dairy, beef and swine production for a large variety of both single- and multiple-trait objectives. Many of these programmes have been highly successful; for example, Kennedy *et al.* (1996) found evidence that national trends in swine increased after breeders were provided with BLUP-based breeding values. There are some examples of research in which BLUP-based selection was used in poultry. For example, Bishop *et al.* (2000) found selection on BLUP-based breeding values for bone quality traits to be successful in reducing the incidence of osteoporosis in a layer flock. Morris (1997) used data from a large broiler flock in a retrospective study to compare predicted response using BLUP-based, index and phenotypic univariate selection, and found that BLUP-based selection gave the greatest response.

BLUP-based selection is used by many commercial poultry breeders (Leeson and Summers, 2000) but has not received the same level of enthusiasm as shown by cattle and swine breeders (Hartmann, 1992). Most selection programmes in poultry are conducted by large breeding companies, and flocks under selection are usually housed together in large contemporary groups, in highly controlled environments. Selection is often conducted within contemporary groups, so that there is little or no overlap between generations. As a result, the advantage that BLUP methods have in differentiating between genetic and systematic effects is less important for poultry than for cattle or swine (Hartmann, 1992). There are additional benefits in using BLUP-based predictions but these do not necessarily apply if the model assumptions do not hold. Critics of BLUP-based selection in poultry have pointed out that the assumptions required for any of the models used in practice never do hold (Fairfull and Muir, 1996). Since systematic effects are less important, the extent to which these assumptions fail, and the implications for BLUP-based vs. phenotype-based predictions, is more important for poultry than for cattle or swine. The next section of this chapter will discuss some of the probable failures in the assumptions implicit in the basic animal model, and the effects that these may have on selection response.

There are also concerns over the application of BLUP-based breeding values, both for measuring genetic trends and with respect to their long-term use, particularly when inbreeding is a concern. The best selection strategy for any situation depends on many factors, including the evaluation method, and some of the more important considerations will be discussed. In practice, it may be difficult to define the objectives of a selection programme completely. Commercial stock is usually the offspring of a cross between two or more lines, which allows for different selection criteria in each line and capitalizes on any beneficial effects of between-line dominance. Selection programmes are conducted within each line separately, usually for many generations and often with no specified end point. Birds not required for within-line breeding are often sold as commercial stock on a

continual basis, which allows the benefits from genetic progress to be realized immediately. Competitive integrated industries such as this would require maximum selection response not only at the end of the programme but also at each generation.

Techniques and Assumptions in the Prediction of Breeding Values

Prediction methods

Phenotypes are used as predictors for breeding values based on the assumption that the breeding value is linearly related to the phenotype:

$$\hat{a}_i = h^2(y_i - \mu) = (\sigma_{ay}/\sigma^2)(y_i - \mu)$$

where y_i is the phenotype of the ith animal, $\hat{a}_i$ is its predicted breeding value, μ is the overall population mean (assumed known), h^2 is heritability, σ_{ay} is the covariance between the breeding value and phenotype (also the additive genetic variance) and σ^2 is the phenotypic variance. Without any further modification, the genetic model implied by this relationship is that of a large number of independent, additive loci, each with small effect. This means negligible dominance and epistatic effects, no mutation, and unlinked loci in gametic phase and Hardy–Weinberg equilibrium. Environmental or measurement variance must also be constant.

Hazel (1943) extended the prediction equation to include family information in order to improve precision:

$$\hat{a}_i = \mathbf{CV}^{-1}(\mathbf{Y_i} - \mu)$$

where $\mathbf{Y_i}$ is a vector consisting of the phenotypes of the ith animal and those of specific relatives, $\mathbf{C} = cov(a, \mathbf{Y}'_i)$ is the covariance between each phenotypic record used and the breeding value, and $\mathbf{V}$ is the variance–covariance matrix of those phenotypes. For both phenotypic and selection index, contemporary group means (μ_j) expressed as deviations from the population mean are used to account for systematic effects if they exist. Note that these methods require that

the population or contemporary group means must be known without error.

Henderson (1984) extended the selection index prediction method to include the estimation of systematic effects, as well as the maximum amount of information from relatives. The basic form of the animal model, for example, is:

$$\mathbf{Y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

where $\mathbf{Y}$ is a vector of records for all recorded individuals, $\mathbf{b}$ is a vector of fixed (systematic) effects such that $E(\mathbf{Y}) = \mathbf{Xb}$, $\mathbf{a}$ is a vector of random breeding values for all individuals in the population, with mean 0 and variance–covariance matrix $\mathbf{A}\sigma_a^2$, and $\mathbf{X}$ and $\mathbf{Z}$ are incidence matrices. $\mathbf{A}$ is the additive genetic relationship matrix for the population, and $\mathbf{e}$ is a vector of random environmental effects with mean 0 and variance–covariance matrix $\mathbf{R}\sigma^2$.

The solutions for the breeding values and fixed effects are:

$$\hat{\mathbf{a}} = \mathbf{CV}^{-1}(\mathbf{Y} - \mathbf{Xb})$$

$$\hat{\mathbf{b}} = (\mathbf{X'V}^{-1}\mathbf{X})^{-1}(\mathbf{X'V}^{-1}\mathbf{Y})$$

which is computed more easily using the mixed model equations of Henderson (1984):

$$\begin{pmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{a}} \end{pmatrix} = \begin{pmatrix} \mathbf{X'R}^{-1}\mathbf{X} & \mathbf{X'R}^{-1}\mathbf{Z} \\ \mathbf{Z'R}^{-1}\mathbf{X} & \mathbf{Z'R}^{-1}\mathbf{Z} + \mathbf{A}^{-1}\lambda \end{pmatrix}^{-1} \begin{pmatrix} \mathbf{X'R}^{-1}\mathbf{Y} \\ \mathbf{Z'R}^{-1}\mathbf{Y} \end{pmatrix}$$

where $\lambda = (1 - h^2)/h^2$. (See Kennedy and Sørensen, 1988, for a more complete explanation.) It should be noted that any environmental, management and genetic effects on the trait must be additive and uncorrelated.

The term BLUP refers to a statistical procedure in which genetic effects are predicted and fixed effects estimated simultaneously; BLUP can be applied to a wide variety of models in breeding applications, including those that account for non-additive inheritance and gametic models. The basic animal model is currently the most widely applied of these models, and much of the concern over the use of BLUP-based breeding values relates to the animal model.

The assumptions underlying any of the three prediction methods described

above are not 'correct' according to current understanding of genetics. Their value, as for models in any field of application, depends on how useful they are for a specific purpose, which in this case is to provide a basis for genetic selection and prediction of response.

Properties of BLUP

Predictions made using BLUP methods are unbiased and have minimum variance among linear unbiased estimators, provided that the model is correct; if it is not, these properties do not hold.

Predictions based on simple phenotype, or selection index, may be biased when several contemporary groups are involved. In addition, most selection programmes are conducted over several rounds of selection and mating, during which time the genetic variance of the trait changes, especially if selection is intense, due to gene frequency changes, gametic phase disequilibrium and possibly inbreeding. Gene frequency changes can usually be considered negligible for traits determined by many loci with small effects, except in the very long term, but gametic phase disequilibrium and inbreeding can change the genetic variance in the short term. Predictions of breeding values and selection response are biased if the evaluation method does not account for these changes.

Predictions based on phenotypic or selection index methods are biased if the index coefficients are not updated, although for non-overlapping generations the phenotypic rankings of the selection candidates, and therefore selection response, are not affected. The animal model accounts for variance changes other than those due to gene frequencies, and BLUP-based predictions are both unbiased and minimum variance if the model is appropriate, provided that the appropriate base population heritability is used and all relevant pedigree information and data are included in the prediction model.

Deviations from the assumptions: problems and modifications of the animal model

Small numbers of loci

Genetic variances change with selection due to gene frequency changes in the short term when there are only a small number of loci, or some loci have large effects. The animal model does not account for this type of change, and BLUP breeding values are biased. Breeding values based on phenotype or selection index methods are also biased, unless the coefficients are updated correctly, though the phenotypic ranks within any generation are not affected.

Several simulation studies of a trait affected by only a small number of loci have compared selection over non-overlapping generations based on animal model BLUP with that using phenotypic selection (e.g. Maki-Tanila and Kennedy, 1986). In general, except for extreme cases, the animal model-based selection response over the short term has been found to be greater than the response with phenotypic selection even for as few as two loci.

Non-additive genetic effects

There are many examples of heterosis or epistasis among line crosses in both growth and reproductive traits in poultry (for example, see Fairfull, 1990). Non-additive genetic effects are likely to exist within lines as well, but the evidence is more difficult to detect. Dominance and some forms of epistasis are associated with inbreeding depression, through either deleterious recessive alleles or loss of overdominance (Lynch and Walsh, 1998). Evolutionary theory predicts that traits that are more closely related to fitness, such as reproductive traits and measures of viability, will tend to have low heritabilities and more non-additive genetic effects (Falconer and Mackay, 1996). Effects of inbreeding depression in poultry are quite large for these traits (Abplanalp, 1974) but also occur for growth-related traits such as body weight (Abplanalp and Woodward,

1967). Non-linear relationships between phenotype and inbreeding level are evidence of directional dominance by dominance (epistatic) effects (Crow and Kimura, 1970) but additive by additive epistatic effects may exist when there is no effect of inbreeding on phenotype.

Commercial animals in poultry, as in many species, are crosses between lines or breeds, partly to capitalize on dominance effects. Offspring performance in crosses between very different, relatively inbred breeds or lines may be predicted sufficiently well from the average of the within-line parent predictions, either phenotypic or animal model BLUP-based, adjusted for any known effects due to between-line heterosis and possibly imprinting.

However, crosses are often made between less diverged lines, and an alternative method that is often used is reciprocal recurrent selection (Pirchner, 1969), under which parents are selected on the basis of a test cross. The method is designed so that, over the long term, alleles at overdominant loci are driven to opposite extreme frequencies in the two lines, and it has the advantage of being easy to apply. The disadvantages are that response is often very slow, particularly when the lines are not much diverged, and that the length of the generation interval is increased, since selection is based on offspring performance. Over-long generation intervals are a major contributer to reduced rates of genetic progress in swine (Kennedy *et al.*, 1996). Madalena and Hill (1972) obtained greater selection response using a subdivide and merge scheme, rather than a single large population, in simulation studies with complete dominance, with the recessive allele at a low frequency. This strategy is also appropriate for only long-term objectives.

An alternative strategy, given by Arnold *et al.* (1992), is used in beef cattle breeding. The method is based on an animal model that includes fixed and random additive line effects, representing pure line effects and general combining ability, and fixed, and possibly random, heterosis effects, representing specific combining ability, and reciprocal effects, if any. Both pure line and cross information is used, where the type of cross is not restricted since it is defined by the proportion of each pure line. This model accounts for dominance effects between lines, but Miller and Goddard (1998) suggest that in non-inbred lines, or those with recent common ancestry, most non-additive variance occurs within lines and should also be accounted for in the model.

Within-line breeding value predictions that do not account for dominance or epistatic effects are biased if these effects exist. The degree of bias tends to increase with the use of more family information, since the covariances among relatives are incorrect; and, as a result, those based on an animal model that does not account for non-additivity are particularly affected (Maki-Tanila and Kennedy, 1986).

An alternative to the basic animal model, expanded to include dominance effects, is given by Henderson (1984), though the interpretation of dominance effects is not clear within the framework of the infinitesimal model. The model has the form:

$$\mathbf{Y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Zd} + \mathbf{e}$$

where the vector of random dominance effects, $\mathbf{d}$, is distributed with variance–covariance matrix $\mathbf{D}\sigma_d^2$, where $\mathbf{D}$ is the dominance relationship matrix and σ_d^2 is the dominance variance. Note that the dominance relationship matrix is not straightforward to calculate when there is inbreeding. Maki-Tanila and Kennedy (1986) used simulations to illustrate the fact that the model does not account for inbreeding depression; Uimari and Kennedy (1990) found that including a regression on inbreeding levels as a fixed effect in the model, as well as accounting for dominance relationships, improved response in simulated populations with dominance. Boer and Hoeschele (1993) used the model:

$$\mathbf{Y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Z}(\mathbf{f}\Delta_1 + \mathbf{d}) + \mathbf{e}$$

where $\mathbf{f}$ is a vector of inbreeding coefficients, $\mathbf{d}$ a vector of random dominance effects, Δ_1 the fixed effect of complete inbreeding depression; and obtained unbiased predictions in simulated populations

with inbreeding. The model is by no means simple to apply. Miller and Goddard (1998) expanded the crossbreed model of Arnold *et al.* (1992) to include both across- and within-line dominance effects, in a 'superbreed' approach.

Although models that include within-line dominance effects have the potential to be useful by reducing the bias in evaluations, practical applications are likely to be difficult for several reasons. First, computer software incorporating these effects is computationally intensive and not currently feasible for other than very small populations. Secondly, more complex models are much more sensitive to inadequacies in the data, such as confounding among genetic and environmental effects, and missing pedigree records. Thirdly, evaluation models that include non-additive effects require the associated variance estimates, which are generally specific to the population. Within-line non-additive variances estimated from field data are difficult to estimate, due to confounding effects, and are unreliable (Barker, 1979).

Johansson *et al.* (1993) suggested an approximation, in which a standard animal model is without dominance relationships:

$$\mathbf{Y} = \mathbf{Xb} + \mathbf{Z_1a} + \mathbf{Z_2c} + \mathbf{e}$$

where the design matrix for the fixed effects, $\mathbf{X}$, includes the inbreeding levels (as a covariate), $\mathbf{Z_1}$ and $\mathbf{Z_2}$ are incidence matrices, and $\mathbf{c}$ is a vector of random full-sib family effects, with mean 0, and variance–covariance matrix $\mathbf{Q}\sigma_c^2$. The model produced unbiased predicted breeding values over ten generations in simulated populations with inbreeding. This type of model, without the regression on inbreeding level, is frequently used to account for non-genetic maternal or litter effects, and requires an estimate of the environmental family variance, σ_c^2. Evaluation models for swine usually include a litter effect, and inbreeding level is now included as a covariate in the model used to predict breeding values for litter size in the Canadian national swine evaluation system. This type of model may be useful in poultry production as well, particularly for selection on reproductive traits, such as egg production, over many generations.

Muir (2000) used simulations to compare the genetic response in small populations selected on phenotype with animal model BLUP-based selection, when the animal model did not include dominance. The trait was simulated using a finite locus model with a relatively realistic genetic architecture, including several chromosomes with linkage between loci within chromosomes. Response was always better using predictions based on the animal model when the trait was simulated with only additive effects, but was better only when more than 10% of the population was selected at five generations, or more than 20% were selected at 20 generations when the trait was simulated with complete dominance at every locus.

Epistasis

Epistasis, the effect of interlocus interactions, was also included in animal models given in Henderson (1984). The theory behind these models originates from Fisher (1918), who described the covariance between different types of relatives in terms of a function of a particular subdivision of the genetic variance, expressed in terms of orthogonal polynomials. Fisher's original subdivision consisted of an additive, dominance (quadratic) and a residual or epistatic part; the division of the epistatic variance was extended by Cockerham (1954) and Anderson and Kempthorne (1954).

Based on this subdivision, the basic animal model can be extended to:

$$\mathbf{Y} = \mathbf{Xb} + \mathbf{Za_1} + \mathbf{Zd_1} + \mathbf{Za_2} + \mathbf{Z(a_1d_1)} + \mathbf{Z(d_2)} + \mathbf{Z(a_3)} + \mathbf{Z(a_2d_1)} + \ldots + \mathbf{e}$$

(Henderson, 1984), where $\mathbf{a_1}$ is the vector of random additive genetic effects, $\mathbf{d_1}$ of dominance effects, $\mathbf{a_i}$ of powers of additive effects and $\mathbf{d_i}$ of powers of dominance effects. For a description of the (co)variance matrix of each component in non-inbred, unselected populations, see Lynch and Walsh (1998). Evaluations based on this model require estimates of variance ratios for each genetic component.

Selection specifically for epistatic effects is unlikely to be productive since favourable combinations are broken apart by recombination. However, predictions of additive values are biased if these effects are not included in the model; in general, the degree of bias increases with the amount of family information used.

Both variance ratios and selection response with epistatic models depend on the initial genotypic frequencies in the particular population, as well as the form of epistasis involved, and may contribute to differing selection response among populations (Quinton *et al.*, 1998). There is no effective way to determine the form of epistasis for a particular trait without genotypic information, but for the purpose of reducing the bias in predicting additive effects (breeding values) a reasonable approximation is to consider only lower-order terms, e.g. only two locus interactions. Van Raden and Hoeschele (1991) included additive by additive effects in an evaluation model, making substantial improvements in computing strategies. There are many practical problems in applications, since the model is computationally intensive, highly sensitive to data inadequacies and dependencies, and requires an estimate of the additive by additive variance, which is likely to be population specific.

Goodnight (1987) suggested that, when epistatic effects exist, there is a greater response from using breeding systems in which within-line selection is performed in many lines, with periodic merging, than from using one large line, because the variance between lines is greater when there is epistasis. The subdivide/merge scheme uses this additional variance. In red flour beetles Schamber and Muir (2001) found that the selection response in the offspring number (a trait associated with epistatic effects) was greater with a subdivide/merge scheme than selection in a single undivided population. Quinton (1999) examined traits with additive by additive effects and concluded that selection among and within crosses between diversely selected sublines was the most effective long-term strategy for both current and future goals.

The advantage of such a scheme in the short term depends on how important the epistatic effects are relative to additive effects. When gene action is additive, greater response, with little difference in inbreeding, is expected from a large, single line, as shown in simulation examples by Smith and Quinton (1993) and experimentally by Goodwill (1974).

Genotype-by-environment interactions

Genotype-by-environment interactions are not uncommon in poultry, as well as in other livestock (see Lynch and Walsh, 1998, for an excellent discussion of the characterization and approaches used in the estimation of these effects). Sheridan (1990) listed environmental factors that differ among or even within poultry farms, many of which can be associated with such interactions. Note that genotype-by-environment interactions can apply to non-additive as well as additive effects. For example, Kennedy and Quinton (1987) found that crosses between two breeds of purebred pigs performed at the mid-parent average in herds with high health status, but above the mid-parent average in herds with low health status.

When there are a small number of specific environments and sufficient genetic connections between environments, evaluations for each individual in each environment are unbiased if calculated from a multiple-trait animal model in which the 'traits' refer to the environments. Alternatively, within-environment predictions could be converted to those for alternative environments, using a prediction equation, as is applied for dairy cattle.

Environments may be random, however, and when they exist, breeding value predictions are biased whether or not family information is used. McMillan (2000) suggested that, in some cases, the environment should be considered specific to each individual, in the sense that an individual has an unique phenotype. For example, broilers with high growth rate are more likely to develop ascites due to the additional stress of growth, but there is no evidence of a genetic correlation between growth rate and

ascites. Simulations with a quantitative trait (growth) and a threshold trait (ascites) associated in this manner produced populations with a structure similar to that found in broiler data. Animal model BLUP-based selection response for the growth trait was greater than that from phenotypic selection, despite the bias due to the fact that individuals with the 'ascites' trait were culled. However, response at 20 generations was greater with phenotypic selection when the comparisons were made at a fixed level of the ascites trait.

Linkage

Breeding value predictions based on more than a single generation of data are biased when loci are linked, unless the method accounts for the recombination rate. Animal model BLUP-based predictions made without considering linkage are biased, and the extent of the bias increases with linkage. It should be noted that phenotypic selection over non-overlapping generations is not affected by bias.

Simulations of replicate populations in which selection was based on animal model BLUP that did not account for linkage were compared in a study by Quinton

(unpublished) with replicates in which selection was based on phenotype. The trait under selection had a heritability of 0.1 and was determined by 64 bi-allelic additive loci, each with the same physiological effect, and with initial frequencies randomly selected from a beta (U-shaped) distribution. Each replicate consisted of 256 individuals, half of each sex, of which 64 females were bred, and produced four offspring each generation. Selection intensity was controlled by the number of sires selected. There were 1000 replicates of each case.

Figures 12.1 and 12.2 show the ratio of average response using animal model BLUP-based selection to that using phenotypic selection after 10 and 20 generations of selection. The ratios decrease as linkage increases, illustrating the increasing disadvantage of selection on standard animal model BLUP evaluations, relative to phenotypic selection. Response is greater for selection based on the animal model evaluations in all cases, but decreases from generations 10 to 20. At generation 10, except for the most intensely selected replicates and those with complete linkage, the relative response showed little effect of selection intensity, whereas at generation 20 the relative response using BLUP decreased as
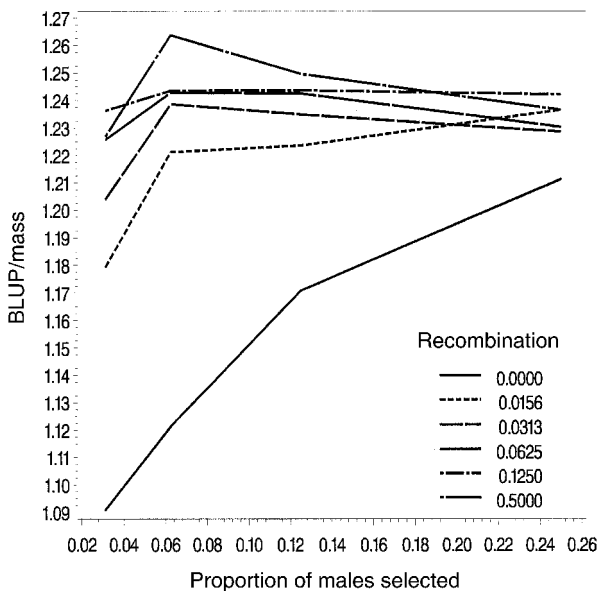
Fig. 12.1. Ratio of average response using standard animal model BLUP-based selection to that using phenotypic selection after ten generations of selection.
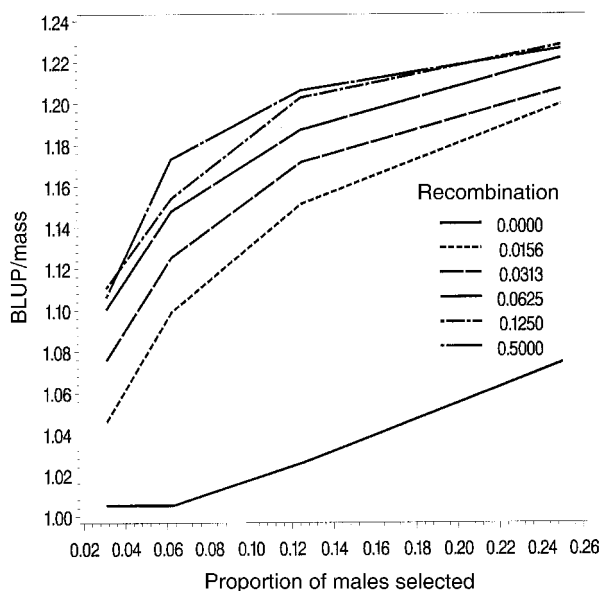
Fig. 12.2. Ratio of average response using standard animal model BLUP-based selection to that using phenotypic selection after 20 generations of selection.

intensity increased, for all recombination rates.

For very long-term selection in linked loci, Hospital and Chevalet (1993) found from simulation results that the optimal selection intensity to maximize ultimate response under phenotypic selection was smaller than 0.5, as was originally predicted for independent loci by Robertson (1960). The optimum selection intensity for BLUP-based programmes will be much smaller than those for phenotypic selection, since the effective population size is reduced more quickly.

Mutation

There are undoubtedly mutational effects on traits under selection which, unless they are very large, remain undetected. These events are rare and are not considered in the time frame of selection in most livestock species. Generation intervals are much shorter in poultry than for other livestock species and changes in the genetic variances of quantitative traits due to many small mutation events appear likely in lines selected over as few as 30 years (Gowe and Fairfull, 1986).

Mutation effects increase genetic variance over time and so standard animal model predictions that are estimated without accounting for the additional mutational variance are biased. Wray and Thompson (1990) described an animal model that accounts for the additional mutational variance and would be appropriate as the basis of selection when the mutational variance is known. In practice, there are few estimates of mutational variance and a more important question may be whether or not favourable mutations are more likely to be lost under animal model BLUP-based selection than under phenotypic selection.

Fixation probabilities of favourable mutations depend on the population size; the effective size of a population under selection depends on the number of parents selected each generation and the method by which they are selected. Since effective population size is reduced more rapidly with BLUP-based selection, the loss of favourable mutations in the long term is a concern. Hill (1985) used simulations to estimate effective population size and fixation probabilities of small to moderately sized favourable mutations in small populations under five selection strategies. Although the methods do not specifically include an animal model, they give an indication as to the effect that including family information in

the selection criterion has on fixation probabilities. Hill (1985) suggested that the most important factor in fixation probability, especially for low heritability, is effective population size, which was largest with within-family selection and smallest with family selection. Fixation probabilities were always smallest with family selection. For higher heritability, with 25% selected, fixation probabilities were higher with within-family and phenotypic selection; with 50% selected, they depended on the size of the mutation.

Effective population sizes are reduced more rapidly as more family information is used, and more rapidly for lower heritabilities, since more weight is placed on family information. Thus, for a given number of parents, fixation probabilities are expected to be lower with animal model BLUP-based selection than with phenotypic selection, particularly for low heritability traits such as egg production.

Mutations are vital for livestock production in the long term, in order to maintain genetic variability and selection potential for new objectives, or to adapt to new environments. It is difficult, however, for breeders to maintain a competitive advantage in the short term without intense selection. Hill (1985) suggested that breeders should maintain both a small line under intense selection and a larger, less intensely selected one, in reserve.

Imprinting

There are several examples of inheritance in which autosomal genetic effects are expressed differently, depending on whether they are inherited from the sire or the dam. Prediction methods that use family information will be biased if this difference is not accounted for in the model. Since more family information is used by the animal model than in most selection indices, the bias is likely to be greater for the standard animal model.

Schaeffer *et al.* (1989) proposed a model that accounts for such effects by including both additive genetic effects expressed regardless of sex of the contribution, and additional effects expressed only when derived from the maternal (or paternal) gamete. The model requires the inverse of additive genetic and gametic relationship matrices, for which simple computation rules are given, and an estimate of additive genetic and maternally (paternally) imprinted variances, expressed as a ratio of residual variance.

Gametic phase disequilibrium

Most poultry populations, prior to the start of a selection programme, have either been previously selected, or are the offspring of recent crosses between selected lines. Dairy cattle populations have also been selected intensely over the years, and show direct evidence of gametic phase disequilibrium, based on molecular methods.

Initial gametic phase equilibrium probably holds only in simulated populations, and although the standard animal model accounts for changes due to selection if the complete pedigree is used, complete records from an equilibrium state are very unlikely. Without specific information as to the form of initial disequilibrium, changes in variances and covariances between relatives cannot be predicted properly.

Problems in Applications

Data structure and confounding

Evaluation models require adequate information if they are to be useful. In general, bigger models need more information in order to distinguish effects; inadequate data will mean an over-parameterized model, which is highly unstable. Animal models can be very complex and, for some data, are unable to separate genetic effects from each other or from non-genetic effects. Models that account for non-additivity are particularly difficult to use in practical situations.

Confounding between systematic and random genetic effects is difficult to measure in large sets of field data. Several methods of assessing connectedness are described by Kennedy and Trus (1993), and individual

results using an approximate method are now supplied to breeders in the Canadian national swine evaluation programme.

Predicted breeding values between individuals without genetic connections are not directly comparable. This is not always apparent from the data or from the solutions, and may be deceiving. The user must always ensure the quality of the data before analysis.

Many breeders interpret favourable trends in predicted breeding values over time to mean that selection has been effective. However, the size of the trend depends on the value of heritability used in the model, and appears larger when too high a value is used. Management effects may show a negative trend in this case. Trends in phenotypic means, as well as in management effects, should always be examined in addition to those in predicted breeding values, since in practice heritability is not only unknown, but can vary – due, for example, to genotype-by-environment interaction, or to undetected scale effects.

Restrictions on parents

The number of birds measured in full- and half-sib families is typically very large, and most breeders apply restrictions to the matings, or the number of related individuals used as parents, to avoid or reduce the rate of inbreeding.

Animal model predictions are not affected by such restrictions, provided that all the data is used in the evaluation model, but the extent to which selection response is reduced by these restrictions depends on the evaluation method. The best strategy choice, including the number of individuals of each sex to be used as parents, depends on the method of prediction, as well as other factors, including the length of duration of the selection programme and the heritability.

Selection for more than one trait

Single-trait objectives in breeding are rare; most breeders select for a large number of traits, using economic values as weighting factors, combined into an overall objective function. Leeson and Summers (2000) listed up to 20 traits commonly used in the broiler industry, for example. Within-line selection on several traits can be applied by independent culling levels, tandem selection, or selection on an economic index of the traits, but Hazel (1943) showed that the expected response for a linear objective function is greatest with selection on an economic index. The economic index accounts for correlations among the traits, and may be generalized to include family information as well.

The application of the selection index is not straightforward over multiple generations, since the genetic variances and covariances among all traits involved are used to construct the index and require regular updating. McMillan *et al.* (1995) showed that covariances among traits may change substantially due to index selection.

Henderson (1984) extended the animal model to multiple traits. Under a multiple-trait animal model, both variance and covariance changes not due to gene frequency changes are accounted for and the construction of the economic index is a straightforward application of the economic objective to the breeding values. Many objectives are linear in the trait means, but there is considerable literature on the application of non-linear and intermediate optimum objectives (e.g. Goddard, 1983; Pasternak and Weller, 1993).

Multiple-trait animal models are used extensively in dairy, beef and pig breeding but are more difficult to apply: they require not only knowledge of both variances and covariances in the base population, but also more powerful computers in order to achieve solutions. Multiple-trait animal models are also more sensitive to inappropriate assumptions and to data problems, such as partial or complete confounding among individuals and traits recorded.

Maternal effects animal models are a particular form of a multiple-trait animal model and are widely used, especially for beef and pigs.

Culling

Populations that are selected intensely may become highly inbred and show reduced levels of fitness. Many breeders practise culling on fitness-related traits throughout the lifetime of the bird and this has been recommended for maintaining reproductive ability in selection of layers (Gowe *et al.*, 1993). Meuwissen *et al.* (1995) recommended selection for production with culling for fitness, based on a simulation study which assumed that production and fitness were uncorrelated.

Culling on the basis of fitness traits that are correlated, or that interact, with those in the selection criterion will cause bias in selection index and BLUP-based predictions (Pollak *et al.*, 1984) if the correlation is not accounted for by using a multiple-trait model. Selection response will be reduced in this case (Mehrabani-Yeganeh *et al.*, 1999). The bias is likely to be more serious with predictions based on several generations of data – increasingly so for low heritability traits, since the (co)variance changes are predicted incorrectly.

Inbreeding

Without mutation, isolated groups of individuals tend to become inbred over time, and the level of inbreeding increases more rapidly in smaller groups. An inbred population is, in effect, smaller than a non-inbred population of the same size since fewer genes are available for selection. Although the offspring of a cross between two inbred populations is not itself inbred, there are fewer genes in the crossed population than in the combined parent populations before inbreeding, and the inbreeding level in the crossed population will increase rapidly over the next two generations of selection to almost the level attained before crossing (Smith and Quinton, 1993).

Populations under selection for a heritable trait become more inbred over time than unselected ones, because relatives of the best individuals are more likely to express desirable values of selected trait(s). Under artificial selection, if the selection criteria or predicted breeding values include information from relatives, they are more similar among relatives than the corresponding phenotypic records. The extent of the similarity increases as information from more relatives is used and is greatest for BLUP-based breeding values, which incorporate data from all known relatives. The partial regression coefficients in the prediction equation are weighting factors; they are smaller, but differ less, for traits with lower heritability. For example, the weights based on using: (i) only the individual's record, (ii) the individual's and its sire's (or dam's) record, and (iii) the individual's, its sire's or dam's, and its full-brother's or sister's record, are shown in Table 12.1.

Correlations among predicted breeding values for family members increase as more family information is used and as heritability decreases. Mates selected on such predictions are more likely to be related; as a result, inbreeding will increase more rapidly, especially if heritability is low. In fact, one strategy for reducing the accumulation of inbreeding suggested by Quinton and Smith (1995) is to use an artificially high heritability in prediction. The effect, however, as with other methods of controlling inbreeding, depends on the situation and the objectives.

Table 12.1. Weight given to information sources in predicting values.

	$h^2 = 0.4$			$h^2 = 0.1$		
	Individual	Sire/dam	Brother/sister	Individual	Sire/dam	Brother/sister
(i)	100%			100%		
(ii)	75%	25%		63%	32%	
(iii)	63%	18.5%	18.5%	53%	23%	23%

Inbred populations are effectively smaller than non-inbred ones and therefore have less potential for future selection. Smith (1967) found that, for a given selection intensity, the selection response increased approximately as the log of the number of individuals tested. As a result, selection response per generation under BLUP-based selection will eventually decline, despite the increase in precision over time. Figure 12.3 shows the average cumulative and Fig. 12.4 the per generation ratio of response under an animal model BLUP-based to phenotypic selection over 100 replicates, simulated using an infinitesimal model with a heritability of 0.1., for a population of 128 males and 128 females. In each generation 64 females were selected, and two offspring of each sex were recorded. Either 16, 32 or 64 males were selected. The cumulative response ratio
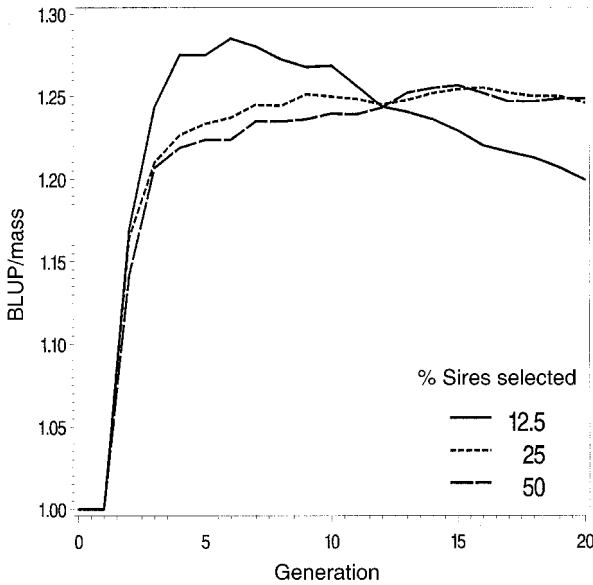
Fig. 12.3. Ratio of cumulative response using animal model BLUP-based selection to that using phenotypic selection.
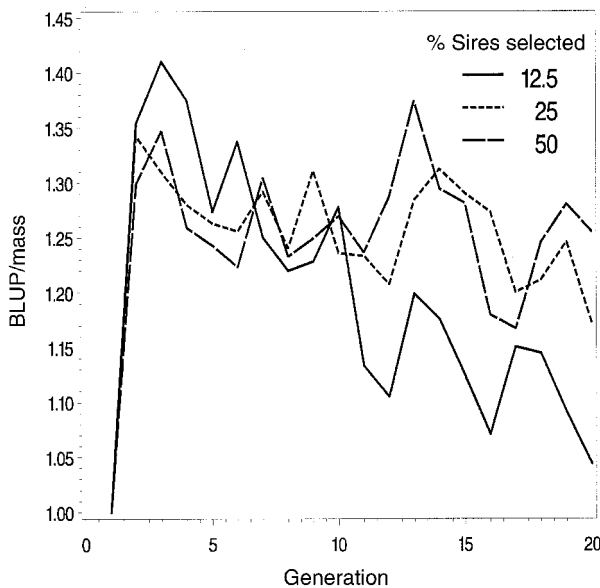
Fig. 12.4. Ratio of response per generation using animal model BLUP-based selection to that using phenotypic selection.

decreases after eight generations only for the most intensely selected replicates, by 13%, whereas the per generation response ratio decreases after five generations, for all selection intensities, by up to 26%.

One of the simplest methods of reducing the increase in inbreeding is to select more parents, at the cost of reduced response. Quinton and Smith (1995) made comparisons among this and other selection/evaluation strategies, such as the use of an artificially high heritability, at the same level of inbreeding in order to standardize potential future selection response. Results from simulated data showed that there was generally little effect of increasing the number of parents selected on cumulative response at higher inbreeding levels, but the best choice of strategy depended on many factors, including heritability, population size and time horizon. Other strategies have been proposed to reduce the rates of increase in inbreeding while maintaining selection response, with some success (e.g. Toro and Perez-Enciso, 1990; Brisbane and Gibson, 1993; Wooliams and Meuwissen, 1993). A practical method, particularly important for poultry populations with very large full-sib families, is to restrict the number of individuals selected from the same family. Morris (1997), using broiler data, compared estimated response to selection on phenotype and selection on predicted breeding values, based on animal model BLUP or selection index, for growth, breast meat yield and egg production. Estimated response using BLUP was greatest, followed by that using selection index, for all traits, whether restrictions were used or not, but the differences among the methods were smaller when restrictions were used.

Although some production traits are not affected by inbreeding, inbred individuals are more likely to express deleterious recessive alleles associated with fitness. Reproductive difficulties can not only prevent a selection programme from continuing, but also interfere with the multiplication of breeding stock for commercial purposes prior to crossing.

Discussion and Recommendations

Breeding values that are predicted using an appropriate animal model BLUP are more accurate than those based on only individual records, and the accuracy increases with the amount of family information available. Selection based on such predictions will show more response, at least in the short term, unless there are serious deviations from the assumptions. Simulation studies have found the basic animal model to be quite robust to deviations from the assumptions, and selection based on predictions from the animal model is generally the most effective strategy for improving the additive genetic value, in the short term. The definition of short vs. long term, however, depends on the initial population size and selection intensity; short term means fewer generations for small populations and/or intense selection. In addition, since BLUP-based predictions are biased when there are deviations from the model assumptions, selection response is reduced – and is reduced more rapidly the more family information is used. As a result, the length of time for which BLUP-based selection is effective is less when there are serious deviations from the model assumptions.

Although breeding value predictions for low heritability traits benefit more from the use of family information, this is at the cost of increased rates of inbreeding, which reduces future response. Biases due to failures in the assumptions are also greater for low-heritability traits. BLUP-based selection is generally more effective than phenotypic selection for these traits, but for a shorter period of time, and it is much more important to minimize any biases. Reproductive traits, such as egg production and related traits, are a particular concern since heritabilities are generally low and they are associated with non-additive genetic effects. It is recommended that BLUP models for such traits incorporate dominance, or that, for more practical applications, the approach suggested by Johansson *et al.* (1993) should be considered.

Models that account for deviations from the assumptions of the basic animal model have been found effective in removing prediction bias in simulated data. However, these are rarely practical for field data, since they are almost always over-parameterized, particularly when a multiple-trait model is required. There is often less risk associated with selection based on a simpler model, despite the prediction bias.

Animal model BLUP-based selection is the best strategy for maximizing genetic gain in the short term, but applications require continual checks on response, management trends, inbreeding levels and changes in the predicted breeding values of individuals as more data is added. Variance and covariance components should also be re-estimated occasionally.

Response to intense selection is likely to be greater when sublines are combined, and animal model BLUP-based selection will be effective for a longer period of time. However, this strategy is inappropriate in the long term, for several reasons.

Favourable mutations are more likely to be lost in selected lines due to the decline in effective population size, whereas overall effective size is at least as large as the total number of sublines, regardless of the selection strategy used within each subline.

Beneficial epistatic effects may be associated with allelic effects that are not favourable individually. When some of these alleles occur at sufficiently low frequencies, selection pressure is against the epistatic effect, and the only possibility for a positive outcome will occur in crosses among stocks with different allele frequencies.

Flexibility is lost for both current and future objectives. Smith (1985) concluded that developing many diverse breeding stocks in a variety of environments is worthwhile in reducing future uncertainty from a national or global viewpoint, although not practical for many breeders. Fairfull and Gowe (1986) and Notter (1999) expressed concern over the declining genetic diversity of poultry stocks. In the past, large numbers of small-scale breeders applied a wide variety of selection criteria under numerous conditions, but the economic advantages for large-scale breeders operating in controlled management conditions have susbstantially reduced the numbers of these and, as a result, the variety of stocks. Smith (1984) proposed conserving many diverse stocks in small numbers, kept as pure lines rather than as gene pools, and using frozen genetic material if necessary. Breeding companies with long-term goals may also benefit from maintaining smaller, more intensely selected lines for maximum early response, possibly using animal model BLUP-based selection, and larger, less intensely selected lines that are selected using individual phenotypes for improved longer-term response (Hill, 1985).

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13 Direct Selection for Improvement of Animal Well-being

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Introduction

In the ongoing debate about the welfare of domestic animals, the two most frequently identified remedial measures involve developing practical improvements to the animal's housing system or increasing its ability to adapt to its environment (Faure, 1980). Unfortunately, these are often wrongly regarded as alternative rather than complementary strategies. Thus, whereas the development of alternative 'welfare-friendly' systems or the modification of existing ones is widely thought to be desirable, genetic selection for increased adaptability is often criticized on ethical grounds. Ideally, an integrated approach should be adopted in order to maximize welfare, productivity and product quality (Faure, 1980; Jones and Hocking, 1999). This chapter will focus on selective breeding as a rapid and reliable way of increasing adaptability.

Selecting for adaptability has been practised since the dawn of domestication. The first phase involved choosing appropriate species for domestication; this was a very restrictive process because only about 20 mammalian and ten avian species were actually domesticated, and some of them with only limited success. Most of the species chosen shared common characteristics, such as the tendency to live in large social groups with hierarchical structures, promiscuous mating, precocial young, low fear reactions and a readiness to accept a wide range of food sources (Hale, 1969). The second phase soon followed and it lasted until the 1950s. This phase, which was characterized by an exaggeration of the favourable characters, was very efficient as is clearly demonstrated by the results of numerous comparisons of domestic animals with their wild ancestors or feral counterparts. The most commonly reported behavioural effects of domestication include reductions in aggression (chickens: Rose et al., 1984; ducks: Desforges and Wood-Gush, 1975b), in fear reactions (chickens: Rose et al., 1984; Jones, 1996; ducks: Desforges and Wood-Gush, 1975a) and in pair bonding (ducks: Desforges and Wood-Gush, 1976). These changes have resulted from both 'natural' and unconscious artificial selection. Natural selection occurred because at an early stage in the domestication process animals that adapted poorly to the domestic environment showed poor reproductive performance; thus they either contributed less to subsequent generations or were eliminated. It was also shown that pheasants performed better when they were housed in the environment (battery cages or outdoor aviaries) in which they had been selected (Faure et al., 1992). Unconscious artificial selection contributed

to the domestication phenomenon as farmers eliminated animals that caused them problems.

Some breeding companies have probably already begun the third phase, which involves conscious artificial selection for adaptability. The need for this approach arose for several reasons.

1. The rearing environment is changing too rapidly for natural selection to be efficient (Faure, 1980). For example, in Europe most laying hens were reared in floor pens until the end of the 1960s; then they were nearly all housed in standard battery cages during the 1980s and 1990s but, as required by a recent EC directive (1999/74/CE), from 2012 they can only be kept in aviaries or enriched cages.

2. The breeding and rearing environments are different. Although details of selection programmes are commercially sensitive, it is understood that many poultry breeders have emphasized the performance testing of individuals in single-bird cages rather than progeny testing (e.g. reciprocal recurrent selection) in multiple-bird cages (see Muir, Chapter 14). While this procedure accelerates genetic progress, it introduces the risk that the birds may be less well adapted to group housing in larger cages, floor pens, aviaries or free-range.

3. If a commercial cross failed to fulfil a farmer's requirements it could be replaced by another, but this possibility is rapidly diminishing with the decrease in the number of breeding companies. Nowadays, virtually all white and brown egg layers, broiler chickens and turkeys are produced by four, six and three companies, respectively (Reffay, 2000).

4. Whereas unconscious artificial selection was possible when farmers were also breeders and were able to identify all their birds, this is no longer the case because breeders keep the birds in flocks of several thousands.

The ethical objections raised by opponents of selective breeding for behavioural traits centre on unfounded claims that it would change the 'nature' of the animals or, even worse, transform them into brainless pieces of meat. This viewpoint derives from the totally erroneous assumption that domestic animals (whether farm or pet) closely resemble their wild ancestors. In reality, any person who has had contact with dogs (the species submitted to the greatest selection for behavioural traits) will know that different breeds have different potentials. These potentials all existed in the common ancestor but some have been intensified in certain breeds while others have been reduced to the point where they are hardly apparent. All studies of behaviour genetics show that the behavioural changes accompanying selective breeding are quantitative rather than qualitative and that it is thresholds of response that have been changed (Price, 1999).

In some circumstances, selection for behavioural traits can be used to counteract the harmful effects of indirect artificial selection or genetic drift. For example, feather pecking in laying hens could result from genetic drift because large differences can be observed between lines with similar selection histories (Muir and Craig, 1998). Excessive aggression can also be an indirect consequence of selection for increased egg production in laying hens (Bhagwat and Craig, 1977, 1978). Currently, attempts to minimize feather pecking and cannibalism involve rearing the birds in small groups in battery cages (Allen and Perry, 1975; Hughes, 1982), housing in dim light (Elson, 1990; Appleby *et al.*, 1992), beak trimming (Craig and Lee, 1990), or a combination of these approaches. However, these 'remedial measures' all have associated welfare problems (Gentle, 1986a,b; Jones and Hocking, 1999) and selection against this behaviour seems likely to provide the most ethically acceptable solution (Muir and Craig, 1998; Jones and Hocking, 1999).

Selection criteria for behavioural or other traits must fulfil at least two major requirements in order to have practical relevance for breeding companies. First, the selected characteristic must not be specific to a particular environment, otherwise its relevance runs the risk of being overtaken by the rapid evolution of housing systems. For example, it is possible to select for reduced

pre-laying pacing by hens kept in battery cages (Mills *et al.*, 1985b) or for a reduced motivation to dustbathe (Gerken and Petersen, 1985). However, such selection programmes will soon be irrelevant in Europe because laying hens will be provided with a nest and a dustbath from 2012. Secondly, the selection process must be affordable. This depends largely on the complexity and cost of the techniques available to measure the particular behaviour pattern as well as the heritability of the character (Faure, 1981a).

Numerous behavioural traits are known to be sensitive to genetic selection in poultry and many show moderate to high heritability (Table 13.1). Here and throughout this chapter a broad-brush approach is taken in considering the effects of selection for behavioural traits. For example, close attention will be paid to physiological measures of stress susceptibility, because changes in this character have been closely linked with some of the selected behaviours (Jones and Hocking, 1999) and they bear particular relevance for the animals' welfare and productivity. Behavioural traits such as fearfulness and sociality (motivation to be near conspecifics) can clearly exert profound effects on poultry welfare (Jones,

Table 13.1. Summary of the selection experiments for behavioural and stress-related traits conducted in different poultry species.

Species	Character	Generation number	h^2	Reference
Fear and stress susceptibility				
Pheasant	Open-field behaviour	1	0.02–0.22	Boyer *et al.*, 1973
Domestic fowl	Open-field behaviour	3	0.00–0.40	Faure and Folmer, 1975
Domestic fowl	Tonic immobility	1	0.78–0.91	Gallup, 1974
Domestic fowl	Tonic immobility	1	0.18–0.32	Campo and Carnicer, 1993
Quail	Tonic immobility	8	0.12–0.28	Mills and Faure, 1991
Domestic fowl	Susceptibility to social stress	6		Gross and Siegel, 1985
Turkey	Susceptibility to temperature stress	3	0.31	Brown, 1968
Quail	Susceptibility to restraint stress	9	0.15–0.33	Satterlee and Johnson, 1988
Social/sexual				
Domestic fowl	Feather pecking	1	0.09–1.04	Bessei, 1996
Domestic fowl	Imprinting	5	0.28–0.32	Graves and Siegel, 1969
Domestic fowl	Dominance ability	4	0.00–0.43	Guhl *et al.*, 1960
Domestic fowl	Dominance ability	5	0.16–0.28	Craig *et al.*, 1965
Quail	Social reinstatement	8	0.06–0.43	Mills and Faure, 1991
Domestic fowl	Sexual behaviour	11	0.14–0.36	Siegel, 1965
Domestic fowl	Sexual behaviour	3		Wood-Gush, 1960
Domestic fowl	Sexual behaviour	2		Tindell and Arze, 1965
Quail	Sexual behaviour	6	0.04–0.29	Sefton and Siegel, 1975
Laying/brooding				
Domestic fowl	Broodiness	5		Goodale *et al.*, 1920
Domestic fowl	Floor laying	6		McGibbon, 1976
Domestic fowl	Pre-laying pacing	2		Mills *et al.*, 1985b
Others				
Domestic fowl	Colour preferences	3	0.03–0.23	Hurnik *et al.*, 1977
Quail	Colour preferences			Kovach, 1978
Quail	Activity	5		Bessei, 1979
Quail	Dustbathing	7	0.18–0.38	Gerken and Petersen, 1985

When more than one value of h^2 was given (calculation at different generations, various methods of calculation) by the authors, only the two extremes are reported in the table. When several papers were published on one selection experiment, only the first is shown.

1996; Faure and Mills, 1998; Jones and Hocking, 1999) but others may also exert important indirect effects. First, environmental constraints often preclude the normal expression of sexual, dustbathing, brooding and laying behaviour and this might result in frustration if the birds are predisposed to show high levels of these behaviours. Secondly, some birds show specific preferences (e.g. for colour) and it is conceivable that the satisfaction of such preferences might improve their welfare (Hurnik *et al.*, 1977).

Fear and Stress Susceptibility

Fear is one of the main emotions that govern an animal's life. In ideal circumstances fear is adaptive, but in reality the restrictions imposed by many farming systems can interfere with a bird's ability to respond in an adaptive fashion. For example, a caged hen cannot run away from danger and chickens on a free-range area lacking in shelter cannot hide from an aerial predator. The expression of inappropriate fear responses, such as panic or violent escape, can waste energy and cause injury, pain or even the death of the bird or its companions (Mills and Faure, 1990; Jones, 1996, 1997). Chickens characterized as fearful (i.e. easily frightened by a wide range of intuitively alarming events) also show poorer egg production, decreased hatchability, reduced food conversion efficiency, lower growth rates and poorer meat quality than do less fearful ones (Mills and Faure, 1990; Jones, 1996, 1997; Hemsworth and Coleman, 1998; Jones and Hocking, 1999). Furthermore, because fear inhibits the expression of all other motivational states (Archer, 1976; Jones, 1996, 1997) its elicitation is likely to compromise the bird's abilities to interact successfully with its companions and with the stockperson, to utilize new resources, and to adapt to changes in its environment. Thus, heightened fearfulness and the expression of fear responses can seriously damage poultry welfare, productivity and profitability. Similarly, despite their ideally adaptive role in maintaining

bodily homeostasis, the corticosteroids may counter-regulate and exert a number of harmful effects, including immunosuppression, reduced growth, decreased reproductive performance and increased deposition of fat (Zulkifli and Siegel, 1995; Jones, 1996). Chronically elevated levels of corticosterone can also increase underlying fearfulness; this may, in turn, exacerbate the adrenocortical stress response and thereby establish a vicious circle (Jones *et al.*, 1988, 2000a; El-lethey *et al.*, 2001).

Clearly, it is imperative that practical ways are found of reducing harmful states, such as fear and chronic distress, from the viewpoints of the birds, the farmers and the public. Although domestication has resulted in increased docility, the clear opportunity for further genetic improvement is illustrated by the considerable diversity in these traits that still exists within and between populations of gallinaceous birds (Faure, 1979, 1981b; Jones and Hocking, 1999). This section focuses on the effects of genetic selection for short tonic immobility fear reactions and for reduced plasma corticosterone responses to brief mechanical restraint. Studies have focused primarily on the Japanese quail, because of its low maintenance costs and short intergeneration interval and because it is considered to be a low-cost and useful model for the domestic fowl (Aggrey and Cheng, 1994; Jones, 1996; Faure and Mills, 1998). It is also an important agricultural species in many countries (Baumgartner, 1994). The considerable phenotypic variation that exists in fear and in adrenocortical responsiveness means that poultry can be selectively bred for contrasting levels of these traits.

A number of successful selection programmes have already been established in the laboratory. Differences in tonic immobility (TI) fear responses to manual restraint were apparent in chickens after genetic selection for just one generation (Gallup, 1974) and divergent selection for low or high ambulation in a novel test arena (Faure and Folmer, 1975; Bessei, 1979; Faure, 1981b,c) was associated with reduced fearfulness in domestic chicks (Faure, 1981c) and Japanese

quail (Jones *et al.*, 1982). More recently, divergent lines of Japanese quail have been selected in France by Mills and Faure (1991) for long (LTI) or short (STI) durations of their TI reactions to manual restraint and in the USA by Satterlee and Johnson (1988) for exaggerated (high stress, HS) or reduced (low stress, LS) plasma corticosterone (C) responses to brief mechanical restraint.

TI is an anti-predator reaction that is positively related to the antecedent fear state (Gallup, 1979; Jones, 1986). This behavioural response was induced by placing a 9–10-day-old quail on its back in a U-shaped cradle and holding it down for 10 s (Mills and Faure, 1991). Upon release, the latency until self-righting was then measured. The risk of potential co-selection for sociality was minimized (Mills and Faure, 1991). Significant behavioural differences between the TI lines were apparent at the first generation of selection. The absence of overlap between the standard deviations of the LTI and STI lines from generation 8 (G8) onwards strongly suggests that they had become distinct genetic lines. Indeed, durations of TI were 216, 51 and 9 s in the LTI, control and STI lines, respectively, at G20 (Faure and Mills, 1998).

The HS and LS quail were selected at Louisiana State University on the basis of their adrenocortical responses to restraint for 5 min in a metal crush cage, a device in which a moveable interior wall can be fixed in place so that it restricts all the birds' movements other than those associated with respiration and slight postural changes (Satterlee and Johnson, 1988). Following pedigree selection for 12 generations, average plasma C levels after restraint in the HS and LS quail were 156% and 54% of the values measured in non-selected controls. Despite occasional relaxation of selection pressure, restraint of G21 quail resulted in circulating C concentrations of 29.0 ± 5.4 and 13.7 ± 4.1 ng ml^{-1} (means ± standard errors) in HS and LS birds, respectively (Jones and Satterlee, 1996). Examination of the lines at G26 showed that divergence had been maintained (13.1 ± 0.1 and 6.8 ± 0.1 ng ml^{-1} in HS and LS quail) while reimposition of selection pressure at G27

resulted in plasma C levels of 14.7 ± 0.3 and 6.3 ± 0.2 ng ml^{-1} in HS and LS birds, respectively (D.G. Satterlee, personal communication). An intermediate plasma C level of 7.7 ± 0.2 ng ml^{-1} observed in random-bred quail at G27 was significantly different from those found in either of the two selected lines.

Despite their intuitive desirability, the selection programmes described above would have little practical relevance in terms of improving the birds' ability to adapt to challenge if the selected responses were specific to the test situations, i.e. manual or mechanical restraint. Encouragingly, though, it was found that selection has modified the quails' behavioural responses to a wide range of intuitively frightening and otherwise stressful events (Table 13.2). Thus, compared with LTI and HS quail, those of the STI and LS lines showed: attenuated tonic immobility reactions (Jones *et al.*, 1991, 1992a); less pronounced inhibition of behaviour during mechanical restraint (Jones *et al.*, 1994a, 2000a; Jones and Satterlee, 1996); less silence and inactivity during enforced exposure to a novel environment (Jones *et al.*, 1991, 1992b); reduced avoidance of novel objects and human beings (Jones *et al.*, 1992a, 1994b); and accelerated emergence from a sheltered area into an exposed, unfamiliar and, hence, frightening one (Jones *et al.*, 1991, 1999b). Simplistically, fear inhibits all other behaviour systems and so a frightened bird would be characterized by silence and inactivity (Archer, 1976; Jones, 1987, 1996). Collectively, these findings clearly demonstrate that selection of the STI and LS lines has been accompanied by reductions in underlying fearfulness. The STI quail also showed lower adrenocortical responses to a mild stressor (ball dropped into home cage) than LTI ones (Launay, 1993), but mechanical restraint elicited similar high levels in both lines (Jones *et al.*, 1994a). It was proposed that this powerful stressor exerted a ceiling effect, thereby swamping any line differences in circulating C levels (Jones *et al.*, 1994a) but a more recent report that restraint induced a greater adrenocortical response in STI than LTI quail (Faure and Mills, 1998)

Table 13.2. Associated effects of genetic selection for low (LS) or high (HS) plasma corticosterone (C) responses to mechanical restraint, short (STI) or long (LTI) tonic immobility fear reactions, or high (HBW) or low (LBW) body weight in Japanese quail.

	Line comparisons		
Measures	LS vs. HS	STI vs. LTI	HBW vs. LBW
Tonic immobility	LS < HS	STI < LTI	HBW < LBW
Restraint-induced inhibition	LS < HS	STI < LTI	HBW < LBW
Inactivity in novel environment	LS < HS	STI < LTI	?
Timidity in emergence tests	LS < HS	STI < LTI	?
Avoidance of novel objects	LS < HS	STI < LTI	HBW < LBW
Avoidance of human beings	LS < HS	?	?
C response to mild novelty	LS < HS	STI < LTI	?
C response to restraint	LS < HS	Variable	HBW < LBW
C response to other severe stressors	LS < HS	?	?
Ease of capture	LS = HS	STI > LTI	?
Sociality	LS > HS	?	?
Osteoporosis	LS < HS	?	?
Growth	LS > HS	STI = LTI	HBW > LBW
Stress-induced fall in meat quality	?	STI < LTI	?
Developmental instability	LS < HS	?	?
Attainment of puberty	LS > HS	?	?

?, untested.

sounds a discordant and cautionary note. Unlike the TI lines, selection of the low plasma C response line (LS) has resulted in an apparent non-specific reduction in adrenocortical responsiveness. Thus, elevations in circulating C concentrations were significantly lower in LS than HS quail not only in response to the selection stressor but also when they were exposed to a wide variety of stressful events. These included manual restraint, cold, crating, social tension, and deprivation of food and water (Satterlee and Johnson, 1988; Jones *et al.*, 1992a, 1994b, 2000a). Catecholamine responses to brief restraint were also lower in LS than HS quail (Satterlee and Edens, 1987). Evidence of cross-species generalization of this phenomenon is provided by a report that genetic selection of turkeys for a reduced adrenocortical response to cold stress was associated with a lowered plasma corticosterone response to heat stress and with decreased 'excitability' (Brown and Nestor, 1974).

A recent study revealed behavioural evidence of habituation to mechanical restraint (the selection criterion) in both LS and HS quail. However, unlike LS quail in which there were no detectable physiological effects of repeated restraint, the HS birds showed experience-dependent sensitization (Jones *et al.*, 2000a). This finding has potentially far-reaching implications for studies of stress susceptibility and psychopathological conditions. First, it is already known that chronic elevation of plasma corticosterone can increase fearfulness (Jones *et al.*, 1988; El-lethey *et al.*, 2001) and damage disease resistance and performance (Gross and Siegel, 1993; Jones, 1996). Thus, if a proportion of the population of commercially important species, such as chickens or turkeys, showed sensitization of the adrenocortical response to stressful events, like HS quail, this could reduce their ability to adapt to chronic stress and thereby compromise their health and productivity. Secondly, our findings demonstrate the importance of considering the background genome of animals that are used for stress research; indeed, they may explain the fact that repeated exposure to stressful stimuli has been variously reported to decrease, to increase or to fail to affect a range of stress responses, including adrenocortical activation (see Jones *et al.*, 2000a). Thirdly, since sensitization of

response to repeated psychosocial stressors may contribute to the development of pathological anxiety in a variety of animals, including human beings (Rosen and Schulkin, 1998), genetic lines like the HS quail might represent useful models for studying this debilitating disorder. Fourthly, such lines might also be used to illuminate the development of depression in human beings, because this condition is associated with stress-induced elevations of plasma cortisol (Tafet *et al.*, 2001).

Many characteristics can be affected by different genes throughout ontogeny and this can compromise trait stability (Nol *et al.*, 1996). However, the fact that line divergence in TI was still apparent at 10 weeks of age in the STI and LTI quail (Launay *et al.*, 1993) and as late as 30 weeks in those of the LS and HS lines (Jones and Satterlee, 1997) suggests that the selected traits are stable. Underlying sociality is likely to be an extremely important behavioural trait in highly social species, such as quail and chickens. Inappropriate levels of this characteristic could exert undesirable effects on all aspects of social interaction, including affiliation, aggression and mating, as well as on a bird's ability to cope with social disruption (Jones and Mills, 1999). Furthermore, a mismatch between underlying sociality and a bird's social environment could elicit chronic distress or a series of acute stress responses, with the associated harmful effects on welfare and productivity (Jones and Hocking, 1999; Jones and Mills, 1999). Thus, low-sociality birds might be poorly suited for housing in large groups or at high stocking densities. When undisturbed same-line groups of 4-day-old chicks were observed in their home cages at regular intervals, it was found that LS quail stayed closer together than HS ones (Jones *et al.*, 2001). LS quail also spent longer than HS chicks near a goal box containing cagemates when they were tested individually in a runway at 11 days of age (Jones *et al.*, 2001; Marin *et al.*, 2001a). These findings demonstrate that underlying sociality is greater in LS than HS quail, because social proximity in the home cage and reinstatement responses in runway tests are positively

related to this behavioural trait (Vallortigara, 1992; Jones and Mills, 1999). Enhanced sociality could be an additional benefit of this type of selection programme because high-sociality birds, like the LS quail, would probably be better suited for housing in large or high-density flocks. It has been shown that 2-day-old broiler chicks that negotiated a T-maze quickly (< 25 s) in order to regain visual contact with their companions subsequently grew faster (Marin *et al.*, 1997, 1999) and showed greater sociality (Jones *et al.*, 1999a) and a lower adrenocortical response to an acute stressor (Marin and Jones, 1999) than their slower (> 75 s) counterparts. Recent results also suggest that rapid negotiation of the T-maze is associated with accelerated puberty and increased egg production in quail of both the LS and the HS lines (Marin *et al.*, 2001b).

In addition to the likely benefits of selection for reduced fear and adrenocortical responsiveness for the birds' welfare, evidence is mounting for divergence in other physiological measures that may have important implications for poultry performance and product quality. For example, stress-induced reductions in meat quality (increased toughness and water loss) were less pronounced in STI than LTI quail (Faure and Mills, 1998). Similarly, growth rate and bone strength were less severely compromised in LS than HS quail following their sequential exposure to a range of stressors (Satterlee and Johnson, 1988; Satterlee and Roberts, 1990). The hypothesis that fearfulness and growth rate were negatively related was further supported by findings that quail that had been genetically selected for high (HBW) rather than low (LBW) body weight at 4 weeks of age (Marks, 1995) showed low and high fearfulness, respectively (Jones *et al.*, 1997). Indeed, these line differences were particularly pronounced. Thus, quail of the HBW line showed shorter TI reactions, less avoidance of a novel object placed near the home cage, increased vocalization and struggling during mechanical restraint, and lower adrenocortical responses to that stressor (Table 13.2) (Jones *et al.*, 1997). Similarly, laying hens characterized as fearful had lower body weights than less fearful

birds (Bessei, 1984a) and fear was greater in hens that had been selected for low rather than high body weight at 8 weeks (Mauldin and Siegel, 1979). However, the relationship between fear and growth rate might not always be straightforward. For example, the fearful line of chickens selected on the basis of their open-field behaviour were heavier than the low-fear line (Faure, 1977) and strains of chickens selected for body weight showed few differences in open-field behaviour (Faure and Ricard, 1977). Both acute and chronic stress can also exert strong negative effects on reproductive performance in a variety of farm animal species (Jones, 1996; Hemsworth and Coleman, 1998). In this context, it was recently discovered that, compared with HS quail, selection for reduced adrenocortical responsiveness has been accompanied by accelerated puberty in both males and females of the LS line (Marin *et al.*, 2001b). Selection for reduced fearfulness could have implications for the ease with which the birds are handled and managed. A cautionary note had been sounded when Japanese quail selected for high locomotor activity in a novel cage (Bessei, 1979) were found to be not only less fearful than those of the low activity line (Jones *et al.*, 1982) but also significantly more difficult to catch from mixed-line groups (Bessei *et al.*, 1983). Such a negative relationship between fear and ease of capture could lead to management problems if it was generalized to include other low-fear lines. Encouragingly, though, no differences were found between LS and HS quail in their ease of capture from mixed-line groups by an unsighted experimenter (Satterlee and Jones, 1997) and STI quail were actually caught more easily than LTI ones (Mills and Faure, 2000).

Developmental instability, which leads to fluctuating asymmetry (FA) defined as small random deviations from symmetry in otherwise bilaterally symmetrical characteristics, is considered to reflect the inability of individual organisms to produce a stable phenotype under given environmental conditions (Parsons, 1990; Møller *et al.*, 1999). It is widely thought that FA is caused by exposure to genetic (e.g. inbreeding, hybridization) and environmental (e.g. hunger,

pathologies, parasitic infestation, social disturbance) stressors (Parsons, 1990; Møller *et al.*, 1999; Thomson, 1999). It was recently found that developmental instability was less pronounced in 32-week-old male quail of the LS than the HS line that had been housed in same-line breeding groups of ten females and five males from 6 weeks of age (Satterlee *et al.*, 2000). In view of the greater fearfulness and adrenocortical responsiveness evident in HS than LS quail, this finding is considered likely to reflect differential susceptibility to chronic social and physical environmental stressors, such as inter-bird aggression, human traffic, cage cleaning, etc. Since both lines have been subjected to a similar amount of inbreeding (genetic stress) it might tentatively be suggested that selection for reduced fear and distress may increase the animals' ability to produce stable phenotypes.

In conclusion, it is particularly striking that genetic selection for one behavioural reaction, one physiological response or one performance measure affected the way the quail responded to a wide variety of stressful events. Furthermore, it is now evident that selection of the LS quail has not only resulted in a non-specific reduction in adrenocortical responsiveness and fearfulness but that it has also been associated with increased sociality, less pronounced developmental instability and accelerated puberty. These findings provide a platform for future studies in more commercially important species. For example, if comparisons of commercial breeds yield sufficient evidence of genetic variation, polar lines could be crossed and the traits of interest measured in the F_2 generation. Selective genotyping of upper and lower quartiles could then precede a search for quantitative trait loci for fearfulness and/or stress susceptibility. This could, in turn, lead to marker-assisted selection to maintain or remove specific gene alleles (Roussot *et al.*, 2001). The overall objective is to increase the birds' ability to adapt to challenge and thereby to improve their welfare, productivity and product quality. Since the birds' behavioural and physiological capabilities are shaped by their environment and

experience as well as their genome (Jones and Hocking, 1999), an integrated approach involving environmental enrichment (Jones, 1996, 2001; Jones *et al.*, 2000b), socialization/habituation to human contact (Jones, 1995) and selective breeding for reduced fear and distress is considered particularly likely to benefit the birds, the farmers and the consumers.

Social and Sexual Behaviour

Feather pecking

Feather pecking and cannibalism are among the most important behavioural problems encountered in commercial egg production. Feather pecking is a major cause of deterioration of the feather cover. This not only represents a welfare issue; it also increases the energy requirements of the birds under moderate and cold climatic conditions. Cannibalism, which may occur after serious feather pecking or even without any overt prior outbreak of feather pecking, can cause considerable damage in layer flocks. Analyses from random sample tests in Germany suggest that problems with cannibalism have tended to increase during the 1990s (Preisinger, 1997).

The direct observation of feather pecking in chickens is time consuming. Hence, few experiments have been carried out to elucidate its genetic background and to develop strategies for eradicating this harmful behaviour in practical breeding programmes. The first information on the potential genetic background of feather pecking appeared when Oettel (1873) reported a strong tendency towards feather pulling in crested chickens. Sanctuary (1934) assumed that feather pecking was inherited and consequently recommended culling any lines that showed a strong tendency to peck. There is now substantial information on the prevalence of feather pecking and cannibalism in different breeds (Hughes and Duncan, 1972; Perry and Allen, 1976; Blokhuis and Beuving, 1993; Kjaer, 1995; Jones and Hocking, 1999; Hocking

et al., 2001a,b). Although they yield some indication of the genetic variation, breed differences do not provide information on the amount of genetic variation within lines, which is the basis of genetic selection. Heritability estimates, as a measure of the additive genetic variation within lines, were first reported by Cuthbertson (1978), who found significant differences in the tendency to feather peck between and within related lines and estimated an h^2 of 0.10 (Cuthbertson, 1978, 1980). The estimate was much higher (0.56) when families that had shown no feather pecking during the observations were discarded from the analyses. Cuthbertson (1978, 1980) also found a low heritability coefficient for a trait described as the tendency to be pecked and, since the genetic groups that showed high frequencies of being pecked also tended to show less feather pecking, she assumed a negative genetic correlation between feather pecking (peckers) and being pecked (peckees).

Bessei (1984b) estimated the heritability of feather pecking using a total of 1200 pullets of four different genetic groups: pure Rhode Island Red, Sussex and their reciprocal crosses. The heritability coefficients varied from 0 to 0.07. In a further study using pullets of a tinted layer strain and only half-sib groups, the heritability was 0.20 for feather pecking and 0.25 for being pecked (Bessei, 1984c). Kjaer and Sørensen (1997) reported heritability coefficients in White Leghorn hens that varied between 0.06 and 0.14 at 38 weeks and 0.35 and 0.38 at 69 weeks of age. For the trait of receiving feather pecks the heritability coefficient was 0.15 at 6 weeks of age but it did not differ significantly from zero at 38 and 69 weeks of age. As an alternative to the visual observation of feather pecking in penned or caged groups of hens, a method was developed to measure feather pecking automatically (Bessei, 1984c). This methodology required hens to be housed in individual cages and a bunch of feathers was presented at the front of the cage. This bunch of feathers was attached to a sensor so that any pecks delivered to the feathers were recorded. A heritability of 0.18 was estimated for this trait using a half-sib analysis of adult tinted

layers. The genetic correlation between pecking at the bunch of feathers and visual observations of feather-pecking activity of the same birds before the onset of lay was close to zero (–0.04).

Laying hens in the Rhode Island Red and Sussex breeds were later identified as either high or low feather peckers and the offspring of the high and low sublines were observed for feather pecking during the rearing period (Bessei, 1997a). The realized heritability, as estimated according to Falconer (1984), was generally lower in the Sussex than in the Rhode Island breed. The estimates varied between 0.09 and 1.04. Although the number of animals used was relatively low, Keeling and Wilhelmson (1997) reported a clear separation of high and low feather peckers in the F_1 generation in a similar experiment but no heritability estimates were reported. A refined method for the automatic measurement of pecking at a bunch of feathers, which allows gentle pecks and vigorous pulls to be recorded separately, has since been developed (Bessei, 1997a; Bessei *et al.*, 1997). Using this technique, a Rhode Island Red line was tested and characterized as two divergent sublines showing high or low rates of vigorous feather pulling (Bessei, 1997b). The offspring of both sublines were raised in deep litter systems and their feather-pecking behaviour was observed visually from 21 to 26 weeks of age; their responses to the feather bunch were later recorded in individual cages. These birds differed significantly in their feather-pulling behaviour and the phenotypic rank correlations, using group means, were 0.82 in the high and 0.61 in the low feather-pulling sublines (Bessei, 1996). However, the differences between the sublines disappeared in later generations when the birds were raised in intermingled groups from 1 day of age (Bessei, unpublished observations).

In summary, the above results suggest that there is sufficient genetic variation for the establishment of selection programmes intended to eradicate feather pecking. However, the magnitude of the heritability coefficient is very low; this is probably due to the fact that feather pecking and cannibalism are highly sensitive to environmental factors, which may, under certain circumstances, obscure the genetic background of this trait.

Kjaer and Sørensen (1997) found a negative genetic correlation between body weight at 52 weeks of age and feather pecking. They concluded that selection for smaller body size, which has occurred under selection pressure for better feed conversion in laying strains, might have been associated with an increased propensity to show feather-pecking behaviour. Conversely, selection for high levels of feather pecking over four generations has resulted in heavier birds (Kjaer, 1999). This is consistent with Bessei's (1984c) report of positive genetic correlations between feather-pecking activity and body weight of pullets at 2 days, 8 weeks and 20 weeks of age, (0.20, 0.66 and 0.57, respectively). It has also been assumed that selection for accelerated maturity and increased egg production may increase the tendency for social aggression, including feather pecking (Craig *et al.*, 1975; Bessei, 1996). On the other hand, the genetic correlation between feather pecking in pullets and the egg output of the same hens was 0.08 and not significantly different from zero (Bessei, 1996). However, the latter finding may have reflected the amount of time that passed between the observation of feather pecking in the pullets and the measurement of reproductive performance. Indeed, when the adult birds were scored in the feather bunch test, a positive genetic correlation of 0.30 was found between pecking at the feathers and egg mass (Bessei, 1996). Further study is required to determine whether or not pecking at a feather bunch is related to feather pecking and cannibalism, particularly in view of some recent findings. A longitudinal comparison of two breeds thought to show differences in feather pecking revealed no detectable differences in pecking/pulling at a bunch of feathers (Hocking *et al.*, 2001b). Furthermore, although severe feather pecking was virtually absent in that study, pecking at the feather bunch did not predict gentle pecking at other birds (Hocking *et al.*, 2001b). Planned development of the apparatus to allow its use in groups of birds coupled with

the ability to identify pecking individuals within that group will address these issues and lead to design improvements.

Social behaviour

The first selection experiments conducted in domestic fowl were designed to modify dominance ability (Guhl *et al.*, 1960; Craig *et al.*, 1965). In both studies, the results showed that the character was easily selected and it could be modified relatively quickly by selection. This result is in agreement with the existence of gamecocks that were obviously selected for very high dominance ability. It was shown that the heightened dominance ability (HDA) was not specific to the testing conditions (pair contests) but that, in mixed groups of the two strains, birds of the HDA strain were statistically dominant over those of the low strain (Craig *et al.*, 1965). The differences between the two strains existed not only in males (the selected sex) but also in females (Craig *et al.*, 1965). The HDA birds were also more aggressive in pair contests as well as in same-strain groups (Craig *et al.*, 1965). This result can probably explain the interaction observed between strain and rearing conditions for laying rate. It was found that, in individual cages, the laying rates of the two strains were similar whereas the low strain showed a better laying rate in multiple bird cages or in groups kept in floor pens (Biswas and Craig, 1970; Craig, 1970). This suggests that selection for reduced dominance ability, and thus probably also for reduced aggressiveness, could be used to obtain birds that would adapt more easily to normal rearing conditions. The target of the selection programme was found to be the sensitivity of the animals to androgens rather than androgen levels per se, because females or capons of the two strains injected with the same quantity of testosterone showed differences in dominance ability of the same magnitude as that observed in intact males (Ortman and Craig, 1968).

Another way to modify social behaviour is to select for increased social attraction. This has been done using an imprinting situation in chickens (Graves and Siegel, 1968, 1969) and in a more naturalistic situation (reaction to conspecifics) in Japanese quail (Mills and Faure, 1991). In the latter case, quail were selected for the distance that they ran on a treadmill in order to maintain close contact with a group of conspecifics. This selection programme resulted in birds showing extreme differences in social reinstatement behaviour not only in the above testing conditions (Faure and Mills, 1998) but also in a test where the criterion was the time spent close to conspecifics (Launay *et al.*, 1991). The line differences persisted whatever the age of the birds (Launay *et al.*, 1993).

In various tests of social motivation using inter-individual distances or the time spent in visual or physical contact, the age of the birds strongly influenced the responses and this variable interacted with the testing conditions. When differences were observed, quail of the high social reinstatement (HSR) line always showed greater social motivation than those of the low line (LSR). In very young animals (1–2 weeks of age) significant differences between the two lines were observed only if the birds were strangers and were submitted only to short tests. All the differences were significant when the quails were 3–4 weeks old, but at 5–6 weeks of age (near adulthood) significant differences only appeared when physical contact was prevented (François *et al.*, 1998, 1999; Delaval, 2000). It was hypothesized that this was due to increased aggression in the HSR line but this was not confirmed experimentally (François *et al.*, 2000). It was, however, observed that non-aggressive pecking was more frequent in the HSR birds (François *et al.*, 2000). Feather pecking is also more frequent in this line (Bilcik and Bessei, 1993). In a sexual situation, the HSR line showed greater proximity between males and females, shorter latencies to copulate, more copulatory responses and greater copulatory efficiency (Burns *et al.*, 1998). Birds of the HSR line are also easier to capture, presumably because they show less fear of humans (Mills and Faure, 2000). If isolated, HSR quail jumped and peeped

more than the LSR birds. In this situation, HSR quail also showed greater increases in plasma corticosterone levels and in heterophil/lymphocyte ratios than the low line (Mills *et al.*, 1993).

One way of explaining all the differences observed between the HSR and LSR lines is to assume that the real target of the selection has been the general ability of the animals to imprint. This could explain not only the greater reinstatement behaviour of the HSR birds but also their more pronounced sexual behaviour and their reduced fear of humans. At first it was thought that the HSR birds would adapt more easily to normal rearing conditions (high densities and large social groups) but the finding that adult HSR quail show more social reinstatement only if social contact is prevented, and that they have a higher rate of feather pecking, might indicate that the LSR line would adapt more easily to intensive rearing conditions. This possibility requires further investigation.

Although no selection programme was conducted, a very simple T-maze test provided a quick and apparently robust measure of social motivation (Marin *et al.*, 1997, 2001c; Jones *et al.*, 1999a; Marin and Jones, 2000). Broiler chicks that negotiated the T-maze quickly in order to regain social contact were found to put on more weight than their slower counterparts in the laboratory and also at a commercial farm (Marin *et al.*, 1997, 1999). However, this finding is inconsistent with the report that quail selected for high social motivation also showed the lowest body weight (Mills and Faure, unpublished data). If the performance in the T-maze test could be selected for, this might offer a quick and cheap alternative to the treadmill test and could thereby offer a useful technique for selecting for appropriate levels of social motivation.

four different experiments. Three were performed with chickens (Wood-Gush, 1960; Tindell and Arze, 1965; Siegel, 1965, 1972; Dunnington and Siegel, 1983) and one with Japanese quail (Sefton and Siegel, 1975; Blohowiak and Siegel, 1984; Yang *et al.*, 1998). In all these experiments the response to selection was evident after just a few generations. These selection programmes had no effect on sexual precocity (Tindell and Arze, 1965; Cook and Siegel, 1972), on natural fertility (Bernon and Siegel, 1981) or on female sexual behaviour (Siegel and Cook, 1975; Dunnington and Siegel, 1983). Sperm production, as measured by the volume (Wood-Gush, 1960) or by sperm concentration (Siegel, 1965), was higher in the low line. It was shown in cocks and in quail that injection of the same dose of androgens to capons of the two lines restored sexual behaviour and the differences between lines (McCollom *et al.*, 1971; Cunningham *et al.*, 1977; Van Krey *et al.*, 1977). This suggests that the behavioural differences are due, at least partly, to differences in the birds' sensitivity to androgens. It was also shown, in one experiment with cocks, that testosterone levels were higher in the high-line birds (Benoff *et al.*, 1978) but this result was not replicated in subsequent generations (Bernon and Siegel, 1983b; De Santo *et al.*, 1983). The finding that the cloacal gland was bigger in the high than in the low line in quail (Bernon and Siegel, 1983a; Blohowiak and Siegel, 1984) seems to indicate that androgen levels were higher in the high line, but there was no direct measurement of androgen levels in this experiment. The high sexual behaviour line of quail is also dominant (Sefton and Siegel, 1975; Bernon and Siegel, 1983a; Blohowiak and Siegel, 1984; Blohowiak *et al.*, 1985; Yang *et al.*, 1998) and heavier (Sefton and Siegel, 1975; Blohowiak and Siegel, 1984) than the low line.

Sexual behaviour

Divergent selection for the level of sexual behaviour in males has been the focus of

Laying and Brooding

Two main problems related to laying behaviour are apparent: (i) the disturbed

pre-laying behaviour shown when no nest is available; and (ii) floor laying, i.e. laying out of the nests even when they are available.

Pre-laying behaviour was first described by Wood-Gush and Gilbert (1969) and further quantified in Wood-Gush (1969). Laying hens often show increasing levels of unrest prior to oviposition. It is known that this unrest is induced by hormonal mechanisms related to ovulation, approximately 25 h before the egg is laid (Wood-Gush and Gilbert, 1969). In caged layers, the unrest is often expressed as stereotypic pacing and intention flight movements. Since these behaviours are thought to be indicators of frustration (Duncan and Wood-Gush, 1972), the pre-laying behaviour of the domestic fowl has been studied extensively. Differences observed between lines provided the first evidence of genetic variation in this behaviour (Wood-Gush, 1972). In the two strains subjected to extensive study, one showed a very high frequency of pre-laying pacing whereas the other strain spent most of the time sitting. The strain showing a high frequency of pre-laying pacing also showed frequent jumping, feeding and drinking bouts during the 10 min before laying (Mills and Wood-Gush, 1985). A selection experiment (Mills et al., 1985b) showed that it is possible to select the two strains for even more extreme phenotypes. Crosses between the two lines indicated additive variation in the expression of sitting behaviour and non-additive variation (dominance of non-pacing over pacing) in the expression of pacing behaviour. There is an indication that the type of pre-laying behaviour is related to heart rate but the results are difficult to interpret, i.e. they could reflect either stress or activity (Mills et al., 1985a). There is also evidence that the birds of the high pacing line may be frustrated by the lack of a proper nest and that those of the low pacing line showed vacuum nesting activities.

Heil et al. (1990) compared the pre-laying behaviour of four White Leghorn lines over three generations. The mean duration of pre-laying activity in these lines varied from 55 to 63 min, the number of escape movements per 5 min from 2.3 to 7.7, and the stance during oviposition (% sitting) from 6 to 20%. Both the genetic and phenotypic variation of these behaviours varied largely. A heritability of 0.33 was observed for stance but heritability values were low for the duration of pre-laying activity and the number of escape movements when the estimate was based on behaviours observed during only one oviposition. However, when the number of ovipositions observed was increased to five, larger h^2 values of 0.12 and 0.09 were observed for the duration of pre-laying activity and the number of escape movements, respectively.

Floor laying is also a heritable character, as demonstrated by McGibbon (1976). This character was selected against when strains were selected in floor pens with trapnests. Nowadays it is not measured, because the birds used for selection purposes are kept in battery cages, thus making an increase in the prevalence of this problem likely.

Broodiness was reported to be dependent on complementary genes (Goodale et al., 1920), on more than one autosomal gene (Punett and Bailey, 1920) and on sex-linked genes (Warren, 1930; Roberts and Card, 1934). Saeki (1957) confirmed the existence of sex-linked and autosomal genes and found low values of heritability ($h^2_s = 0.16$; $h^2_m = 0.06$) for this character. Broodiness has been nearly eliminated from modern commercial strains.

Activity

Locomotor activity in chickens is expressed as walking, running, jumping, hopping and flying. Under intensive husbandry conditions, such as conventional cages, the expression of these activities may be seriously compromised. The wild ancestors of chickens spend a considerable proportion of their time budget in locomotor activities (Collias et al., 1966). Even under intensive conditions, hens spend about 20% of the time walking (Bessei, 1983). Furthermore, domestic chicks can move up to 7.5 km within 6 h in a treadmill cage (Garren and

Shaffner, 1954). It has often been assumed that locomotor activity as such is genetically fixed and that a certain level of activity is maintained under all environmental conditions. Locomotor activity in chickens has been studied in different housing systems (Bareham, 1972; Hughes and Black, 1974, 1977; Eskeland, 1976; Kivimäe, 1976; Süs, 1976) and when the birds were fed mineral-deficient diets (Wood-Gush and Kare, 1966; Bessei, 1978). Some information on the genetic background of locomotor activity has been provided from comparisons of egg-type breeds and hybrid strains (Wood-Gush, 1972; Black and Hughes, 1974; Hughes and Whitehead, 1979; Savory and Mann, 1997) and egg-type with broiler strains (Savory, 1975) but there is relatively little information concerning the amount of variation in this character within lines.

The heritability of locomotor activity was estimated by Jezierski and Bessei (1978). They measured the activity of individual laying hens of two different White Leghorn strains (Mount Hope and German Leghorn) that had been raised either in deep litter compartments or in cages for 9 h on each of two consecutive days in a shuttle box. This study revealed significant effects of rearing systems and lines. The German Leghorn birds were more active than the Mount Hope ones and birds reared in cages were more active than those reared in floor pens. The heritability of locomotor activity calculated on the basis of paternal half-sibs was 0.18. The genetic correlation between body weight and locomotor activity was positive ($r = 0.31$). However, heavier breeds of chickens are generally found to be less active than lighter ones; this relationship is clear when layer and broilers strains are compared (Savory, 1975). Genetic studies on locomotor activity in different lines of Japanese quail (*Coturnix japonica*) have been carried out by Saleh and Bessei (1980).

Locomotor activity was measured in males and females of a light laboratory strain maintained at the University of Hohenheim and of a commercial broiler strain obtained from France. The birds were placed individually in experimental cages for 60 min and locomotor activity was calculated as the numbers of times they broke a photobeam bisecting the cage. Males were generally more active than females and birds of the broiler strain were more active than those from the light laboratory breed. The heritability estimates for locomotor activity were 0.17 for the light and 0.04 for the heavy strain. The genetic correlations between activity and body weight were negative in the light strain ($r = -0.30$) and positive in the heavy strain (0.31). Gerken (1991) measured the locomotor activity of lines of Japanese quail that had been selected for high and low dustbathing activity and found that the heritability coefficients were 0.19, 0.01 and 0.10 for the sire, dam and sire-plus-dam components, respectively. The genetic correlation between locomotor activity and dustbathing activity was positive ($r = 0.39$) whereas activity and body weight at 12 weeks were negatively correlated ($r = -0.21$).

In two other experiments, reported by Bessei (1979), two lines of quail and their reciprocal crosses were tested for locomotor activity in the equipment described above. While activity measures in the crosses were similar to the means of the pure strains in one experiment, the mean scores of the reciprocal crosses showed considerable deviation from those of the pure strains in the second experiment, indicating the existence of non-additive genetic effects in the latter case. Bidirectional selection for locomotor activity in Japanese quail for five generations resulted in a significant differentiation of the selected lines for their activity index (105.0 vs. 57.5), with the unselected control line occupying an intermediate position (85.7) (Bessei *et al.*, 1983). When underlying fearfulness was assessed in these lines using commonly employed methods such as emergence, open-field, response to a bell and tonic immobility tests (Jones, 1987, 1996), the birds from the active line consistently demonstrated lower levels of fear than those from the inactive one; the control-line birds showed intermediate values (Jones *et al.*, 1982). Novelty is a potent fear elicitor and fear inhibits activity (Gray, 1971; Jones, 1996). Therefore, the above findings suggest that the selection criterion may have

reflected fear of the novel test cage as well as or instead of underlying differences in general activity levels.

In summary, the studies of locomotor activity in chickens and quail show that there is considerable genetic variation for this trait, which could be amenable to genetic selection. Although heavier lines of chickens are generally less active than lighter ones, the genetic correlations estimated within lines in both chickens and quails reveal that the opposite relationship holds true in some cases.

Dustbathing

Dustbathing in laying hens has been considered as a typical example of a genetically fixed and invariable behaviour (Wennrich and Strauss, 1973; Vestergaard, 1980). Since standard battery cages do not provide the substrate necessary for the birds to perform dustbathing, it was assumed that the hens might suffer under these conditions, though the 'out of sight, out of mind' argument might also apply. Regardless of this debate, Gerken (1983) selected two lines of Japanese quail for high and low frequencies of dust-tossing behaviour, a component of dustbathing. The experiment continued to G17 (Gerken, 1991) and the relationships between dustbathing and other behaviours and performance traits were determined. After three generations the lines diverged significantly (7.9, 13.6 and 16.2 dust tosses for the low, control and high dustbathing lines, respectively). Although the selection was based only on the behaviour of males, these differences were also expressed in the females. The heritability estimate for the selection trait was 0.28, and the realized heritabilities of 0.18 and 0.38 in the high and low dustbathing lines indicated an asymmetrical response to selection. The selection programme not only modified the frequency of the selected trait but also all the related characteristics of dustbathing behaviour, such as total time spent dustbathing, the latency to start and the intensity of dustbathing as well as vacuum dustbathing shown on a wire floor (Gerken, 1991). The genetic correlations between dustbathing, egg production, fertility and body weight were generally low. The birds of the high dustbathing line consistently showed better plumage and less pronounced fear reactions as measured in a pencil test (Gerken, 1991).

Discussion and Conclusions

A question that is often asked concerning the possible consequences of genetic selection for increased adaptability in poultry is whether or not it will improve productivity. There are three possible scenarios.

1. Selection for adaptability might exert detrimental effects on production. Although it is hypothetically possible that selection for adaptability might be accompanied by indirect and detrimental effects on production, to the best of our knowledge this scenario has never been reported in poultry. Indeed, it could be argued that the likelihood of its occurrence is low if the selection environment and the production environment are similar. Furthermore, even if this undesirable association did become apparent it would probably be infrequent because increased adaptability implies a greater likelihood that the organism will cope successfully with extraneous challenges that might otherwise impair performance.

2. Selection for adaptability may have no effect on production. This scenario can become apparent when artificial selection for production characteristics has been unconsciously associated with indirect selection for adaptability. In this case, selection for fitness characteristics during the early phases of domestication improved the animals' ability to adapt until a point was reached at which these criteria became independent. In other words, reproductive performance was restored to pre-domestication levels but the welfare of the animals was not necessarily assured at this point. The indirect selection for reduced fear that accompanied domestication provides a good example, because highly fearful wild

animals would have shown poor reproductive performance in captivity. This is strongly supported by the clear reductions in fear reactions shown by domestic animals compared with their wild or feral counterparts (Desforges and Wood-Gush, 1975b; Rose *et al.*, 1984). However fear reactions are still a major problem in many circumstances (Mills and Faure, 1990; Jones, 1996, 1997; Hemsworth and Coleman, 1998).

3.   Selection for adaptability may unconsciously exert positive effects on production. By definition, this scenario can occur only in cases where either no direct selection pressure for production characteristics has been imposed or when the change in adaptability only becomes apparent in the production environment and not in the breeding environment. Three examples are immediately apparent. First, even though co-selection for production traits was not imposed during the development of the STI and LTI quail (Mills and Faure, 1991), the low-fear birds showed less pronounced reductions in meat quality after the preslaughter imposition of stressful stimuli (Mills *et al.*, 1997). Secondly, bone strength and growth in the low stress (LS) quail were less seriously damaged by sequential exposure to a range of stressors than in HS birds (Satterlee and Johnson, 1988; Satterlee and Roberts, 1990). Thirdly, inappropriate levels of underlying sociality could compromise every aspect of social interaction and thereby damage welfare and performance through the elicitation of chronic stress (Jones and Hocking, 1999; Jones and Mills, 1999). In most breeding programmes the birds are housed in individual cages, which precludes the expression of social behaviour. Social interactions can only occur in colony cages or in social groups housed in floor pens. Interestingly, lines selected for divergent social behaviour (Craig *et al.*, 1965) showed no differences in their laying rates when tested in individual cages but the more tolerant, low dominance ability line performed better in floor pens or collective cages (Biswas and Craig, 1970; Craig, 1970).

The present review of genetic selection for behavioural traits shows that, except

for feather pecking and cannibalism, rapid responses were observed in all the selection experiments. The associated heritabilities were moderate to high and this illustrates the potential for further genetic improvement of adaptability. In recent years several relatively short tests have been used as selection criteria in artificial breeding programmes for behavioural traits (Mills and Faure, 1991; Bessei, 1996) or at least they have been identified as potential criteria (Satterlee and Johnson, 1988; Marin *et al.*, 1997). The execution of these tests requires a little more working time than does the measurement of body weight but much less than the assessment of laying rates. Moreover, the continued development of appropriate automation could easily reduce the time required to carry out these tests and thereby decrease the associated costs.

Breeding programmes intended to improve adaptation and welfare should focus on characters that are likely to be influential in all current and future rearing environments. Since the precise nature of future husbandry systems is uncertain, any selection programme that fails to meet this criterion may well prove to be without value. Thus, if artificial selection is to be effective in improving animal welfare, it should be directed at changing underlying motivational states rather than simply suppressing undesirable symptoms of those motivations. The significant reduction in underlying fearfulness that accompanied selection of quail for shorter TI fear reactions or decreased adrenocortical responses to mechanical restraint (Jones *et al.*, 1991, 2000a; Faure and Mills, 1998; Jones and Hocking, 1999) suggests that such a breeding programme is likely to meet the above criteria.

The potential eradication of 'undesirable' stereotyped pacing by artificial selection provides an example where greater caution should be exercised. When housed in battery cages, hens of certain strains show stereotyped pacing behaviour in the prelaying period whereas those of other strains show increased sitting and vacuum nest building. Both sitting and stereotyped pacing are sensitive to genetic selection under experimental conditions (Mills *et al.*, 1985b)

and so it should be possible to eliminate pacing behaviour in commercial lines. However, with respect to welfare, eradicating pacing would be of little value if the birds then simply expressed their frustration in some other potentially harmful way. On the other hand, if it was demonstrated that birds which show sitting behaviour are better able to adapt to the absence of a nest in battery cages and that birds showing pacing behaviour are not, then eliminating stereotyped pacing would be likely to improve welfare.

The use of genetic selection to improve an animal's ability to adapt to its environment should not be considered if the same effect could be produced by slight transformations of the environment. On the other hand, it might be argued that practical environmental interventions intended to minimize the elicitation and expression of certain harmful behavioural states are not always immediately apparent. Cases in point include feather pecking and cannibalism as well as fear. Current environmental strategies used to reduce the occurrence and spread of feather pecking and cannibalism either cause ethical concerns (housing in battery cages) or they may themselves damage the birds' welfare. For example, beak trimming, which is effective in reducing damage caused by feather pecking and cannibalism, can cause chronic pain (Gentle, 1986a,b) and housing the birds under dim light can lead to environmental impoverishment and eye abnormalities such as dim-light bupthalmos (Manser, 1996; Jones and Hocking, 1999). Encouragingly, though, some environmental manipulations can lead to significant benefits. First, for instance, the provision of simple enrichment/pecking devices made of white string not only maintained the birds' interest for lengthy periods (Jones and Rayner, 2000; Jones et al., 2000b; Jones, 2001) but also reduced feather pecking in an experimental line showing high levels of this behavioural vice and decreased pecking-related feather damage in caged layers at a commercial farm (Blokhuis et al., 2001; Keeling, McAdie, Blokhuis and Jones, in preparation). Secondly, fear of novelty can be reduced by the provision of appropriate environmental enrichment (Jones and Waddington, 1992; Jones, 2001) and regular visual contact with human beings alleviated chicks' and laying hens' fear of people (Barnett, et al., 1994; Jones, 1995) – at least in the laboratory.

In conclusion, genetic selection is becoming widely accepted as the quickest and most reliable method of eliminating harmful characteristics and promoting desirable ones in farm animals (Mench, 1992; Craig and Swanson, 1994; Jones, 1996; Faure and Mills, 1998; Jones and Hocking, 1999). There are cases where selection to increase the bird's ability to adapt to its social and physical environment should be applied as soon as possible but there are others where more research on the consequences of selection and on environmental alternatives is required. In view of the beneficial effects of practicable environmental enrichment and human contact (see above) and the fact that 'genetic effects on behaviour are sometimes abolished or reversed by minor variations in environmental background', in rodents at least (Wurbel, 2001), an approach that integrates genetic and environmental strategies is the most likely to satisfy the requirements of the birds, the farmers and the public.

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14 Indirect Selection for Improvement of Animal Well-being

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Introduction

Adaptation and domestications

In natural environments some behaviours, such as alertness, flightiness, pecking and aggression, are desirable because of the need to avoid predators or to establish exclusive territory and thereby secure limited hunting and foraging areas for the bird and its offspring. However, in a domesticated setting extreme alertness and flighty behaviour lead to wasted energy and injuries in attempting to escape from otherwise harmless stimuli when predators are not present; or aggressiveness in obtaining desired resources may result in the failure of low-status birds to receive adequate necessities even when enough is supplied for all (Craig and Muir, 1998). Domestication is a method of adapting an animal to a production environment (multiple-bird cages or pens) and stress is a manifestation of how well the bird is adapted to that environment. A bird that is genetically adapted to cages should not be stressed nor have its well-being compromised (Siegel, 1981, 1995). This conclusion also results from the observation that, through natural selection, species have evolved that prefer environmental extremes. Birds that are adapted to the Arctic are highly stressed by environments that might be considered optimal for poultry, and vice versa.

Examples of behavioural problems include: susceptibility to hysteria in large groups; amount of time spent pacing before laying; incidence of beak-inflicted feather loss; cannibalistic deaths; fearful or panic responses; cannibalism; feather pecking; and inter-individual aggression (Appleby *et al.*, 1992; Craig, 1994). These behaviours have major impacts on productivity and yield. Current methods of animal breeding ignore competitive effects in their selection objective. Even worse, if higher-producing animals tend to be more competitive, the effect of selection is to increase competition. Chapter 5 discusses behaviour problems associated with selection for increased production in poultry. Given that competitive interactions have a major impact on performance, a breeding programme that takes competition into account would be of foremost importance in the genetic improvement of poultry.

Direct selection to reduce undesirable behaviours

Direct selection on behaviour is one method of addressing this problem (Craig and Muir,

1998; Faure *et al.*, Chapter 13). Criteria that have been used in assessment of well-being in chickens include overt indicators (bodily injury, morbidity, weight loss, non-moult feather loss, death and mean days survival of groups), physiological and immune responses, productivity and behaviour. With the exception of overt indicators, difficulties of interpretation of the various criteria lead to the conclusion that, as far as possible, multiple indicators should be used (Craig and Muir, 1998). However, direct selection requires measurement of those behaviours on individual birds and a multi-trait selection programme. As such, measurement costs and selection intensity must be diverted to improvement of those traits. Commercial breeders are under economic constraints that may prevent their ready adoption of programmes to address those problems. Behaviours that are easily identified (such as broodiness) or that leave traces (such as feed consumption) are more amenable to selection than those that must be measured by time-consuming observations (e.g. pre-lay pacing, fear-related and aggressive behaviour) (Craig and Muir, 1998).

Direct selection could also be disappointing if such selection reduced efficiency of production (Craig and Adams, 1984; Craig and Muir, 1998). Wegner (1990) suggested that welfare could be improved through adaptation by selecting against frustration, restlessness and stereotyped pacing before laying, and selecting for a greater tendency to sit during the pre-laying period. However, direct selection on either behavioural or physiological objectives should be viewed with caution. The intended results may not be as expected. For example, Webster and Hurnik (1991) showed that traits associated with non-aggression, such as sitting and resting, were negatively correlated with productivity. Similarly, Gross and Siegel (1985) were successful in selecting lines of birds for high and low plasma corticosterone in response to social strife, but further testing (Siegel, 1993) showed that the birds did not differ in their corticosterone response to a non-social stressor. Siegel concluded that genetic selection altered the perception of

the animal to stress rather than involving the general adaptation syndrome (GAS) directly. Even worse, Siegel (1993) noted that the low line in a low-stress environment was more susceptible to infections from endemic bacteria and external parasites, while the high line in a high-stress environment was more susceptible to viral infections.

Furthermore, the link between behaviour and stress can be misinterpreted. For example, Duncan and Filshie (1979) showed that a flighty strain of birds that exhibited avoidance and panic behaviour following stimulation returned to a normal heart beat sooner than a line of more docile birds, implying that docile birds may be too frightened to move. Therefore, is flightiness good or bad for well-being?

Stress and production

The relationship between stress and production supports the notion that production may be a good indicator of stress. Siegel (1995) pointed out that stress has important consequences for the bird's well-being, especially those that affect energy and mineral metabolism and interactions with the immune system. Social or behavioral environments can also activate stress responses in birds, just as physical stressors do. Results may manifest themselves in reduced growth in juveniles, reduced reproductive capacity in adults and increased food consumption (Craig and Muir, 1998). However, there is a hidden danger in selection for productivity on an individual basis. Although the highest-producing individual in a group may not be stressed because it is high in the peck order, as a result of aggressive behaviour, others in the group lower in the peck order may be highly stressed. Supporting this conclusion, Lowry and Abplanalp (1970, 1972) showed that strains selected under floor flock conditions became socially dominant to both those selected in single-bird cages and unselected controls. Thus, selection based on individual productivity may select for individuals

high in the peck order and result in greater aggression and lower flock productivity.

Indirect selection to reduce undesirable behaviours

An alternative to direct selection on behaviour is group selection. Group selection does not increase costs or divert selection intensity and is based on the hypothesis that when productivity of the group is high, behavioural stresses of the group must be reduced or absent. Group selection does not require measurement of behaviour traits, but rather indirectly improves desirable behaviours, while improving productivity and adaptability of birds to production environments. Siegel (1989) considered adaptability to be an individual's ability to adapt to its environment. He concluded that individuals that adapt have a higher probability of contributing genes to subsequent generations than those that do not. While this conclusion is true, it embodies the heart of the problem: it still emphasizes the individual's performance. What if an individual adapts to its environment by eating its cagemates? The individual's survival would be maximized, along with its productivity, but what of that of the group? This chapter will examine theory and application of group selection to poultry breeding.

Group Selection Background and Theory

The importance of selection based on family group in the evolution of social behaviour was discussed by Hamilton (1964). Recognizing this problem, Griffing (1967) extended classic population genetic models to include competitive effects. Griffing (1967) recognized that, with competition, the usual gene model for a given genotype must be extended to include not only the direct effects of its own genes, but also the associative contributions from other genotypes in the group. In this theory, first the conceptual biological model must be

extended to define the group and secondly the usual gene model must be extended to include not only the direct effect of the individual's own genes, but also associative contributions from other genotypes in the group.

Griffing (1967) showed that, in the presence of interacting genotypes, the expected change in the mean ($\Delta\mu$) from individual selection of intensity i in a population with a phenotypic standard deviation of σ is:

$$\Delta\mu = \left(\frac{i}{\sigma} \right) \left[{}_d\sigma_A^2 + {}_{(da)}\sigma_A \right]$$

where ${}_d\sigma_A^2$ is the additive variance of the direct effects and ${}_{(da)}\sigma_A$ is the additive covariance between direct and associative effects. If the covariance is negative, as occurs when there is competition for a limited resource, then selection based on individual performance can have a reverse effect on the mean, i.e. positive selection will reduce rather than increase the mean. This is because a gene that has a positive direct advantage for the individual has a negative associative effect on the group. These results are contrary to the theories advanced by classic quantitative genetic theories and require a rethinking of selection programmes.

In contrast, if the group is defined as the unit selection, then:

$$\Delta\mu = \left(\frac{i}{\sigma} \right) \left[{}_d\sigma_A^2 + {}_{(da)}\sigma_A^2 + {}_a\sigma_A^2 \right]$$

where ${}_a\sigma_A^2$ is the additive variance for associative effects. In this case, because all the individual terms are squared, $\Delta\mu$ is always positive. Thus, transferring selection from the individual to the group ensures that the population mean will not decrease.

Griffing (1967) also showed that, with group selection, it is possible to select for an allele that has a negative direct effect but positive associative effect, i.e. altruistic or self-sacrificing traits. Griffing (1967) further noted that, as group size increases, associative effects take on an increasingly dominant role in determining the consequences of selection and implied that, even for weakly competitive conditions, a negative response to selection can occur. Griffing (1976) showed that the efficiency of selection is greatly increased if the group

is composed of related individuals, particu-
larly as group size increases.

Experimental Examination of Competitive Theory

The theories of Griffing (1967) provide
several testable hypotheses and predictions.
In the presence of competition: (i) indi-
vidual selection for increased production
will result in increased aggressiveness; (ii)
individual selection may fai or result in a
negative response to selection, whereas
group selection will always result in a
positive response; and (iii) associative
effects are a greater problem in larger
groups.

Individual selection for increased production will result in increased aggresssiveness

Research has shown that selection to
improve productivity based on individual
bird productivity is associated with
increased aggressiveness. Choudary *et al.*
(1972) compared four commercial lines of
poultry and found that the line which had
the highest number of eggs per hen per day
had the lowest number of eggs per hen
housed, due to high mortality. Craig *et al.*
(1975) demonstrated that stocks selected for
part-record egg production on an individual
basis exhibited more social dominance
and aggressiveness following flock (group)
formation during adolescence. Bhagwat and
Craig (1978) found that social dominance
increased in response to selection for egg
mass. Craig and Lee (1989) detected a
strong genotype-by-beak-treatment inter-
action for egg mass per hen housed among
three commercial lines. From 32 to 36
weeks of age, the genotype that produced
the greatest egg mass with beak treatment
produced the least with intact beaks. The
re-ranking was shown to be due to mortality
from beak-inflicted injuries. Stock selected
for increased productivity had greater
feather loss than the unselected control
when kept in three-bird cages (Lee and
Craig, 1981).

A corollary to this hypothesis is that the
reverse should also be true, i.e. selection for
behaviour, desirable or undesirable, should
have the opposite effect on production. Craig
et al. (1965) and Craig and Toth (1969)
showed that hens selected for high social
dominance had lower rates of egg pro-
duction and higher mortality than did hens
of the same line selected for low social
dominance. Craig (1970) found that the high
social dominance line withstood crowding
less well than the low social dominance
line. However, in single-bird cages, egg pro-
duction of the high line was superior to that
of the low. Biswas and Craig (1970) also
showed that the high strain hens had much
lower production than the low line in floor
pens or multiple-bird cages but were equally
productive in single-bird cage. Craig (1994)
showed that selection for social dominance
will reduce performance when hens are
housed in a large group, but performance is
increased when they are housed singly.

Individual selection inferior to group selection in the presence of competitive effects

Goodnight (1985) showed that the leaf area
of *Arabidopsis thaliana* would respond to
group but not individual selection. Wade
(1976, 1977) and Muir (1977) demonstrated
with *Tribolium castaneum* that a negative
group response could be obtained from pos-
itive individual selection for productivity.
Muir and Schinckel (2002) observed that
selection for increased 6-week weight in
quail using standard best linear unbiased
predictor (BLUP) procedures resulted in a
negative response to selection even though
the heritability of the trait was greater than
30%.

Competitive effects are a greater problem in larger groups

Muir (1985) observed a significant genotype-
by-cage-environment interaction for num-
ber of days of survival in a random-bred
population of White Leghorns between nine-
bird and one-bird cages but not between

four-bird and one-bird cages, even though birds in the nine- and four-bird cages were housed at the same density. Muir *et al.* (1992) later showed that this interaction was mainly due to re-ranking of genotypes, indicating that the best performers in one cage environment were poorer in the others.

Selection for Group Productivity

The first successful experiment with group selection in poultry was initiated in 1981 using a synthetic line of White Leghorns to improve adaptability and well-being of layers in large multiple-bird cages by use of a selection procedure termed group selection (Craig and Muir, 1996a,b; Muir, 1996; Muir and Craig, 1998). With this procedure, each sire family was housed as a group in a multiple-bird cage and selected or rejected as a group. Group size in the first two generations was nine (413 cm^2 per bird); in the next four (G3, G4, G5 and G6) group size was 12 (362 cm^2 per bird). Birds were not beak-trimmed and lights were at high intensity so as to allow expression of genetic variation for aggression, feather pecking and cannibalism. Production was measured to 60 weeks of age in the first four generations and to 72 weeks in the last two. The criterion of selection was initially egg mass, which was computed as the product of eggs per hen housed and egg weight. In later generations an index giving equal weight to eggs per hen per day and days survival was used.

Direct responses

Muir (1996) reported that after six generations, in comparison with the unselected control, annual percentage mortality of the selected line (KGB) in multiple-bird cages decreased from 68% in the initial generation to 8.8% in the sixth generation. Mortality in the sixth generation of the selected line in multiple-bird cages was similar to that of the non-selected control in single-bird cages (9.1%). Annual days survival

improved from 169 to 348 days and rate of lay improved from 52 to 68%. Annual egg mass improved from 5.1 to 14.4 kg per bird. The dramatic improvement in liveability demonstrates that adaptability and well-being of these birds were improved by group selection. The similar survival of the selected line in multiple-bird cages and the control in single-bird cages suggests that beak-trimming of the selected line would not further reduce mortalities, which implies that group selection can eliminate the need to beak-trim. Corresponding improvements in rate of lay and egg mass demonstrated that such changes can also be profitable.

Craig and Muir (1996a) compared the selected and control lines with a commercial line, Dekalb XL (DXL), in G7, again housed in either single-bird or 12-bird cages. Management conditions were the same as in previous generations except that birds that died were replaced with extra birds of the same line. Performance was measured from 20 to 58 weeks of age. In single-bird cages, performances in terms of eggs per hen housed, eggs per hen per day, egg weight and egg mass were significantly greater for the DXL than for the KGB line, which was in turn greater than the unselected control, and mortality was zero for all three lines. However, the reverse was seen in 12-bird cages, with the KGB line superior to the DXL line for eggs per hen housed, egg mass, and eggs per hen per day. The most remarkable difference was for mortality (Fig. 14.1): the DXL line had an 89% mortality at 58 weeks of age as compared with 20% for the KGB line and 54% for the control. The comparison with the DXL is particularly important because this line constituted one of the resources used to establish the control line from which the KGB was established. The DXL line was the result of continued selection for improved productivity based on individual bird performance. While the comparison is not exact, productivity and survival in relation to the control line demonstrate that continued selection on individual productivity will improve productivity when competitive interactions are absent, as in a single-bird cage, but can result in a negative response to selection in a group setting.
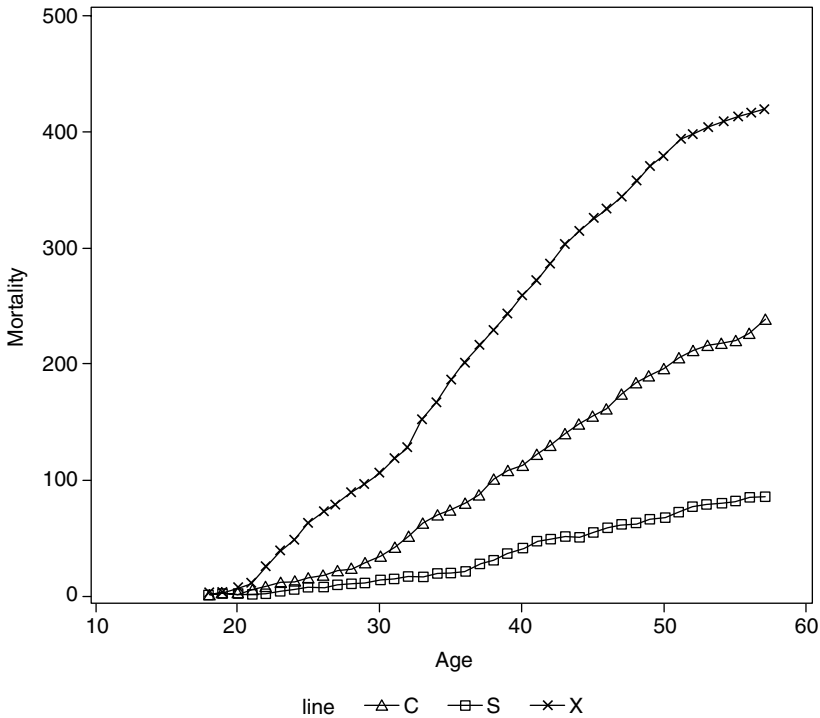
Fig. 14.1. Cumulative mortality of the commercial (X), control (C) and KGB (S) lines to 56 weeks of age.

Correlated physiological and behavioural responses to group selection

In the same study, Craig and Muir (1996a) observed that feather scores did not differ in single-bird cages among genetic stocks, but in 12-bird cages the KGB line had significantly better feather scores than the other lines. Observations of the birds' agonistic activity in the 12H cage environment revealed that the KGB stock had fewer agonistic acts than the control stock from which it was derived, and both control and KGB lines had less agonistic activity than the DXL stock (Craig and Muir, 1996b).

These lines were subjected to the stress of housing at about 17 weeks, to cold stress at 36 weeks and to heat stress at 47 weeks of age. Blood physiology and egg production were monitored before, during and after each of these periods (Hester *et al.*, 1996a,b).

Packed cell volume immediately after housing indicated that the KGB line adapted to the new watering system more quickly than the other lines. During cold stress, the DXL and control lines showed an increase in heterophil:lymphocyte ratio in 12-bird cages while the selected line did not. Egg production before, during and after stress indicated that the KGB line withstood social, handling and environmental stress better than the control and in some cases the DXL line. Similar observations with heat stress showed that the KGB line withstood heat stress better, as indicated by a lower mortality than the control or DXL lines.

Cheng *et al.* (2001a) established a reverse (low group productivity group, LGPS) line for comparison in the eighth generation. The HGPS hens had significantly higher percentages of blood lymphocytes and CD4(+):CD8(+) ratios of circulating T cells. In contrast, the LGPS hens exhibited

eosinophilia and heterophilia and greater heterophil:lymphocyte ratios. The concentrations of plasma IgG were also significantly higher in the LGPS hens. The KGB hens exhibited a greater cellular-mediated immunity, while the LGPS hens had heterophilia and a greater heterophil:lymphocyte ratio. These results suggest that genetic selection for group productivity also altered the birds' immunological and haematological systems.

Cheng *et al.* (2001b) also found that group selection altered the birds' neuroendocrine homeostasis. In single-bird cages the LGPS hens had greater blood concentrations of dopamine, serotonin and epinephrine than the KGB hens. In contrast, the KGB hens tended to have a higher level of blood corticosterone than the LGPS birds. The line differences of blood concentrations of dopamine (DA), serotonin and epinephrine were in the directions expected for better coping ability of the KGB line. These results suggest that selection for group productivity alters neuroendocrine homeostasis, and these changes may correlate with a bird's line-unique survivability and ability to cope with domestic environments (Cheng *et al.*, 2001a,b).

Cheng *et al.* (2003) showed that, in response to the different social treatments, plasma DA concentrations were differently regulated in each line. Compared with the KGB hens, the LGPS hens had greater levels of plasma DA in single-, two- and ten-bird cages. The greater plasma DA concentrations of the LGPS hens could be linked to selection-related reorganization of behaviour, such as cannibalism and aggression. Conversely, the KGB hens had significantly lower concentrations of plasma DA, which could be associated with their sedate and passive behaviours, better and quicker adaptation to various stressors (Hester *et al.*, 1996a,b,c) and higher production (Craig and Muir, 1996a,b). Collectively, genetic selection has created lines with significantly different phenotypes, each of which has unique characteristics in physical indexes, domestic behaviour, responsiveness to stressors and immunity (Cheng *et al.*, 2001a,b).

Incorporating Competitive Effects in Breeding Programmes with Individual Selection

An alternative to group selection that incorporates competitive effects in the breeding objective, but allows individual selection and without measuring additional behavioural traits, was recently developed by Muir and Schinckel (2002). This method requires that productivity of each individual be measured and pen mates identified. The essential feature is to incorporate a second random effect in the mixed model equations to account for associative effects of cage mates as well as the usual direct effects of the bird's own genes. Solutions of the mixed model equations yield breeding values for the direct and associative effects of each individual. One can then select for direct effects and against associative effects using an optimal index to maximize group performance.

The method was tested using Japanese quail as a model. The birds were selected for 6-week weight based on either standard animal model BLUP (AM-BLUP) ignoring competitive effects, or one incorporating competitive effects (CE-BLUP), for 28 cycles (hatches). Results showed that selection using AM-BLUP failed to yield any response to selection and was trending less than zero. CE-BLUP gave significantly better results with significant genetic gains. The lack of response to AM-BLUP was a result of a negative correlated response of associated effects, indicating an increase in competitive effects. Selection based on CE-BLUP increased direct while reducing associative effects.

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15 Genetic Diversity and Conservation of Poultry

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Introduction

The 20th century progress in poultry production, advances in basic and applied poultry science, and contributions of poultry to basic biology and biomedicine were made possible to a large extent because of the intensive selection, characterization and study of specialized populations of poultry. Population development was possible because of the breed base inherited by scientists at the turn of the 19th century, most notably chicken breeds, providing the opportunity for selection for a very wide range of purposes; these resources contributed directly to many significant agricultural and scientific advances of the 20th century. A challenge now exists to conserve the genetic reservoir of existing poultry populations in order to afford the opportunity to respond to future research and production needs and for continued selection-based progress during the 21st century.

Poultry biodiversity refers to the extant genetic variants within and among poultry species (chicken, turkey, quail, duck, goose, pheasant) distributed around the globe. Indigenous and local breeds, as well as specialized populations of each species developed by modern industry, small-scale traditional farms, academic and governmental research institutions and fanciers, provide great value to humankind. The existing global poultry biodiversity represents a vital genetic reservoir with enormous potential for contributing to major innovations in food production, biotechnology and basic or applied research significant for the health of humans and animals. Decision making regarding conservation of genetic variation within and among specialized populations should rely on composite information including phenotype, the historical record and molecular genetic variation.

The core objective for conservation of farm animal genetic resources is to maintain access to the adaptive genetic potential of each species and, further, to maintain the current collection of valuable research resources (Notter, 1999). The practical management of genetic resources for long-term conservation should be based on genetic principles such as: (i) maintenance of population heterozygosity; (ii) conservation of alleles; and (iii) conservation of allele combinations. The application of these principles and development of conservation strategies for the varied poultry genetic resources will differ accordingly. Thus, there is not one solution for all of the various poultry categories.

An important overall guiding principle is that poultry biodiversity should be maintained so that future advances and improvements can occur in response to the likely changing human and animal production

needs of the future. The rationale for conserving genetic diversity is that genetic variation enables both adaptive evolutionary change and artificial selection, making breeding for improvement and adaptation to disease and environmental factors possible. The current genetic reservoir is the basis for future economic, scientific and sociocultural opportunities.

Poultry Biodiversity

Biological diversity (biodiversity) can be defined as all forms of life, including all species and genetic variants within species and all ecosystems that contain and sustain diverse forms of life (CAST, 1999). The status, conservation and utilization of biodiversity are issues of global scale and significance (O'Brien, 1994; CAST, 1999). The accelerated pace of diminishing biodiversity (species extinction; destruction and alteration of habitats and ecosystems) results primarily from the growth and development of industrialized societies. Central to protecting biodiversity is the field of conservation genetics, which integrates population genetics and molecular genetics such that molecular genetic variation within and among populations is assessed, interpreted and managed to conserve genetic diversity.

Chicken and other poultry species are of significant value around the world contributing to humankind in many ways. These animal groups: (i) provide for human nutrition (animal protein in the form of meat and eggs); (ii) serve as a research model for studies in vertebrate biology, animal and human health; (iii) provide cells and eggs for pharmacologically important purposes (human vaccine production, biotechnology applications); (iv) serve as a research model for the 'other' 9000 avian species; and (v) contribute to human leisure activities (hobby breeding and, although illegal in many places, cock fighting) and provide a universal source of humour and merriment associated with poultry antics.

The historical record regarding the domestic chicken provides an interesting perspective: thousands of years of domestication, adaptation and global dispersal (Wood-Gush, 1959; Crawford, 1990), several hundred years of intensive breed selection, 70 years of selection for production improvement and research purposes, 20 years of discussion on imperatives for conservation and preservation of poultry genetic resources (CAST, 1984; Crawford, 1990, 1997; Delany and Pisenti, 1998; Christman and Hawes, 1999; Notter, 1999; Pisenti *et al.*, 1999; Ruane, 1999) and about 15 years of data accumulation on molecular genetic variation within and among poultry populations (first molecular diversity studies: Hillel *et al.*, 1989; Kuhnlein *et al.*, 1989).

Poultry biodiversity refers, then, to the totality of genetic variants found within the very specialized group of birds defined by their size (*pou*: small animal) and domestication purpose (raised for meat or eggs). The species typically considered include the chicken, turkey, duck, quail, goose and pheasant. This chapter focuses predominantly on chickens and, to a lesser extent, turkeys. General genetic nomenclature and examples of categories of poultry (species, subspecies, race; breed, variety; stock, strain, line; inbred, crossbred, etc.) are reviewed in Delany and Pisenti (1998). Considerations for maintaining poultry biodiversity are not based on concern for worldwide extinction of these species per se, as is the case for many exotic birds. Rather, concerns about diminishing poultry biodiversity and the need for management plans that conserve and preserve extant genetic variation are based on: (i) accelerated loss of specialized research populations used for human and animal research (Pisenti *et al.*, 1999); (ii) consolidation of primary breeder companies (Hunton, 1998; Arthur and Albers, Chapter 1) resulting in the loss of industry foundation populations based on business management decisions (similar to the loss of research populations, but different decision makers) unencumbered by oversight and with no opportunity for 'rescue'; (iii) possible erosion of genetic potential in industry stocks as a result of decades of intensive selection; and (iv) replacement of locally adapted breeds found on small farms and in villages

around the world with modern industry stocks, reducing the global genetic reservoir available in these breeds and loss of populations that may be of future value.

Poultry Genetic Resources

Conservation and preservation

Conservation genetic theory emphasizes the evolutionary significance of the genetic structure of species, the distribution of genetic variation within and between species being of critical evolutionary significance. The genetic resources of a species exist at two fundamental levels: genetic variation between individuals within a population; and genetic differences between populations. Efforts to conserve genetic resources must consider both levels (Allendorf, 1983). The amount and distribution of genetic variation for any genetic population or resource is subject to five forces: genetic drift, mutation, recombination, migration and selection. In some cases, these forces are intentionally and intensively fashioned by breeders and researchers; in other cases (locally adapted populations) these forces act under natural circumstances.

Preservation is often discussed in the same context as conservation; however, preservation deals with maintenance of species without regard to their population characteristics. Thus, in the case of poultry, this would mean simply ensuring that the various species remain in existence in some form, in some location. Cryopreservation is an important part of a conservation programme and is the most visible activity for many farm animals – e.g. a priority focus of the National Animal Germplasm Program of the United States Department of Agriculture (USDA) – perhaps because it is the most easily organized programme to implement: inventory, prioritize, collect and freeze samples in liquid nitrogen. The strategy is largely the same, regardless of species or resource, for many animal systems. However, in the case of poultry, the utility of cryopreserved specimens for meaningful use (i.e. population restoration) is poor as tested for various research lines and untested and thus unknown for breeds and industry populations. Furthermore, only male gametes can be preserved, because the female gametes (yolk-laden oocytes) cannot be frozen. It is hoped that cryopreservation of various entities (e.g. primordial germ cells, Stage X embryos, tissues and DNA) will be of use, presuming that advances in research and biotechnology will make such samples valuable for conservation efforts.

Conservation, in comparison with preservation and cryopreservation, focuses on the development of programmes for the long-term retention of specific breeding communities (populations) *in situ* (as opposed to *ex situ*, in zoos and in the freezer) with regard and appreciation for their specialized genetic and phenotypic distinctions from other populations. Much has been discussed regarding conservation strategies for small and endangered natural populations, whether in a natural setting or in zoos. However, where there are multiple species, numerous small and large populations possessing unique genetic features, and a variety of stakeholders, as is the case of poultry, a single strategy is neither realistic nor warranted. Each set of resources requires a conservation management programme tailored to maintain and conserve that resource. On a global scale, the Food and Agriculture Organization of the United Nations (FAO) has invested heavily in the effort to prod nations to conserve farm animal genetic resources. The FAO defines genetic resources as those populations that show the highest genetic differences within a species and/or show unique alleles or allelic combinations.

The major categories of poultry genetic resources (see Delany and Pisenti, 1998; Pisenti *et al.*, 1999; Weigend and Romanov, 2001) include: (i) experimental research lines (developed and characterized for research purposes); (ii) industry stocks (foundation and pure line populations selected intensively for reproduction and growth traits); and (iii) domesticated and wild breed populations (native and locally adapted, standards, hobby and fancy exhibition).

Experimental research populations

Poultry are an invaluable resource for biological study. Specialized research genetic populations of chicken, turkey and quail developed since the 1930s include random-bred, inbred, congenic, long-term selected, mutant and cytogenetic stocks. Poultry genetic research resources have been utilized in research producing fundamental discoveries in biology (e.g. vertebrate developmental biology), biomedical research (e.g. oncogenesis, vaccine development and production) and agricultural sciences (e.g. genetic basis for disease resistance and susceptibility) (Delany and Pisenti, 1998; Pisenti *et al.*, 1999).

The status and loss of research populations of poultry in North America was documented in a report developed by the Avian Genetic Resources Task Force (Pisenti *et al.*, 1999). The report surveyed scientists at major academic and governmental institutions and documented 230 chicken, 65 quail, 20 turkey, six waterfowl, and two gamebird *living* stocks. An additional 40 stocks are available only in the form of cryopreserved semen or pre-blastula embryos (these stocks were dropped with the closure of Agriculture Canada's Center for Food and Animal Agriculture Research, Ottawa, Canada). Significantly, in North America more than 200 experimental genetic research populations were eliminated between 1988 and 1998 (Somes, 1988; Pisenti *et al.*, 1999). The combined effects of researcher retirements, institutional programmatic shifts, reduced funding base for animal agricultural research and rising animal care costs all contributed to the losses. Many of the researchers surveyed indicated that their stocks are at risk over the long term and expected that the stocks would be dropped when their research programmes were no longer active. No safety net exists; researchers are still in the position of scrambling to get another researcher and institution to agree to take the population(s). The individual researcher has become the de facto conservator of these genetic resources.

Modern production stocks

The stocks of chickens contributing to the global production of meat and eggs are managed and in a sense 'designed' by the primary breeder companies in response to consumer desires and marketplace economics. Elite lines of birds are placed under intense selection for performance traits to create the grandparent or parent lines that are subsequently crossed to create the production populations that supply the market. The phenotypes of these birds are strikingly specialized. To achieve their high performance of rapid juvenile growth in broilers (42 days to market weight) and high rate of egg production in layers (300 eggs per year) they require specialized management and controlled environmental conditions. Many of the turkey, layer and broiler populations available today were developed during the first half of the 20th century from breeds that possessed growth and reproduction traits of obvious value for production objectives.

Knowledge regarding the current status of industry populations is not in the public domain. Not surprisingly, due to the proprietary nature of such information, there is no existing inventory that describes the features or the number of lines maintained by each company. What has been well documented is that the consolidation of primary breeder companies has resulted in an overall decrease in the number of companies (see Arthur and Albers, Chapter 1); anecdotal information indicates an associated decrease in the total number of elite purelines. Forty years ago (around 1960) the USDA listed 132 primary breeder firms of egg-type stock participating in the random-sample egg test, whereas in 1996 only five firms were listed and these were owned by three large multinational layer companies (Pisenti *et al.*, 1999). Pollock (1999) reported six major primary breeders and a similar number of smaller breeders controlling the 35–40 pure lines that supply the 400 billion market broilers (i.e. birds sold for consumption); at the present time, two major companies supply stock globally.

The concerns regarding industry populations and biodiversity include the following:

1. Industry stocks have a restricted genetic base. Most chicken layer and broiler stocks were developed during the 1930s. The breed and strain sources utilized were often common among the breeders. For example, white-egg layer lines derive predominantly from the Mt Hope farm strain (Williamstown, Massachusetts, USA) of Single Comb White Leghorns, which originated from a very small number of importations from the Italian port of Leghorn in the late 19th century. Relative to other breeds, these White Leghorns were prolific egg producers even under inhospitable circumstances (winters of the northeastern USA). Brown-egg layer lines derive from several dual-purpose breeds developed in the USA (dual-purpose breeds were developed from Asiatic × British breeds selected for body type and egg production characteristics). The broiler populations derive primarily from strains of dual-purpose White Plymouth Rock (used for female lines) and White Cornish (male lines). The White Cornish derive from the British Cornish Indian Game breed, which in turn was derived from Malay and Azeel breeds of India. These breeds exhibited a thick compact body type with a high proportion of breast meat. Commercial turkey breeding started early in the 20th century and utilized the Bronze variety (eventually the Broad-breasted Bronze) as well as Standard Bronze, Bourbon Red, Narragansett and White Holland. However, the dark pigmented bird was replaced (during the 1960s) and the industry breeding populations are now based solely on the Large White variety (Christman and Hawes, 1999).

2. The number and structure of primary breeder companies, supplying broiler grandparent and layer parent lines globally, have altered drastically since the 1950s. Shaver (1999) reported that industry restructuring during the 1950s and 1960s resulted in the loss of small pedigree poultry breeders, leaving only about 25 breeders. This was followed by numerous company mergers in the 1980s and 1990s, resulting in fewer than a dozen firms worldwide. Thus, currently almost all of the successful poultry breeder companies exist primarily as merged multinational firms that provide chicken germplasm (grandparent and hybrid parent lines) globally. Retention or termination of lines is necessarily based on profitability and management decisions. In 2000, there were seven main companies supplying broiler breeding stock (Leeson and Summers, 2000) and now it is estimated that two firms supply three-quarters of the broiler world market share. Three multinational companies control chicken egg-layer breeding stock and three companies control the majority of the commercial turkey primary breeder stocks (Christman and Hawes, 1999). A valuable asset for maintaining poultry genetic diversity lies within the smaller primary breeder companies, perhaps numbering half a dozen, that supply a much smaller market share (2% or less) (Pollock, 1999).

3. The intensity and duration of selection can result in the loss of genetic variation (loss of alleles). The concept of a 'healthy genetic reservoir' implies genetically heterogeneous populations that can respond to directional selection whereby phenotypically superior animals are chosen for breeding (or can respond to natural selection, e.g. an unanticipated disease outbreak). Directional selection, when applied to genetically heterogeneous populations, typically results in the mean phenotype of the population changing over subsequent generations, in the direction of the selection. If the population has previously been subjected to long-term selection for the trait in question, a 'selection wall' or lack of response may result. If a population is genetically uniform, the mean phenotype cannot be altered because allele variation is required for progress (at the extreme, in an inbred homozygous line, the only source for new alleles and allele combinations is mutation). Breeders suggest that there is no evidence for lack of selection response for growth and reproduction trait selection (Emmerson, 1997; Emsley, 1997) even though many stocks have been closed and under selection for as many as five to seven decades (Hunton,

1998; Pisenti *et al.*, 1999). Others argue that current testing and selection methods will result in genetic improvement reaching a plateau in the near future (Wheeler and Compian, 1993).

Long-term maintenance of genetic diversity is important because it provides options and opportunities for the challenges of the future. The future is unpredictable, challenges are likely to include new diseases or more virulent recurring diseases, environmental changes, societal changes related to animal welfare (e.g. housing; see Christman, 1998) and consumer preferences, as well as expansion of poultry-related nutritional demands for a global society that includes many who live in non-industrialized nations with little infrastructure for intensive animal production (Sheldon, 2000). Clearly, breeding companies have a vested interest in maintaining genetic diversity for sustainability; as private entities, though, they do not necessarily accept the label or responsibility of being the curators and conservators of these globally important stocks. Therein lies the problem from a conservation point of view.

Indigenous, locally adapted and standardized breeds of poultry

The breeds developed during the 18th and 19th centuries were the genetic base for the research and production stocks described above and it is no exaggeration to state that the breeds of the time provided the raw material for the positive progress seen during the 20th century in terms of research and production. Currently, in developed countries, the devoted poultry fanciers and the small-scale hatchery businesses are the conservators of poultry breeds and varieties. Breeds in developing, non-industrialized countries have even more precarious underpinnings. These are at risk from combined effects of displacement (due to habitat changes) and replacement (by modern production industrial stocks) (Sheldon, 2000). Loss of these locally adapted breeds is an important biodiversity

issue as such breeds are an important genetic reservoir, many developed over thousands of years, and successful in extreme and unusual environments with limited veterinary and management input (Hall and Bradley, 1995). These indigenous breeds hold alleles and allelic combinations important for small-scale production scenarios and have unknown potential for large-scale production through their utilization in future biotechnology applications. Poultry breeders of improved industrial stocks have avoided utilizing older-style breeds (a practice of plant breeders) largely because of the disadvantages and problems associated with introgressing entire genomes into already improved stocks. The future ability to transfer small pieces of genomes routinely, although on an unpredictable time line, may afford new opportunities for utilization of standardized and locally adapted breed genomes. Regardless of their use for future biotechnological applications, it is important that these breeds be preserved and conserved in their own right for their contribution to the societies and traditions where they were developed.

Many locally adapted breeds and varieties have already been lost: Stromberg (1996) provided a partial listing of 58 extinct chicken breeds and the FAO (2000) listed over 30 extinct chicken breeds (as reported by particular countries around the globe), with greater numbers at the critical and endangered status levels and large amounts of missing data for particular countries. Documentation of local and standardized breeds by the FAO (including risk assessment) is growing, but it remains incomplete, largely as a result of the degree of country contribution of information. For example, to date in the Domestic Animal Diversity – Information System (DAD-IS, http://dad.fao.org/), China lists 12 breeds and none are listed for the USA even though the American Poultry Association lists about 150 breed standards (American Poultry Association, 1998). The American Livestock Breeds Conservancy (ALBC) routinely conducts census inventories of mid-level poultry breeds and varieties having economic and historic significance in the USA (Christman and Heise, 1987).

A recent update places 31 poultry breeds/ varieties (chickens, ducks, geese, turkeys) in the critical and rare categories and another 12 in the watch category, based on criteria such as the number of breeder females and primary breeder flocks (American Livestock Breeds Conservancy, 2001). Christman and Hawes (1999) argued that the reliance of the turkey industry on a few strains of the Large White variety, coupled with the decline of small-scale turkey raising, has marginalized most turkey varieties to the point where they are rare, with many near extinction. The total percentage of avian breeds at risk of being lost increased from 51% in 1995 to 63% in 1999 (FAO, 2000), indicating both an alarmingly high number and the continued trend of loss. Note that the FAO risk statistics include consideration of more agricultural avian species than just traditional poultry (e.g. pigeon, emu, cassowary, ostrich, various quail, guineafowl, cormorant).

Evaluation of Genetic Variation Within and Between Poultry Populations

Conservation activities and priorities

A critical initial activity for the development of conservation management programmes is to establish descriptive inventories of the relevant genetic resources, including status information. A second important activity is the development of integrated assessments of genetic variation within and among populations. To this end, the FAO has proposed a project entitled 'Measurement of Domestic Animal Diversity' (MoDAD). The purpose of MoDAD is to promote comparative molecular descriptions, incorporating standard molecular markers to assess breed and population genetic diversity; for example, a standard set of chicken microsatellite markers has been proposed for use, including ADL158, 171, 176, 210, 267 and MCW1, 4, 73 (Wimmers *et al.*, 2000). Unfortunately, many populations are disappearing even before inventory/phenotypic characterization and molecular analysis can begin. A third

activity is to prioritize the genetic units for conservation and the final activity is to implement an appropriate conservation strategy. Ruane (1999) reviewed a list of criteria for selecting breeds and populations for conservation once an inventory and genetic variation have been documented. The criteria include: (i) degree of endangerment; (ii) species of the breed; (iii) adaptation to a specific environment; (iv) possession of traits of current or future economic value; (v) possession of unique traits that may be of scientific interest; (vi) cultural or historic value; and (vii) genetic uniqueness.

At the present time, the concept of genetic uniqueness is largely based on calculation of genetic distance using molecular assessment. Genetic diversity research focuses on establishing allelic diversity, heterozygosity and genetic distance. The validity of the genetic data collected (and calculated gene frequency, heterozygosity and distance values) for accurately describing genetic diversity depends to a great degree on the sample size, the number of loci and their representation of the genome, validity of the population(s) and population size (Ruane, 1999). The FAO recommends guidelines for microsatellite-based genetic diversity projects including the use of 25 animals per breed (population) and 25 to 30 microsatellite marker loci, having four to ten alleles per locus (reviewed in Ruane, 1999). It has been suggested that loci selected for study should include those exhibiting both high and low numbers of alleles to prevent bias. A concern regarding microsatellite markers for genetic distance studies is that microsatellites are neutral loci, and thus do not reflect loci under selection (natural or artificial). Another consideration regarding microsatellite studies is the contribution of 'null alleles', i.e. no amplification due to deletion or alteration of the primer binding sequence resulting in misclassification of heterozygotes. Given limited resources for breed conservation on a worldwide scale, Ruane (1999) argued that there is overemphasis on genetic distance studies and that other methods, including basic phenotypic data, should be used to establish conservation priorities. Considering the high number

of local breed populations worldwide, this view should be given serious consideration. Wimmers *et al.* (2000) found that a phylogenetic tree constructed from microsatellite data resulted in the clustering of chicken breed populations on a country-specific basis. Thus, for locally adapted breeds, an initial conservation strategy might best operate by prioritizing a specific number of breeds from each country, or at least on a continent basis, in lieu of or until genetic variation analyses can be conducted.

Population genetics nomenclature

Genetic diversity analysis, regardless of assessment method, is based on population genetic theory. A review is provided below of the terms and concepts of population genetics useful for diversity analysis and consideration (Schonewold-Cox *et al.*, 1983; Hedrick, 1985; King and Stansfield, 1997).

- *Allelic frequency*: the percentage of all alleles (gene variants) at a given locus in a population gene pool represented by a specific allele (also called gene frequency). If the frequency is 1 (100%), the allele is fixed in the population and there is no variation, and thus no basis or opportunity for selection of alternative alleles.
- *Artificial selection*: the process of choosing parents of the following generation on the basis of one or more heritable traits, or of preventing individuals from reproducing on the basis of one or more heritable traits. Selection (natural or artificial) alters allele frequency by reducing the likelihood that one or more genotypes will contribute to the next generation. The intensity of selection is the degree to which particular genotypes make a lesser contribution to the next generation (than as per Hardy–Weinberg expectations).
- *Effective population size (N_e)*: the size of an ideal population that would have the same rate of increase in inbreeding (or decrease in genetic diversity) by genetic drift as the population being studied (N_e is usually much less than the true size of the population).
- *Genetic diversity*: the heritable variation within and between populations of organisms largely determined by mutation, genetic drift, migration (gene flow) and selection (natural and/or artificial).
- *Genetic distance*: a calculated value based on allelic (Nei, 1972) or genotype frequencies (Hedrick, 1971) (other methods incorporate correlations among allelic frequencies at different loci) used to evaluate the amount of genetic variation shared among groups and to construct phylogenetic trees. Various assumptions (Hardy–Weinberg equilibrium and allele mutation models) are part of the different distance measure methods.
- *Genetic drift*: the chance changes in allelic frequency that result from the sampling of gametes from generation to generation. This occurs in all populations; effects are obvious in very small populations.
- *Hardy–Weinberg equilibrium*: the prediction of genotype frequencies (frequencies at which a genotype will be observed in the population) on the basis of allele frequencies in the population. It assumes that mating occurs randomly in a large population with no selection, migration, mutation or genetic drift.
- *Heterozygosity*: the sum of the frequencies of the heterozygous genotypes of the population at a particular locus. Heterozygosity is usually calculated from the expected Hardy–Weinberg genotype frequencies. The expected heterozygosity is an important measure of genetic variation (often summed over a number of loci).
- *Heterosis*: change in phenotypic mean of individuals resulting from a cross of two genetically different lines compared with the average phenotypic value of the individual parent lines. Usually there is an increase in phenotypic value, most noticeable in survival or fitness traits.

- *Heterozygote*: an individual having different forms of a gene (alleles) at a locus on homologous parental chromosomes.
- *Homozygote*: an individual having the same form of the gene at a locus on each homologous parental chromosome.
- *Inbreeding*: the mating of related individuals, associated with the loss of alleles.
- *Inbreeding depression*: reduction in phenotypic value (most noticeable in fitness traits) due to inbreeding of a normally outbreeding population.

Genetic-based threats to populations are due to the slow erosion of genetic variation by drift, lowering of fitness due to inbreeding (loss of alleles) and phenotypic effects of inbreeding depression. Selected populations (e.g. commercial elite lines) may face the added threats of accelerated drift by the intense selection constraints on gamete selection and founder 'bottlenecks'. What have the research studies to date provided in the way of assessing the extant genetic variation in commercial, breed and research stocks?

Molecular genetic variation assessment in poultry

The first poultry diversity studies focused on morphological characters (phenotype), allelic protein variants (allozymes) and blood group polymorphisms (reviewed by Weigend and Romanov, 2001). The revolution in molecular biology and genome analysis set the stage for the development of a number of molecular marker methods (DNA-based) for the characterization of genetic variation within and among poultry populations. Examples of markers used in diversity studies include: endogenous virus (ev) loci; random amplified polymorphic DNA (RAPD) (Smith *et al.*, 1996); minisatellite and microsatellite markers (Tables 15.1 and 15.2); restriction fragment length polymorphisms (RFLP) of single genes or gene families such as the MHC (Zhu *et al.*, 1996c; Sacco *et al.*, 2001); and the ribosomal DNA (rDNA) complex (Delany,

2000). Mitochondrial DNA sequences have also been used to study relationships within/ among poultry (Akishinonomiya *et al.*, 1994). Most of the diversity studies in poultry focus on chickens and turkeys (experimental research, commercial and/or breed populations) utilizing minisatellites and microsatellites. Tables 15.1 and 15.2 review the main diversity studies to date.

Genetic diversity by minisatellite analysis

The majority of the poultry genetic diversity studies in the literature assess genetic relationships using DNA fingerprinting (DFP) of minisatellite loci. For details about DFP and other molecular genetic markers, see Aggrey and Okimoto (Chapter 23, this volume). Briefly, hypervariable minisatellite regions are tandem repeats of short DNA segments that include a core repeat motif of 15–100 bp in length (Jeffreys *et al.*, 1985a,b). The allelic variation is due to: (i) variation in the number of tandem repeats and (ii) point mutations in the flanking restriction enzyme recognition sites. Genomic DNA is digested with restriction enzymes (common enzymes: *Hin*fI, *Alu*I, *Hae*III, *Msp*I) and size-separated using agarose gel electrophoresis. The DNA is transferred by Southern blotting to nylon membranes, which are hybridized with minisatellite probes; common probes include: Jeffreys 33.6, 33.15 (Jeffreys *et al.*, 1985a,b); M13 (Vassart *et al.*, 1987); R18.1 (Haberfeld and Hillel, 1991); and various di- and tri-oligonucleotide repeats, e.g. $(CAC)_n$. Samples for diversity studies are analysed as single individuals or as mixes. The technique produces a 'banding pattern' of 20 to 40 bands in the size range of 2–30 kb. The basis for establishing genetic relationships is whether a band is held in common in pair-wise comparisons (band sharing) and by calculation of band frequency (from band intensity) in pooled samples.

The DFP technique enjoyed much initial popularity but has been largely abandoned due to several disadvantages. DNA fingerprinting is labour intensive and typically only a relatively small number of individuals can be examined accurately;

Table 15.1. Review of genetic diversity studies in poultry by DNA fingerprinting (DFP) analysis.

Reference	Genetic resource Type (no. lines or groups)	Species	DFP method parameters	Summary results, general conclusions and interesting features
Hillel *et al.* (1989)	Commercial Broilers (1) (Cornish × White Rock) Layers (1) (White Leghorn) Source not indicated: Duck (1) (Muscovy); turkey (1)	Chicken Duck Turkey	Individual samples $n = 4$/group *Hinf*I Jeffreys 33.6 M13	Bandsharing (BS) within: broilers 0.21, layers 0.25, turkeys 0.27 ducks 0.64

Kuhnlein *et al.* (1989)	Research
	Inbred
	  WG – White Leghorn
	Line-bred
	  Cornell K
	  Cornell S
	  AgCanada Strain 7
	  Meat-type – New Hampshire
	  French Broiler (2)
		Chicken	Individual samples
			$n = 6$/group
			*Msp*I
			M13
				Index of genetic distance calculated based on DFP patterns
				Historical record of the lines matched the calculated distance
				Largest distance between WG and New Hampshire
				Genetic variability within strains: 0.24 inbred, 0.51–0.52 all
				others (calculated by band frequency)

Kuhnlein *et al.* (1990)	Research – White Leghorn AgCan 7 (no selection) AgCan 8 (selected: egg traits) AgCan 9 (selected: egg traits) S (selected: MDV susceptibility) WG (inbred derived from AgCan 9) 6sub3 (inbred: MDV resistance) 7sub2 (inbred: MDV susceptible) Note: Agriculture Canada lines were dispersed, frozen as semen or embryos, or dropped in 1997; many of the lines listed are no longer in existence (Delany and Pisenti, 1998).	Chicken	Individual samples $n = 6$/group *Msp*I M13	Mutation rate calculated: 0.0017 per locus per generation BS: Inbreeding coefficient 0.44 AgCan 7 0.026 0.51 AgCan 8 0.103 0.52 AgCan 9 0.126 0.69 S 0.3 0.81 WG 0.762 1 6sub3 > 0.98 1 7sub2 > 0.98 BS (or band frequency) reflects degree of inbreeding

Reference	Breed/description	Species	Sample	Results
Haberfeld et al. (1991)	Commercial Broilers (1)	Chicken	Individual samples n = 20 HinfI Jeffreys 33.6 (GTG)5 and (GT)12 Hillel R18.2	BS unrelated broilers = 0.25 0.25–0.27 depending on probe BS value of chickens was typically less than sheep, cattle or human
Dunnington et al. (1991)	Research White Plymouth Rock (2) selected for high or low juvenile weight White Leghorn (2) selected for high or low antibody response F1s of crosses between selected lines with each breed	Chicken	DNA mixes n = 20/mix HinfI Jeffreys 33.6 Hillel R18.1	BS corresponds with degree of relationship: 0.67–0.71 between parent lines and F1 offspring 0.53–0.58 between the related high/low selected lines within each breed 0.06–0.21 between the breed lines DNA mixes work well
Haberfeld et al. (1992)	White Plymouth Rock lines (2) crossed Parentals (low body weight; bantam), reciprocal F1s and backcrosses	Chicken	Blood or DNA mix n = 10/mix HinfI Jeffreys 33.6	Band frequency data generated from band intensities Band intensity and genome content highly correlated (0.996)
Siegel et al. (1992)	Wild Red Jungle Fowl (1) (Malaysia) Commercial White Leghorns (2) Broiler crosses (2) Research Random-bred control (2) Domestic Jungle Fowl (1) (experienced a bottleneck)	Chicken	Sex-specific blood mixes n = 3–5/mix Pooled DNA HinfI Jeffreys 33.6	BS results: Within JF 0.21 JF compared with all others 0.08–0.29 Among layer stocks 0.39 Among broiler stocks 0.57 (highest reported of any study) Controls vs. all: 0.13–0.22 Genetic tree constructed

Table 15.1. *Continued.*

Reference	Genetic resource Type (no. lines or groups)	Species	DFP method parameters	Summary results, general conclusions and interesting features
Dunnington *et al.* (1994)	Parental pure lines White egg layers (16) Broilers (11 dam; 9 sire) Wild Jungle Fowl Research Random-bred controls (2)	Chicken	Blood mixes *n* = 10/mix *Hin*fl Jeffreys 33.6 Hillel R18.1	'Adequate' level of genetic variation: BS 22–32% within or between commercials BS 7–9% commercials with JF BS 15–16% commercials with random-bred controls
Grunder *et al.* (1994)	Research Chinese breed and Synthetic strains (4) Selected and unselected compared	Goose	Individual *n* = 6/group DNA mix *n* = 15/mix *Msp*I M13	Genetic distances measured by three methods Inbreeding coefficients calculated Inbreeding increased with selection BS selected:unselected Values: 0.27:0.45 (Chinese breed) 0.15:0.32 (Synthetic strain)
Plotsky *et al.* (1995)	Research Inbred lines (13) of different breed origin and selection strategy	Chicken	Individual *n* = 4/line *Hin*fl Jeffreys 33.6 Hillel R18.1	Compared DFP with RAPD-PCR method, concluded DFP is a better measure to detect DNA-level diversity BS values ranged from 0.1 (different breeds) to 0.95 (common genetic background)
Lamont *et al.* (1996)	Commercial Egg-type lines (2 parental plus various crosses)	Chicken	Blood mix *n* = 6/mix *Hin*fl Jeffreys 33.6 Hillel R18.1	BS among parental egg lines: 0.31–0.33
Meng *et al.* (1996)	Commercial Beijing White Leghorn purelines (9) 'Market' chickens (*n* = 7 birds purchased)	Chicken	Individual *n* = 12/group DNA mix *n* = 30/mix *Hin*fl Jeffreys 33.6 a-globin 3'HVR M13	BS within lines: 0.50–0.63, all probes BS among lines: 0.15–0.50, all probes BS within market birds: 0.253, all probes

Reference	Species	Sample/methods	Comments
Tixier-Boichard et al. (1996)	Chicken	Research Rhode Island Red (2) lines divergently selected for residual food consumption Fayoumi breed	Individual $n = 33$ DNA mix $n = 7–9$/mix *HinfI* or *HindIII* Jeffreys 33.5 Hillel R18.1 EAV	BS within line: 0.9 BS among line: 0.82–0.86 Lines differ widely in the trait and correlated traits; yet only a low proportion of the genome differs Calculated inbreeding higher than from pedigrees BS RIR to Fayoumi: 0.17–0.3
Zhu et al. (1996a)	Turkey	Research Random-bred control and selected lines (6) Commercial Primary breeder lines (2 sire and 3 dam lines)	Individual samples $n = 18$/group *HaeIII* Jeffreys 33.6	BS and band frequencies used to estimate genetic diversity and distances Average BS among commercials: 0.26 (range 0.21–0.33) BS within commercials: 0.39–0.49 BS within research: 0.42–0.62 Commercial turkey lines show similar BS values as commercial chicken lines
Zhu et al. (1996b)	Turkey	Research 6 lines used in study listed above	Individual samples $n = 18$/group *HaeIII* Jeffreys 33.6	BS within lines: 0.42–0.62 Inbreeding coefficients: range 2.5–45% Correlation coefficient (BS and inbreeding) 0.992 Argues inbred lines bias the linear relationship between parameters because of differences in mutation rates between outbreds and inbreds (i.e. to reach same level of inbreeding, the outbreds have more mutations because of longer time taken to achieve the same inbreeding level)
Ponsuksili et al. (1998)	Chicken	Research Inbred (1) Commercial Broiler strain (1) Rhode Island Red layer (1) White Leghorn layer (1) Exotic breeds (8)	Individual or pooled $n = 7–15$/group; no mix info *AluI* or *HinfI* Oligonucleotide probes	BS within: Highest: inbred line 0.94; lowest: broiler 0.32 Range for other stocks: 0.39–0.67 Greatest genetic distance between inbred and broiler lines

Table 15.1. *Continued.*

Reference	Genetic resource Type (no. lines or groups)	Species	DFP method parameters	Summary results, general conclusions and interesting features

Ye *et al.* (1998a)	Research
	  Selected lines (2)
	Commercial
	  Primary breeder lines (10)
		Turkey	Individual samples
			n = 18/group
			*Hae*III
			Jeffreys 33.6
				BS:
				Within dam lines: av. 0.39
				Within sire lines: av. 0.48 Genetic distance:
				Among dam lines: av. 0.21 0.64
				Between sire lines: av. 0.27 0.57
				
				Genetic distance of research and commercial lines: 0.749, 0.810
				Research lines possess genetic variation different to the commercials
				Comparisons among commercial turkey lines indicate reasonable genetic variation, with levels similar to that found among chicken lines
				Notably, homogeneity (as per BS) within lines is greater than between

Ye *et al.* (1998b)	Research
	  Random-bred control (1)
	  Divergently selected lines (6)
		Quail	Individual samples
			n = 16/line
			*Hae*III
			Jeffreys 33.6
				BS ranges:
				Within: 0.384 (control) to 0.525 (selected, low 4-week body wt)
				Among the selected lines: 0.230–0.308
				Selection increased genetic homogeneity within lines
				Genetic distance values between lines: 0.277–0.628
				(for homogeneous populations, distance = 1)

Table 15.2. Review of genetic diversity studies in poultry (chicken) by microsatellite (MS) analysis.

Reference	Genetic resource (no. popns)	MS method parameters	Summary results, general conclusions, interesting features
Crooijmans et al. (1996)	Commercial Broiler (9) Layer (6)	17/19 loci Pools of 60/group Compared individ. to pools	Heterozygosity Av. no. alleles/locus Broiler: 53% 3.6 Layers: 27% 2.0 Moderate MS polymorphism in commercials Line-specific alleles observed Heterozygosity greater in broilers than layers
Takahashi et al. (1998)	Native Japanese breeds (10) Commercial White Leghorn (2)	8 loci n = 12–24/group	Av. no. alleles/locus: 5.6 Tree created with three main Japanese breed clusters Data fit historical record Japanese vs. Leghorn, distinct, excepting two Japanese breeds
Vanhala et al. (1998)	Commercial White Leghorn hybrid (3) Broiler hybrid (1) Breeds Finnish Landrace (3) Rhode Island Red (1)	9 loci n = 12–31/group	Heterozygosity Av. no. alleles/locus Genetic distance 0.29 (layer) to 2.4 (layer) to 0.12 (layers) to 0.67(broiler) 5.7 (broiler) 1.17 (RIR to layer) Tree shows three main clusters: 1. Broiler and Rhode Is. Red with one Finnish Landrace 2. Two Finnish Landraces 3. Three commercial layers
Zhou and Lamont (1999)	Research Inbred lines (23) from White Leghorn, Red Jungle Fowl, Fayoumi, Spanish	42 loci n = 2–3/line	Heterozygosity: 0–0.123 2–6 alleles per locus Line-specific alleles Tree created with four clusters Calculated proportion shared alleles for distances
Kaiser et al. (2000)	Commercials Broilers (2)	57/59 loci n = 6/group	Av. no. alleles/locus: 2.8, 2.9

Table 15.2. *Continued.*

Reference	Genetic resource (no. popns)	MS method parameters	Summary results, general conclusions, interesting features
Wimmers *et al.* (2000)	Local breeds Asia, Africa, S. America and zoo populations (23)	22 loci 405 chickens, no. per group not indicated	Heterozygosity: 0.45–0.71 2–11 alleles per locus Local populations have generally higher heterozygosity values than commercials Based on genetic distances (distinctiveness) resources ranked for conservation Tree shows four main clusters, fitting along country origin; Nigerian most distant from Bolivian populations
Romanov and Weigend (2001)	Native breeds Ukraine (5) and Germany (15) Jungle Fowl in captivity (3)	14 loci *n* = 6–23/group	Av. no. alleles/locus: 11.2 Tree shows three main groupings Jungle Fowl show a specific allele distribution
Weigend and Romanov (2001) (review)	Breeds Native, unselected; standardized Jungle Fowl Commercial Broilers, layers Research Selected, inbred	25 loci Pools of 50 individuals	Heterozygosity: 0.11–0.66 Jungle Fowl, unselected breeds, broilers exhibit higher heterozygosity than breed standards, research lines, layers Heterozygosity was negatively associated with genetic distance.

gel reproducibility problems (lane-to-lane variation both within and between gels) make the pair-wise comparisons problematic. A major assumption underlying the band comparisons is that bands equal in size represent the same allele for a locus (vs. a size allele from a different locus that migrates to the same position in the gel). Furthermore, there is no exact knowledge of how many loci or how many alleles are present. A major advantage of the DFP technique is that sequence information is unnecessary. DNA fingerprinting was the method of choice for diversity studies in poultry (reviewed in Table 15.1) from 1989 to 1998 but has been largely replaced by microsatellite analysis.

Genetic diversity by microsatellite analysis

Microsatellite markers are reviewed in detail by Aggrey and Okimoto (Chapter 23). Briefly, microsatellite DNA consists of tandem repeats of a 2–5 bp core motif with unique flanking sequences, which are used to develop primers for PCR amplification of the locus. PCR amplicon size usually ranges from 100 to 300 bp. Advantages of microsatellites for diversity studies include the following:

- Results are locus-specific.
- The loci are highly polymorphic (many alleles).
- Loci are evenly/randomly distributed across the genome. Interestingly, the poultry genome is reported to be deficient in microsatellite frequency relative to mammals (Primmer et al., 1997).
- Co-dominant inheritance of these locus-specific markers means that allele frequency data is easily generated.
- Techniques are relatively straightforward (easily used and evaluated).
- Large numbers of individuals can be handled.

Disadvantages include the need for expensive equipment (thermocyclers, imagers or sequencers) and sequence data (for primer development). The contribution of primer sets and sequences supplied by the US Poultry Genome Mapping Project and European counterparts has been enormously significant in this regard, as development of microsatellite libraries can be costly. Because of the large amount of sequence information available for chickens, it is common to attempt to use chicken microsatellite primers for studies in other species – but this approach has met with limited success (Liu et al., 1996; Pang et al., 1999; Reed et al., 2000). Kayang et al. (2000) reported development of microsatellite markers for Japanese quail. The results of microsatellite diversity studies in poultry are reviewed in Table 15.2.

Molecular genetic variation within and among research, commercial and breed populations

Collectively, the results of both DFP and microsatellite diversity studies suggest that there exists a strong genetic reservoir among the various categories of poultry genetic resources (research, industry, breed populations). Thus, looking at the totality of genetic resources, significant genetic variation remains; this is not surprising given the wide range of phenotypic variation observed among the resources. What emerges, however, is a general trend for reduced genetic variation within specific populations.

By DFP analysis, the degree of genetic variation among turkey commercial lines was similar to that observed among chicken populations (Zhu et al., 1996a,b). Evidence was also provided that turkey research populations contain 'different' variation as compared with industry lines (Ye et al., 1998a). Not surprisingly, for both chickens and turkeys, directionally selected populations were found to contain less genetic variation as compared with their random-bred controls (Kuhnlein et al., 1990; Grunder et al., 1994; Ye et al., 1998b). Most of the fingerprint studies in chicken, with a few exceptions (Dunnington et al., 1994), found that layers, in general, exhibited less overall genetic variation than broilers.

The power of the microsatellite data lies with the numbers: increased numbers of individuals sampled, increased number of loci, plus exactness of allele information. Thus, the microsatellite data from the last 4 years is well suited to addressing questions regarding the hierarchy of variation among the various poultry genetic resource categories – chickens and turkeys, specifically. Collectively, the data suggest that the number of alleles per locus is greater in broilers than in layers and that the heterozygosity values of broilers are typically higher (double) than that of layers. A consensus is emerging that the wild jungle fowl and unselected local breeds have the highest amount of variation, followed by the broiler, breed standard and dual-purpose breed populations (which include the brown-egg type birds). The various research lines and white-egg layer populations exhibit the lowest level of genetic variation. Many research lines share in common the Single Comb White Leghorn breed base of the layers, and many are purposely designed to lack genetic variation. Studies of coding loci contribute a valuable perspective, providing information on loci that may be pushed in one direction or another by selection. Interestingly, both Ye *et al.* (1998a) (MHC studies in turkey commercials) and Delany (2000) (rDNA studies in chicken commercials) reported evidence for greater genetic uniformity both within and among industry lines using functional loci than that seen when using neutral non-coding loci.

What genetic mechanisms maintain genetic diversity in selected populations?

Although the white-egg layers appear to possess less overall diversity compared with the broilers, all of the commercial populations exhibit genetic variation. Furthermore, by all accounts, the lines appear to continue to respond to selection. What maintains the genetic variation, especially given the narrow breed (Single Comb White Leghorn) and variety base (Mt Hope strain) and decades of selection applied to layer populations? The large population sizes of the commercial pure lines provide benefit. Muir (1997) reported that a layer operation may maintain on the order of 10,000 birds; if ten lines are in operation, population sizes could be about 1000 per line, with an effective population size of about 800 – well within the number suggested as 'healthy' (Frankham, 1999). Simulation studies suggest that as population size increases, under a constant selection index, the inbreeding rate will be less, as will be the subsequent inbreeding depression (as opposed to the results when population size decreases); thus, short-term consequences of inbreeding may be negligible (Muir, 1997). Small population size leads to inbreeding, loss of genetic diversity and increased risk of extinction. These outcomes have been shown empirically by the numerous species extinctions and in model systems (Frankham, 1999). Thus, maintaining large foundation populations is an important strategy in conserving commercial genetic resources. In that light, avoidance of 'stud males', as practised by other animal industries, will be key, so that effective population sizes remain large. Although about 50% of the broiler pure lines make use of artificial insemination (AI), using AI as to tool to minimize the number of elite sires is considered unwise because it would serve to reduce genetic variation and increase genetic drift (Pollock, 1999). Hopefully, that view will be maintained in the future. There may be incentive to resolve some of the reproduction issues of broiler breeder males (especially in unfavourable climates, which can exacerbate existing problems) through advanced reproductive technologies and assistance (Singh, 1999); this could potentially have a negative effect on genetic diversity.

The contribution of other genetic forces in maintaining diversity of chicken populations is unclear (e.g. mutation and recombination), given expected rates and time scale. One could postulate that the arrangement of the avian genome ($2n = 78$ in chicken and quail, $2n = 80$ in turkey) into numerous small linkage groups

(about 40% of the genome is organized into microchromosomes) may provide the advantage of increased combinatorial association of linkage groups. Perhaps the combinatorial variation of many small linkage groups in concert with very large population sizes underlies the continued response to selection – new combinations of alleles creating new opportunity for response to selection. Cahaner *et al.* (1996) proposed the interesting scenario that intense selection pressure over the long term (decades) has eliminated most of the detrimental recessive alleles. The argument was offered that heterosis effects are actually negligible in commercial crosses and heterozygosity resulting from crosses provides little advantage for performance traits. Furthermore, these authors proposed that the positive performance results in offspring of crosses are the result of additive effects of unique combinations of alleles, not heterozygosity at each locus per se.

The contribution of mutation to the generation of new genetic variation and its effects on genetic drift are unknown for poultry populations. Emsley (1997) suggested that 'persistence of moderate levels of heritability despite generations of selection calls for re-examination of the importance of mutation in our breeding populations'. Is the mutation rate significant enough to have impact, given the time scale of consideration? Model systems (e.g. *Drosophila*) suggest that the answer is no (Frankham, 1999). Little information on poultry mutation rates exists; based on DFP inheritance patterns, Kuhnlein *et al.* (1989) calculated a mutation rate of 0.0017 in chickens. Another type of genetic reshuffling, i.e. recombination, could also impact genetic variation and allele combinations. Genetic linkage and chiasmata frequency data provide evidence for high rates of chicken recombination (cross-over events per unit DNA) as compared with mammals (1 cM ~ 500 kb in chickens; 1 cM ~ 1000 kb in mammals) and recent data suggest that microchromosomes have a higher rate of recombination than macrochromosomes (Schmid *et al.*, 2000).

Conservation of poultry resources: who is in charge?

The data to date certainly support the notion that genetic diversity exists within and among the current genetic resources, although to varying degrees. The greatest challenge lies in the current inability to prevent losses resulting from management decisions (at academic institutions and primary breeder companies) and the losses of locally adapted breeds of developing countries. For all the decades of discussion, we remain reliant on individual researchers and company policies to conserve the valuable research and industry resources. At the breed level, we are reliant for conservation on the interest of individuals, breed organizations and commercial hatcheries. For the locally adapted breeds, the situation is also precarious. In many cases, the populations just happen to 'be there' and if they provide only negligible progress in supplying a stable protein source, especially in developing nations, over time the local populations will likely be replaced with modern industrial stocks (Gueye, 2000; Sheldon, 2000; Tadelle *et al.*, 2000; Safalaoh, 2001).

What is the role for cryopreservation in conservation of poultry diversity?

Cryopreservation, given its current application in poultry, can neither replace nor provide insurance for the living collections. Poultry are at a disadvantage as compared with many mammalian species because of the lack of a capacity to preserve both male and female gametes. Furthermore, the value of semen cryopreservation varies with species and genetic population, expertise in semen handling is required, and the best recovery efficiencies result from intramagnal inseminations, which are not favoured by animal care committees. Given the current technology, genetic lines possessing single gene mutations could be preserved as semen and the mutant gene presumably resurrected (although perhaps not in the same genetic background, known

to have a large effect on mutant phenotype). Also, congenic lines could be restored by semen cryopreservation, if the inbred parent line exists to provide the female gamete and background genotype. However, selected random-bred control and industry populations could not be expected to be revived with semen cryopreservation alone. Primordial germ cells (PGCs) can be frozen, thawed and inserted into appropriate-age embryos and subsequently produce gametes from the donor PGCs (Tajima *et al.*, 1998). Similarly, Stage X embryos can be frozen, thawed and mixed with compromised (irradiated) recipient Stage X embryos, and gametes of the donor genotype can be produced and used for breeding (Etches *et al.*, 1997). For both PGC and Stage X embryo manipulations, technical expertise and specialized tools (e.g. source of gamma irradiation to compromise the embryo recipient) are required to implement these techniques successfully. At present, overall efficiency for producing animals through PGC and embryo manipulation remains low.

Strategies for maintaining poultry genetic diversity

What should the goals be for maintaining poultry genetic diversity, especially given the broad range of categories, number of populations and different purposes/intents for the populations? Conservation strategies should be based on one or more of the following genetic principles:

1. Maintain alleles (conserve all alleles possible).
2. Maintain heterozygosity (keep alleles in heterozygous state for long-term survival of a population).
3. Maintain allelic combinations (as in breeds, it is the combination that defines the unique phenotype).

At the breed level, it is the combination of alleles that defines the breed and that requires conservation. Populations should remain independent and not be interbred. The data to date suggest that populations isolated geographically are genetically diverse and thus breeds should be conserved and managed on a 'community' basis (continent, region, state). Each community needs to identify its most valued resources for priority conservation. The approach should be based on phenotype and purpose, especially in developing nations where the breeds provide sustenance of eggs and meat, rather than waiting for molecular genetic variation studies. For developed nations, the near-term expectation is continued reliance on individuals and breed organizations to maintain the breed allele combinations that keep a Silkie, or any other breed, unique. Loss of breeds is expected to continue, due to increased urbanization and decreased exposure to and interest in hobby breeding.

Regarding the genetic research resources, updated inventories should be maintained (Pisenti *et al.*, 1999). An immediate goal should be the collection and cryopreservation of semen from the single-gene mutation stocks. Long-term survival of research genetic stocks requires institutional and government support. Unless and until stable financial intervention is established, there will no doubt be continued erosion of the research resources.

For the commercial populations, total diversity is improved by keeping more genetic lines (studies indicate between-line variation is higher than within-line; thus, maintaining more lines equates with more total diversity). Therefore, it would be of great conservation benefit (and entirely unrealistic) for the industry to remain at its present status and not consolidate further. Continued contributions from smaller primary breeder firms should be encouraged by government incentives. Foundation and pure-line populations should be kept distinct with limited interbreeding. Lines should continue to be managed with large population sizes. Use of stud males should be avoided. A number of researchers have suggested that a strategy to maintain commercial population(s) would be to divide and isolate subpopulations followed by periods of crossing. Such a strategy in concert with moderate selection intensity would prevent genetic drift and maintain allelic

diversity (Muir, 1997; Notter, 1999). Industry analysts consider that this subdivide-and-merge strategy is impractical (Hunton, 1997). Another strategy proposed to conserve the alleles (W. Muir, 2001, personal communication) is through the development of a series of highly inbred industry-derived stocks, which could be maintained in small numbers (four to five individuals per line) as has been accomplished with research inbred lines (Pisenti *et al.*, 1999). Reconstitution of populations by crossing the lines could take place in the future, as needed. It seems most probable, however, that poultry breeders will continue along the selection strategies favoured by company breeding policy and history as driven by consumer demand and economics; companies are unlikely to shift to focus on conservation strategies without broad incentives. A series of national inventories should be instituted encouraging anonymous listing of industry resources with associated inbreeding and genetic trends. Studies measuring genetic distances of breeds and industry stocks should continue.

Summary

An improved knowledge base is available regarding the molecular genetic variation of selected segments of chicken genetic resources, and to a lesser extent turkey genetic resources, with a clear paucity of information in the other species. Also, for some resources, valuable inventories are available or under development (Pisenti *et al.*, 1999; FAO, 2000; American Livestock Breeds Conservancy, 2001). Development and implementation of an organized conservation programme for any single segment of current genetic resources remains to be established. The de facto conservators continue to be those entities that use the resources, the scientists, commercial companies (primary breeder companies and hatcheries), local breeders and hobbyists, who continue to develop and maintain poultry genetic resources. Zoos in some countries are the conservators for locally adapted breeds of chicken (Romanov and Weigend, 2001). The breadth of poultry genetic resources is extensive; thus, the degree of genetic diversity within and among various segments remains reasonable. It is important that the creative opportunities embedded in these extant poultry genetic resources should not be lost through lack of attention and foresight. Most stocks are at risk due to management decision making, whether it be academic, commercial or local government planning. The need for conservation is as important as ever and yet the financial resources to support it remain severely limited.

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16 Progress and Prospects in Resistance to Disease

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Introduction

Avian infectious diseases are costly for the poultry industry. They increase management costs, lead to production losses and raise humane concerns. Avian infectious diseases are also a concern for humans, since chickens may be carriers for pathogens that affect human health. Bacterial pathogens such as *Salmonella* or *Escherichia coli* may cause ill health in humans; viral pathogens may evolve in chickens, cross species barriers and infect humans (Campitelli *et al.*, 2002). The outbreak of avian influenza in Hong Kong in 1997 is an example: it was contained by a territory-wide slaughter of 1.5 million chickens (Chan, 2002).

The major tools to combat avian diseases are antibiotics and vaccines. There are concerns that the large-scale application of antibiotics may contribute to the evolution of antibiotic-resistant microbes. As a result, several countries have placed restrictions on its usage (Hofacre *et al.*, 2001; Quirk, 2001). Efficient vaccines in conjunction with proper management may be the method of choice but whether it is possible to eradicate an avian disease completely is questionable, since improving management may not be justified by the production losses. As a consequence virulence of pathogens increases, requiring the development of new vaccines (Witter, 1998).

An adjunct in controlling avian disease is the development of strains of chickens that are resistant to disease. Selection can be conducted at the phenotypic level or, alternatively, at the DNA level. Powerful technologies are now in place to study the network of genes involved in the host defence to pathogens. Patterns of genetic variations that affect disease resistance can be identified and used to develop strains of poultry that are more resistant to disease. Selection for disease resistance will be an ongoing process. Similar to the development of pathogens resistant to vaccination, new variants will evolve that can overcome host resistance. However, combining the selection for host resistance with vaccination and improved management strategies may lead to the eradication of at least some avian diseases.

The chapters that follow contain detailed accounts of the progress made in elucidating mechanisms involved in disease transmission, characterization of the host response to pathogens and the identification of genetic variations that affect disease resistance. This chapter will discuss some these results and some recent observations made in our laboratory that emphasize the complexity of host–pathogen interactions. The human genome project has revealed that vertebrates may

have as few as 26,000 to 35,000 translated genes – a number that is surprisingly small. As a consequence, genes must interact to achieve the phenotypic complexity of higher eukaryotes. Hence, the whole is greater than its part and explaining phenotypic values as the additive effects of variations in single genes may not be adequate.

Phenotypic Selection for Disease Resistance

Host defences can be improved by direct selection for resistance to pathogens. Many examples where such selection has been successfully applied are described in subsequent chapters. In particular, several inbred lines that differ in disease susceptibility were established in order to identify genes by linkage analysis (see Bumstead, Chapter 18). It is of practical importance to establish whether selection against a specific pathogen alters susceptibility against other pathogens. If the selective gain is due to the loss of a pathogen-specific receptor, it may not affect the susceptibility to other pathogens. Similarly, changes in the types of major histocompatibility complex (MHC) surface protein may alter the spectrum of the antigens presented to the immune system and hence change the spectrum of pathogens that evoke an efficient immune response. In contrast to selection for immune responsiveness (see below), only a few strains selected for resistance to a pathogen were also tested for susceptibility to other pathogens. Hartmann *et al.* (1984) measured mortality from Marek's disease (MD) in three pairs of strains divergently selected for avian leukosis virus (ALV) resistance. In two pairs the ALV resistance was correlated with MD resistance, while in the third pair the ALV-resistant strain had a lower MD mortality rate. Hence, the susceptibility spectrum to pathogens other than the one used for selection may vary in different populations. This may be due to random drift during selection and/or reflect differences among the genetic determinants in the original base populations.

Base populations used in most divergent selection experiments were produced by intermating strains from a variety of different origins in order to increase the genetic variability in the base population. Whether direct selection for disease resistance in modern commercial pure lines will also lead to rapid gains has not been reported. In addition, the effect of selection in pure lines on the resistance in hybrid lines has to be tested. It is to be expected that the success and consequences of divergent selection for disease resistance may vary between different commercial strains of chickens.

Selection for disease resistance may not be feasible at the commercial level, since it requires large-scale exposure to pathogens. Further, challenge on an industrial scale may contribute to the spread of disease and may be ethically unacceptable.

Selection for Immune Responsiveness

An alternative to resistance towards a pathogen is selection for immune responsiveness (see Lamont *et al.*, Chapter 22). Such selection does not require containment facilities and may provide simultaneous protection against a wide variety of pathogens. Six detailed selection experiments are described (see Lamont *et al.*, Chapter 22). These experiments comprised single-trait selections based on the antibody (Ab) response to sheep red blood cells (SRB) or *E. coli* vaccine. Multiple-trait selections were based on an index combining Ab response, cell-mediated immune response and/or phagocytic ability. The selection experiments indicated the presence of genetic determinants that differentially affect parameters of the Ab response, such as the kinetics, the route and time of antigen administration, the type of antigens, as well as the primary vs. secondary Ab response. Less detailed information is available for the cell-mediated immune response, which may be as complex as the Ab response. The results indicate that the genetics of the immune response are complex and that it may be difficult to devise a selection

protocol that is optimal for resistance to a wide range of diseases.

Since the protocols and the genetic backgrounds differed in these experiments, it is difficult to generalize the results. Nevertheless, selection gains had been achieved and the gain in Ab responsiveness resulted in an increased resistance to a series of viruses and other parasitic agents. Susceptibility to some bacterial infections increased or was not affected, indicating that the gain of resistance may not be uniform. Detrimental effects of a highly responsive immune system such as the potential for autoimmune disorders have not been reported.

Identification of Genes Affecting Disease Resistance

Genetic architecture of complex phenotypes

Complex traits (i.e. traits with low penetrance or non-Mendelian traits) are determined by networks of many different genes. These networks are flexible and buffer phenotypes from the effects of stochastic, environmental and genetic change. By serendipity, genetic variations that have a selective disadvantage when combined with certain gene combinations may be maintained in a breeding population because they have selective advantage with combinations of other genes.

Genetic networking has been documented in mice, where the phenotype of many spontaneous or engineered mutants is dependent on the genetic background. Several modifier genes that suppress the mutant phenotype, cause novel phenotypes or change the spectrum of phenotypic effects have been identified (Nadeau, 2001). Even apparently monogenic traits are subject to modulation by modifier genes as exemplified by the phenotype–genotype relationship in thalassaemia, a human blood disorder (Weatherall, 2001). An example in chickens may be the ALV receptor, where the apparent clear-cut relationship between

receptor mutations and susceptibility to ALV infection appears to be strain dependant (see Bumstead, Chapter 18).

It is important to realize that the lack of phenotypic consequences of a mutation in one genetic background but not another does not simply reflect the presence of redundant pathways. Rather, altering a gene leads to modification of the genetic network where old connections are altered and new connections established (Greenspan, 2001). Only certain combinations of alleles in different genes may lead to a breakdown of the network and result in qualitatively new phenotypes.

Assuming that trait distributions within a breeding population are to a large extent determined by genetics, some mutations are expected to affect the variance rather than the mean of a distribution. Further, one may predict that interactions between genes are common and that many genetic variants are pleiotropic and will alter trait correlations. An example of an altered trait correlation is the somewhat unexpected observation that selection for immune responsiveness decreases body weight. It may indicate that some genes are components of networks that determine growth as well as those that determine the immune response.

The term 'disease resistance genes' has two meanings. On the one hand, disease susceptibility genes may be referred to as genes that, when manipulated, may affect disease resistance (i.e. they are part of networks that determine disease resistance). On the other hand, the definition of disease susceptibility genes may be restricted to those genes that naturally segregate for variants with differential effects on disease resistance. It is not to be expected that these two classes of genes are identical.

In chickens, analyses are restricted to natural genetic variants since efficient methods to manipulate the chicken genome are not available. Such variants have to escape selection imposed by fitness or by the breeder. They may accumulate in genes that are recessive or genes that can 'hide' because modifier genes mask the phenotypic effects of large fluctuations in gene expression.

Mapping of disease resistance loci by linkage analysis

The basic strategy in mapping chromosomal segments that harbour quantitative trait loci (QTL) is to follow the coinheritance of markers and traits over two generations of matings (one round of recombination). A putative QTL located near a marker is coinherited with that marker, whereas a gene located distant to the marker will be separated due to recombination. From the map positions of the markers, the marker genotypes and the quantitative trait measurements, one can then infer the map position of the putative QTL. QTL mapping in its simplest and most powerful version requires inbred strains of chickens that differ in the quantitative trait to be analysed. The two strains must be homozygous for contrasting marker alleles such that the descent and recombination events of DNA segments can be traced.

Such linkage studies have been conducted for resistance to Marek's disease virus (see Cheng, Chapter 21), salmonellosis (see Bumstead, Chapter 18) and coccidiosis (see Pinard-van der Laan *et al.*, Chapter 19). In addition, QTL affecting Ab response to Newcastle disease virus and *E. coli* have been mapped (see Lamont *et al.*, Chapter 22).

Linkage studies are very labour intensive. Extensive breeding is required to establish inbred lines with contrasting phenotypes to carry out the crosses required for linkage analysis and to determine marker genotypes and quantitative trait values. Further, the resolution that can reasonably be obtained is limited. Hence, the QTL region may contain many genes and direct identification of the causative mutation may not be possible. However, in order to apply marker-assisted selection successfully, the identification of the causative mutations (or at least of tightly linked markers) is necessary. Otherwise the phase between marker alleles and the putative quantitative trait mutation may differ among individuals of a non-inbred parental strain (i.e. gametes carrying one of the marker alleles will segregate for the quantitative trait allele).

An additional caveat is that the lines used for QTL mapping are created by divergent selection and inbreeding and therefore have novel genetic backgrounds that are very different from the parental background. QTL mapping is based on the effect of a mutation averaged over a genetic background created by mating these two lines. This artificially created genetic background may deviate from the genetic background in the strain of interest and the contribution of a QTL identified in the cross may have a relatively minor contribution in the original population. Further, due to a different array of genetic variations in the starting population or genetic drift during selection, different arrays of QTL may be identified in duplicate experiments.

Identification of disease resistance loci by association

In linkage analysis, QTL are identified by measuring their coinheritance with markers over two generations and by using recombination frequencies between markers to pinpoint the QTL locations. Association studies are simpler: they entail the identification of markers associated with traits using populations that have been propagated by random mating for at least 20 generations (Cardon and Bell, 2001). The rationale is that repeated matings randomize the linkage between markers and putative quantitative trait mutations. Hence, those markers that are associated with traits are markers that are either tightly linked to a quantitative trait mutation or are even responsible for the trait itself.

Association studies require fewer assumptions than linkage analyses. However, when large numbers of markers are analysed, the multiplicity of tests may lead to significant associations by chance. Despite this problem it has been argued that even genome-wide association studies are more powerful than linkage analyses, particularly for traits with low penetrance (Risch, 2000). In order to reduce the multiplicity of tests (and magnitude of the task), one may

restrict the analysis to candidate genes. Candidate genes are genes that may potentially be involved in disease resistance. Such choices may be based on the known biological properties of the gene products, phenotypes associated with mutant alleles in other biological systems or by virtue of their location in a chromosome segment harbouring a QTL as determined by linkage analyses.

Candidate genes may also be selected on the basis of expression profiling at the RNA or protein level (see Cheng, Chapter 21). An example is the change of RNA expression profiles in cells infected with MD virus (Liu *et al.*, 2001a). Altered expression of these genes may be a requisite for successful viral propagation or reflect the activation of host defences. Hence, mutations in these genes may affect MD resistance. An example of the identification of a candidate gene by proteomics is the demonstration of an interaction between the MD viral protein SORF2 and the growth hormone (GH) and the subsequent finding that a marker in the GH gene affects resistance to MD (Liu *et al.*, 2001b). It confirms previous reports that markers in the GH gene are co-selected with selection for disease resistance (Kuhnlein *et al.*, 1997) and are associated with immune responsiveness (Aggrey *et al.*, 1996).

Genes Implicated in Disease Resistance or Immune Responsiveness

So far variations in only a few genes have been implicated in affecting disease resistance. The MHC(B) locus involved in antigen presentation was among the earliest loci to be identified. It has been studied extensively, but the genes and sequence variations that give rise to different MHC(B) haplotypes are still unknown (see Plachy *et al.*, Chapter 17). More precisely defined are the genes *tva* and *tvb* that code for the ALV receptor (see Bumstead, Chapter 18). Other genes such as *INF*γ or *Nramp* have also been identified as modulators of disease resistance. Kuhnlein *et al.* (1993) reported the induction of immune tolerance to ALV by the endogenous viral genes *ev*-6

and *ev*-9. Further, selection for multiple traits of immune responsiveness in outbred strains led to a decrease of the frequency of *ev*-6 and *ev*-9 (Lamont *et al.*, 1992). *Ev*-6 was shown to be highly expressed in bursal cells, indicating that it may be related to the functionality of B cells (Ewert and Halpern, 1982).

Analysis of markers in genes of the growth hormone (*GH*), growth hormone receptor (*GHR*) and insulin-like growth factor I (*IGF*1) revealed associations with the Ab response to attenuated Newcastle disease virus (NDV) and delayed type hypersensitivity (DTH) response to avian encephalomyelitis virus (AEV) and phytohaemagglutinin (PTH) (Aggrey *et al.*, 1996). It indicates that the GH axis plays an important role in the immune response and that the genes can be separated into two groups: *GH* and *IGF*1 affect the DTH response, while *GHR* affects the Ab response. Ornithine decarboxylase (ODC) was also found to affect the Ab response. ODC is the rate-limiting enzyme in polyamine synthesis and hence cell division. In contrast, no associations were observed for markers in genes that affect energy metabolism (mitochondrial haplotypes and the two isoforms of phosphoenolpyruvate carboxykinase (*PEPCK*-M and *PEPCK*-C), pivotal regulatory enzymes in gluconeogenesis (Kuhnlein, unpublished results).

Gene Interactions

Disease susceptibility and immune responsiveness are complex traits and interactive effects between genes are to be expected. Evidence for such interaction can be gained by direct comparisons of trait distributions in the genotypic classes formed by the combinations of genotypes of individual genes. This is indeed observed for genes affecting the immune response. An example shown in Chapter 32 (Fig. 32.5) is the association of *IGF*1 and GH genotypes with the delayed hypersensitivity response to AEV. It shows that the effect of the genotype of one gene is dependent on the genotype of the other

gene. Similarly, interactive effects between the ODC and the GHR genotypes were observed for the antibody response to NDV (Kuhnlein, unpublished results) and between GH and GHR for the delayed hypersensitivity to AEV (Aggrey *et al.*, 1996). Another example is the interaction of the MHC haplotype and the GH genotype in MD resistance. Challenge tests in a commercial strain indicated that an association of a GH marker genotype with several MD traits was only observed in conjunction with one of two segregating MHC haplotypes (see Cheng, Chapter 21).

Demonstration of gene interaction requires large sample sizes. Since a gene segregating for two alleles will give rise to three different genotypes, two polymorphic genes will give rise to nine and three genes to 27 different genotype combinations. Hence, extension of this type of analysis to more than two genes requires breeding to create an adequate number of the individuals with the genotype combinations of interest.

In most cases the identities of genes that modify the phenotype associated with a primary gene are unknown. Nevertheless, assuming that a trait distribution of a strain is primarily determined by individual genotypes, it can be shown that segregation of a modifier gene increases the variance of the trait distribution. Hence, the variance of the trait distribution in different genotypic classes may differ, depending on whether they are subject to the action of modifier genes. An example is given in Fig. 16.1. It shows normality plots of the Ab response to NDV in two genotypic classes of ODC. Significant differences are observed in the variance but not the mean of the two distributions. In addition, the difference in variance is dependent on the GHR, since it is only observed in conjunction with one of the two GHR marker genotypes.

Trait distributions can be used to split populations into groups with different ensembles of genotypes. Specifically, populations may be split into individuals that have trait values above and individuals that have trait values below the median. These two groups may comprise different modifier genes and hence alter the association of a gene with a second trait (Feng *et al.*, 1997). An example is shown in Fig. 16.2. The population was split into two groups on the basis of juvenile body weight and both groups were analysed for association of the GHR marker genotype with the Ab
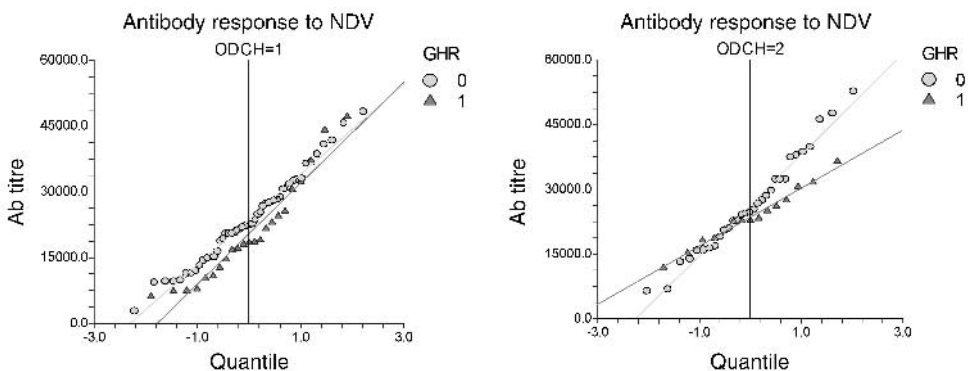
Fig. 16.1. Influence of ODC and GHR marker genotypes on the distribution of antibody titres against Newcastle disease virus. Hens from a non-inbred strain of White Leghorn strain were challenged with attenuated NDV virus and antibody titres were measured 28 days post-infection. The genetic markers were *Hin*dIII RFLP in the *ODC* gene and the *GHR* gene, respectively. ODCH = 1 designates the *Hin*dIII+/– genotype and ODCH = 2 the *Hin*dIII+/+ genotype. For *GHR* (haploid), 0 designates the *Hin*dIII– and 1 the *Hin*dIII+ genotype. The probability plot indicates that the *GHR* genotype affects the variance among individuals of the ODCH = 2 genotype (variance-ratio test: $P = 0.016$) but not among individuals of the ODCH = 1 genotype (variance-ratio test: $P = 0.387$). The means were not significantly different (*t*-test).
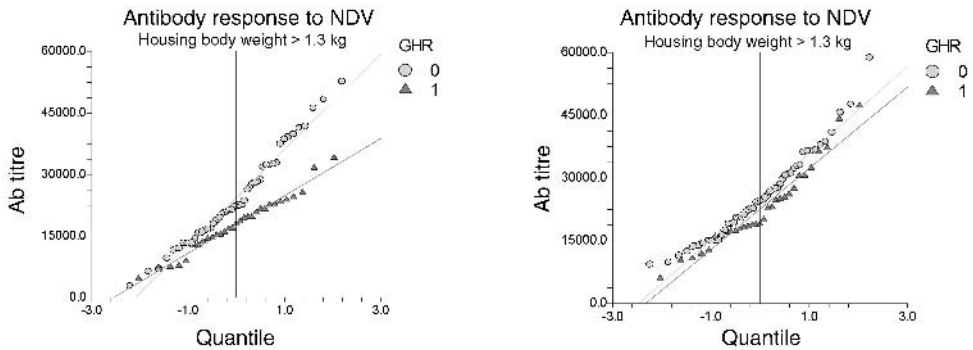
Fig. 16.2. Influence of the GHR genotype on the antibody titres against Newcastle disease virus in chickens above and below the median juvenile body weight. The data set is the same as described in Fig. 16.1. In chickens above the median body weight the GHR receptor genotype has a significant effect on the antibody response (Wilcoxon rank-sum test, $P = 0.015$) and on the variance (variance-ratio test, $P = 0.002$).

response towards attenuated NDV virus. The association was only significant for individuals with body weights below the median.

Another manifestation of gene networks is pleiotropy. Genes may be part of overlapping networks that affect different traits. Hence, genetic variations in such genes may affect more than one trait. Divergent selection for Ab responsiveness affects the juvenile body weight and onset of egg laying (see Lamont *et al.*, Chapter 22). Chickens from strains selected for a high Ab response have a lower body weight and mature later than chickens from strains selected for a low Ab response. Similarly, we observed a negative correlation between the Ab response to attenuated NDV and body weight in a non-inbred strain of White Leghorn chickens (see Fig. 16.3). Further, the GHR and ODC markers associated with the Ab response markers also affect body weight and onset of sexual maturity, respectively (see Kuhnlein *et al.*, Chapter 32). The link between Ab responsiveness and juvenile body weight appears genetically determined, since mitochondrial markers and markers in *PEPCK-C* affect the body weight but not the Ab response (Kuhnlein, unpublished data).

Marker-assisted Selection

The ultimate test for the usefulness of genetic markers is to carry out a selection
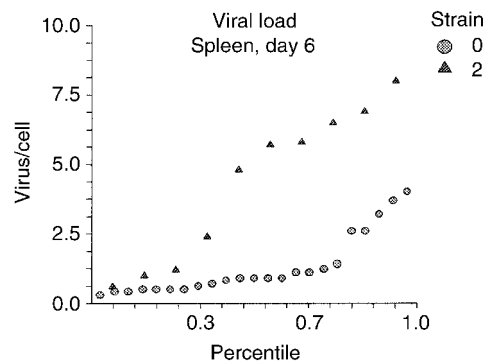
Fig. 16.3. Distribution of the viral titres in the spleen at 6 days post-infection. Three-weeks-old unvaccinated chickens were challenged with the RB1-B strain of MDV by intraperitoneal injection. The two strains were developed from the White Leghorn strain 7 (Gowe *et al.*, 1993) and selected for homozygosity at the markers in the *GHR* (*Hin*dIII RFLP) and *GH* (*Sac*I RFLP) genes. The restriction sites are absent in strain 0 and present in strain 2.

experiment. We have developed two lines differing in markers in the *GHR* and *GH* genes that had previously been identified as being co-selected with resistance to MD-induced tumour formation and/or mortality (Kuhnlein *et al.*, 1997; Feng *et al.*, 1998; Linher *et al.*, 2000). After five generations, chickens of the two strains were challenged with MD virus and viraemia was measured in the bursa, spleen and thymus. At 6

days post-infection, the average splenetic viraemia in strain A was 70% lower than in strain B. No significant differences were observed in the bursa or the thymus.

Analysis of the parental strain revealed that the antibody response to NDV was higher in individuals with the GHR genotypes present in strain A than in those with the GHR genotype of strain B (Aggrey *et al.*, 1996). Hence, an increased Ab responsiveness may be responsible for reduced viraemia.

However, the interplay between the MD viraemia and the immune system may be more complex. The two strains may differ in the kinetics of viral proliferation, rather than in the number of viral particles produced during the course of infection. Such a situation would be similar to the observation by Weigend *et al.* (1997), who reported that selection for immune responsiveness might alter kinetics, rather than the absolute amount of the immune response.

An additional caveat is that the GH and GHR genotypes associated with low viraemia are those that are co-selected with susceptibility rather than resistance to MD-induced tumours and/or mortality. It may be related to the observation that the DTH response to AEV is lower in strain A than in strain B. Hence, the cell-mediated immune response may be more important in preventing MD tumors and mortality than the Ab response.

Prospects

Knowledge about the architecture of complex traits is still in its infancy. Initially it was thought that variations in complex traits may be adequately explained as the additive effect of variation in single genes. However, studies in mice indicate that for complex traits gene interactions are the major determinants of phenotypic variations. As a consequence, analysis of the genetic underpinning of complex traits requires the simultaneous analysis of the effect of combinations of allelic variations in many genes, rather than the analysis of one gene at a time. It also implies that

additive models are inadequate descriptors of the genotype/phenotype relationship.

Association studies are based on minimal assumptions. Their essential goal is to identify the sequence variations in the entire genome and search for patterns that are correlated with phenotypes. Although it may sound prohibitive at the current stage, the rapid development in technology may make such whole genome scans possible.

Pattern recognition has to be based on statistics (such as cluster analysis) as well as biological considerations. Biological clues may narrow the search for patterns to groups of genes, rather than the entire genome. Such groups of genes may be identified by virtue of correlated expression using expression profiling at the RNA or protein level, or by searching for physical interaction between proteins. Additional clues will come from comparisons with other species where research for disease resistance is more advanced.

Current knowledge about genetic determinants of disease resistance is anecdotal. One must be prepared to find that associations in one strain may not be found in another strain. Ironically, while expected from gene interaction, observations that are strain specific have been classified as spurious and hence flawed. In addition, most analyses have been restricted to genes that are translated and an entire class of non-translated genes that may affect gene regulation has been ignored (Eddy, 2001).

Despite the paucity of knowledge, programmes for selection at the DNA level can be established. Using simple association analyses, markers associated with disease resistance can be identified and selected for in the particular strain of interest. The newly established strain can then again be subjected to a screening process and selection at the DNA level can be continued. What is not known at present is how such markers would affect disease resistance in a strain with a different genetic background. Further, it is possible that, depending on the sequential order of gene selection, one may end up with arrays of genes and at end points with different degrees of susceptibility. The

situation may be similar to tumour formation, where natural selection for proliferation may lead to phenotypically similar but genetically dissimilar cancer cells.

Currently it cannot be predicted how selection at the DNA level will affect other traits. As an example, selection for alleles of the *GH* and *GHR* genes described above not only reduced splenetic MD viraemia but also reduced body weight. We have also observed that, even in a random-bred population, many markers are at linkage disequilibrium despite being located on different chromosomes. It indicates that certain marker combinations affect fitness. It is therefore to be expected that selection at a single DNA locus will lead to a remodelling of the genetic composition of the entire genome.

Despite the lack of a comprehensive picture, it should be recognized that it is possible to identify markers that affect immune responsiveness and/or disease resistance and achieve gains by selection at the DNA level. It should also be recognized that selection at the phenotypic level is a viable alternative.

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17 Genetics of the Immune System

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Immune System: Innate and Adaptive Immunity

The immune system is an adaptive defence system that evolved in phylogenesis to control an organism's integrity. Immunity is the state of protection from infectious disease. There are two main components of the immune response: innate immunity (mostly non-specific) and acquired (specific) immunity. Both are able to recognize self and non-self. Acquired immunity does not occur independently of innate immunity and depends on many non-specific (physiology, environment, etc.) and specific (e.g. genetic control) factors. This chapter will deal with the most important genetic factors, represented by three recognition systems: immunoglobulin (Ig) receptors, T-cell receptors and major histocompatibility complex (MHC) genes. The emerging modulators, the cytokines, will also be discussed. There are many other genes capable of regulating the immune response but they are mostly, as yet, poorly defined in the chicken. For endogenous viruses see Bumstead (Chapter 18).

Generation of the Chicken (Avian) Immunoglobulin Repertoire

An old enigma of (immuno)genetics was the apparent impossibility of explaining the Ig repertoire by the presence in the genome of sufficient individual genes to encode thousands of different antibodies (Ab), since the numbers required would clearly exceed the entire genome's capacity. The finding that the genetic information encoding the tremendous diversity of Ab molecules is stored in a relatively small number of germline Ig gene segments was one of the greatest achievements of (molecular) immunology (Tonegawa, 1983). During B-cell development, variable (V), diversity (D) and joining (J) genes undergo somatic recombination to generate a primary Ig gene repertoire.

A variation of this mechanism was first demonstrated in chickens (Reynaud *et al.*, 1985; Thompson and Neiman, 1987) and also later in certain mammalian species such as rabbits and sheep (Knight and Becker, 1990; Motyka and Reynolds, 1991). In the chicken a diverse set of Ig is created mainly by intrachromosomal gene conversion of the single variable genes of Ig heavy and light

chains. Recombination of this limited number of (V, D, J) genes appears to serve only to activate Ig expression in a B-cell-specific fashion, while Ig diversity is created by a unidirectional transfer of sequence information from a number of pseudo-V elements to the single rearranged functional V element (for review of this topic, see Reynaud and Weill, 1996). The developing B cell completes the recombination events necessary to express Ig molecules prior to migration towards the bursa of Fabricius – a central lymphoid organ present in all birds but not in mammals. Unmodified functionally rearranged Ig molecules are directed against a bursal-specific antigen. This serves as a positive signal for clonal expansion, while cells that do not functionally rearrange an Ig molecule die by apoptosis. Thus, the initial role of the bursa is to expand Ig-positive B cells to provide a large precursor population. Between day 18 of embryogenesis and the 6th week of age these cells undergo the gene conversion process within the bursa, leading to Ig diversification as well as negative selection of potentially autoreactive cell clones. Selected cells are finally allowed to emigrate from the bursa to the periphery.

Chicken T-cell receptors

The production of monoclonal Ab (mAb) raised against a variety of functionally important chicken T-cell molecules homologous to mammalian molecules – CD_3, CD_4, CD_8 and three lineages of T cells, named for the order in which they appear in the embryonic thymus: TCR1 (γδ T cells), TCR2 (αβ T cells expressing a family of Vβ1 genes) and TCR3 (αβ T cells expressing a second family of Vβ2 genes) – was the starting point for a detailed analysis of TCR genes and T-cell development in the chicken (Chan *et al.*, 1988; Chen *et al.*, 1989). Analysis of the chicken TCR genes and their protein products revealed divergent evolution at the level of nucleotide (nt) sequence, but conservation of important structural features of the TCR polypeptides in avian and mammalian species. In contrast to Ig genes,

chicken TCR genes undergo a similar set of events in the generation of their diversity as those in mice and humans. The bulk of the chicken TCR repertoire is generated by combinatorial joining of individual members of the V, D and J gene families. The only marked genetic difference is the relative simplicity of the chicken TCR loci compared with those of a typical mammal. There are only two Vα and Vβ families in the chicken, compared with the 20–30 families in both mice and humans (Cooper *et al.*, 1991; Chen *et al.*, 1996; Dunon and Imhof, 1996). T-cell differentiation and function are remarkably similar in birds and mammals, including separation of T-cell development into two discrete pathways characterized by the expression of αβ or γδ TCR, thus indicating a relatively ancient separation of these T-cell lineages during vertebrate evolution. In mice and humans, γδ T cells comprise only approximately 5% of the peripheral blood and splenic T cells, while in chickens (and also rabbits, cattle and sheep) γδ T cells represent 30–50% of the peripheral T-cell pool (Cooper *et al.*, 1991; Hein and Mackay, 1991; Su *et al.*, 1999). It is interesting that the 'γδ-low species' have a high degree of diversity of *TCR-V* genes, while 'γδ-high species' all have limited diversity. This pattern is similar to that found for Ig variable region (*Ig-V*) genes. The chicken *TCR-V* genes seem to have the lowest diversity of all examined species and also the most restricted *Ig-V* gene repertoire yet found in vertebrates. Cell-surface or secreted molecules expressed by invading pathogens and/or self-stress response proteins induced upon infection are recognized by γδ T cells. Antigen recognition by γδ T cells clearly does not correspond to the paradigm of the classical peptide–MHC–TCR interaction for αβ T cells (reviewed by Davis and Chien, 1995).

Recent studies suggest that γδ T cells recognize protein antigens directly without any requirement for antigen processing and can also recognize a variety of non-peptide antigens such as Ig (Poccia *et al.*, 1998). Sequence analysis suggests that TCR γ or δ sequences are the most ancient members

of the Ig superfamily, implying that the ancestral immune cell was like a modern γδ T cell. It is reasonable, therefore, that indirect MHC-restricted antigen recognition is a derived characteristic of αβ T cells, while γδ T cells exhibit the primitive condition of direct antigen binding (Richards and Nelson, 2000). An important role for γδ T cells in the intestinal mucosal immune response to infection by *Eimeria* species has been suggested (Lillehoj, 1994). Using a unique quail-chick chimera model, Cotley *et al.* (1987) demonstrated that the chicken thymus is colonized by haematopoietic precursors in three waves, beginning on embryonic days 6, 12 and 18, respectively. Adoptive cell transfer into congenic chickens showed that colonization of the periphery by mature T cells is entirely thymus dependent, and TCR γδ and αβ cells leave the thymus in independent waves, in which γδ cells always precede αβ cells by 1–2 days (Dunon and Imhof, 1996).

Cytokines

Cytokines are the modulators of the immune response, controlling and coordinating both innate and acquired immunity. They have profound effects on the duration and direction of immune responses, which explains why many pathogens, including certain avian viruses such as Marek's disease virus (MDV) and fowlpox, have evolved or captured genes whose products interfere with host cytokines in an attempt to subvert the host's immune response. Cytokine genetics are of interest for several reasons. To what extent is the wide panel of cytokines seen in mammals replicated in a non-mammalian vertebrate? Is there synteny of genetic location for avian cytokines with their mammalian homologues? Are there polymorphisms in the regulatory regions of avian cytokine genes that correlate with particular disease resistance/susceptibility profiles in inbred lines of birds? There are still notable gaps in the repertoire of chicken cytokines as compared with mammals (Table 17.1). The availability of a chicken bacterial artificial (BAC)

Table 17.1. Comparison of mammalian and chicken cytokines.

Mammalian	Chicken
Type I IFN (IFN-α, -β, -ω, -τ)	Type I IFN (IFN-α, -β)
Type II IFN (IFN-γ)	Type II IFN (IFN-γ)
IL-1–IL-13, IL-15–IL-23	IL-1β, IL-2, IL-6, IL-8, IL-15, IL-18
Colony stimulating factors (3)	(0)
Tumour necrosis factors (3)	(0)
Transforming growth factors β1–3	Transforming growth factors β2–4
Chemokines (> 50)	Chemokines (8)

library (Crooijmans *et al.*, 2000) and several chicken expressed sequence tag (EST) libraries (Abdrakhmanov *et al.*, 2000; Tirunagaru *et al.*, 2000), along with the likelihood that the chicken genome will be completely sequenced within the next several years, provides the tools to try to fill some of these gaps. However, it may be that the chicken has a reduced repertoire for some groups of cytokines. This is illustrated by recent work looking at chicken chemokines (Hughes *et al.*, 2001), which identified three novel chicken CC chemokines that mapped to three chromosomal regions corresponding to (syntenic with) regions containing homologous chemokines in humans. It appears that the major branching of the chemokine family occurred before the divergence of birds and mammals. The chicken genome seems to contain representatives of, but not as many members as, most of the mammalian CC chemokine clusters. In humans and mice the CXC chemokine cluster and the CC chemokine macrophage inflammatory protein (MIP)-like and monocyte chemoattractant protein (MCP) clusters have greatly expanded. It remains to be seen if this is the case in chickens, or whether, as for the MHC (see below), the chicken clusters are simpler and contain fewer genes. Synteny of genetic location has also been shown for interleukin (IL)-2 (Kaiser and Mariani, 1999), IL-8 (Kaiser *et al.*, 1999) and interferon (IFN)-γ (Guttenbach *et al.*, 2000), but does

not extend to the type I IFNs, which are on autosomes in mammals but on the Z (sex) chromosome in the chicken (Nanda *et al.*, 1998).

In mammals, there is an increasing raft of literature showing that cytokine gene promoter polymorphisms are linked to disease resistance/susceptibility profiles. There are sequence polymorphisms in the chicken IFN-γ promoter between inbred lines of chickens, which correlate to resistance to *Escherichia coli* infection (Kaiser *et al.*, 1998). Mammalian cytokine genes have been involved in disease resistance profiles. As genes for new chicken cytokines become available, it should become clear whether they confer similar resistance profiles, like their mammalian homologues. If so, the resistant phenotypes could then become part of a disease-resistance breeding strategy.

MHC(B) of the chicken

From an evolutionary point of view, the most striking characteristic of the MHC is the high polymorphism of some MHC loci: these loci are the most polymorphic known in the animal kingdom. MHC genes show high rates of non-synonymous (amino acid changing) substitutions in the antigen (peptide)-binding site (Hughes and Hughes, 1995) and long persistence times of alleles in evolution (Klein, 1987). Diversity at the population level is thought to arise via interactions between host MHC gene products and parasites, leading to balancing selection: either frequency dependent (rare allele advantage) or overdominant (heterozygote advantage). While biochemically and serologically recognizable polymorphic antigens had been considered as candidates for components of the disease resistance system since their discovery, the first potential clues to the mechanism of pathogen control was provided by the MHC restriction phenomenon (Doherty and Zinkernagel, 1975). Because MHC allelic products vary in their ability to present different antigens, in a population exposed

to a variety of pathogens such differences could give rise to heterozygote advantage (overdominant selection) that would enhance diversity at the MHC loci. The fact that selection is specifically focused on the sequence diversity of the peptide-binding region of class I MHC genes (Hughes and Hughes, 1995) provides strong support for Doherty and Zinkernagel's idea. There are several other explanations for MHC polymorphism, such as mating preferences and selective abortions (Potts and Wakeland, 1993), which may be more or less important in some taxonomic groups of vertebrates, but host–parasite competition seems to be the most universal force.

Blood group system B: from serological patterns to the discovery of avian MHC

The chicken MHC was originally described as blood group system B. Four blood group systems, A, B, C and E, are considerably polymorphic in the chicken, but from the very beginning it has been clear that the polymorphism of the B system exceeds that of all the other systems (Briles, 1962). At least 30 *B* alleles have been identified with certainty within rather narrowly based genetic stocks of inbred and partially inbred lines (Briles and Briles, 1982; Simonsen *et al.*, 1982). A difference between donor and recipient in the B system was shown to be sufficient for rapid rejection of a skin graft (Schierman and Nordskog, 1962). Similarly, only the B system is involved in strong graft vs. host (GvH) reactions. These functions, known to be controlled by the MHC in mammals and linked to the chicken B blood group locus, raised this chromosomal region to the status of the chicken MHC(B). Additional support was the first formal proof for the existence of the MHC(B)-linked immune response genes (Günther *et al.*, 1974; Plachy *et al.*, 1992).

The availability of inbred lines prompted a systematic search for genetic recombinants within the MHC(B) (Hála *et al.*, 1976, 1981a; Skjodt *et al.*, 1985). In all, more than 25,000 informative typings have

been performed using serological analysis, and in some cases also mixed lymphocyte reactions and restriction fragment length polymorphisms (RFLP), with only nine recombinants identified (reviewed by Plachy *et al.*, 1992). All recombinants were similar in that the crossing-over event separated a serologically detectable *B-G* gene, apparently not involved in graft rejection and clearly different from all already known MHC genes, from the chicken analogs of class I (*B-F*) and class II (*B-L*) MHC genes. The map distance between the *B-G* gene and the rest of MHC(B), or perhaps the proper MHC of the chicken, was estimated on the basis of pooled data to be 0.037 cM. The surprisingly low frequency of recombination within the chicken MHC(B) (at least one order below the frequency within *H-2*), and particularly the failure to detect any recombinants between class I (*B-F*) and class II (*B-L*) loci, led to the speculation that the whole MHC(B) occupies a much shorter chromosomal segment than does *H-2* or *HLA*. Alternatively, Skjodt *et al.* (1985) suggested that the lack of class I/class II recombinants may be due to intermixing of these genes.

The biochemical analysis of the MHC(B) antigens was pioneered by Ziegler and Pink (1976). The first genetic recombinant found in Prague (Hála *et al.*, 1976) soon brought these workers together, and the resulting three-locus model (*B-F*, *B-L* and *B-G*) for the MHC(B) was described (Pink *et al.*, 1977). Antigenic products of two different loci were identified within the genetically defined *B-F* region of the recombinant MHC(B) haplotype. B-F antigens presented on both erythrocytes and lymphocytes are homologous to mammalian MHC class I molecules. They comprise the 40–43 kDa heavy (α) chain, which is a transmembrane glycoprotein non-covalently associated with the invariant polypeptide of 11–12 kDa, the avian β_2-microglobulin homologue (coded by an MHC-independent locus). B-L antigens, expressed in lymphocytes but not in erythrocytes, appeared to be homologous to MHC class II molecules with two associated transmembrane chains, α (30–32 kDa) and β (27–30 kDa) (Pink *et al.*, 1977).

The second genetic region, *B-G*, appeared to comprise only one locus, encoding the B-G antigens, the expression of which seemed to be confined only to erythrocytes. No histocompatibility function was ascribed to the *B-G* region by early studies (reviewed by Plachy *et al.*, 1992). The B-G antigens have been rather enigmatic from the beginning. They could have been considered simply as blood group antigens fortuitously associated with the MHC, but some properties classified them as class IV histocompatibility antigens (Simonsen, 1981). The *B-G* genes exerted an extreme polymorphism, characteristic of MHC genes, and strong linkage disequilibrium (gametic association) with the *B-F* alleles (Simonsen *et al.*, 1980). Although the B-G antigens have approximately the same relative mobility on reducing SDS-PAGE gels as class I antigens, there is no evidence that they are associated with β_2-microglobulin. B-G products seem to carry no carbohydrate moieties, which alone sets B-G apart from MHC antigens of other classes. The amount of B-G present on erythrocytes is several times greater than the amount of class I (B-F) antigen (Salomonsen *et al.*, 1991a).

Search for MHC(B)-associated traits

There are many experimental examples of the association of particular chicken MHC(B) haplotypes with resistance to tumour diseases induced by infectious viruses, including different strains of Rous sarcoma virus (RSV), a prototype of the group of small retroviruses, and Marek's disease virus (MDV), a herpesvirus (Plachy *et al.*, 1992). This contrasts with a relative paucity of experimental examples of clear associations between disease resistance and certain MHC alleles in mammals. This difference was later ascribed to the presence of only a single dominantly expressed class I gene in many chicken MHC(B) haplotypes, while several expressed class I genes are always present in *H-2* or *HLA* haplotypes. This would explain the frequent directly observable MHC(B)-disease associations in the chicken (Kaufman *et al.*, 1995b).

In recombinant congenic lines, genes controlling resistance to the progressive growth of RSV-induced tumours were located in the *B-F/B-L* region of the chicken MHC(B) (Plachy and Benda, 1981). T-cell-mediated immunity has a decisive role in resistance to tumour progression, especially in Rous sarcomas (Schat, 1987; Plachy *et al.*, 1992). It is interesting that there is good correlation between expression level of the class I (B-F) antigen and response to RSV within MHC(B) haplotypes (a higher expression level is found in MHC(B) haplotypes of the regressor lines) (Kaufman and Wallny, 1996). On the other hand, Kaufman *et al.* (1995b) found an inverse correlation between the level of class I expression and resistance to Marek's disease. The *B21* haplotypes, which confer the highest degree of resistance, have the lowest class I expression. The authors explain this contradiction by assuming that high expression of the B-F class I antigen elicits maximum cytotoxic T-cell activity, while low expression is compatible with maximum activity of natural killer cells. It is presently not possible to distinguish between viral-specific and oncogenic-specific immunity in virus-producing tumours such as Rous sarcoma.

In addition to replicative genes, RSV carries the viral oncogene v-*src* that is solely responsible for malignant transformation.

The growth of tumours is facilitated by continuous spread of the virus and oncogene to neighbouring cells by reinfection. To avoid these complications, molecularly cloned v-*src* oncogenes were used to induce virus non-producing tumours (Fung *et al.*, 1983; Svoboda *et al.*, 1992). The MHC(B)-dependent patterns of v-*src* DNA- and RSV-induced tumours were analogous and v-*src*-specific immunity, in tumour regressors, had a protective effect against RSV challenge (Plachy *et al.*, 1994; Svoboda *et al.*, 1996).

The protective activity of v-*src*-derived DNA vaccines against growth of v-*src* DNA- and RSV-induced tumours in congenic chickens has been investigated (Plachy *et al.*, 2001). For vaccination a non-oncogenic v-*src* construct was employed, which, in addition to v-*src* point mutations, contained a stretch of 12 new amino acids

differing from the c-*src* proto-oncogene and representing a characteristic feature of RSV v-*src* oncogene alteration. Whereas the CB (chicken congenic) line B-F12 MHC class I molecule recognized the antigenic peptide within this v-*src*-specific sequence, the CC line B-F4 molecule did not (Hála *et al.*, 1999). CB line chickens were protected by the vaccine, to a high degree, against oncogenic v-*src* challenge. Cytotoxic T lymphocyte (CTL) responses were demonstrated *in vitro* and also by adoptive transfer of immune cells both to the syngeneic host and to CC line chickens rendered tolerant to CB cells. In the CC line chickens, tumour growth was retarded after a low-dose DNA vaccination was administered to immature recipients, while higher amounts of DNA vaccine in immunocompetent chickens had an enhancing effect (Plachy *et al.*, 2001). V-src protein peptides that bind (with low stringency) the CC line MHC class I molecule are, contrary to the situation in the CB line, almost identical to those of endogenous c-*src*. DNA vaccine-driven expression of such peptides early in ontogeny can activate potentially self-reactive T-cell clones of a low avidity for autoantigen (c-*src* peptide) positively selected in the thymus (Goldrath and Bevan, 1999). Such T cells are then rescued and recognize foreign antigens (Surh and Sprent, 2000). The activation of (peripheral) T cells exhibiting a weak cross-reactivity for these self-peptides could be easier in young CC chickens, because later in adult animals most of the potentially (auto) reactive clones are anergized or eliminated.

The abolition of tolerance to self-peptides of c-*src* has been inferred from experiments with chickens regressing v-*src*-induced tumours in another chicken experimental system (Halpern *et al.*, 1996). In our system we have shown that the CC (but not the CB) chickens are prone to an auto-immune reaction, as measured by T-cell-mediated GvH splenomegaly induced in syngeneic hosts by lymphocytes of thymectomized donors (Plachy *et al.*, 1989).

Studies on the functional relevance of the B-G are less conclusive. Three (probably related) immunological phenomena can be ascribed with certainty to the B-G molecules.

These are the adjuvant effect, the preferential response and the presence of natural Ab against B-G in a wide range of species, including mammals (reviewed by Kaufman and Salomonsen, 1992; Plachy *et al.*, 1992). In the adjuvant effect, B-G molecules mediate the antibody response to poorly immunogenic molecules (including the B-F) present on the same cell (Hála *et al.*, 1981b) or on a liposome (Salomonsen *et al.*, 1991b). The preferential response to B-G means that antibody response is faster and higher titres are reached compared with other erythrocyte antigens. This is valid for both chicken allo-antibodies and mouse mAb (Longenecker *et al.*, 1979; Hála *et al.*, 1981b). These phenomena may be explained by the presence of natural Ab to B-G molecules in unimmunized animals, including chickens and several mammals (Longenecker and Mosmann, 1980; Neu *et al.*, 1984).

Since B-G antigens are expressed on bursal stromal cells (Salomonsen *et al.*, 1991a), another explanation of the above-mentioned phenomena concerns the role of B-G molecules in B-cell selection. B cells can be selected in a positive way: rearranged germ line Ig would bind self B-G and be stimulated to continue gene conversion, while those that were no longer bound would be free to emigrate; or in a negative way: B cells with rearranged germ line Ig would be inactivated by binding the self B-G. In any case, the surviving B cells would probably cross-react with closely related allogeneic B-G molecules. GvH splenomegaly, acute skin graft rejection and resistance to the progressive growth of RSV-induced tumours are unambiguously under the genetic control of the B-F/B-L region. Experiments with large numbers of chickens carrying recombinant MHC(B) haplotypes on a homozygous genetic background have shown that some interaction (or cooperation) of the B-G molecules with those of the *B-F/B-L* region may significantly influence these immunological traits (reviewed by Plachy *et al.*, 1992; Plachy and Hála, 1997).

One of the not fully understood but clearly MHC(B)-controlled traits is the characteristic ratio of CD4 to CD8 cells in the peripheral blood of congenic chicken lines (Hála *et al.*, 1991; Malin *et al.*, 1993). A typical CD4:CD8 ratio of 2.5 is associated with the *B12* and *B12r1* haplotypes (sharing the *B-F/B-L12* region), and a ratio of 0.6 with the *B4* and *B4r1* haplotypes (sharing the *B-F/B-L4* region). As the CD4:CD8 ratio is similar between parental inbred lines and recombinant lines sharing the *B-F/B-L* region, the conclusion was drawn that this trait is under the genetic control of this region.

From the three-locus model for the chicken MHC(B) to the sequence of a small but multigene complex

The contemporary picture of the chicken MHC(B) is shown in Fig. 17.1. The core region – the proper MHC(B) – is only 92 kb long and contains 19 genes, making the chicken MHC roughly 20-fold smaller than the human MHC (Kaufman *et al.*, 1999b). The region is remarkably compact, containing 'essential' MHC genes found in mammalian species, but also lacking many of the genes expected from the MHC of mammals (*LMP*, class II α and most class III region genes). Furthermore, genes that have never been found in mammalian counterparts are present, including the *B-G* and newly described NK-cell receptor genes. Two *B30* genes, orthologous to those found within the class II region of human MHC, were located in close proximity to the 'classical' chicken *B-G* gene (Kaufman *et al.*, 1999b; MHC Sequencing Consortium, 1999). B30 has a non-Ig-type domain found in a transmembrane protein butyrophilin (BTN), the major protein associated with fat droplets in the milk of many mammals (Jack and Mather, 1990). BTN is composed of the intracytoplasmic B30 domain, linked to a transmembrane segment followed by two extracellular Ig-like domains, the distal Ig domain being similar to those of the myelin oligodendrocyte glycoprotein (MOG) (Gardinier *et al.*, 1992) and the B-G (Miller *et al.*, 1991). This Ig-like domain is also similar to that occurring in molecules of the B7 family, some members of which (CD80, CD86) are major costimulatory molecules of activated B cells that interact with CD28

molecules on T cells (Henry *et al.*, 1997). *BTN*, *MOG* and *B7c* genes are closely linked to the class I MHC genes. All of these genes appear to be related by having at least some of their domains derived, by exon shuffling, from a common ancestor (Henry *et al.*, 1997; Klein and Sato, 1998). MHC class I and class II molecules appeared in evolution only in jawed vertebrates, while some Ig-superfamily genes are known even in bacteria (Kuma *et al.*, 1991). MHC molecules are composed of two types of domains that differ profoundly in their tertiary structure: the Ig-like domain (ILD) and the peptide-binding domain (PBD). It is hard to imagine a simple way of changing ILD (two sheets
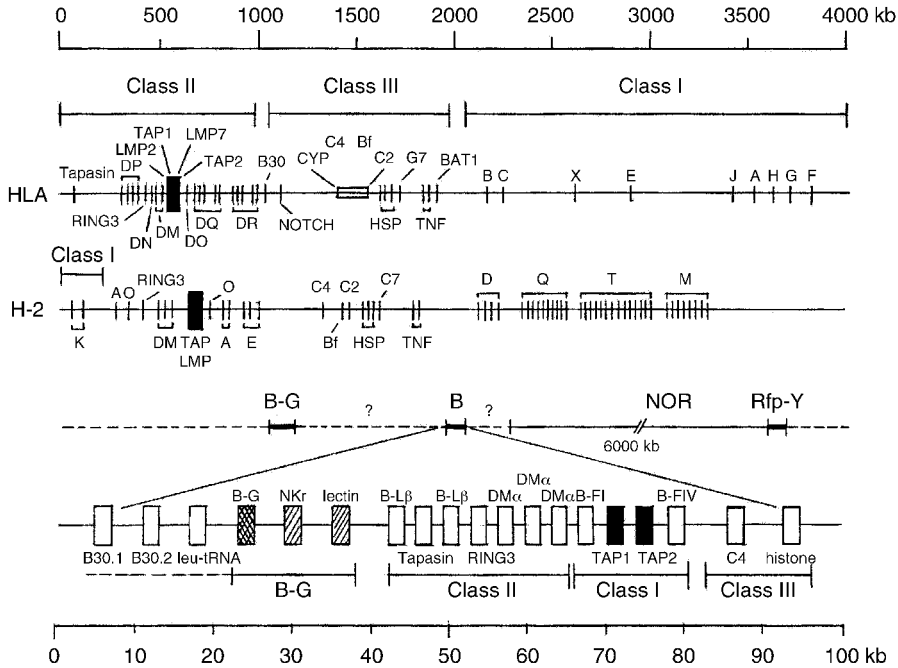
Fig. 17.1. Comparison of the structure of the human (*HLA*), mouse (*H-2*) and chicken (*B*) major histocompatibility complexes.

A simplified chart of MHC maps representing a compilation of the data published in several papers.

Black boxes, genes of the transporter associated with antigen processing (*TAP*) and 20S proteasome β-type-subunit low-molecular-mass protein (*LMP*). These genes were probably translocated from the class I region (if the chicken MHC is accepted as having a more ancient structure) to the class II region in the mammalian lineage. The *LMP* genes were lost from the MHC(B) or the whole genome in chickens.

Cross-hatched box, class IV gene peculiar to the avian MHC.

Hatched boxes, genes of the lectin-like natural killer receptors. These genes have never been located in any known mammalian MHC.

?, reflects an uncertainty in the overall organization of the chicken MHC(B) microchromosome, namely the location of the other *B-G* genes and the class II α genes (5 cM away from the MHC(B)). Other complement genes perhaps also lie outside the sequenced MHC(B) region (*Bf* is 12 cM away from the *Rfp-Y*).

Other gene symbols: *BAT1*, nuclear RNA helicase; *Bf*, complement alternative pathway serine protease (factor B); *B30*, non-Ig-type domain found in a transmembrane protein butyrophilin involved in lactation; *CYP*, cytochrome P450 superfamily; *C2*, complement classical pathway serine protease; *C4*, complement thioester-containing proteins; *G7*, valyl-tRNA synthetase; *HSP70*, heat shock proteins 70; *NOTCH4*, 'notched' wings (*Drosophila*) homologues; *RING3*, nuclear kinase; *Tapasin*, MHC class I binding protein (class I molecule could associate with TAP via this tapasin bridge, or might interact with both tapasin and TAP); *TNF*, tumour necrosis factor.

The class I or II region was probably transposed across the class III region in mammalian MHC.

For further explanation, see text.

of antiparallel β strands sandwiched tightly against each other) to PBD (a groove formed by α-helical ridges above a base of β strands). These considerations make plausible the idea that the ILD and PBD were originally encoded by different genes and that they came together by the process of domain shuffling, i.e. exon shuffling at the DNA level (Klein and Sato, 1998). The close linkage of the B7 family proteins (including the chicken B-G) with MHC class I genes, and the derivation of at least some of their domains by exon shuffling from a common ancestor, suggests a B7-like protein as a candidate for the ILD donor for MHC proteins (Klein and Sato, 1998). However, the identity of the PBD donor is unknown. Some candidates, such as heat shock protein (HSP), transporter associated with antigen processing (TAP) or low molecular mass protein (LMP), apparently bind peptides or proteins in a different manner, with tertiary structures unrelated to the PBD of MHC molecules (Joachimiak, 1997; Klein and Sato, 1998).

B-G antigens, their position in the evolution of the MHC and their immuno-regulatory functions still remain rather enigmatic. The extracellular portion of the B-G antigen is an Ig-like domain, which has no structural motifs for a peptide-binding site. The cytoplasmic region consists of multiple short repeats of 21 nt, which encode heptad amino acid repeats with the characteristics of α-helical coils (Kaufman and Salomonsen, 1992). It is not clear whether B-G protein size variations are due to different genes or alternative splicing (or both). The cytoplasmic tails of the B-G dimer presumably wind around each other and could also interact with other molecules. Some of the B-G molecules that have been found on lymphocytes and stromal cells, unlike B-G products expressed on erythrocytes, are apparently glycosylated and probably form disulphide-linked multimers (Miller *et al.*, 1990; Salomonsen *et al.*, 1991a). Some indisputable B-G effects on histocompatibility reactions and close linkage disequilibrium with class I (the *B-F* genes) raise the possibility of B-G functioning as a costimulatory molecule. The

capacity to down-modulate and ultimately to terminate immune responses is a critical point in regulation of the vertebrate immune system (Lanier, 2001). An expanding family of immune inhibitory receptors can be identified by a consensus amino acid sequence, the immunoreceptor tyrosine-based inhibitory motif (ITIM), present in the cytoplasmic domain. Inhibitory receptors are type II proteins with the N terminus of the molecule inside the cell. Signal transduction, initiated by ligand binding and hence clustering of the receptor molecules, is mediated by an src-family kinase that phosphorylates the ITIM. The conservation of ITIM in immune receptors during vertebrate evolution is evidenced by their presence in the chicken NK-cell receptors (NKRs). These latter are encoded by genes (*NKr*, *lectin*) within the chicken MHC(B) (Kaufman *et al.*, 1999b). NKRs are transcribed in chicken NK-cell lines but not in other haematopoietic cell types. *NKR* genes have never been located in any other known MHC. Their presence within the chicken MHC(B) might explain the association of certain *B* haplotypes with resistance to Marek's disease.

The central portion of the chicken MHC(B), the *B-F*/*B-L* genetic region, is represented by an especially compact stretch (roughly 44 kb) of 11 genes (*B-Lβ*–*B-FIV*). These genes have average intron sizes of 200 nt and intergenic distances (excluding promoters) as small as 30 nt, resulting in genes that are one-third the size of their mammalian homologues (Kaufman *et al.*, 1999b). No repetitive elements or repeats are present within this region. There is little change in base composition over the region, as well as the entire chicken MHC, providing no evidence for the striking isochore structure that correlates with the class I, II and III regions of the human MHC (MHC Sequencing Consortium, 1999). The chicken class II region encodes classical class II β genes, as well as *DMα* and *DMβ* genes, which appear to be necessary for correct loading of peptide on to class II molecules (Fling *et al.*, 1994), and the *RING3* gene that encodes a nuclear kinase (Thorpe *et al.*, 1996). In contrast to the mammalian MHC, in which closely linked class II α and class II β gene

pairs encode expressed heterodimers of MHC class II molecules, no classical class II α genes were found within the chicken MHC(B). Nor were class II genes related to the mammalian *DN/DZα* or *DOβ* genes found within the chicken MHC. The only non-polymorphic classical class II α gene was located 5 cM away from the chicken MHC(B) (Kaufman *et al.*, 1995a). Further departure from the organization of mammalian MHC is evident from the presence of the chicken *TAP* genes between two class I (*B-F*) genes. In mammals, *TAP* genes are present in the class II region, where they are intermingled with proteasome (*LMP*) genes (MHC Sequencing Consortium, 1999). The *LMP* genes were not found within chicken MHC at all (Kaufman *et al.*, 1999b). The absence of *LMP* genes might explain why some chicken class I (B-F) molecules bind peptides with acidic residues at the carboxy end (Kaufman *et al.*, 1995b). Mammalian class I molecules only bear peptides that end in hydrophobic or basic amino acids, as a result of the LMP molecule's action, which shifts the proteolytic specificity of the proteasome from acidic to hydrophobic and basic residues (Pamer and Cresswell, 1998).

In many of the MHC(B) haplotypes studied, the *B-FI* is a 'minor' class I gene with low expression due to disruption (or deletion) of the enhancer A region (an NF-κB binding site). The *B-FIV* is a 'major' class I gene with intact enhancer A in all haplotypes (Kaufman *et al.*, 1999a). Similarly, the second *B-Lβ* gene (next to *RING3* and *DM*) is the dominantly expressed class II gene. In contrast to the overall organization of the chicken MHC(B), the primary and, by inference, secondary and tertiary structure of class I and class II gene products have been remarkably conserved between birds and mammals. The same holds true for the intron–exon structure. Moreover, the promoter regions of class II (*B-Lβ*) genes have transcription-regulating elements organized similarly to those in mammalian species (Zoorob *et al.*, 1990). It is interesting, however, that the proximal promoter boxes S, X and Y (crucial for initiating class II transcription in mammals) are well conserved in the *B-Lβ* genes, but their deletion had

no significant effect on promoter activity. It seems that a different regulatory mechanism may have been developed in birds, and the S, X and Y boxes may represent mere remnants of evolution (Chen *et al.*, 1997). The promoter structure of the *B-FIV* gene has both homologous structures (interferon responsive element, cAMP responsive element and CAAT box), but also significant differences, to the mammalian class I promoter. *B-FIV* has a tandemly repeated Sp-1 binding site motif at the site of the mammalian TATA box. The absence of a TATA box in the *B-F* promoter is surprising and results in two transcription initiation sites and hence heterogeneity at the 5′UTR. Furthermore, the presence of S, X and Y boxes, typical for mammalian class II genes, in the chicken class I gene is intriguing from the phylogenetic point of view. One explanation would be that the common ancestor of both class I and class II genes contained these elements, which have been lost in the mammalian but not in the avian class I lineages during evolution (Kroemer *et al.*, 1990).

Other genes (*leu-tRNA*, *tapasin* and *histone*) similar to those found within the human MHC were also found within the chicken MHC(B). The chicken *tapasin* gene is located between two class II (*B-Lβ*) genes, while the human and mouse *tapasin* gene is 100 kb centromeric to the class II *β* genes. The lack of a large class III region, present in most mammalian MHC, within the chicken MHC was confirmed. Only one gene of the class III region, the complement component *C4*, was found outside the chicken class II (*B-Lβ–DMβ*) and class I (*B-FI–B-FIV*) regions. This difference in organization may also explain the compactness of the chicken MHC, because the highly polymorphic class II and class I genes are not separated by a huge class III region. Furthermore, the order of regions in the chicken MHC(B) probably represents a primordial MHC organization, suggesting that the mammalian MHC arose by rearrangement (Kaufman *et al.*, 1999a).

A model for MHC evolution has been proposed in which the MHC class III region is the 'primordial immune complex', with its members giving rise to classical MHC molecules (Salter-Cid and Flajnik, 1995).

The class III region certainly existed long before class I and class II genes, but the types of immune responses in which the class III and class I/II genes are involved appear to be totally different (Klein, 1997). Whether any of the genes of the ancient linkage group contributed to the creation of the class I and class II genes is an open question, but it was not any of the known genes that now reside in the class III region. These considerations favour the idea that only genes encoding class I and II molecules constitute the proper MHC (Klein and Sato, 1998). It is evident that linkage of several genes affecting antigen presentation (*TAP*, *LMP*, *DM* and perhaps *HSP*) to the MHC has been maintained throughout evolution. The close proximity of *TAP* and class I genes in the chicken MHC(B) even suggests the possibility of coevolution, driving the use of a single dominantly expressed class I molecule with peptide-binding specificity determined by the TAP molecule (Kaufman *et al.*, 1999a). Whether or not *TAP* or *LMP* genes are necessary for MHC class I function, they are unrelated structurally and seem to be of independent evolutionary origin.

Chromosomal location of the MHC(B) and the Rfp-Y *locus – a second MHC*

Certain avian species are unique in that the genome is fragmented into many microchromosomes; therefore, aneuploids for these elements may have few or no deleterious biological consequences. Such is the case for trisomies of chromosome 16. This microchromosome contains all of the ribosomal ribonucleic acid (*rRNA*) genes in the nucleolar organizer region (NOR). The chromosome is acrocentric with a small but discernible short arm. The NOR occupies most of the long arm. Aneuploid chickens with three nucleoli in somatic cells displayed three serologically defined MHC(B) haplotypes, thus establishing genetic linkage of the MHC(B) and NOR (Bloom *et al.*, 1987).

Individuals trisomic for microchromosome 16 also transmit three *Rfp-Y* haplotypes of a putative second chicken MHC (Miller *et al.*, 1996). Despite localization on the same microchromosome, haplotypes of the *Rfp-Y* assort independently of the MHC(B) system. This is probably because they are divided by the NOR, a highly repetitive sequence, which should be highly recombinogenic. Thus, from the genetic point of view, MHC(B) and *Rfp-Y* could be considered as independent MHC loci. The *Rfp-Y* system, however, is considered to be an MHC-like region rather than the second MHC (Kaufman and Wallny, 1996). There is no report of rapid allograft rejection due to the *Rfp-Y*. RFLP reported for the *Rfp-Y* locus describe polymorphisms outside the coding region, and *Y-Lβ* genes of clusters II/IV and III, mapping to the *Rfp-Y*, show very low polymorphism (Zoorob *et al.*, 1990; Miller *et al.*, 1996). The *Y-Lβ* genes were classified to family III, the *B-Lβ* genes to family II. The expression levels of *Y-Lβ* and *Y-Fα* genes are lower than those of *B-Lβ* and *B-Fα* genes (Kaufman and Wallny, 1996). Recent studies have shown that *Rfp-Y* incompatibility did not induce significant one-way mixed lymphocyte reaction, and that *Rfp-Y*-incompatible skin grafts were rejected at speeds that resemble rejection of minor histocompatibility antigens (Pharr *et al.*, 1996). The *Rfp-Y* class I locus *Y-FV* exhibits polymorphic restriction fragments and is expressed in many organs (Afanassieff *et al.*, 2000). However, amino acid substitutions occur in *Y-FV* sequences at highly conserved sites critical in anchoring the ends of peptide antigen in classical class I molecules, making it unlikely that *Y-FV* molecules bind peptide in an analagous manner. Some evidence suggests a contribution of the *Rfp-Y* genotype to the incidence of Marek's disease and the fate of Rous sarcomas (Wakenell *et al.*, 1996; LePage *et al.*, 2000) – traits typically under control of the chicken MHC(B). The mechanism underlying this putative *Rfp-Y*-mediated disease resistance is not known.

What is still missing to complete the picture of the chicken MHC(B)?

The complete picture of the chicken MHC is still elusive mainly because of the uncertain location of other putative *B-G*

genes (Salomonsen *et al.*, 1991a) outside the sequenced 'core' MHC(B) region (Kaufman *et al.*, 1999b). Their existence had been suggested by earlier genetic studies (Briles *et al.*, 1983; Miller *et al.*, 1988), which observed a significantly higher frequency of recombination between the *B-G* and *B-F* markers in crosses of different breeds of chickens in comparison with results obtained from crosses of inbred lines (Plachy and Hála, 1997). The reason for this high frequency of recombination may be due to differences in more distal (from the 'core' region) *B-G* genes between chickens of the unrelated breeds used. These distal *B-G* genes may, conversely, be non-polymorphic in lines used in other laboratories.

Is the chicken MHC(B) a universal model for avian MHC?

As discussed above, the chicken MHC(B) seems to consist only of the genes that are absolutely essential (Kaufman *et al.*, 1995b). The chicken belongs to the order Galliformes and the MHC of three other galliforms, the ring-necked pheasant (*Phasianus colchicus*), turkey (*Meleagris gallopava*) and Japanese quail (*Coturnix japonica*), are similar to the more thoroughly investigated chicken. However, the Japanese quail is slightly exceptional within this group, clearly having more class I and class II genes than the chicken (Shiina *et al.*, 1999).

The general validity of the 'minimal essential' model for birds, however, has been challenged by recent studies in songbirds (Passeriformes) (Edwards *et al.*, 1995, 1999; Hess *et al.*, 2000; Westerdahl *et al.*, 2000) suggesting that passerines have more complex MHC, with many more MHC class I and II genes than galliforms. We should be aware of the fact that the morphological homogeneity of the class Aves, due to flight adaptation, does not necessarily correspond to the genetic homogeneity of this taxon. The evolutionary distance between Galliformes and Passeriformes is relatively large (more than 90 million years, as assessed from mitochondrial DNA (Härlid *et al.*, 1997)). Interestingly, passerines probably diverged early during avian evolution and represent a basal group in the avian phylogenetic tree (Edwards *et al.*, 1999; Härlid and Arnason, 1999). The passerines, then, would not have an expanded MHC but, rather, the chicken and other galliforms would have a reduced MHC (Westerdahl *et al.*, 2000). Different relationships of the orders within the class Aves and mammals are illustrated by contrasting histories of MHC class II β genes (Edwards *et al.*, 1995). The evolutionary tree of avian class II β genes reveals that orthologous relationships have not been retained as in mammals and that MHC class II genes in songbirds and chickens had very recent common ancestors within their respective groups. In other words, while some mouse and human class II gene sequences cluster together, none of the songbird (*B-Lβ* exon 3) sequences fall inside the cluster of chicken genes. Thus, although the cloned songbird sequences likely stem from multiple genes, none of them is orthologous to any of the chicken genes (Edwards *et al.*, 1995).

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18 Genetic Resistance and Transmission of Avian Bacteria and Viruses

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Introduction

Genetic variation in resistance to disease is a widespread phenomenon observed in most if not all species. However, the mechanisms by which different pathogens reproduce and propagate themselves are very diverse, and it would be surprising if the host genes affecting disease resistance were not equally diverse. At present a number of factors limit understanding of disease resistance: even in model species, such as mice, not all of the genes involved in immune function have been identified or characterized, and the structure and function of few non-immune genes involved in infection have been identified. Since the frequency of variants in these genes in commercial flocks is largely unknown, and since studies of the epidemiology of infections are generally lacking, it is difficult to determine the selective pressures that bear on resistance genes or their importance in the chicken's response to disease.

The first genetic differences in the susceptibility of chickens to disease were observed long ago (Asmundson and Biely, 1932; Hutt and Scholes, 1941; Smith, 1956), though these comprised relatively simple comparisons between the susceptibility of different breeds. However, developments in molecular biology, better genetic resources and latterly techniques for genomic mapping have considerably extended and refined knowledge of the range of genetic resistance in chickens, and in the case of the avian leukosis virus receptors have come very close to explaining the mechanisms of resistance at a molecular level. In a number of cases it would now be possible in principle to utilize linked markers for the selection of resistance genes, but identification of the causative genes remains very difficult. Even in the case of the major histocompatibility complex (MHC)-associated resistance to Marek's disease, (one of the earliest and strongest examples of variation in resistance to infectious disease in any species), it is still not clear which gene or genes within the complex are responsible for resistance.

Essentially, studies of genetic resistance in chickens fall into three categories: (i) studies aimed at improving the general immunocompetence of the chicken (see Pinard-van der Laan *et al.*, Chapter 19); (ii) studies aimed at selecting for increased resistance directly in outbred flocks or populations; and (iii) studies comparing the susceptibility of different chicken genotypes to particular diseases, discussed here.

Resistance to Avian Leukosis Viruses

The family of avian leukosis and sarcoma viruses (ALSVs) are able to cause a variety

of neoplastic diseases in cells of the myeloid lineage, as well as reducing growth rate and productivity in birds with subclinical infection (Calnek, 1968; Gavora *et al.*, 1980). Birds that are infected in the embryo from their parent or soon after infection can become tolerant carriers and shedders of infection, whereas birds infected later in life are more transiently infected. Through elegant genetic and viral interference studies it was discovered during the 1950s that different subgroups (A–E) of the virus existed, which exhibited different host ranges in chickens, corresponding to the presence or absence of putative viral receptors (*tv* loci) for each of the subgroups (Rubin, 1965; Payne and Biggs, 1966; reviewed in Weiss, 1993). Subgroups A and C appeared to have their own specific receptors, subgroups B and D to share a receptor and subgroup E to partially share the B/D receptor. The situation was further complicated by the presence of endogenous retroviruses of subgroup E, which in some cases express viral proteins and can either cause tolerance to viral proteins or cause blocking of subgroup E (reviewed in Crittenden, 1991). It is possible that some endogenous viral genomes may affect egg production or even susceptibility to other diseases (Aggrey *et al.*, 1998).

Analysis of viral genomes of the different subgroups identified the viral determinants of receptor specificity within the envelope genes of the viral subgroups (Bova *et al.*, 1986, 1988; Holmen *et al.*, 2001) and Young *et al.* (1993) succeeded in isolating the receptor for ALSV subgroup A by transfecting monkey COS cells with chicken genomic DNA and identifying transfectants that were susceptible to an ALSV carrying a selectable marker gene. The gene encodes a cell-surface glycoprotein with homology to the mammalian low density lipoprotein receptor. Confirmation that this gene is responsible for the phenotypic differences in resistance to ALSV-A was provided both by transfecting genetically resistant chicken embryo fibroblasts to demonstrate transfer of susceptibility and by genetically typing a restriction fragment length polymorphism (RFLP) within the coding sequence of the gene in a segregating F_2 cross between line 7_2 (resistant to ALSV-A) and line 6_3 (susceptible to ALSV-A). In the 93 birds tested in this cross there was exact co-segregation of the RFLP, making it highly probable that the variation in the receptor is responsible for the differences in resistance (Bates *et al.*, 1998). Although the difference seen in the coding sequence of the receptor gene in these lines lies within the viral binding domain (Rong and Bates, 1995; Zingler *et al.*, 1995) and viral entry can be blocked by antibody to the receptor (Bates *et al.*, 1993), it does not appear that variation in the viral binding portion of the gene is responsible for the differences in susceptibility. In other chickens, and in other transfection studies, there was no general correspondence between inheritance of the arginine and threonine residues seen for line 7_2 or the corresponding histidine and isoleucine residues seen in line 6_3. Resistance or susceptibility to ALSV-A and the causative difference responsible for the resistance remains unclear (Bates *et al.*, 1998).

Subsequently, using a similar approach, the receptor for the B and D subgroups of ALSV was also identified (Brojatsch *et al.*, 1996) and shown to map to the same genetic location as *tvb* (Smith *et al.*, 1998). As had been expected on the basis of viral interference, the B and D viral subgroups share a common receptor that Brojatsch *et al.* referred to as cytopathic ALSV receptor 1 (CAR1). CAR1 shows homology to mammalian tumour necrosis factor receptor-related proteins TRAIL-R1 and TRAIL-R2, which are implicated in triggering apoptotic cell death, providing an explanation for the cytopathic effects seen in infection by ALSV subgroups B and D (Weller *et al.*, 1980). Subsequent studies comparing the infectivity of transfected cells showed that this gene also provides the receptor for ALSV subgroup E (Adkins *et al.*, 1997) but that a specific variant of the gene containing a cysteine residue at position 62 is required for this subgroup (Adkins *et al.*, 2000). The *CAR1* gene therefore appears to exist in three allelic forms: a resistant non-permissive form *tvb^r*; *tvb^{s1}*, which permits infection by ALSV subgroups B, D and E; and *tvb^{s3}*, which permits infection

only by subgroups B and D. Since the human TRAIL receptors and a homologous turkey gene tvb^T all possess the cysteine residue of tvb^{s1}, Adkins *et al.* (2000) argued that tvb^{s3} may have evolved in chickens as an adaptation to the presence of endogenous subgroup E viruses, possibly allowing the chicken to preserve the *CAR1* gene function while avoiding cell death (Adkins *et al.*, 2001).

The presence or absence of the appropriate receptor greatly affects cellular susceptibility to the virus, but other factors affect the likelihood that birds will subsequently develop neoplasms. The factors that affect this susceptibility appear complex and may depend on the properties of the particular viral strain (Crittenden *et al.*, 1972) but it is likely that the MHC plays some part in resistance to neoplasia (Bacon *et al.*, 1980).

Recently a new form of ALSV has been identified within commercial meat-type flocks (Payne *et al.*, 1991). This apparently new virus subgroup, J, has a novel envelope protein, which has homology to that of the E-51 element of the endogenous avian virus (EAV) family of chicken endogenous retroviruses (Bai *et al.*, 1995). The ALV-J subgroup shows a distinct tissue tropism, suggesting that it utilizes a distinct cellular receptor, and in contrast to the classical ALSV subgroups no chickens showing cellular resistance to ALSV-J infection have so far been discovered (Payne, 1998). However, the incidence of myeloid leukaemogenesis differs considerably among chickens of different genotypes, ranging from none in lines 15I and N to 17% in line 0 and a commercial line, and appears to correspond to the sensitivity of bone marrow cells to transformation by the virus (Arshad *et al.*, 1997).

Resistance to Marek's Disease

Despite the dramatic success of attenuated vaccines, Marek's disease remains one of the most significant diseases of chickens in both economic and welfare terms, and a significant cause of mortality and morbidity. The disease is caused by a herpesvirus, Marek's disease virus (MDV), whose genome has been sequenced (Tulman *et al.*, 2000), and the complex pathogenesis of the disease has been elucidated gradually (reviewed in Calnek, 2001). Many investigations have addressed aspects of the host immune response to the disease, identifying aspects of innate and acquired immunity involved in this response, and potential mechanisms for immune evasion by the virus (reviewed in Schat and Markowski-Grimsrud, 2001). From the very early days it was recognized that there are large genetic differences in resistance to the disease (Cole, 1968) and many investigations have been carried out to characterize the nature of this resistance. The apparent progressive increase in virulence of the virus (Witter, 1997) has prompted continuing interest in the mechanisms of genetic resistance, both as a means of protection (Witter, 1998) and to provide insights into viral pathogenesis. There has recently been an excellent review of resistance to Marek's disease by Bacon *et al.* (2001) and here the focus will only be on specific aspects of recent progress.

Early investigations showed that the MHC plays a major role in resistance to Marek's disease (Hanson *et al.*, 1967; Longenecker *et al.*, 1976; Briles *et al.*, 1977) and the association between particular MHC haplotypes and resistance to Marek's disease remains the clearest known example of such a relationship between the MHC and an infectious disease in any species. The association was refined to the classical portion of the MHC containing the class I and class II β presentation by Briles *et al.* (1983), which has now been extensively sequenced (Kaufman *et al.*, 1999). Despite a rapidly increasing understanding of the nature of the chicken MHC, the mechanism underlying the MHC-associated resistance to Marek's disease remains unknown (see Plachy *et al.*, Chapter 17).

Despite the strong contribution of some MHC haplotypes, it is clear that other genes also have a large effect on the overall level of resistance to Marek's disease. This is particularly evident when comparing the inbred lines 6_3 and 7_2, developed at the Avian Disease and Oncology Laboratory, East Lansing (Michigan, USA) by Stone

(1975). Although these lines are both homozygous for the same MHC haplotype, they differ greatly in their resistance to a wide range of MDV strains (Pazderka *et al.*, 1975), and analysis of crosses between these lines therefore offers a possible route to identifying other genes involved in the resistance process. Vallejo *et al.* (1998) exploited this possibility by carrying out a genetic mapping experiment in an F_2 cross between these lines. Chickens were challenged with 2000 pfu of the JM strain MDV at 1 week of age and monitored for evidence of disease until 10 weeks old, when they were examined post mortem for evidence of neural or visceral lesions. Resistance was scored in terms of a range of parameters, including viraemia and the numbers and types of organs found to be affected by either tumours or neural lesions at necropsy. A panel of microsatellite markers was used to genotype the birds, and association was compared between markers and the parameters of disease, both individually and in combination. From these comparisons 14 chromosomal regions were identified (Vallejo *et al.*, 1998; Yonash *et al.*, 1999) that showed association with one or more aspects of resistance even though marker coverage of the genome was incomplete at 70%. In general, resistance was dominant and although the effects of individual regions were small in total they accounted for 75% of the variation between the parent lines.

To provide a quantifiable measure of differences in resistance to MDV, Bumstead *et al.* (1997) developed a quantitative PCR assay to measure the replication of MDV in infected cells or tissues. When the levels of MDV in peripheral blood lymphocytes were measured in this way, it was seen that levels of virus in line 6_1 birds were considerably lower than those in line 7_2 birds, suggesting that early viral replication corresponded to the later incidence of tumours and mortality in these lines. Comparison of viral levels in an F_2 population confirmed this relationship, since on average birds that developed tumours had substantially higher levels of virus from early in infection (Bumstead *et al.*, 1997; Bumstead, 1998). This strongly

suggests that control of the early replication of MDV is an important factor in the outcome of the disease, and comparison of viral loads in other resistant and susceptible lines has shown a similar relationship between resistance and low levels of viral replication (Bumstead, 1998, and unpublished results). Despite this general relationship in these experiments, some birds that survived had high levels of virus, and some birds with low levels of virus died; to some extent this may be due to inaccuracies in the sampling or assay, or birds dying from causes other than their MDV infection, but it remains possible that other factors may affect the development or growth of tumours later in infection. This would be consistent with the apparent multifactorial nature of resistance.

Using levels of viral load and tumour development as measures of susceptibility, Bumstead (1998) attempted to map resistance in a line $(6_1 \times 7_2) \times 7_2$ backcross population. A combination of conventional microsatellite markers, together with additional targeted markers generated by representational difference analysis (RDA) following the approach described by Wain *et al.* (1998), was used to genotype the animals of this population. Analysis identified a region of chromosome 1 showing strong association with resistance, accounting for approximately half of the difference in viral replication between the parent lines, and this relationship was confirmed through testing an independent F_2 population. Since the association of the chromosome 1 region was seen for viral replication at various time points as well as for later mortality/tumour development, it seems that the mechanism of resistance in this instance operates through control of viral replication rather than later control of tumour development. Comparison of genes lying in this region in chickens with human and mouse indicated that it corresponds to regions of conserved synteny on human chromosome 12 and mouse chromosome 6. Strikingly, in the mammalian species these regions contain the human and murine natural killer (NK) cell gene clusters, which contain multiple lectin-like cell surface proteins important in controlling the activity of NK cells (Yabe

et al., 1993; Forbes *et al.*, 1997; reviewed in Moretta *et al.*, 2001). In mice, resistance to the murine herpesvirus cytomegalovirus (CMV) (which has a similar phenotype of reduced viral replication in the spleens of infected animals) has been mapped to the NK cell region (Scalzo *et al.*, 1990, 1995) and shown to be due to polymorphism within the *Klra8* gene within the NK lectin cluster (Brown *et al.*, 2001; Lee *et al.*, 2001). Since NK cells have long been thought to play a role in the early response to Marek's disease (Sharma, 1981; reviewed in Schat and Markowski-Grimsrud, 2001), genes affecting their activity would be likely candidate resistance genes. However, it has yet to be shown that chickens have a similar NK cell-regulating cluster to that of mammals; and other genes within or close to this region in mammals, such as *CD4* and genes of the TNF receptor family, are also possible candidate genes.

In a complementary approach to determining the genes underlying these areas of association with resistance, Liu *et al.* (2001a) used a DNA microarray to assess differences in transcription level of a panel of 1200 genes in uninfected and MDV-infected peripheral blood lymphocytes of lines 6_3 and 7_2. A number of genes were identified that showed twofold or greater differences in expression between the two lines, including known immunologically relevant genes such as *TCR-β*, MHC class I, interferon-γ and immunoglobulin light chain, but also many genes of currently unknown function. Relating the genomic positions of the differentially regulated genes to their genomic location identified cases where the genes coincided with regions associated with resistance to MDV, notably in the case of lymphotactin on chromosome 1 in a resistance region identified by Yonash *et al.* (1999) and on the edge of that identified by Bumstead (1998).

In another novel approach to the identification of resistance genes, Liu *et al.* (2001b) sought to utilize the interactions of a gene from the virus itself to identify host genes involved in the infection process. Recognizing that the viral *SORF2* gene is inactivated in non-oncogenic strain RM1 (Jones *et al.*, 1996) and hence may be important in viral oncogenicity, Liu *et al.* (2001b) expressed the *SORF2* gene in a yeast two-hybrid assay to screen a splenic cDNA library to identify chicken genes that bind to the SORF2 protein, a gene thought to be involved in host specificity (Tulman *et al.*, 2000). The assay identified chicken growth hormone as interacting with SORF2, and this was confirmed by co-immunoprecipitation and histological co-localization. Chicken growth hormone (*GH*) was among the genes identified as being differentially upregulated in the microarray experiments of Liu *et al.* (2001a) and had previously been shown by Kuhnlein *et al.* (1997) to display distorted allele frequencies in lines of chickens selected for resistance to Marek's disease. A polymorphism in the fourth intron of *GH* was used to genotype a chicken population infected with MDV, and an association was seen between alleles at the *GH* locus and the number of tumours and length of survival, although only when the population was partitioned into MHC classes.

Resistance to Infectious Laryngotracheitis Virus

Infectious larynotracheitis virus (ILTV) is an avian herpesvirus that causes mild to severe respiratory infections in chickens (Hanson and Baghust, 1991). Infection is largely limited to the respiratory tract; virus is seldom detected in other tissues and, unlike MDV, is not a cause of neoplasia. Loudovaris *et al.* (1991a) compared the susceptibility of three inbred lines to a range of ILTV inoculated intratracheally at 4 weeks of age. The levels of mortality of the three lines differed, with line J_1 consistently having least mortality and line M_1 greatest mortality, irrespective of dose. In these experiments there did not appear to be differences in the level of anti-ILTV antibodies and it was suggested that MHC-associated cellular responses might be responsible for the differences in mortality. When cultured macrophages of these lines

were infected *in vitro* with either a virulent or an attenuated strain of ILTV, a greater proportion of the macrophages from the resistant lines J and N were found to be positive for ILTV antigen (Loudovaris *et al.*, 1991b). Macrophages from F_1 chickens also showed the higher level of ILTV uptake, while chickens from a line $M \times N$ backcross population showed a bimodal distribution pattern, with the macrophage populations from approximately half the chickens having the high level of ILTV uptake of the resistant parent strain, and half the lower uptake of the susceptible parent. This suggests that the differences in ILTV uptake in macrophages, and possibly the differences in resistance seen in the birds of these lines, may be due to a single dominant resistant gene. Poulsen *et al.* (1998) demonstrated differences in resistance to ILTV in F_1 crosses between three inbred lines. Birds of the Hy-Line SC F_1 hybrid were less susceptible across a range of doses than those of Hy-Line TK or F_1 birds of a cross between East Lansing lines $15I_5 \times 7_2$. This was particularly the case for challenges following vaccination with a low dose of pathogenic ILTV and better survival corresponded to the production of much higher levels of ILTV-specific antibodies in the SC line, suggesting differences in the immune response between the lines. Although the parent lines differed in MHC haplotype in these experiments, no segregating crosses

were investigated and the possible role of the MHC is unclear.

Resistance to Infectious Bursal Disease

Infectious bursal disease, sometimes referred to as Gumboro disease, is an acute cytolytic infection of pre-B lymphocytes caused by a small bisegmented RNA virus, infectious bursal disease virus (IBDV). The disease is rapid in onset and is particularly severe in young birds, where the developing B cells of the bursa are severely affected. The disease can cause mortality and morbidity but the depletion of the B-cell population can also result in an inability to respond to vaccination or to other infections. Although birds that survive infection are able to restore their B-cell population and recover their normal immune function during the infection, their B-cell response is severely impaired. Resistance to infectious bursal disease has been described in comparisons of inbred lines by Bumstead *et al.* (1993), who showed differences in survival between inbred lines ranging from 0 mortality in several White Leghorn lines to 80% mortality in a Brown Leghorn line, and that these differences correlated with the degree of destruction of the bursal cells in these lines (Fig. 18.1).

Crosses between resistant and susceptible lines showed that resistance was
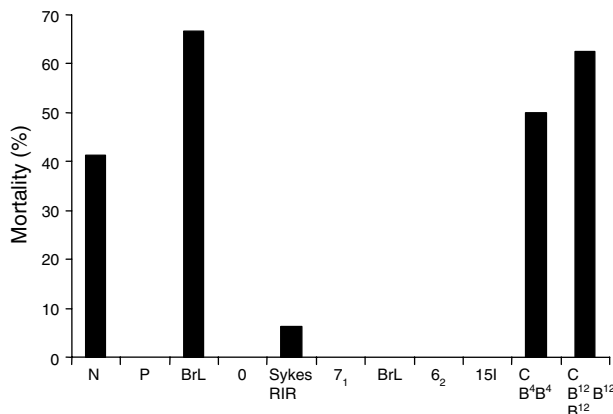
Fig. 18.1. Mortality in 3-week-old chickens infected intranasally with IBDV strain CS89.

heritable and inherited in a dominant autosomal fashion. Other outbred lines and samples from commercial flocks showed intermediate levels of susceptibility.

It is not possible to quantitate levels of infection by IBDV in a straightforward manner such as a plaque assay, but a quantitative PCR method has recently been developed that allows a degree of quantitation and this has been used to compare the replication of IBDV in resistant and susceptible lines (Moody *et al.*, 2000). Surprisingly, it was possible to quantitate levels of IBDV in the blood of infected birds over the course of infection, and results of such an experiment are shown in Fig. 18.2. These experiments show the rapid course of infection, with levels of viral RNA falling below detectable amounts 5 days after infection, though later post-mortem examination of the bursae of the birds showed continuing severe damage. Although levels of IBDV in the blood are much lower than those seen in bursal tissue, the results indicate that the differences in mortality seen in these lines are reflected in lower levels of virus in the resistant animals

and that in segregating crosses measurement of viral load correlated significantly with mortality. These are preliminary results but they suggest that genetic resistance restricts the viral replication in some as yet unknown way. Given the extreme tissue specificity and very short time scale of the infection, it seems likely that the resistance may be due to a small number of genes rather than to global differences in immune response. This would be consistent with the results of crosses.

Resistance to Infectious Bronchitis

Infectious bronchitis is a respiratory disease caused by the coronavirus infectious bronchitis virus (IBV). In young chicks the principal effect of the disease is to cause damage to the trachea, which can lead to secondary bacterial infections by a number of bacteria, tracheal blockage and death by suffocation (Cunningham, 1970; Springer *et al.*, 1974). The virus also gives rise to a short-lived systemic infection involving the visceral organs,
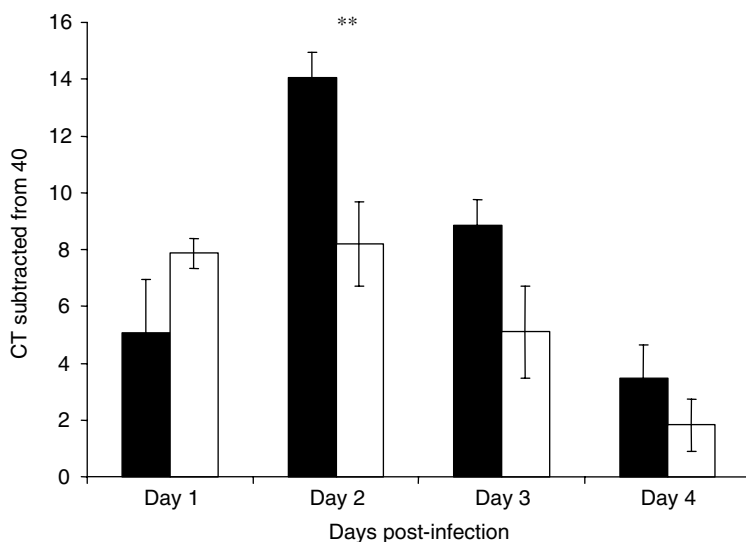
Fig. 18.2. Quantification of IBDV RNA in 50 µl of whole blood extracted from chickens over the first 4 days following infection of 3-week-old birds with IBDV F52/70 using the multiplex TaqMan assay. Mean cycle threshold values have been standardized and are expressed subtracted from 40 (the negative end point) with standard error bars. Higher values represent higher levels of IBDV RNA. Key: ■, Brown Leghorn; □, Line 6_1; **, $P \leq 0.01$. (A. Moody and N. Bumstead, unpublished results.)

and in laying hens can cause decreased egg production and malformation of the eggs. Differences in susceptibility to IBV were investigated by Bumstead *et al.* (1989), who compared differences in susceptibility between inbred lines of chickens inoculated with IBV. In order to reflect secondary bacterial infection, IBV was inoculated together with a cocktail of pathogenic strains of *Escherichia coli*. Resistance was measured in terms of mortality. The inbred lines that were tested differed considerably in their susceptibility, whether infected with IBV alone or in combination with pathogenic *E. coli* (Fig. 18.3).

Resistance was inherited in a dominant fashion, with the inbred line 7_2 being particularly susceptible, and showed no association with the MHC. When the same lines of birds were infected intravenously with pathogenic *E. coli* alone, small differences in susceptibility were detected. However, these effects were small in comparison with the differences seen for IBV infection and there was no correlation between the lines resistant to IBV and those resistant to

E. coli, indicating that the differences in resistance seen for the combined infection were not due to its bacterial component.

In mice, genetic resistance to the murine coronavirus murine hepatitis virus (MHV) has been shown to be due in part to the presence or absence of functional biliary glycoproteins that function as its receptors, leading to differences in rates of viral replication, though variation in one or more other genes also appears to affect the ability of the virus to replicate (Buschman and Skamene, 1995). Differences in susceptibility to coronaviruses in pigs also appear to be due to the presence or absence of receptors for transmissible gastroenteritis virus (which, like MHV, is gut-tropic) and the related porcine respiratory coronavirus (Delmas *et al.*, 1993). Experiments to measure the levels of replication of IBV in visceral tissues showed limited differences in the levels of virus recovered from tissues of infected birds, though there was a quicker recovery from infection in resistant animals (Cook *et al.*, 1990; Otsuki *et al.*, 1990). However, virus was recovered from the visceral organs
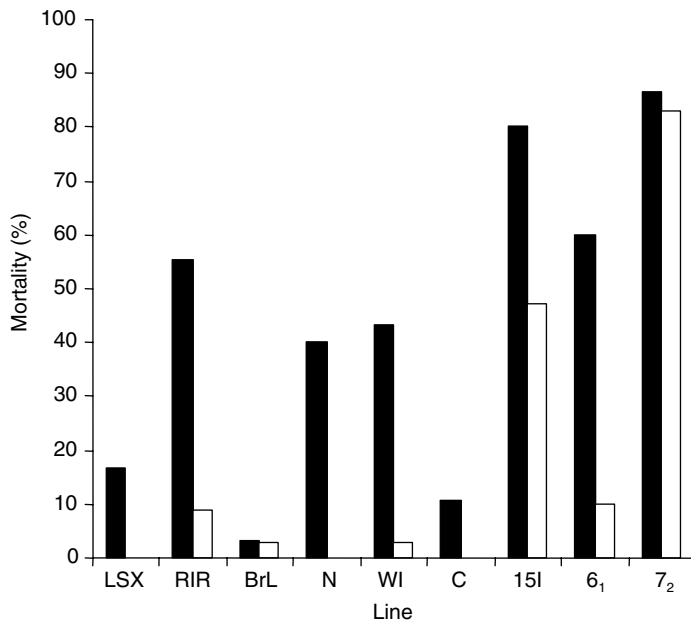
Fig. 18.3. Mortality of nine lines of chickens infected with a pool of Massachusetts serotype IBV strains at 2 weeks of age. Key: ■, mortality following infection with IBV and a cocktail of pathogenic *E. coli*. □, mortality following infection with IBV alone. (From Bumstead *et al.*, 1989.)

of all birds, indicating that the virus can penetrate the trachea and replicate in resistant as well as susceptible birds, and no differences in levels of viral replication were observed when tissue from resistant and susceptible birds was infected *in vitro*. This suggests that the differences in mortality observed are due to differences in the level or nature of the local response to infection in the trachea, rather than the presence or absence of viral receptors as in the mouse and pig.

Resistance to Salmonella

Salmonella infection is a major cause of disease and mortality in poultry and also a principal cause of human food poisoning, through bacterial contamination either of the carcass or in eggs. There are three distinct aspects to salmonella infection.

1. Invasive strains can cause severe typhoid-like disease, especially in young chickens, leading to high levels of mortality in some cases. This is particularly the case for the avian-specific serotype *Salmonella gallinarum*, though other salmonellae can cause systemic infection and mortality in young chicks.

2. In older birds, salmonellae that are less pathogenic to birds (notably *S. typhimurium* and *S. enteritidis*) can cause colonization of the gut and lead to carcass contamination by the bacteria and subsequent human infection, without causing evident disease in the chicken.

3. Hens at laying age can transmit salmonella within eggs, potentially leading to a further source of human food contamination.

The relationships between these aspects of infection are not well defined and vary with the *Salmonella* serotype. So far, the visceral infection of chickens has been most studied in terms of genetic resistance, since this is the most easily defined and accessible aspect of the infection. Studies on possible differences in the extent and duration of gut colonization and egg contamination have now begun.

Differences in the susceptibility of chickens to salmonellosis were first described by Hutt and Scholes (1941). Smith (1956) also described differences in susceptibility between strains. These early studies simply compared the susceptibility of outbred breeds of birds and it was not clear that these differences were heritable. In 1988 it was shown that there are genetic differences in the mortality of day-old chickens among inbred lines of chickens following oral or intramuscular infection. This initial study (Bumstead and Barrow, 1988) demonstrated that the mechanism of resistance was effective against a range of strains of *S. typhimurium* of varying virulence and was later extended to show that the inbred lines resistant to *S. typhimurium* are also resistant to *S. gallinarum*, *S. pullorum* and *S. enteritidis* (Bumstead and Barrow, 1993). This suggests that the resistance mechanism may apply to all *Salmonella* spp., though it is not known whether it extends to the other facultative intracellular bacteria. Resistance was autosomally inherited, fully dominant and not associated with the MHC; and in these crosses it was consistent with the inheritance of a single major gene (Bumstead and Barrow, 1988).

Studies on the mechanism of resistance indicated that resistant birds were resistant to intravenous as well as oral or intramuscular infection, and that under these circumstances the inoculated bacteria were very rapidly phagocytosed, particularly by cells in the spleen and liver. Growth curves for the bacteria in these organs show more rapid growth in the susceptible animals (Fig. 18.4), ultimately reaching very high levels, the breakdown of the organisation of the organs, systemic release of the *Salmonella* and death. In contrast, in the resistant birds, replication of the *Salmonella* was reduced, and reached a peak at 4 days after infection, after which numbers of *Salmonella* declined.

These early differences in the growth of *Salmonella* during infection suggest that the resistant birds are in some way better able to control early replication of the *Salmonella* within the phagocytic environment, until a secondary response can be developed that can progressively eliminate the infection,
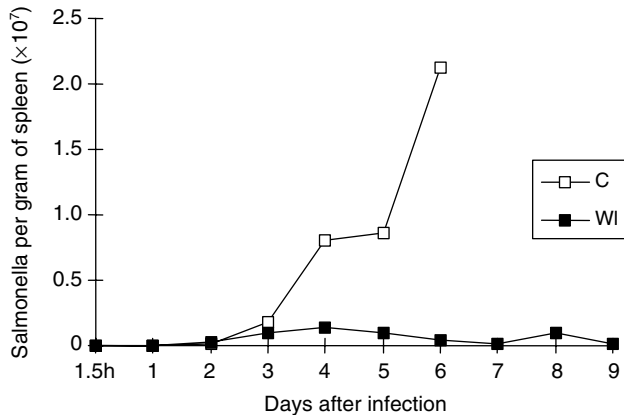
Fig. 18.4. Growth of *S. typhimurium* in spleens of lines C and Wl following intravenous infection. All birds of line C had died before day 7 whereas line Wl other than those sampled survived to the end of the experiment.

whereas susceptible birds may be overwhelmed by the infection before the response can be mounted. Preliminary *in vitro* studies support this, indicating that splenic macrophages from resistant genotypes cultivated *in vitro* are better able to contain the replication of pathogenic salmonella, and that this correlates with an ability to mount a greater respiratory burst following infection (P. Wigley, P.A. Barrow and N. Bumstead, unpublished).

The differences in pathology in resistant and susceptible chickens resemble those seen in mice for resistance and susceptibility associated with the *Ity* locus. In mice, the gene responsible for this resistance has been identified (Vidal *et al.*, 1993) and shown to be a macrophage protein associated with the transfer of cations into the phagocytic vacuole. The gene was originally named Natural Resistance Associated Macrophage Protein 1 (*NRAMP1*) and has recently been redesignated as *SLC11A1*. A single amino acid change within the protein coding portion of the gene is responsible for the large difference in susceptibility seen between *Ity* resistant and susceptible mice (Malo *et al.*, 1994), though the effect of this change on gene function is not yet clear (Marquet *et al.*, 2000). The chicken gene corresponding to *NRAMP1* has been genetically mapped to chicken chromosome 7 (Hu *et al.*, 1995), in a region of conserved synteny with humans and mice (Girard-Santuosso *et al.*, 1997).

Several studies have investigated whether polymorphism in the chicken gene is responsible for the observed differences in susceptibility. Hu *et al.* (1995) sequenced the cDNA corresponding to the coding sequence of the gene from several susceptible and resistant chicken lines. These studies showed a number of non-synonymous base changes between these lines. In crosses between a resistant and a susceptible line, there was a limited association with variation in *NRAMP1* and also with a marker for a second murine resistance gene, *LPS* (Hu *et al.*, 1997), but these associations accounted for only a portion of the differences between the parental lines. Girard-Santuosso *et al.* (2002) investigated the effects of possible *NRAMP1* polymorphism in more outbred birds and again found a significant association between variation in the *NRAMP1* region and susceptibility to visceral salmonellosis.

In an attempt to map the genes responsible for resistance to salmonellosis directly in chickens, Mariani *et al.* (2001) carried out genomic mapping experiments using crosses between line 15I (highly susceptible) and line 6_1 (highly resistant). Susceptibility was assessed by bacterial counts in the spleen following infection of 2-week-old birds since this provides a more humane and also more quantifiable measure of the response of individual birds. In these experiments there was little association of
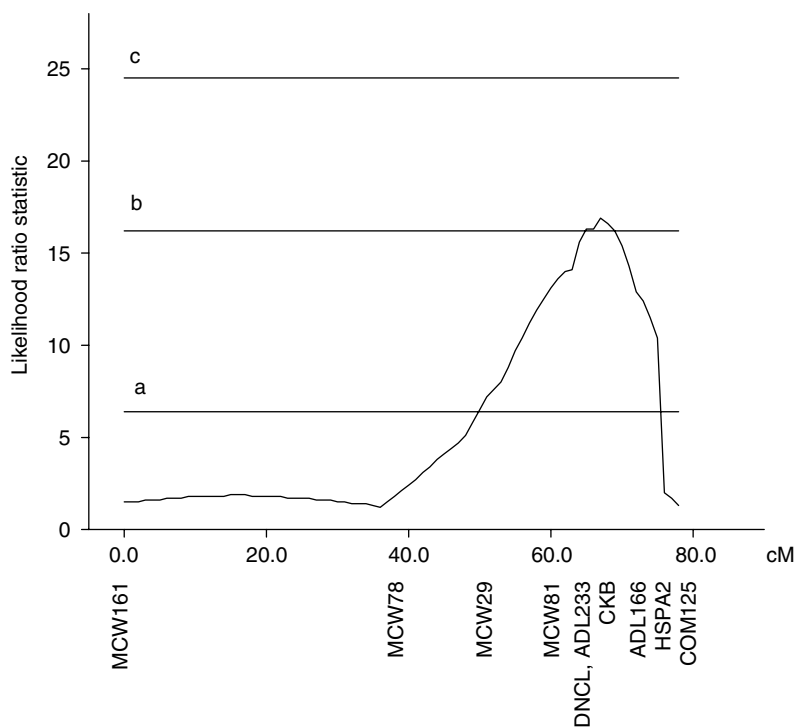
Fig. 18.5. Association between markers on chicken chromosome 5 and numbers of *S. typhimurium* in the spleens of infected animals. Lines a–c correspond to suggestive, significant and highly significant likelihood thresholds calculated using MAPMANAGER QT (Manly and Olson, 1999). From Mariani *et al.* (2001).

resistance with *NRAMP1* or other regions corresponding to known bacterial resistance genes in mice, but markers in the distal region of chicken chromosome 5 showed a strong association with resistance, which was confirmed in other crosses between these lines (Fig. 18.5). Several genes have now been mapped in this region in chickens, and these show conserved synteny with telomeric regions of human chromosome 14 and mouse chromosome 12 (Boyd *et al.*, 2002). So far no candidate resistance gene has been identified within this region.

In contrast to the considerable progress that has been made toward identifying the genes responsible for resistance to visceral infection, relatively little work has been directed to possible genetic differences in susceptibility to gut colonization. Preliminary work suggests that such differences exist and can be demonstrated reproducibly between lines of chickens, but that experimental variation in the level of colonization

of individual chickens is high, making genetic experiments difficult (Fig. 18.6).

Although the sporadic nature of egg contamination from infected birds makes experimental investigation difficult, there is some evidence that there are differences between lines in the degree of shedding to eggs. Lindell *et al.* (1994) compared four strains of commercial laying hens inoculated orally with *S. enteritidis* phage type 8 and identified differences in the incidence of *Salmonella* contamination of both eggs and faeces between the lines but found no differences in levels of infection of the visceral organs in the four strains.

At present it is not clear whether there is a relationship between susceptibility to visceral infection and greater gut colonization or egg contamination. Girard-Santuosso *et al.* (1998) assessed levels of salmonella in the visceral organs and caecae of four chicken lines intravenously infected with *S. enteritidis* phage type 4. *Salmonella* were
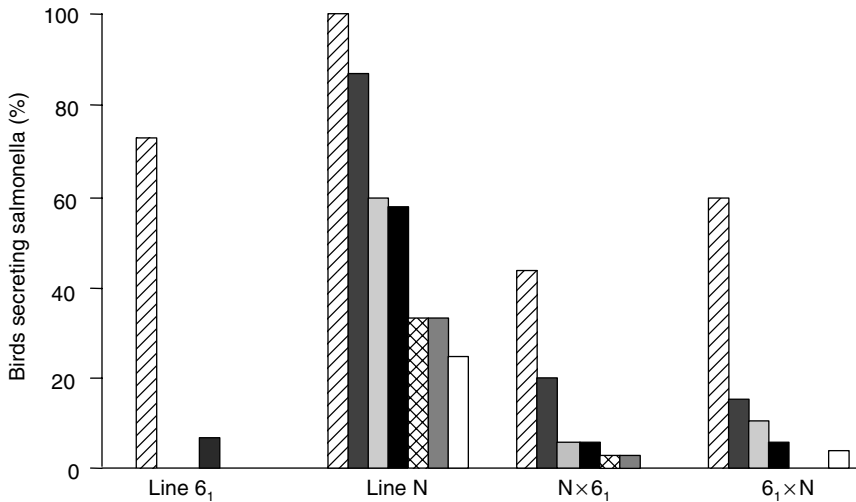
Fig. 18.6. Percentage of birds secreting salmonella at weekly intervals following oral infection with *S. typhimurium* (P. Barrow and N. Bumstead, unpublished results).

detected in the caecae from day 3, indicating that recycling of bacteria from visceral organs to the gut occurs, and differences between the lines in the levels of bacterial colonization were observed for spleen, liver and caecae, though not for the gonads. In this experiment, lines B13 and PA12, which had lower levels of infection of the liver, also had lower levels of caecal contamination, suggesting that there may be some relationship between the two aspects of infection. At 13 weeks, however, the inbred lines that showed the clearest difference in extent of colonization were both resistant to visceral infection, suggesting that these differences are due to some other mechanism.

Resistance to Other Bacterial Infections

Relatively little work has been done to investigate differences in genetic resistance to other bacterial infections, though some information is available regarding *Pasteurella multocida*, *Staphylococcus aureus* and *Campylobacter*. *P. multocida* causes fowl cholera, a contagious disease affecting domesticated and wild birds, which at high doses can cause mortality in chickens (Brogden *et al.*, 1978). Lamont *et al.* (1987) identified differences in

survival following inoculation with the highly virulent *P. multocida* strain X73. Mortality following infection of 3-week-old chickens with *Pasteurella* was compared among sublines of the Iowa State S1 line and F_2 crosses between the sublines. Chickens that were homozygous for the major histocompatibility complex (MHC) haplotype B^1 survived better than B^{19} homozygotes, especially at the lower dose level, and increased survival was particularly associated with inheritance of the B-G region of the B^1 MHC. The design of these experiments was directed principally to identify possible MHC-associated effects and the results provide a rare example of MHC-associated differences in susceptibility to a bacterial disease. It is not clear what other genes are involved.

Cotter and Taylor (1991) compared the susceptibility to *S. aureus* of a chicken line selected for small bursal size with a related unselected line. In neonatal chicks and in older birds, the mortality of the line selected for small bursal size was significantly less than that for the unselected line and the development of morbidity was slower.

Campylobacter infections of chickens are principally of concern as a source of human food contamination from chickens, though in some circumstances they can be

pathogenic in chickens. King *et al.* (1993) used a model of embryonic infection to compare the susceptibility of inbred lines of chickens to a range of *Campylobacter* isolates and showed significant differences in embryonic survival, though there were no differences in the ability of adult birds of these lines to produce antibodies in response to *Campylobacter* infection. More recently there has been some indication of differences in colonization by *Campylobacter*: Stern *et al.* (1990) compared levels of caecal colonization of chicks of three outbred commercial lines infected orally with *Campylobacter jejuni* when 2 days old. The incidence of campylobacter infection in the cacae of the birds after 6 days ranged from 2/60 to 19/60 birds.

Summary and Future Perspectives

It is clear from very many studies that there are large genetic differences in susceptibility to most (if not all) diseases of chickens and that these could, in principle, contribute to the control of disease. It is also apparent that different diseases are influenced by different resistance genes and mechanisms, rather than a single common mechanism such as increased immunological activity, though it is possible that some inbred lines or individual birds may suffer from a generally impaired immune response through fixation of deleterious mutations in genes of the immune system. Elimination of such birds could be beneficial.

An interesting but little studied area is that of potential differences in response to vaccines. This is an important aspect of variability, since more homogeneous or enhanced responses could significantly affect the efficacy of vaccines at the level of both the individual and the flock. The results of Bacon and Witter (1993, 1994) and Bumstead *et al.* (1993), for vaccines against Marek's disease and IBDV, respectively, provide evidence that such differences exist and may in part relate to MHC haplotype (Juul-Madsen *et al.*, 2002). This is likely to be particularly important in the response to recombinant vaccines, as Ross *et al.* (1996) observed in the varied responses of inbred lines of chickens to a recombinant herpesvirus of turkey virus carrying MDV glycoprotein B.

Comparisons with other species, particularly humans and mice, have proved very helpful both in the identification of genes of the immune system and more recently in suggesting possible candidate genes through comparison of the surprisingly extensive regions of conserved synteny seen between chickens and mammals (see Burt, Chapter 29). These comparisons can only grow in power as sequencing of the human and mouse genomes reaches completion and the characterization of their genes increases. This may provide a means of identifying resistance genes relatively directly (Qureshi *et al.*, 1999); however, while these comparisons have been very powerful in identifying chicken equivalents of mammalian genes involved in immunity or pathogenesis, it is less clear whether polymorphisms that contribute to differences in susceptibility are equally conserved. For example, polymorphisms in the *NRAMP1* and *TLR4* genes have large effects on susceptibility to salmonellosis in mice, but variants in these genes in chickens have much smaller effects; conversely, the polymorphism seen in the chicken *SAL1* region appears to have no equivalent in mice.

The development of genomic mapping technology in chickens has opened up exciting new possibilities for locating the genes responsible for these differences in susceptibility, and has been used to great effect by Vallejo *et al.* (1998) and Yonash *et al.* (2001) in respect of resistance to Marek's disease and survival rate, respectively. In the absence of clear candidate genes from other species, progress from identification of the genomic location of a mapped gene to its isolation and identification remains very difficult. The approach adopted by Liu *et al.* (2001b) of identifying host genes that interact with those of the pathogen may be very helpful in complementing mapping information to identify the causative genes, particularly as the genomes of most poultry pathogens have now been sequenced and their gene functions are

being rapidly characterized (e.g. MDV: Tulman *et al.*, 2000; fowlpox: Afonso *et al.*, 2000; *S. typhimurium*: McClelland *et al.*, 2001). Similarly the development of DNA microarrays for the chicken will allow the comparison of transcription patterns in resistant and susceptible birds; it is likely that many genes will be differentially expressed as downstream consequences of a polymorphism in a resistance gene, but comparison of the map locations of the differentially expressed genes with the results of mapping experiments may point to the underlying causative gene (Liu *et al.*, 2001a).

Although the interactions between ALV and its receptors illustrate the extraordinary complexity that may underlie even apparently straightforward mechanisms of resistance and substantial technical hurdles remain, the accelerating technical developments in genomic mapping and in the analysis of host–pathogen interaction offer real hope that the genes and mechanisms responsible for the differences in resistance can be identified, and will shed new light on the mechanisms of host response to infection and provide new possibilities for controlling disease.

Acknowledgements

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19 Genetic Resistance and Transmission of Avian Parasites

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Introduction

Avian coccidiosis is becoming increasingly difficult to control for the poultry industry as a consequence of two factors: resistance to chemoprophylaxis and confined rearing conditions (Stephen *et al.*, 1997). Although in-feed medication of broilers has been commonly used to control coccidiosis in the past decades, drug-based control measures are costly to the industry and are becoming increasingly ineffective as the resistant strains of *Eimeria* develop. Furthermore, increasing public concern over the presence of chemical residues in the food supply is demanding a non-drug-mediated control strategy for poultry diseases. In view of rapid development of new biotechnology in veterinary science, novel control strategies using genomics, molecular biology and immunology would offer an alternative way to prevent the spread of coccidiosis in the future.

The genomics approach includes mapping the quantitative trait loci and the identification of candidate genes that control coccidiosis resistance and susceptibility. Molecular and immunological methods include vaccination, immunostimulation with cytokines, and development of chicken strains with increased innate immunity. For any of these measures to be successfully applied in the commercial setting, a fundamental understanding of avian genomics and the host intestinal immune system is required. Increasing evidence for the protective role of the local immune response and various effector molecules, as described in recent reviews (E.P. Lillehoj *et al.*, 2000; Yun *et al.*, 2000a), provides optimism that novel means to control coccidiosis will be found in the near future.

The aim of this chapter is to present an introductory summary on immunopathology of avian coccidiosis and strategies to combat the disease. Evidence for genetic resistance to coccidiosis will then be presented. In reviewing experiments studying genetic resistance, the different criteria used for assessment of disease resistance will be discussed and attention will be drawn to the effect of major histocompatibility on resistance. Finally, strategies and the first results of two quantitative trait loci (QTL) mapping experiments will be presented.

Immunopathology of Avian Parasites

Avian coccidiosis is caused by protozoa belonging to the genus *Eimeria*, a member of the family Eimeriidae that belongs to the

subphylum Apicomplexa. Coccidial parasites replicate via a precisely programmed life cycle, which includes three phases: sporogony, merogony and gametogony. Oocysts shed in the faeces of infected birds undergo sporogony in the environment to produce infective sporozoites. Sporozoites ingested by feeding birds invade the intestinal epithelium and undergo merogony (asexual reproduction) resulting in the release from one sporozoite of about 1000 merozoites, sometimes repeating this stage two to four times. Gametogony (sexual reproduction) occurs when merozoites develop into either microgametes (male) or macrogametes (female). Fertilization of macrogametes by microgametes results in the formation of a zygote encased by a thick outer wall impervious to the harshest of environmental conditions and subsequently excreted. Once outside the host, oocysts remain viable for long periods of time before being ingested and initiating the cycle again. In view of the complex life cycle of the coccidia, it should not be surprising that host immune responses to these parasites are also complex. Following coccidial infection, both antibody and cell-mediated immune responses are activated and cell-mediated immunity plays a major role in disease resistance (Lillehoj and Trout, 1996; E.P. Lillehoj *et al.*, 2000).

Eimeria spp. are highly host specific. Rarely does one species of *Eimeria* complete an entire infectious cycle in more than one host species, though exceptions have been noted under experimental conditions (Augustine *et al.*, 1991). The underlying mechanisms of host specificity are not well understood but most likely include genetic, nutritional, biochemical and immune factors (Mathis and McDougald, 1987). In addition to host specificity, a given *Eimeria* parasite only infects particular cell types, tissues and organs within a given host. These observations suggest distinct intraspecies and interspecies antigenic diversity among *Eimeria* parasites, which has important implications for vaccination programmes.

Not all *Eimeria* spp. develop at the site of invasion. Some species are transported, by host cells, from the site of invasion to the site of development. Early reports suggested that macrophages (Van Doorninck and Becker, 1956; Doran, 1966) or intraepithelial lymphocytes (IELs) (Al-Atar and Fernando, 1987; Fernando *et al.*, 1987) were responsible for sporozoite transport, though the identity of the cells was not confirmed. Large numbers of macrophages were observed in the intraepithelial region but they only rarely contained sporozoites. Extracellular sporozoites were rarely seen in tissue spaces and no developmental stages (trophozoites or meronts) were observed in IELs. Because IELs are a heterogeneous population consisting of B cells, T cells and natural killer (NK) cells (Lillehoj and Cheung, 1992), the nature of the IEL responsible for *E. acervulina* sporozoite transport was identified using a panel of monoclonal antibodies detecting chicken leukocyte subpopulations (Lillehoj *et al.*, 1988a; Cheung and Lillehoj, 1991). Following primary infection, most sporozoites were seen inside CD8^{+} lymphocytes (Trout and Lillehoj, 1994, 1995), indicating that these cells are responsible for sporozoite transport. Significant numbers of sporozoites were also detected inside macrophages. However, macrophages are phagocytic in nature and it is possible that different mechanisms exist for sporozoite invasion of CD8^{+} cells. Following secondary infection, there was an accumulation of sporozoites in CD8^{+} cells, suggesting that sporozoites were unable to exit these cells to complete their journey to crypt epithelial cells. Furthermore, when CD8^{+} cell-depleted chickens were infected with *E. acervulina* or *E. tenella*, there was, on average, a 55% decrease in oocyst production during a primary infection (Trout and Lillehoj, 1996). These data further suggest a role for CD8^{+} lymphocytes in sporozoite transport and are in agreement with observations that, in birds immune to *E. maxima* or *E. tenella*, sporozoite transport and/or transfer from IEL to crypt enterocytes is inhibited (Rose *et al.*, 1984; Riley and Fernando, 1988). This inhibition of sporozoite transfer to crypt epithelial cells during secondary infection could indicate that sporozoites are unable to exit once inside activated CD8^{+} T cells.

The details of the sporozoite invasion and subsequent translocation are not fully defined. That *Eimeria* spp. tend to be very site selective suggests that sporozoites from different species recognize different host cell structures during the invasion process. Shortly following infection, *E. tenella* sporozoites can be seen invading cells of the intestinal (or caecal) surface epithelium (Van Doorninck and Becker, 1956). Some species complete their development in the surface epithelium, while other species develop in endothelial cells of villus lacteals, the lamina propria (LP), or the epithelium of the crypts. A number of factors influence the ability of sporozoites to invade epithelial cells, including whether or not the host was previously exposed to the parasite and therefore has developed an immunity.

Pathogenicity of avian coccidiosis depends upon the multifactorial nature of aetiology, which involves complex interactions between host and parasites and depends on, for example, the coccidian species or strains, the infecting dose, the presence of other pathogens, the genetic background, sex, age and immunity status of chickens, the management strategy and stress. The pathology of coccidial infection varies with species of *Eimeria* and the host but some features are common to all infections. Clinical signs include lethargy, depression, decreased intake of food and water, and decreased body weight gain or actual weight loss. The water and mucus content of faecal material is increased and blood or diarrhoea may be present. Gross pathological lesions may include grey or white nodules or striations on the lumenal surface of the intestine, and haemorrhagic enteritis may be noted. Microscopically, a pericryptal infiltrate of mononuclear cells and granulocytes is often seen, accompanied by oedema and a thickening of the mucosa. In chickens, a large percentage of the cellular infiltrate is composed of CD8^{+} lymphocytes, which are sometimes visible as large aggregates in the crypts and LP (Trout and Lillehoj, 1995). All symptoms associated with a combination of intestinal function modification and correlating with the schizont stage of the parasite are clearly related. *E. tenella* induces haemorrhage, which can result in blood losses of up to 10% of body weight. The death of animals is preceded by hypothermia, depletion of carbohydrate stores, acidosis and renal tubule dysfunction (Ruff, 1978).

There has been increased understanding of coccidia-induced immunopathology and acute phase proteins associated with coccidia. Most major enteric parasites, including coccidia, invade the intestinal mucosa and induce a certain degree of epithelial cell damage and inflammation. Extensive damage leads to diarrhoea, dehydration, weight loss, rectal prolapse, dysentery, serious clinical illness and, at times, mortality (Cook, 1988).

There is an increased recognition of the importance of oxidative stress as an initiator of signal transduction in biological processes (Forman and Cadenas, 1997; Tanaka *et al.*, 2000). External or internally generated free radicals, such as superoxide and nitric oxide, or changes in the redox potential of cells (Droge *et al.*, 1994; Lawrence *et al.*, 1999; Suzuki and Ford, 1999; Rahman and McNee, 2000; Sen, 2000) can lead to production of second messengers and transcription factors that up-regulate genes expressing antioxidant factors, including enzymes that counteract oxidative stress (Fialkow and Downey, 1997). Oxidative stress is an important regulator of immunity (Crawford, 1999; Li and Karin, 1999; Driscoll, 2000) and an important component of host–parasite interactions (Murray *et al.*, 1985; Hughes *et al.*, 1989; Assreuy *et al.*, 1994; Fernandes and Assreuy, 1997). Production of free radical species accompanies infection by murine coccidia (Ovington and Smith, 1992; Ovington *et al.*, 1995) and all species of avian coccidia (Allen, 1997a,b; Allen and Lillehoj, 1998). Expression of enzymes that control glutathione and other cellular antioxidant components are regulated by cytokines and other biological messenger molecules elaborated during the immune response. Reduced weight gain and increased feed conversion are major characteristics of avian coccidiosis and can be accounted for by nutrient malabsorption (Ruff, 1978). However, reduced feed intake due to

anorexia is also involved and it is possible that inflammatory cytokines, such as IL-1, may be related to decreased feed intake and can also mediate growth depression (Klasing *et al.*, 1987). It has been proposed that the immune and neuroendocrine systems are linked through networks of common receptors and ligands to modulate disease resistance, metabolism and growth (Kelley *et al.*, 1994; Johnson, 1997, 1998; Johnson *et al.*, 1997). In particular, the expression of leptin, a peptide that homes to the hypothalamus and causes depressed feed intake and reduced energy expenditure, is up-regulated by inflammatory cytokines. Finck and Johnson (2000) suggested that hyperleptinaemia induced by cytokines is an integral part of the acute phase response and necessary for immunocompetence. Assessment of leptin and muscle protein turnover, 3-methylhistidine (Fetterer and Allen, 2000) and growth response IGF-1 during coccidiosis should provide insights into the regulation of weight gain during coccidiosis. Application of genomic analyses over time courses of infection to investigate the enzymes catalysing both the oxidative response to parasite invasion as well as the host enzymes that counteract oxidative stress could provide important clues to innate resistance and development of immunity to coccidiosis, and may provide QTL useful in identifying resistant chicken strains.

Strategies Against Coccidiosis

Avian coccidiosis is a major intestinal parasitic disease of poultry, affecting nutrient absorption and animal growth, and costs over US\$1 billion annually worldwide. Two methods are currently used to control this disease – antibiotics in feed and vaccination with live attenuated parasites – but both have drawbacks. Anti-coccidial drugs are expensive and their effectiveness is hindered by widespread parasite drug resistance and the high cost of new drug development. Moreover, consumer concern about drug residues in the food supply may eventually force the industry to eliminate this practice. Problems associated with

antigenic variation of field strains and the effect of live parasites on the immunocompromised host pose limits to the vaccination approach. Thus, novel strategies to control coccidiosis are needed, but will only be realized after a systematic and detailed analysis of host–parasite interactions at the molecular and cellular levels is completed. In particular, there is a need to increase fundamental knowledge on the basic immunobiology of the events associated with parasite invasion and intracellular development as well as parasite biology and metabolism. Immune responses to coccidia are extremely complex and different effector mechanisms may be involved, depending on the species of *Eimeria*, stage of parasite development, prior host exposure, the nutritional status of infected chickens and the genetic makeup of the host (Lillehoj and Lillehoj, 2000). Additional basic research is needed to ascertain the detailed immunological and physiological processes that mediate protective immunity.

The advent of new molecular techniques to manipulate the genomes of host and pathogens and an enhanced understanding of interactions between the gut-associated immune system and peripheral lymphoid organs will enable new approaches to vaccination against *Eimeria* to be realized in the near future. In view of the absence of alternative control methods for avian coccidiosis, genomics research (at the levels of both host and parasite) holds the greatest potential to reduce and hopefully eliminate this disease from commercial poultry flocks. Although tremendous success in the improvement of commercial chicken growth, reproduction and feed efficiency has been accomplished using classical genetic breeding techniques, selection of commercial poultry stocks for improved disease resistance using similar breeding techniques has been unsuccessful due to technical difficulties (Gavora, 1990). It is impossible to measure disease-resistant phenotypes without introducing pathogens into chickens, an impractical procedure with significant negative effects on poultry production. Although selection based on progeny tests may be used to avoid this negative impact, as demonstrated by selection of

broiler strains with enhanced antibody responsiveness to *Salmonella enteritidis* (Kaiser *et al.*, 1998), this is a labour-intensive, time-consuming and costly approach. Moreover, lack of a clear understanding of the mechanisms of protective immunity against most avian diseases makes genetic selection of stocks with enhanced disease resistance very difficult. DNA marker technology avoids many of these problems, making it easier to select animals with superior performance for resistance to particular diseases of commercial importance.

While the successful application of various genomic and immunological strategies will not be possible in the near future, vaccination strategies using several live coccidian vaccines are widely used in commercial poultry production (Immuncox, Livacox, Coccivac). Since pathogenicity occasionally predominates over immunogenicity, live vaccines may introduce new species or unexpected pathogens into a flock. Vaccination with live attenuated coccidian parasites avoids some of the problems associated with pathogenic field strains. Attenuating the pathogenicity of coccidia has been accomplished by selection of precocious strains (McDonald *et al.*, 1986), *in vivo* embryo passage (Shirley and Long, 1990) and irradiation (Jenkins *et al.*, 1991, 1997; Gilbert *et al.*, 1998). Studies using a variety of approaches, including gamma radiation, have shown that early stages of *Eimeria* in the host cells play a critical role in the development of protective immunity against coccidiosis (Jeffers and Long, 1985; Jenkins *et al.*, 1991). Intracellular metabolism, but not sporozoite invasion, may be necessary for resistance to develop (Jenkins *et al.*, 1991).

Several alternative methods of immunization with live *Eimeria* vaccines have been investigated in attempts to circumvent these problems, including *in ovo* vaccination, co-administration of coccidiostats with vaccines and multiple low-dose inoculations to young chickens over a long period of time (trickle immunization). *In ovo* vaccination and co-administration of coccidiostats with *Eimeria* oocysts both failed to induce protective immunity against avian coccidiosis (Long and Jeffers, 1982; Watkins *et al.*, 1995).

In contrast, trickle immunizations with *E. acervulina* (Galmes *et al.*, 1991) and *E. tenella* (Nakai *et al.*, 1992) were successful in conferring upon chickens resistance to subsequent challenge with the homologous parasites. The ability of multiple low-dose immunizations of *Eimeria* to protect against heterologous challenge has also been investigated (Augustine and Danforth, 1990; Augustine *et al.*, 1991). In contrast to the studies with homologous parasite challenge, only partial immunity to coccidiosis was seen in chickens recurrently inoculated with *E. adenoeides* and challenged with *E. tenella*.

Identification of parasite life-cycle stages and development-specific antigens inducing protective immunity is a critical step in recombinant vaccine development. In the case of *Eimeria*, recombinant forms of parasite surface and internal antigens have been investigated as vaccine candidates. Sporozoites are the preferred parasitic form for preparation of recombinant vaccines because they are relatively easy to obtain and blocking their activities should theoretically prevent infection. Cell surface antigens are logical components of vaccines because of their direct role in host–parasite interactions. A cDNA encoding an immunogenic region of a 22 kDa surface protein of *E. acervulina* sporozoites was cloned and expressed (Jenkins *et al.*, 1989). The recombinant protein (MA1) induced significant *in vitro* activation of T lymphocytes obtained from chickens inoculated with *E. acervulina* (Lillehoj *et al.*, 1988b). A cDNA (MA16) from *E. acervulina* encoding an immunogenic region of a surface antigen shared between sporozoites and merozoites was cloned, expressed in *Escherichia coli* and shown to activate T lymphocytes from *E. acervulina*-immune chickens *in vitro* (Castle *et al.*, 1991). Intramuscular immunization with a recombinant p250 surface antigen of *E. acervulina* merozoites or oral inoculation with live *E. coli* expressing p250 resulted in antigen-specific T cell and humoral responses and conferred significant reduction in intestinal parasitism (Lillehoj *et al.*, 1988b; Kim *et al.*, 1989). Vaccination with *E. coli* expressing a recombinant protein is

more effective than immunization with the protein alone, since bacteria growing in the intestine continuously express the recombinant protein, thus providing antigenic stimulation over an extended period of time.

DNA vaccines employ genes encoding immunogenic proteins of pathogens rather than the proteins themselves. They are directly administered in conjunction with appropriate regulatory elements (promoters, enhancers) permitting the encoded DNA sequence to be expressed in its native form and thereby recognized by the host's immune system in a manner that simulates natural infection. DNA vaccination requires gene transfer and expression of the antigen in tissues accessible to the immune system, such as the skin and mucosal surfaces. Two methods of administering DNA vaccines are used: (i) injection of the DNA alone; and (ii) introduction into tissues by particle bombardment (gene gun)-mediated gene transfer. Chickens parenterally immunized with the cMZ-8 cDNA produced higher antibody titres and T lymphocyte responses compared with oral inoculation (Castle *et al.*, 1991). H.S. Lillehoj *et al.* (2000) and Song *et al.* (2000) observed immune protection manifested by significantly reduced faecal oocyst shedding following subcutaneous vaccination with a cDNA (3-1E) encoding an *E. acervulina* protein inducing interferon (IFN)-γ production (3-1E). Further protection was obtained when the 3-1E cDNA was administered in conjunction with cDNAs encoding chicken IFN-γ or IL-15.

Lymphokines mediate intercellular signals during normal immune responses and have thus been investigated as vaccine immunopotentiators for avian coccidiosis (H.S. Lillehoj *et al.*, 2000). Few chicken lymphokines homologous to their mammalian counterparts have so far been described (IFN-γ, interleukin-2, interleukin-15, transforming growth factor and tumour necrosis factor), of which IFN-γ has received the most attention as an immunomodulator (Yun *et al.*, 2000b). The chicken IFN-γ gene was cloned (Song *et al.*, 1997), transfected into chicken fibroblast cells and inhibited intracellular development of *E. tenella* following *in vitro* infection (Lillehoj and

Choi, 1998). An identical effect on *E. tenella* development *in vivo* was observed after administration of recombinant IFN-γ protein to chickens prior to challenge with virulent parasites. Recently, a 19 kDa recombinant *E. acervulina* protein (3-1E) stimulating native IFN-γ production by chicken spleen cells was expressed in bacterial and eukaryotic vectors and the recombinant protein purified to homogeneity (H.S. Lillehoj *et al.*, 2000). One-day-old chicks intramuscularly immunized twice with 1.0 μg of purified 3-1E protein in incomplete Freund's adjuvant were significantly protected from oral challenge with *E. acervulina* oocysts as measured by subsequent faecal oocyst shedding. Co-administration of 3-1E recombinant protein with cDNAs encoding chicken IFN-γ or IL-2/15 led to further enhancement of *Eimeria*-specific immunity. The authors speculated that the protective effect of 3-1E vaccination was mediated by general elevation in immune status as a result of increased IFN-γ production or increased IFN-γ coupled with a specific cellular immune response against the 3-1E recombinant protein. These results raise the exciting possibility of using IFN-γ immunoprophylactically to control coccidiosis in commercial poultry flocks.

Evidence for Genetic Resistance to Avian Parasites

Before searching for specific genes that might control resistance to coccidiosis, evidence for genetic variability has been proved by the feasibility of selection for resistance traits and by major variations in level of resistance between lines of different genetic background.

Selection

The most straightforward way to bring evidence for genetic resistance was to test whether selecting for or against resistance to coccidiosis was possible. Early reports (Rosenberg *et al.*, 1954) brought a clear and

positive answer to this question. Johnson and Edgar (1982) gave the following extended description of a divergent selection for acute caecal coccidiosis (*E. tenella*). From a parental Auburn strain (line A) kept during the experiment as the non-selected control line, two lines were derived. One line was selected for resistance to *E. tenella* (line R) for 14 generations and one line was selected later for susceptibility (line S) for seven generations. The selection criterion was mortality until 216 h after infection of chicks inoculated at 2 weeks of age with *E. tenella*. In line R, selection tended to reach a plateau after six generations only and relaxing the selection could reverse the progress obtained. Selection for susceptibility appeared less efficient. Difference in mortality between contemporary selected lines was about sixfold. These results together could suggest that a relatively small number of genes is involved.

Because of the practical and ethical implications of this kind of experiment, such selected lines were rare though they are highly valuable for looking for genes controlling resistance (e.g. Johnson and Edgar, 1986; see section 'By analysis within outbred lines', this chapter) and for studying resistance mechanisms (e.g. Quist *et al.*, 1993).

Comparison between lines or families

The importance of the host genetics on the outcome of the disease has been evidenced repeatedly by large variations for resistance to coccidiosis between lines (e.g. Long, 1968; Martin *et al.*, 1986; Bumstead and Millard, 1987, 1992; Lillehoj *et al.*, 1989; Nakai *et al.*, 1993; Caron *et al.*, 1997; Pinard-van der Laan *et al.*, 1998) and between sires within a random-bred line (Mathis *et al.*, 1984). This last study is one of the rare analyses of genetic variability and estimation of genetic parameters for resistance to coccidiosis. Heritabilities for resistance to *E. tenella* and *E. acervulina* were estimated in offspring from 18 sires infected by both species and were found

to be moderate (between 0.08 and 0.30 for mortality, weight gain and packed cell volume).

Jeffers *et al.* (1970) compared 15 egg-type lines for resistance to *E. tenella* and found large variations between the lines for survival. When crossing the lines, five of the nine crosses showed significant heterosis and in four of them maternal effects were shown. Heterosis and maternal effects were reported by Pinard-van der Laan *et al.* (1996) when crossing the most resistant and susceptible lines from the screening test.

'Classical' resistance criteria and correlations between traits

Obviously, there is not one 'disease resistance' trait but several criteria linked to resistance. In contrast to other quantitative traits, such as growth and egg production, disease resistance is usually difficult to measure. In avian coccidiosis, it is almost impossible to measure genetic resistance directly in a feasible way, especially when dealing with individuals. Thus the experimental studies quoted in this chapter have often considered several criteria, such as body weight gain, lesion score or mortality, in order to approach the complexity of resistance. Attempts have been made to standardize infection conditions in the sense that these measures are performed after chickens have been inoculated with an equal amount of oocysts to reflect the resistant or susceptible status of the individuals (e.g. Martin *et al.*, 1986; Lillehoj and Ruff, 1987; Lillehoj, 1988; Lillehoj *et al.*, 1989; Nakai *et al.*, 1993; Bumstead *et al.*, 1995; Caron *et al.*, 1997; Pinard-van der Laan *et al.*, 1998).

Attention should be drawn to the interpretation of 'correlations' according to the experimental design. Correlations, in a narrow sense, are estimated from multiple measures on individuals within a population, and this has been done in very few studies. Comparisons between average observations across lines should be interpreted as associations only.

Mortality is a natural criterion for disease resistance and selection for this trait

was efficient (see section 'Selection', this chapter). However, mortality is a binomial trait and thus difficult to discriminate, and moderate infections do not always cause death, so that assessment of resistance becomes quite impossible when only this criterion is used. Subclinical infections are especially important from a production point of view. Jeffers *et al.* (1970), when studying 15 unselected lines infected with *E. tenella*, showed no clear association between mortality and weight gain. Albers and Verheijen (1992) confirmed these results on five commercial broiler lines inoculated with *E. tenella*, as did Bumstead and Millard (1987) on seven inbred or partially inbred lines. In the latter study the authors reported negative correlations between mortality and weight gain within lines, concluding that the correlation was more genetic than phenotypic in origin, but the genetic background of these lines is not ideal for such estimations. Mathis *et al.* (1984) estimated negative phenotypic and genetic correlations between mortality and weight gain in an outbred population.

Clearly, one or two parameters measured in chickens with coccidiosis do not truly reflect the genetic resistance of an individual (e.g. Bumstead and Millard, 1987; Caron *et al.*, 1997) and as well as mortality when observed in the trial, other resistance traits are classically measured such as lesions score or body weight gain. Specific lesions are described for each *Eimeria* species and are scored from 0 (no lesion) and 1 (mild) to 4 (severe) (Johnson and Reid, 1970). Although observation of lesions in a bird is a clear and specific symptom of a given coccidiosis, several reports tend to show that importance of the lesion (and the amount of oocyst output) is not necessarily an indicator of severe health status or affected growth of the animal, as reviewed by Williams and Catchpole (2000). When comparing five outbred lines, Pinard-van der Laan *et al.* (1998) found that the most resistant line for *E. tenella* had the least severe lesions and that, per line, animals suffering the most severe lesions (score 4) showed higher depression of their weight gain than did birds with a lesion score of 3. Mathis *et al.* (1984)

estimated lesions positively and negatively correlated with mortality and weight gain, respectively. It can be concluded that lesion is an important trait to analyse but its significance may vary according to whether it is observed in resistant or susceptible birds.

Although body weight seems a valuable resistance trait – because (from the broiler breeder's point of view), in the absence of death, it is the ultimate economic trait that is affected – there is much controversy on the relevance of weight gain as the major resistance criterion to be considered. Some results on the relationship between weight gain and other criteria have already been quoted in this paragraph. Based on variation between congenic lines, Caron *et al.* (1997) observed little association between body weight gain and lesion scores and between gross and microscopic lesion scores. It may be considered that body weight gain is not a sensitive measurement in coccidiosis, since it usually requires large inoculation dosages to produce a response (Conway *et al.*, 1990). In addition, genes controlling growth may influence body weight gain more than genes governing disease resistance. For example, in a recent study (Zhu *et al.*, 2000), there was a high correlation between body weight gain and initial body weight. If weight gain is the only parameter for genetic analysis of coccidiosis resistance, we may end up mapping genes that control growth rather than genes that control coccidiosis resistance. Mathis *et al.* (1984) concluded, in agreement with other studies, that the resistance traits analysed did not depend on body weight. Finally, weight gain measured at different moments may reflect different traits. For example, Albers and Verheijen (1992) showed large variations between five lines infected with *E. tenella* for weight gain 1 week after infection (which is the most used criterion) but differences in weight gain disappeared 3 weeks after infection, probably because of compensatory growth. More generally, these authors pointed out large variations not only for resistance to acute infection but also for capacity to recuperate after infection. In this study, these two aspects were probably not associated and more definitive research is needed on this

second aspect, which is highly relevant for production.

Blood parameters such as packed cell volume (PCV, or haematocrit) or serum discoloration are interesting indirect measurements of resistance because they are harmful and allow the kinetics of infection to be followed. Serum discoloration has been shown to come from both decreased food consumption and decreased availability of a carrier molecule (Ruff, 1978). Plasma carotenoid level has been found to be a very sensitive parameter in avian coccidiosis and it is considered an excellent indicator of intestinal physical integrity (Conway *et al.*, 1990). Body weight gains were correlated with PCV and plasma coloration in both sexes of different outbred chicken lines (Pinard-van der Laan *et al.*, 1998) and with plasma carotenoid in broilers (Conway *et al.*, 1990).

Obviously, there is a lack of reliable estimates of phenotypic and genotypic correlations based on valid experimental design to enable conclusions to be drawn about any correlation between classical criteria extensively used so far.

'Immunological' resistance criteria and correlations between traits

Oocyst production has been used as the indicator of susceptibility to coccidiosis in mice (Rose *et al.*, 1996) and in chickens (Bumstead and Millard, 1992). Though this parameter appears to be widely used in avian coccidiosis, it is difficult to remove the technical error in sampling and counting. Therefore, inclusion of other correlated parameters may better reflect the true status of genetic resistance to coccidiosis in an individual. Based on individual measures of resistance to *E. maxima*, Zhu *et al.* (2000) found that oocyst production, $NO_2^- + NO_3^-$ levels at 6 days after infection and carotenoid levels were correlated to body weight gains. Plasma nitric oxide (NO) level at 6 days after infection was positively correlated with oocyst output (Zhu *et al.*, 2000), which agrees with a report that high-oocyst-output SC chickens showed higher $NO_2^- + NO_3^-$ levels than low-oocyst-output TK chickens (Allen and Lillehoj, 1998).

Other examples show different results: from between-line comparisons, Pinard-van der Laan *et al.* (1998) found no correlation between oocyst production and the other criteria of resistance to *E. tenella* (weight gain, mortality, carotenoids and lesions) as found by Bumstead and Millard (1987) for *E. tenella* and *E. maxima*. Thus, correlations reported between oocyst shedding and other resistance traits are rather inconsistent and it is doubtful whether oocyst production alone should be taken as a resistance criterion. Results are likely to vary in relation to factors such as the *Eimeria* species or the dose used.

To date, there are no other reports about correlation between IFN-γ and other parameters. In a recent study (Zhu *et al.*, 2000), IFN-γ level measured at 9 days after infection was positively correlated to the level of NO assessed at day 6 after infection. It appeared that IFN-γ was correlated to oocyst output and body weight gain at 6 days after infection but the correlations were not significant ($P = 0.14$ and 0.08, respectively).

Use of an index of resistance criteria

In view of the complexity of mechanisms involved, considering several of these criteria quoted above simultaneously seems an attractive approach. This strategy has been applied for a viral disease, Marek's disease. For better assessment of the disease status of individuals in a large-scale genetic study, disease indices were used to obtain more information in a QTL mapping study of Marek's disease (Vallejo *et al.*, 1998). In the case of coccidiosis, Zhu *et al.* (2000) also used infection indices and suggested that this may be a better reflective indicator of genetic resistance or susceptibility to coccidiosis (Table 19.1). The infection indices in general displayed much higher correlations with the correlated parameters than those among the parameters. The contents of the indices may vary according to the infected species and more research is needed to build relevant indices. Further investigations into the relationships among the parameters and including new parameters will greatly improve the

Table 19.1.   Correlation coefficients (*r*) between infection indices and measured parameters from male and female chickens infected with 10^4 *Eimeria maxima* oocysts (modified from Zhu *et al.*, 2000).

Parameters[b]		GN	GC	GI	GO	GNC	GNI	GNO	GCI	GCO	GIO	GNCI	GNCO	GNIO	GCIO	GNCIO
							Infection indices calculated from different parameters[a]									
O = Oocyst	*r*	0.55*	0.73**	0.51*	**0.87****	0.70**	0.56**	0.79**	0.72**	**0.88****	0.79**	0.70**	**0.83****	0.75**	**0.85****	**0.82****
G = BWG6-9	*r*	**-0.95****	**-0.90****	**-0.84****	-0.87**	**-0.92****	-0.87**	-0.89**	-0.86**	-0.81**	-0.82**	**-0.88****	-0.86**	-0.85**	-0.80**	-0.84**
N = NO6	*r*	**0.95****	0.75**	0.74**	0.77**	**0.89****	**0.89****	**0.90****	0.76**	0.72**	0.76**	**0.87****	**0.85****	**0.88****	0.74**	**0.84****
C = CRTN9	*r*	−0.63**	**−0.90****	−0.52*	**−0.83****	**−0.83****	−0.56*	−0.78**	**−0.80****	**−0.93****	−0.73**	−0.76**	**−0.88****	−0.71**	**−0.86****	**−0.82****
I = IFN9	*r*	0.47+	n.s.	**0.84****	0.45+	0.44+	0.75**	0.49*	0.71**	n.s.	0.76**	0.68**	0.45+	0.71***	0.65***	0.64**

[a]DI = Σ { (individual value of a parameter – mean of the parameter in the group) × C/SD}, where C is a factor. The factor is 1 for parameters positively correlated to oocyst counts and –1 for those negatively correlated to oocyst counts, e.g. GNCIO = (oocyst – mean)/SD – (BWG6-9 – mean)/SD + (IFN9 – mean)/SD + (NO6 – mean)/SD.

The letters G, N, C, I and O indicate that parameters BWG6-9, NO6, CRTN9, IFN9 and oocyst output, respectively, were included in the infection index calculation.

[b]BWG6-9, body weight gain between between days 6 and 9 p.i.; Oocyst, faecal oocyst production between days 5 and 9 p.i.; CRTN9, plasma carotenoid levels at day 9 p.i.; NO6, plasma levels at day 6 p.i.; IFN9, plasma interferon-γ at day 9 p.i.

P values of *r*:

+*P* < 0.10

*P < 0.05

**P < 0.01

n.s., not significant.

understanding of genetic resistance to coccidiosis and help to build up more powerful QTL mapping designs and further apply their results.

Genes Influencing Resistance to Avian Parasites

As shown previously, the influence of host genetics on resistance to coccidiosis is well documented. A number of studies have tried to look further into elucidating the genetic control of resistance by considering the effect of particular genes that can be identified so far. The most studied genes are the major histocompatibility genes, candidates of choice in disease association studies. When selecting for high and low resistance to acute caecal coccidiosis, Johnson and Edgar (1986) showed large differences for MHC frequency for given alleles between the selected lines, some alleles being present in one or the other line. Changes in frequency may be due partly to the selection.

Major histocompatibility complex (MHC) genes

By analysis within outbred lines

Accurate effects of MHC on resistance are best estimated in outbred lines, in families segregating for different MHC haplotypes, provided that all possible genotypes are present and represented in several families, that families are large enough and that family effect is properly taken into account.

To date, no such study has reported any substantial effect of MHC, indicating that MHC may have a direct or linked effect but is far from explaining the whole genetic variability observed for resistant traits. Pinard-van der Laan *et al.* (1998) compared five outbred lines for resistance to experimental infection with *E. tenella* and estimated MHC effects in one of them, a White Leghorn line, segregating for B^{15}, B^{19} and B^{21} haplotypes. The overall effect of MHC was not significant. However, when testing

contrasts between genotypes, $B^{15}B^{21}$ birds lost less weight than $B^{15}B^{19}$ birds, and $B^{15}B^{21}$ or $B^{15}B^{15}$ birds showed less severe lesions than those carrying $B^{21}B^{21}$. In a partially inbred line tested for resistance to *E. tenella* and segregating for B^4 and B^{12} haplotypes, Bumstead and Millard (1987) found no effect of MHC on oocyst production or weight loss but showed that the B^{12} haplotype was associated with a higher mortality than the B^4. In meat-type chickens originating from an F_2 cross between lines selected for high and low *E. coli* response, Uni *et al.* (1995) tested the effect of MHC RFLP types on resistance to *E. acervulina* and reported that birds possessing the 'K' haplotype had less severe lesions and higher plasma carotenoid levels than birds having the 'F' or 'A' haplotype.

A few studies have analysed the MHC effect on acquired resistance in outbred lines. In a non-inbred line of New Hampshire chickens segregating for B^{23} and B^{24}, Clare *et al.* (1985) detected no effect of MHC on acquired immunity to *E. tenella* (weight gain, delayed wattle reaction, spleen weight) but $B^{23}B^{23}$ birds had less severe lesions than $B^{24}B^{24}$ birds.

All of these studies collectively indicate some association of some given alleles on resistance traits that may vary according to the genetic background, the dose used, etc. They should often be repeated with larger families. The need for large-scale experiments, with segregation of all possible genotype combinations from sufficient families and spread within genotype and families between tested and control birds, is crucial. Also more experiments conducted in outbred resources should focus on acquired immunity and analyse immunological measures in a more advanced way.

By comparison between congenic lines

The influence of MHC types on resistance has been also assessed by comparing congenic lines, i.e. those that share a common genetic background but differ for MHC types. Caron *et al.* (1997) evaluated the influence of the B complex on disease resistance, susceptibility and immunity to *E. tenella* in 12 congenic lines. In their first

experiment, naive chickens were challenged with a high (10^4) oocyst dose and caecal lesion scores were evaluated. Lesion scores in B^3B^3 chickens were observed to be significantly lower than all other genotypes, while B^2B^2 chickens displayed the highest lesion scores. In the second experiment, chickens were vaccinated with 500 oocysts and challenged 14 days later with a high oocyst dose. Compared with unvaccinated controls, B^3B^3 and B^QB^Q chickens had significantly lower lesion scores than other genotypes. Brake *et al.* (1997) developed a model to simulate natural immunity to *E. tenella* in $B^{19}B^{19}$, $B^{24}B^{24}$ and $B^{30}B^{30}$ congenic lines. $B^{19}B^{19}$ chickens displayed the greatest level of immune protection; within this group, antigen-specific lymphocyte proliferation developed earlier in immune challenged birds compared with non-immune controls. Lillehoj *et al.* (1989) compared several congenic lines and inbred lines, also allowing comparison between lines sharing the same B type but with different genetic background. Lines were tested for innate and acquired resistance to *E. tenella* and showed wide variation for both traits, meaning that both MHC and non-MHC genes play a role in control of resistance to coccidiosis. Interestingly, some lines were more susceptible for both primary and secondary infections, whereas others appeared to be susceptible to innate infections but resistant to acquired ones.

Other studies found associations between some B types and acquired immunity to *E. tenella* across congenic lines or crosses between them (Clare *et al.*, 1985; Clare and Danforth, 1989; Lillehoj *et al.*, 1990; Nakai *et al.*, 1992; Bumstead *et al.*, 1995) and *E. maxima* (Bumstead *et al.*, 1995). By taking these studies together when some identical B types have been compared (for example B^2 and B^5), results are far from identical, indicating that B types may have an effect – especially on acquired resistance – but it may very well be a linked effect, and/or MHC genes interact with background genes.

The availability of MHC congenic chicken strains has also proved useful for investigating the influence of genetics on particular aspects of acquired immunity to

avian coccidiosis, such as lymphocyte subpopulations and cytokine production. Lillehoj and Bacon (1991) studied intestinal T-cell subpopulations in two MHC congenic strains of chickens, B^2B^2 and B^5B^5, and demonstrated significantly increased numbers of αβ TCR$^+$CD8$^+$ and γδ TCR$^+$CD8$^+$ intestinal IELs after primary infection with *E. acervulina* in B^2B^2 but not B^5B^5 chickens. Following secondary infection, however, no differences were noted between the two host strains. B^2B^2 birds also produced significantly fewer oocysts than the B^5B^5 strain, indicating a correlation between protective immunity following primary immunization and increased numbers of CD8$^+$ cells. Two genetically divergent strains of inbred chickens, SC (B^2B^2) and TK ($B^{15}B^{21}$), displaying different degrees of disease pathogenesis upon infection with *E. tenella* or *E. acervulina*, have also been studied to identify important components of acquired immunity to coccidiosis. In general, SC chickens are more resistant than TK chickens to *Eimeria* infection. Intestinal IEL T cell development was shown to differ in SC and TK chickens (Lillehoj and Chai, 1988). The ratio of γδ TCR$^+$ to αβ TCR$^+$ cells in the intestine gradually increased after hatching, reaching ratios of 3.4 and 4.3 by 12 weeks in TK and SC chickens, respectively. By comparison, the ratio of γδ TCR$^+$ to αβ TCR$^+$ LPLs in SC chickens was 1.2. In both strains, CD8$^+$ IELs increased until 4–6 weeks after embryonic development and subsequently declined as chickens aged.

In a subsequent study, the dynamics of intestinal T-cell subpopulations bearing CD4, CD8 and TCR surface markers were investigated following primary and secondary *E. acervulina* infections of SC and TK chickens (Lillehoj, 1994). CD4$^+$ intestinal IELs increased 7 days after primary infection in SC and TK chickens and, in the latter strain, the number of αβ TCR$^+$CD8$^+$ cells increased shortly after challenge infection. Following secondary infection, a significantly higher number of αβ TCR$^+$CD8$^+$ intestinal IELs were observed in SC chickens, which manifested lower oocyst production compared with TK chickens. This study suggested that the increased number of αβ

TCR⁺CD8⁺ IELs in SC chickens following secondary infection might contribute to their enhanced protective immunity to *Eimeria* compared with TK chickens.

Another immune parameter that has been studied with respect to coccidiosis in SC and TK chickens is cytokine production. In particular, Choi *et al.* (1999) observed IFN-γ and tissue growth factor (TGF)-β4 transcripts from caecal tonsil and splenic lymphocytes to be more abundant in SC chickens following *E. acervulina* infection, suggesting that production of both cytokines is not only genetically controlled but also influences cell-mediated immunity to coccidiosis. Genetic differences in host intestinal response and IFN-γ production were studied following *E. tenella* infection in two inbred strains (SC and TK) that display different patterns of susceptibility to coccidiosis (Yun *et al.*, 2000c). Flow cytometric analyses of caecal tonsil lymphocytes demonstrated greater numbers of CD4⁺ and TCR1⁺ cells in SC chickens and elevated numbers of CD8⁺ and TCR2⁺ cells in TK chickens following primary infection. IFN-γ mRNA expression was significantly increased in caecal tonsil and intraepithelial lymphocytes at days 6 and 8 after primary infection, respectively, in SC compared with TK chickens.

The mechanism by which IFN-γ exerts its immunological effects may involve production of NO, a highly reactive free radical. Dimier-Poisson *et al.* (1999) observed NO synthesis in chicken macrophages treated with IFN-γ-containing culture supernatants from reticuloendotheliosis virus-transformed lymphoblastoid cells and concanavalin A-stimulated spleen cells. Furthermore, addition of an NO synthase inhibitor together with either supernatant was able to inhibit IFN-γ production. In contrast, Allen and Lillehoj (1998) noted minimal involvement of NO production in the immune response to challenge by *E. tenella* in SC and TK chickens and hypothesized that this reactive compound may be only one of several anticoccidial responses to infection. Further studies by Allen (1997a) suggested that NO might actually promote development of intestinal lesions and thereby contribute to disease pathology rather than protect against

parasite invasion. Thus, while significant increases in NO production were seen in chickens infected with *E. tenella* compared with uninfected controls, its production correlated with the time when mucosal damage was highest, suggesting that intestinal lesions develop in coccidiosis in part due to the host's response to parasite invasion.

Zhang *et al.* (1995a,b) investigated the effect of a TNF-like activity on the pathogenesis of coccidiosis in SC and TK chickens. Peripheral blood macrophages from both strains produced circulating TNF-like activity in a time- and dose-dependent manner following primary, but not secondary, infection. Treatment of chickens with TNF antibody resulted in partial abrogation of *E. tenella*-induced body weight loss in SC but not in TK chickens. Chicken IL-2 and IL-15 genes share DNA sequence homology to both mammalian IL-2 and IL-15 and stimulate T-cell growth (Lillehoj *et al.*, 2001). To investigate the role of IL-2 in *Eimeria* infection, 3-week-old chickens were orally infected with *E. tenella* and mRNA expression quantified by PCR in intestinal T cells. SC and TK inbred chicken strains displayed differential protective immunity to coccidiosis, SC being more resistant and TK susceptible to disease. The association between IL-2 and disease phenotype as assessed by cytokine quantification in serum, duodenum, caecum and spleen cell cultures of SC and TK chickens experimentally infected with *E. tenella* showed that, following primary infection, SC and TK strains produced equivalent amounts of IL-2 in all sources examined. However, after secondary infection, SC animals displayed significantly greater IL-2 levels in serum and the duodenum compared with strain TK (Li *et al.*, 2002). Thus, IL-2 production following reinfection with *Eimeria* may be an important factor contributing to the genetic differences in coccidiosis between SC and TK chickens.

Non-MHC genes

Studies looking for the effect of other non-MHC genes on resistance to coccidiosis

are rather scarce. This is probably due to a lack of potential candidate genes so far. Alloantigen systems other than the B system were studied for their possible effect on resistance: Martin *et al.* (1986) showed that a line selected for high antibody response to sheep red blood cells was more resistant to *E. tenella* than the low-responder line, that within the high line the D blood group showed some effect on mortality and that within both lines some alleles of the I blood group were associated with mortality and within the high line with lesion score. No effect of alloantigen groups on weight gain was noticed.

A few other studies have tested whether a gene present in a given line, and presenting interesting effects for breeding is associated with resistance to coccidiosis. For example, the naked-neck gene (*Na*) may be beneficial for production in high temperature conditions (Fraga *et al.*, 1994): Berrio *et al.* (1991) reported a positive effect of *Na* on growth, mortality and haemoglobin level in chicks inoculated with *E. tenella*; and a higher resistance to *E. necatrix* associated with *Na* was also reported by Banga Mboko (1996) in Dahlem-Red chicks. Some experiments have studied the effect of the dwarf (*DW*) gene on resistance to coccidiosis: Zulkifli *et al.* (1993) found a negative effect of *DW* on lesions due to *E. tenella* but the effects of infection were rather mild; Haas *et al.* (1975) reported no association between *DW* and resistance to *E. necatrix, E. acervulina* or *E. brunetti*; nor was any effect of *DW* on resistance to *E. tenella* found by Pinard-van der Laan *et al.* (1998), who used segregating families for *DW* (the other two studies compared lines). So far, then, there has been no proof of any significant effect of the dwarf gene on resistance.

Since many studies have reported interaction effects between MHC and non-MHC genes (see section 'Major histocompatibility complex (MHC) genes', this chapter), it can be hoped that future studies searching for genomic regions associated with resistance traits will discover some of these non-MHC genes and give more insight on interactions between genes.

Specific vs. General Resistance to Several Parasite Species

Although the problem of resistance to several *Eimeria* species is of high relevance, the way it is approached experimentally remains delicate and some results may be as doubtful as those concerning correlation between several resistance criteria. 'General' resistance may be assessed by multiple infections occurring either at different moments or simultaneously. The latter case seems of greater interest: a report by Williams *et al.* (1996) showed that 95% of the coccidiosis cases observed in 22 farms in France were due to simultaneous infections, regardless of the type of farm management. Even in cases of mixed infection, with species having their specific site of replication, the infection may affect the same traits, such as lesions or weight gain, though the mechanisms and thus the underlying genetic control may differ. Moreover – depending of the mix of species – competition or inhibition of a given trait such as oocyst production may occur (Williams, 1999). A genetic analysis may then be quite difficult to interpret. Regarding the genetic control of site of infection, Johnson and Edgar (1982) showed that, at high doses, birds selected for resistance to coccidiosis did not show the same site of infection as did the susceptible birds. The authors concluded that genes controlling tissue specificity may be: (i) identical or complementary to the genes controlling the level of resistance to coccidiosis (acute *E. tenella* in this case); and (ii) in small numbers.

One of the few genetic studies analysing concurrent infection (Mathis *et al.*, 1984) estimated the correlation between resistance to *E. tenella* and to *E. acervulina*. Lesions for specific species were moderately but positively correlated between each other and were highly correlated to total lesion scores; the authors concluded that the latter parameter would be a good criterion for resistance to coccidiosis.

A number of other studies have compared several lines for resistance to different species: *E. tenella* and *E. maxima* (Bumstead

and Millard, 1987) or *E. tenella* and *E. acervulina* (Ruff and Bacon, 1989; Albers and Verheijen, 1992). No clear association was found but, once again, more research is needed to study, in a proper way, resistance to multiple species.

Factors Influencing Resistance to Avian Parasites

Other non-genetic factors influence the outcome of the disease, such as age, sex or dose. It is important to mention these factors here because they should be taken into account in any genetic study and moreover have sometimes been reported to interact with genetic factors.

In general, young animals are more susceptible to coccidiosis and more readily display signs of disease, whereas older chickens are relatively resistant to infection (Lillehoj, 1998). Young animals that recover from coccidiosis may later be able to compensate partly for growth loss, but their growth potential remains severely compromised.

Studies of the effect of sex on immunological or pathological responses often show inconsistent trends, and the interactions between hormonal and immune systems are still not clearly understood. As far as coccidiosis is concerned, reported effects of sex (when taken into account) are indeed various. When comparing the resistance of different lines to *E. tenella* and to *E. maxima*, Bumstead and Millard (1987) showed that males and females responded similarly to infection. Pinard-van der Laan *et al.* (1998) found that there was no effect on four of five lines studied for weight gain, plasma coloration or lesions due to *E. tenella* and that females showed a lower resistance than males in relation to weight gain in only one White Leghorn line. Mathis *et al.* (1984) did not find any significant effect of sex but a significant sex-by-sire interaction. In response to *E. tenella*, Martin *et al.* (1986) and Johnson and Edgar (1982) also reported a lower growth rate from females and in the latter study the difference between sexes increased with the dose; but mortality of males was higher at

low dose. Zhu *et al.* (2000) reported significant sex effects and sex-by-dose interaction effects on infection with *E. maxima*.

Effect of dose is likely to be the major factor, because it may lead to either subclinical or clinical infection. Most studies show that increasing the dose causes increasing mortality, lesions and oocyst shedding and decreasing weight gain and several blood parameters such as PCV or plasma carotenoids (e.g. Lillehoj and Ruff, 1987; Lillehoj, 1988; Conway *et al.*, 1993; Yvoré *et al.*, 1993; Zhu *et al.*, 2000). Effect of dose might affect not only the severity of symptoms but also the kinetics of infection and so dose and time should ideally be studied in parallel. Some classical parameters will not be modified by low doses whereas others seem to be very sensitive and may be very good indicators of mild infections: plasma carotenoids and lipids are such parameters in the case of *E. acervulina*, *E. tenella* and *E. maxima* infections (Conway *et al.*, 1993; Yvoré *et al.*, 1993). Interaction between genetic background and dose was reported by Pinard-van der Laan *et al.* (1996), who showed that maximum differences for lesions, weight gain and plasma coloration between a resistant and a susceptible line could be obtained by an intermediate dose of *E. tenella*. Johnson and Edgar (1982) reported that high dose caused clearly different symptoms between two selected lines.

More generally, multiple genetic and non-genetic factors might influence the course of the disease. Other factors such as management or nutrition have been proved to be highly relevant regarding coccidiosis and interesting research is being performed, which will not be discussed in the context of the chapter. Obviously, an integrated epidemiological approach (Graat, 1996) is needed to combat coccidiosis in the most efficient way.

Genomics for Searching for Resistance Genes to Avian Parasites

Disease resistance or susceptibility to avian parasitic diseases, such as coccidiosis, can

be considered a quantitatively inherited trait, because chickens display continuous variations of sickness after infection and the trait is probably controlled by polygenes. Unlike other production traits, it is very difficult to improve disease resistance using a classical approach. It is impossible to measure the phenotype without introducing pathogens into the chickens – an impractical procedure with significant negative effects on poultry production. Progeny tests may be used to avoid this negative aspect, but this method is tedious, time consuming and expensive. Moreover, lack of a clear understanding of the mechanisms of disease resistance and the difficulties of measurement of the trait make genetic selection more difficult. DNA marker technology can greatly facilitate this genetic selection: QTL and genes underlying disease resistance can be identified using DNA markers and statistical analysis. Once the genes are identified, the undesired alleles can be removed from breeding stocks by using marker-assisted selection (MAS).

QTL mapping

Strategies for identifying genes underlying disease resistance

Currently, two approaches – linkage and association analysis – are used to map genes underlying disease resistance. Linkage mapping analyses the co-segregation of markers and trait loci based on linkage disequilibrium within families; association analysis studies the associations between alleles and phenotypes using linkage disequilibrium at the population level. Elston (1992) proposed a cost-effective two-stage strategy for gene discovery: in the first stage, linkage analysis is applied to identify potential chromosomal regions using widely spaced markers (approximately 20 cM) and relatively low stringency of significant threshold; in the second stage, densely spaced markers located at the chromosomal regions of interest are used to pinpoint the loci/genes by association analysis.

Inbred lines with significantly divergent phenotypes are more desirable resource populations for linkage mapping than outbred populations. Unfortunately, no inbred lines have been developed based on resistance to coccidiosis. An alternative approach is to design mapping experiments based on crosses between populations that differ widely in traits of interest to produce the F_1 generation, which is then intercrossed to generate F_2 or back-crossed to parental lines to produce backcross generations (Soller and Andersson, 1998).

Unlike other production traits, methods of assessing coccidiosis severity are not well established, as explained above. Historically, body weight gain, intestinal lesion score, feed conversion, plasma constituents and oocyst shedding have been measured in infected chickens from various genetic backgrounds to determine their resistance or susceptibility status but these measurements are only weakly correlated amongst themselves (Bumstead and Millard, 1987; Caron *et al.*, 1997; Idris *et al.*, 1997; Zhu *et al.*, 2000) and a single trait constituting the best indicator of disease resistance remains to be determined.

For traits with larger measurement error, such as oocyst counts, the average of multiple measurements can increase detection power. The consistency of *Eimeria* challenge strains and dosages should be taken into consideration for experiments extended over a period of time. Possible covariates, such as age, sex, hatch and some environmental factors, should be documented and included in statistical models to account for the phenotypic variation if statistically significant (Almasy and Blangero, 1998). Continuous phenotypes that provide more information are preferable to dichotomous phenotypes.

Two examples of strategies and protocols aiming at finding genetic markers of resistance to coccidiosis and their first results will be now presented. The first experiment was conducted at the USDA Parasite Biology, Epidemiology and Systematics Laboratory (USA) on *E. maxima* and the second at the INRA Department of Animal Genetics (France) on *E. tenella*.

Identifying QTL for resistance to coccidiosis: example 1

In the USDA experiment, two commercial broiler lines displaying different susceptibilities to coccidiosis were selected as resource populations to map for QTL associated with resistance to coccidiosis. Chickens from these two lines were crossed to produce F_1, and the F_1 birds were intercrossed to produce 314 full-sib offspring (Zhu *et al.*, 2003).

To map loci associated with resistance to coccidiosis, the F_2 chickens were orally inoculated with 10,000 sporulated *E. maxima* oocysts at 4 weeks of age. The chickens were weighed at days 0, 3, 6 and 9 postinoculation (p.i.). One faecal sample per bird was collected during the period from days 5 to 9 p.i. The oocysts in the faecal samples were counted using methods described by Lillehoj and Ruff (1987). Blood samples were also collected from each bird in sodium EDTA on days 0, 3, 6 and 9 p.i. to measure plasma interferon-γ levels with an enzyme-linked immunosorbent assay developed by Yun *et al.* (2000c). In addition, $NO_2^- + NO_3^-$ and carotenoid concentrations were estimated, using the methods described by Allen (1997b).

Statistical analysis of phenotypic data showed that oocyst shedding appeared to be a good indicator of resistance to *E. maxima* (Zhu *et al.*, unpublished data). First, oocyst shedding was independent of both sex and body weight at the time of challenge. Secondly, oocyst shedding displayed the highest correlation with body weight gain after the acute phase (days 6 to 9 p.i.) of the disease in females. Thirdly, oocyst shedding was significantly correlated with the decrease of plasma carotenoid concentration between day 3 and day 9 p.i. in both males and females. After all, this measurement is unique to coccidiosis and more relevant to the disease pathogenesis than other parameters. The oocyst count takes the entire infection process into account. These results indicate that oocyst shedding is a quantitative measurement of sickness due to the infection of *E. maxima*. However, the measurement of resistance to coccidiosis still needs further investigation.

Sequential oligogenic linkage analysis (SOLAR), Version 1.6.6, developed at the Southwest Foundation for Biomedical Research, San Antonio, Texas, was used to perform QTL linkage analysis. Variance component-based multipoint quantitative-trait linkage analysis was implemented in the package according to methods reported by Almasy and Blangero (1998). Based on the current chicken consensus linkage map (Groenen *et al.*, 2000), 119 microsatellite markers were selected to cover approximately 75% of the chicken genome with an average marker interval of 25 cM.

Marker LEI0101 (259 cM on chromosome 1) displayed a logarithm of the odds (LOD) score of 1.55 associated with oocyst shedding in the single-point linkage analysis. To balance Type I and Type II errors, a relaxing threshold ($P = 0.0023$ or LOD = 1.75 for 400 markers) was suggested to allow one false positive per genome scan (Rao and Gu, 2000). According to the threshold, the LOD score indicates that this locus may be a good candidate locus for further investigation. Because the resource population used in this experiment was outbred, the linkage phases between genes underlying quantitatively measured trait and linked markers and informativeness of marker loci may vary from family to family. Lynch and Walsh (1998) suggested that the association between markers and traits must be evaluated separately for each parent. Some families in this study may not be informative for the QTL of interest. The QTL detection power can be increased significantly if non-segregating families are removed.

When the single-point linkage analysis was based on the data of four families with an LOD score > 0 at LEI0101, the LOD score at the marker locus increased to 3.02. Taking the number of markers used into consideration, the LOD score is significant according to the guidelines suggested by Lander and Kruglyak (1995). Multipoint linkage analysis with 1 cM increments based on the four selected families also revealed a significant LOD score of 3.46 at 252 cM on chromosome 1. The heritability associated with this locus was estimated to be 0.54. By comparing

the means of oocyst shedding of different genotypes, the genetic effect at this locus based on the largest family was mostly additive. In summary, oocyst shedding is a good and unique measurement of genetic resistance to coccidiosis caused by *E. maxima*. The results indicate a QTL close to LEI0101 on chromosome 1 associated with resistance to coccidiosis in chickens. However, a replication of the experiment is necessary to validate this QTL.

Due to limitation of sample size, the 95% confidence limits, e.g. 1 LOD support interval (Lynch and Walsh, 1998), for a statistically significant QTL identified with linkage mapping are usually larger (30 cM in this experiment). Increasing marker density alone will not substantially decrease the confidence interval, and the sample size required to achieve a smaller confidence interval usually is impractical. Association mapping, based on the population-level linkage disequilibrium between alleles of markers and genes of interest, can be used to reduce the required sample size significantly (Risch and Merikangas, 1996) and allows the localization of genetic effect to specific genes.

Two experimental designs can be used in association mapping: one uses samples of unrelated individuals based on case-control design; the other uses unrelated families or pedigrees based on transmission and linkage disequilibrium. In the case of coccidiosis, phenotyping is tedious, costly and usually limited by resources available. The number of phenotyped individuals should be minimized. Additionally, pedigrees are relatively easy to obtain in poultry. Therefore, experiments using unrelated families are more desirable than those based on unrelated individuals. In this design, parents are genotyped and their sibs are genotyped and phenotyped. According to simulation, increasing the number of sibs can improve detection power but little gain is produced if the number of sibs is more than five (Abecasis *et al.*, 2000; Monks and Kaplan, 2000). Therefore, increasing the number of families, unlike linkage analysis, creates more power than having large full-sib families in association mapping.

In addition, unrelated families allow implementation of allelic transmission in the analysis, which make this design more robust than that of unrelated individuals. Sampling schemes, such as choosing individuals with extreme trait values in analysis, can increase sensitivity of detection (Risch and Zhang, 1995).

Because association analysis is based on linkage disequilibrium at the population level, densely spaced markers (< 1 cM) must be used in order to detect the genes of interest. Unfortunately, the current chicken linkage map cannot provide such marker resolution. The current project of contig construction of the chicken BAC library will provide information to develop such markers for association mapping (J.B. Dodgson, personal communication). Another basis in association analysis is allelic identity-by-descent. Microsatellite markers that are very useful in linkage mapping may not be so in association mapping because of high mutation rate (Callen *et al.*, 1993) and homoplasy (confounding) due to the fact that the genotyping is size-based (Weber and Broman, 2000). The marker density of microsatellite markers may not be enough for association mapping. In contrast, markers based on single nucleotide polymorphism (SNP) can serve association mapping well. The SNP is the most abundant form of genetic variation in the genome, comprising 90% of all known polymorphisms in human (Collins *et al.*, 1998). The density of SNP is approximately 1 per 1000 base pairs. Although SNP markers are mostly biallelic and less informative than microsatellite markers, they are more frequent and much more stable. In addition, SNP markers are based upon known genomic sequences, and the genotyping of SNP markers is sequence-based. These features make SNP markers very useful for association mapping based on linkage disequilibrium.

Due to the limitation of sample size and linkage disequilibrium, association mapping may not be able to localize the genetic effect to a specific gene. Transcript mapping using bioinformatics tools can be used to annotate gene sequences in the target areas to find the possible causes of genetic effects.

Identifying QTL for resistance to coccidiosis: example 2

In the INRA experiment, a similar strategy of using an F_2 cross was used, except that the two resource lines were not commercial lines but experimental ones displaying extreme phenotype for resistance to coccidiosis, and specifically to *E. tenella*.

In order to identify a resistant line and a susceptible line, we first screened several experimental lines for resistance to *E. tenella* (Pinard-van der Laan *et al.*, 1998). Five outbred lines were challenged with high dose (1.5×10^6 oocysts) at 4 weeks of age and several criteria were measured: mortality, lesions, body weight gain and plasma coloration. One Egyptian line, called 'Fayoumi', was clearly the most resistant, with no mortality, few lesions and 30% reduction of body weight gain, as compared with a White Leghorn line that showed 27% mortality, severe lesions and a reduction of 85% in growth rate. The difference in feed conversion between the two lines was huge. Differences in oocyst production after primary challenge between the Fayoumi and Leghorn lines were not observed but, interestingly, the Fayoumi also showed higher resistance after secondary infection, with specially no oocyst production (Laurent *et al.*, unpublished data). The two lines were compared for resistance to another species, *E. acervulina*, which is the most widespread species after *E. tenella* on most French farms. Again, the Fayoumi was more resistant than the Leghorn line as far as growth being affected. Also, the Fayoumi birds showed much less negative effect of infection on their body temperature, as already observed with *E. tenella*. All this information is highly beneficial for QTL mapping projects and for further practical use, because it is hoped to identify QTL not only for a restricted resistance trait or only for a specific coccidiosis species.

The following steps to identify and validate QTL for resistance are performed (Fig. 19.1). To increase the power of the QTL mapping design, the two candidate lines were challenged again with different doses of *E. tenella* in order to find the optimal dose,

which would maximize the difference in resistance between the two lines. Four doses were tested and the difference was greatest by using a slightly lower dose (5.0×10^4) (Pinard-van der Laan *et al.*, 1996). The lines were further crossed to produce an F_1 generation and then F_2 crosses. In the F_1 cross, heterosis for resistance to *E. tenella* was clearly shown since the F_1 birds were as resistant as the resistant Fayoumi line. Maternal effect was also shown, the birds originating from Fayoumi dams being more resistant than those from White Leghorn dams. Finally, six F_1 males were each mated to five F_1 females to produce 860 F_2 birds, which have been all tested and measured for the different resistance criteria.

The next step was to type the F_2 population with anonymous markers (microsatellites) to identify QTL. More than 300 microsatellite markers were tested on F_1 fathers and among them 154 were kept as being informative in the experimental cross. These 154 markers met the required standards as far as genome covering and technical quality were concerned. In order to limit the number of typing to be performed, the technique of selective genotyping has been applied (Darvasi and Soller, 1992) since the animals displaying the most extreme phenotypes contribute the greatest amount of information on the marker–QTL linkage. The 15% most susceptible and the 15% most resistant animals of the F_2 (i.e. a total of 260 F_2 birds) were genotyped. The choice of animals was performed within mother families, based on the weight gain criterion. After this selection, two groups displaying two non-overlapping distributions for this resistance criterion were obtained. These two groups show high differences for lesions as well and so identification of QTL for lesions will also be possible. The results are now being analysed.

Evidence in the experimental cross for genomic regions controlling resistance to coccidiosis will be a major step in the understanding of the genetic control of disease resistance and, in parallel, will suggest ways of achieving a better understanding of the resistance genes and the specific immune mechanisms involved. To apply these
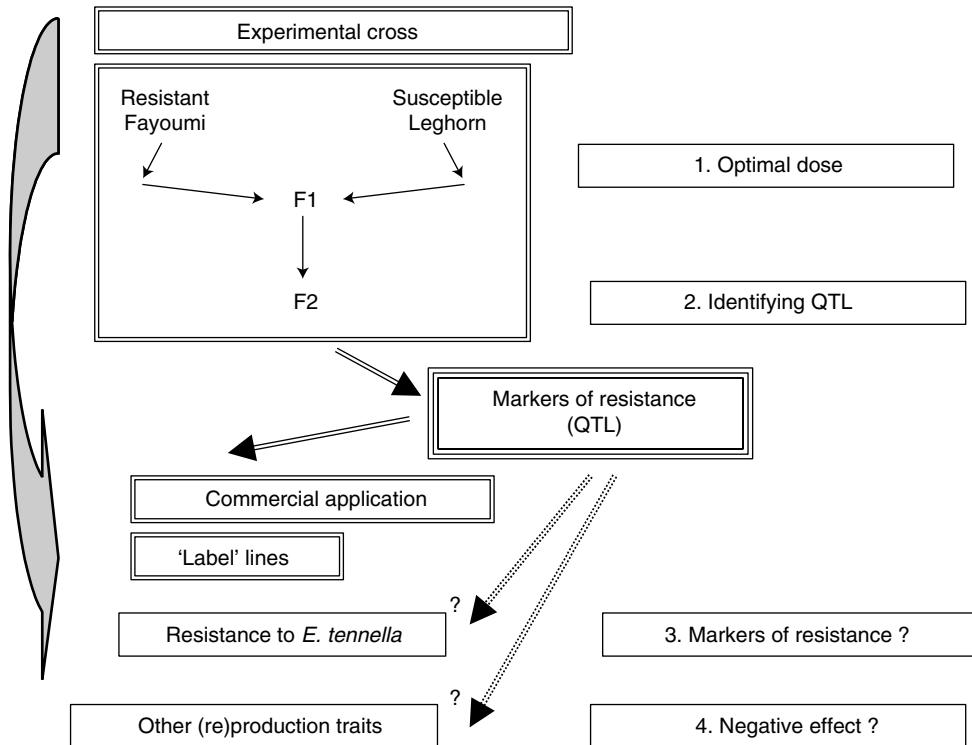
Fig. 19.1. Strategy for detection of genetic markers for resistance to coccidiosis (*E. tenella*) in an experimental cross and its validation in a commercial line.

results in practice, validation of the identified QTL is required by means of the next two steps (3 and 4 in Fig. 19.1). Two different commercial lines of the 'label' type will be tested, firstly to determine whether the QTL found in the experimental F_2 cross also confer significantly higher resistance in the commercial lines and, secondly, whether or not these QTL are associated with any negative effect on other important economic traits, such as growth, in order to validate potential interest for breeders.

DNA microarray analysis – candidate genes

In addition to DNA marker technology, DNA microarray is another high-throughput tool for genomic study. This technique allows simultaneous monitoring of the expression of a large number of genes. The hypothesis of using the technique is that the expression levels of genes will be different between resistant and susceptible chickens if these genes are associated with disease resistance. The genes with significant differences can be used as candidate genes. The markers developed from these genes can be used in association mapping to test the association between the genes and phenotypic variance. Currently, thousands of chicken expression sequence tags (ESTs) from various tissues are available for microarray analysis.

In the case of coccidiosis, RNA samples can be prepared from chicken intestine before infection and at different times after infection. The goal of the experiment is to examine the dynamic changes of gene expression in the time course of an infection. A 'loop' design proposed by Kerr and Churchill (2001) can be used to partition these experimentally. In this design, each sample is labelled separately with two dyes, 'red' or 'green'. Sample 1 with red dye and Sample 2

with green dye are combined for use in one array, and Sample 2 with red dye is paired with Sample 3 with green dye. Finally, Sample N with red dye and Sample 1 with green dye are paired to close the 'loop'.

To compare the differences between resistant and susceptible chickens, a Latin square design, also called a dye-swap experiment (Kerr *et al.*, 2000), can be used. All RNA samples prepared from resistant and susceptible chickens are used to make cDNA labelled with 'red' and 'green' dyes. The red-labelled cDNA from resistant chickens and green-labelled cDNA from susceptible chickens are used in one array, and the dye assignments are reversed on another array. Replication of microarray experiments is necessary because of the inherent noise of microarray data (Lee *et al.*, 2000). The replication can be achieved at spot and/or array levels. An internal control, such as a known DNA sequence from distantly related species and its corresponding labelled cDNA probe, could be used to check the quality of the microarray and to normalize the data (Zhou *et al.*, 2000).

Once array data are obtained, there are two major approaches for identifying the genes with different expression. One is based on the ratio of compared samples and the other is according to statistics. Although twofold difference has been widely used as the threshold of differences, it is considered that using ratio alone does not use all the information from array data. Appropriate statistical analysis can provide more information than using ratio alone. Once genes with differential expression have been identified, they can serve as candidate genes for other genetic studies.

In summary, two-stage analysis is a cost-effective approach to localize genes underlying disease resistance. Linkage analysis can be used to identify chromosomal regions potentially associated with traits of interest, and association analysis can be applied to pinpoint the genes responsible to the phenotypic variations. However, current chicken breeding stocks have been undergoing intensive selection and rapid population expansion due to increasing demand. The resolution that these populations can

provide in association analysis is unknown. Microarray analysis allows high-throughput and systematic screening for candidate genes.

Conclusion: Use and Limits of Genomics to Control Resistance to Coccidiosis

Evidence of genetic variability for resistance to coccidiosis was well proven long ago and projects for identifying genetic markers are finally now reaching their concrete phases and giving their first results. Moreover, advances in bioinformatics should provide additional state-of-the-art tools for gene discovery. Knowledge about unknown genetic markers linked to resistance may already be used in practice since MAS is becoming the ultimate goal in poultry breeding. However, a number of restricted conditions should be required in applying these results:

- Markers should be closed enough to the putative loci to allow reliable MAS. It is not known whether the density of markers used so far will be sufficient and it is likely that additional work will often be necessary to meet the requirements.
- Alternatively, QTL are identified directly within commercial lines or from an experimental cross. In the latter case validation in a commercial setting is still needed and the same markers might be not found or might not give the same effects. One solution is introgression assisted by markers, which might be applied if there is sufficient economical value. The optimal number of generations will depend on the number of genes that are of potential interest.
- In all cases, genetic markers will be used only if: (i) they significantly increase resistance; and (ii) they do not depress other important breeding traits.
- Finally, but not least, is the question of resistance to multiple diseases, which so far is difficult to predict. If the use of genetics as a way of combating diseases

has the advantage over other ways, such as vaccination, of persisting and even increasing over generations, it brings together the risk of increasing at the same time the susceptibility to other diseases.

Overall it is clear that, although very much desired for economic as well as ethical reasons, the use of genetics for controlling resistance to coccidiosis will also have its own predicted direct and less easily predicted indirect costs or benefits for breeders. It is likely that the use of genomics will be beneficial if used rationally: the choice for its application will result from economic compromises and strict evaluation of the risks towards the different diseases a bird may face in a given environment and the efficiency of the ways to combat them. Moreover, it can be hoped that, thanks to advanced current research, relationships between resistance to different diseases will be better understood from a genetic and a mechanistic standpoint and therefore better monitored. Clearly, in the near future, the use of genetics should become one way of combating parasites, in synergy with other ways (vaccination, nutrition, environmental control). Best results will come from an epidemiological view of the parasites and additional research is still needed to combine all approaches optimally.

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20 Selection for Disease Resistance: Conventional Breeding for Resistance to Bacteria and Viruses

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Introduction

Breeding for disease resistance in poultry has a long history. Resistance of animals was selected naturally at the beginning of commercial selection, when no protected areas were available. However, the efficiency of such a method was limited as pathogens varied and the lack of knowledge did not always make it possible to distinguish between unrelated diseases, as for example when Marek's disease and lymphoid leukosis were grouped as lymphomatosis. Animals from primary breeders are now reared in protected buildings and no longer in contact with pathogens. As for other selection criteria, several questions have to be answered before there can be any practical application (Payne, 1973).

The existence and importance of genetic variability have to be investigated first. Documenting differences in resistance between breeds or lines is usually the initial step. This requires observing a limited number of animals but does not conclusively demonstrate the existence of genetic variability. Estimating heritability makes it possible to predict what could be expected from selection. This analysis of genetic variability largely benefits from advances in methods of estimation of genetic parameters for normally distributed traits (see Besbes and Ducrocq, Chapter 9) and all-or-none traits (such as mortality/survival), which are very frequent for such analysis.

Choosing the exact measurement of resistance is probably the most important question. A distinction is usually made between resistance to infection or true resistance, when the host genotypes can preclude invasion by the pathogens, and partial resistance, when pathogens may enter the animal but do not result in severe disease (Gavora, 1990). Resistance to carrier-state (i.e. ability of the animal to eliminate a pathogen) should also be considered to prevent transmission of pathogens from apparently healthy animals to other animals or human beings. Natural exposure used to be the most common method but is less repeatable than experimental inoculation, which involves choosing the route and dose of inoculation. The characteristics of the pathogen must also be considered, as they will influence the dose and length of observation.

As it is not possible to rear inoculated animals with uncontaminated animals, selection has to be on collaterals or less frequently on progeny. This also increases the selection costs. Identifying indirect

selection criteria such as immune response could preclude the need for inoculation of a pathogen, but only if genetic correlation with resistance is high enough. Estimating correlation between resistance and economic traits is of major importance for commercial breeders because it will allow prediction of the extent to which selection for increased resistance will reduce the gain in the other criteria. The commercial value of selection will depend on this and on the significance of the disease. Typically, breeding for resistance has been intensified whenever losses were increasing and other means of control seemed ineffective.

The identification of resistance genes is the final step. It will be facilitated by the development of genomics and the existence of susceptible and resistant poultry lines. Selection will in turn take advantage of marker genes or of the genes themselves. A brief summary of current knowledge on this topic will therefore be included in this chapter.

Resistance to Mareks's Disease

Marek's disease (MD) is an economically important disease of chickens characterized by the development of lymphoid tumours in various tissues and organs, including nerves, muscles and viscera (Gavora and Spencer, 1979), which caused high mortality in North America and other parts of the world before the disease was recognized under its present name. Early publications on genetic resistance or susceptibility to fowl paralysis, avian leucosis (Hutt and Cole, 1948) or lymphomatosis (Heisdorf *et al.*, 1947) probably in fact dealt with MD or a combination of MD and lymphoid leukosis (LL) viruses. Cole (1968) was one of the first authors to use the term Marek's disease in connection with the development of MD and LL resistant and susceptible lines. A review of historical research work to identify MD and LL as two separate disease complexes can be found in Calnek and Witter (1997). LL, caused by a vertically transmissible retrovirus, has been eradicated from most commercial pedigree populations, whereas MD, caused by a herpesvirus that is not egg transmitted, is a continuing threat to the poultry industry.

When the first MD vaccines became available (about 1970), the need for continued selection for resistance had to be re-evaluated. Since then it has been learned that vaccination is never 100% effective and that genetically resistant chickens respond better to vaccination (Gavora, 1990). Also, the tendency of the MD virus (MDV) to mutate to more virulent forms (Witter, 2001a,b) is another strong argument to continue breeding for improved MD resistance. Indeed, the goal of improved genetic resistance is not to eliminate the need for vaccinating day-old chicks, but to lower mortality in the case of field infection before vaccinal immunity becomes effective. Mortality due to MD in broilers is usually negligible; culling on the growing farm and condemnations in the processing plant can be minimized by proper cleaning and disinfections between batches. Where commercial broilers are grown to higher weights (40 days of age or more) in areas of high poultry concentration and a history of significant condemnations due to MD, prophylactic vaccination has become standard practice, increasingly using *in ovo* techniques to save labour. The need for reduced susceptibility to MD in the case of field infections is more obvious for layers and parents of meat-type chickens than for commercial broilers. The interest of the research community in MD seems to be as lively as ever, as documented in the proceedings of the Marek's symposia in 1978 in Berlin (Biggs, 1980), 1984 in Ithaca (Calnek and Spencer, 1985), 1988 in Osaka (Kato *et al.*, 1988), 1992 in Amsterdam (Dutch Branch of the World Poultry Science Association, 1992), 1996 in East Lansing (Silva *et al.*, 1996) and 2000 in Montreal (Schat *et al.*, 2001).

Evolution of MD incidence with time

Table 20.1 summarizes results from five German random sample testing stations for

Table 20.1. Mortality statistics from German random sample tests for laying hens, 1974–1998 (source: original reports of 111 tests).

Tests starting in years	No. of tests	No. of entries	Total mortality (%)		% MD and LL (20–72 weeks)
			0–20 weeks	20–72 weeks	
1974–1978	25	505	1.8 (0.1–5.0)	7.1 (3.6–10.0)	1.8 (0.2–6.3)
1979–1983	24	496	2.0 (0.1–4.9)	5.3 (2.0–8.2)	0.5 (0.1–1.8)
1984–1988	24	426	1.2 (0.2–4.4)	4.5 (2.7–7.2)	0.3 (0.0–1.3)
1989–1993	24	421	1.7 (0.1–8.3)	5.3 (2.3–11.0)	0.4 (0.0–4.2)
1994–1998	14	245	1.2 (0.3–3.4)	5.5 (2.1–8.6)	0.1 (0.0–0.2)

the 25-year period from 1974/75 to 1998/2000, covering a total of 111 tests and 2093 entries with about 100–160 hens each. Data from earlier years would have been difficult to compare because of the transition from floor to cage management and the gradual introduction of MD vaccination. Although causes of death were usually not recorded for the rearing period, inspection of the original reports indicates that losses due to the 'classical' form of MD were practically zero. Only one out of 111 tests had unusual mortality before 8 weeks of age, apparently due to a staphylococcus infection that only affected some White Leghorn entries. The only case with high mortality between 9 and 20 weeks was explained by a severe outbreak of gumboro.

Correct differentiation between LL and MD during the laying period is doubtful in these data: some stations reported only MD, others only LL, some tried to divide the mortality between these two causes. Inspection of the original reports suggests that most of the MD mortality during the laying period was in fact LL. Typically, the incidence varied considerably between strains, and specific white-egg strains had a higher incidence of tumour mortality across several stations and years. Elimination of some strains and LL eradication in others has changed the pattern of mortality during recent decades and the incidence of MD and/or LL in German random sample tests for layers has become negligible.

Such data from past random sample tests may have limited value for a critical assessment of the MD situation in different regions at different points in time, but comparable field data are not available. In practice, we are confronted with cases of MD breaks in different parts of the world. They usually result from insufficient biosecurity, sometimes combined with poor efficacy of the vaccine (vaccine virus and storage of a given batch), compromised ability of the chicks to develop immunity (e.g. due to chicken anaemia virus (CAV) or gumboro infections) and/or very virulent field strains of MD. Every MD break is a reminder that commercial chickens are more or less susceptible to MD and that the complex prophylactic programme has to be further tightened up (Voss *et al.*, 1997). Benefits from increased biosecurity will, of course, not be limited to lowering the risk of MD mortality.

Differences between and within strains

In one of the first publications documenting strain differences in MD mortality, Cole (1968) compared cockerels of ten strains of commercial layers from two subsequent random sample tests against the susceptible Cornell S strain. He found significant correlations between resistance assessed in different years by different challenge methods (inoculation vs. exposure) in males as well as between resistance of males challenged by inoculation and naturally exposed females. Brown-egg layers tend to be less susceptible to MD than White Leghorns, but specific White Leghorn crosses may actually be more resistant. In the strain comparison reported by Flock *et al.* (1976), three white-egg strains (A, B, C) had higher MD mortality than the brown-egg control (D), but one white-egg strain (E) was more

resistant in three out of four challenge treatments. In a more recent study, Flock *et al.* (1992) reported data demonstrating that not only White Leghorns but also brown-egg strain crosses will exhibit high MD losses if they are not protected by vaccination and challenged by virulent field strains of MD: cumulative losses to 18 weeks were 51.1 and 54.6% (as intended by the challenge) but continued and reached 92.6 and 92.8% at 28 weeks, compared with 100% of the SPF White Leghorn controls at 24 weeks of age.

The results from a recent MD challenge test (Table 20.2) illustrate the high level of mortality that may be encountered in unvaccinated chicks exposed to a virulent field strain, but also the variable mortality of two single crosses of White Leghorns (A × C, A × D) following different experimental combinations of vaccine batches and their application. The chicks in this test were pedigreed progeny of 80 sires of a common male line, with a maximum of eight sons and 20 daughters per sire and mating type for genetic analysis, the remainder being used to compare the efficacy of different vaccination schemes against a severe MD challenge by contact exposure to chicks previously injected with a highly virulent field strain.

In this case, one of the experimental treatments gave the best results for A × C, but across all treatments A × D had 6% lower

mortality. Escudero Gomez *et al.* (1991) reported a highly significant reversal of ranking for MD mortality in two White Leghorn pure lines in two subsequent generations, without any apparent explanation. Strain comparisons are mainly of internal value for breeding companies who need to know where their own products stand relative to major competitors. A single well-designed test may yield more information than a large volume of field observations without standardization of conditions. If a breeder decides on the basis of competitor comparisons to increase selection pressure for MD resistance, it will generally be within-line selection, perhaps by splitting lines into sublines to be selected on different criteria (Flock, 1974). It is unlikely to find a more resistant line that will also improve the overall merit of a successful multiple strain cross.

Selection for MD resistance in experimental lines

Early attempts to improve genetic resistance against MD started with 'natural exposure', i.e. placing day-old chicks in facilities with older virus shedders. Later, the challenge techniques were improved by intranasal and/or intraocular application of a standard

Table 20.2. Results of MD challenge test involving two pedigree White Leghorn single crosses (A × C, A × D) and several experimental combinations of vaccines and their application (Lohmann Tierzucht, 2000, unpublished).

			Mortality (%)	
Treatment	Sex	*n*	A × C	A × D
Control (unvaccinated)	Male	575	90.8	90.0
Rispens	Male	364	61.4	53.0
Rispens	Female	1568	63.9	55.5
Rispens + HVT	Female	1569	53.9	51.7
Exp. A	Female	809	58.3	47.1
Exp. B	Female	890	60.0	53.7
Exp. C	Female	198	58.6	51.5
Exp. D	Female	198	13.1	22.2
Exp. E	Female	200	33.0	22.0
Exp. F	Female	198	34.0	39.8
Exp. G	Female	198	67.7	55.5
Average (vaccinated)	Female	5828	56.0	50.1

dose of whole blood from clinically sick birds. Depending on the virulence of the challenge virus, mortality rates between 30 and 60% were usually achieved. Challenge by intraperitoneal (i.p.) injection of whole blood or suspensions of fresh tumorous gonads from clinically sick birds was also tested, but later discontinued because the mortality rates exceeded the optimum of 50% and it was not clear whether injection might circumvent some mechanism of natural defence.

Of the MD selection experiments conducted at several locations, the work of Cole (1968) at Cornell has been the most extensive and most widely recognized. In this selection programme, pedigreed cockerels were injected i.p. at 2 days of age with a suspension of fresh tumorous gonads (JM material) from older chicks. Results of selection for susceptibility (S line) or resistance (C and K lines) were later tested on the basis of female chicks intentionally exposed to naturally occurring strains of viruses for Marek's disease and lymphoid leukosis. From the results, Cole (1968) concluded that the challenge of cockerels with JM material was effective in ranking families on their resistance to naturally occurring MD, whereas the correlation with LL mortality was low. Published shortly before the first vaccines against MD became available, the discussion of this selection experiment contains several critical questions that may determine the success of other MD selection programmes: (i) correlation between resistance to one specific isolate of field virus and some or all other MD viruses; (ii) correlations between MD resistance and other traits of economic importance; and (iii) correlation between improved MD resistance in one strain and commercial crosses involving this strain.

MD selection experiments involving commercial lines and estimation of genetic parameters

The extent to which results of MD challenge trials with inbred lines or other experimental strains should be applicable in commercial strains of layers or broilers remains an open question. This section will therefore concentrate on experience with MD challenge tests involving commercial strains of layers. Von Krosigk *et al.* (1972) summarized results from MD challenge tests between 1961 and 1968, involving 18 tests in three countries (USA, Spain, Germany) with a total of 37,998 progeny of 2504 White Leghorn sires. Average heritability for MD resistance transformed to 50% mortality was 0.14. It was 0.10 for exposure by contact, compared with 0.20 for i.p. injection. The genetic correlation between both measures was only 0.38, suggesting that contact exposure may be more efficient for breeding programmes. Genetic correlations between MD mortality (to about 20 weeks) and total laying house mortality (20–68 weeks of age) in non-challenged half-sibs estimated from this large data set were positive (+0.36), contributing to a desirable correlation between MD mortality and housed-hen egg production (–0.18), while correlations with body weight, egg weight and shell strength were insignificant. However, the results of a 5-year selection experiment summarized in Table 20.3 show a disadvantage in laying house mortality (11.9 vs. 8.4%) of the MD-selected sublines compared with the main lines selected on a production index. Based on a smaller data set, Friars *et al.* (1972) observed a much higher realized heritability (0.67 ± 0.30) in a broiler female line after i.p. inoculation, which they attributed to the efficacy of family selection. Hartmann and Sanz (1971) compared MD mortality of broiler type chicks involving 13,000 chicks from ten pure lines and 21 cross-line combinations, using different types of challenge in four experiments. Their main conclusion was that cross-lines tend to be less susceptible to MD than pure lines and that cross-line liveability can best be predicted from pure lines under conditions of contact exposure, whereas correlations involving i.p. challenge were lower. In a subsequent test involving 4000 pedigree chicks of three crosses, they found a rather low heritability (0.06) and a low genetic correlation (+0.26 ± 0.19) with 8-week body

Table 20.3. Cumulative selection response in 5 years to index selection vs. MD selection (Flock *et al.*, 1975).

Cross	No. males[a]	MD-Mort. (%) 1–22 weeks	No. females[b]	Mortality (%) 20–68 weeks	Egg no. per hen housed	Egg weight (g)	Shell strength (Newtons)
A × B	1332	54.7	2552	9.4	269.4	59.1	39.8
B × A	1622	55.6	2803	7.5	268.8	58.9	41.9
Index-selected	2954	55.2	5355	8.4	269.1	59.0	40.8
A′ × B′	210	32.4	426	16.4	245.1	56.6	41.5
B′ × A′	258	37.2	447	7.5	252.2	57.2	43.6
MD-selected	468	34.8	873	11.9	248.6	56.9	42.5
A × B′	293	50.9	769	10.6	261.8	59.1	41.0
B × A′	408	35.3	773	8.8	258.4	57.4	42.1
Index*MD	701	43.1	1542	9.7	260.1	58.2	41.6

[a]Males MD challenged by exposure.
[b]Females vaccinated and reared without deliberate exposure to MD.

weight, suggesting that fast-growing broilers may be more susceptible to MD (Hartmann and Sanz, 1974).

Data in Table 20.3 are based on results of a 5-year selection experiment involving two White Leghorn lines under reciprocal recurrent selection (RRS) published by Flock *et al.* (1975). Selection based on survival of cross-line daughters and half-sibs following contact exposure lowered MD mortality in reciprocal crosses (A′ × B′ and B′ × A′) compared with 'controls' selected on a production index under conditions of no MD exposure (A × B and B × A). In this selection experiment, MD mortality of males under conditions of contact exposure to a field virus was reduced by approximately 20% within 5 years (three generations of progeny testing), while their sisters laid about 20 eggs less per hen housed compared with the index-selected main lines (five generations of sib testing) in an environment with normal disease control. The index was constructed to hold egg weight and shell strength constant at a competitive level. The loss of egg weight in the MD-selected sublines could be interpreted as mainly due to natural selection, and the difference in shell strength as a result of the strong negative correlation between egg weight and shell strength. Somewhat unexpected was the difference in adult female mortality, giving the index-selected main lines an advantage of 3.5% (8.54 vs. 11.9). Combinations of

MD-selected sublines and index-selected main lines were generally intermediate. The crosses with reduced susceptibility to MD were clearly not competitive under conditions of reasonably effective MD control by vaccination and avoidance of early exposure to field infections.

While most of the results are within expectations, the significant difference in MD mortality between the reciprocal crosses of index-selected males × MD-selected females (A × B′ vs. B × A′) was unexpected. Apparently only subline A′, but not subline B′, had responded to selection for MD resistance. Unfortunately allele frequencies of MHC-related blood groups were not monitored during the experiment, but later analyses revealed that line A had predominantly B^{15} (from the literature thought to be related to MD susceptibility), whereas line B segregated for B^{19} and B^{21} at almost equal frequencies. If B^{21} had been a major factor contributing to MD resistance in these lines, the changes in frequency should have occurred in line B. This puzzling result may be explained by looking at the MD mortality (at least in this genetic background) of pure-line vs. cross-line progeny of heterozygous B^{19}/B^{21} sires summarized in the Table 20.4.

The conclusion from these results was that selection for the B^{21} allele can help to reduce MD susceptibility in this particular White Leghorn pure line, but variation in the

Table 20.4. MD mortality in pure-line and cross-line progeny of sires with different genotypes for *B* alleles (modified after Flock, 1993).

Genotype of sire	Cross-line daughters – 1978	Pure-line daughters	
		1978	1991
B^{21}/B^{21}	33.0	19.0	8.2
B^{21}/B^{19}	30.9	26.8	17.8
B^{19}/B^{19}	40.5	37.6	25.3

frequency of other genes must also be exploited to make the commercial cross relatively more resistant.

Practical implications

The selection experiment cited above was terminated after MD vaccines became available. Obviously, selection for MD resistance will not contribute significantly to reduced mortality in the field unless it is practised within commercial strains with a significant market share. The success of a breeding plan to reduce the susceptibility to MD in a particular population depends on the genetic parameters of the source lines and the design of the challenge experiments. Several options that may be considered for challenging pedigreed chicks are outlined below.

FEMALE OR MALE CHICKS. Sex differences have been reported from several experiments, with variable results: males tend to die at a slightly lower frequency or later than females (for example, compare lines 2 and 3 in Table 20.2). Males have often been used for challenge tests for the simple reason that they are available as by-products of pedigree hatches. If the objective is to improve the liveability of females, as in laying stocks and broiler breeders, it is important to verify that the correlation between male and female mortality is high. In particular, late forms of MD, affecting different organs, may have a genetic correlation between sexes significantly below 1.0.

CHALLENGE OF UNVACCINATED VS. VACCINATED CHICKS. Until several years after the advent of MD vaccines, it was commonly agreed that challenging unvaccinated chicks was a reasonable approach for testing genetic resistance. Most researchers in this field now agree that test chicks should get a complete vaccination programme during the MD challenge tests, especially to eliminate gumboro and chicken anaemia agent (CAA) as sources of variation. If chicks are vaccinated against MD before the challenge, the use of two different types or sources of vaccine may produce interesting additional information if full-sibs are assigned to different treatments. Of recent interest is the question of whether low frequency MD losses observed in some growing farms may also have a genetic basis or are simply due to 'missing' chicks at vaccination.

CHALLENGE AFTER ONE OR TWO WEEKS. Instead of exposing day-old chicks to infected litter, they can be challenged by intranasal and/or intraocular application of field virus at a later age, preferably before 2 weeks of age to get a high enough average level of mortality to expose genetic differences between families. In contrast to most US trials designed to compare different MD vaccines, which use i.p. challenge at 5 days of age to obtain the desired average mortality at an early age, breeders prefer a milder challenge by contact and a longer observation period.

CHOICE OF THE CHALLENGE VIRUS. For interpretation of the results it is desirable to identify the field virus used for the challenge tests. As more virulent strains are found in different parts of the world, it is important to know whether relative resistance to one type of virus also conveys resistance to other types of virus. Mortality close to 100% has been observed in challenge experiments with unvaccinated chicks exposed to very virulent MD strains. Apart from biosecurity considerations, geneticists prefer to work with MD field strains that produce mortalities in the range of 30–50%.

CHOICE OF THE OBSERVATION PERIOD. Most early challenge experiments concentrated on the 'classical' form of MD. Mortality and lameness with neural lesions were observed between approximately 8 and 18 weeks of age; survivors were sacrificed before reaching sexual maturity. For genetic improvement, breeders today prefer extended periods of observation, i.e. until the weekly mortality becomes negligible, though the heritability may decrease if the mortality exceeds 50% (Flock *et al.*, 1992).

CROSS-LINE OR PURE-LINE CHICKS. Differences between pure lines and reciprocal crosses between them have been found in many challenge tests. Challenge tests involving pedigree crosses of commercial lines are likely to produce more genetic progress in the long run than indirect selection based on pure-line data that may appear superior in short-term experiments. Using artificial insemination, cross-line and pure-line progeny can be produced simultaneously from the same selected sires, whereas progeny testing of selected females with a sufficient number of progeny has practical limitations.

Outlook for conventional selection

In more than 30 years since the introduction of MD vaccination, the scientific community has contributed a wealth of specific information toward the understanding of Marek's disease but no concept has yet been found for successful control comparable to LL eradication. Theoretically, it should be possible to eradicate MD on a regional basis, because the virus is not vertically transmitted: chicks from any parent flock placed in an isolated environment without MDV will not exhibit any MD mortality. This has been demonstrated many times – for example, by one of the German random sample testing stations, Neu-Ulrichstein, which decided not to vaccinate until the need became apparent. Unfortunately field strains of MD have become ubiquitous. They survive in vaccinated flocks and even in empty poultry houses for many months, they are easily

transmitted horizontally and they tend to mutate to more virulent forms. Since biosecurity is seldom perfect on commercial farms, improved genetic resistance remains a goal for egg-type chickens and broiler breeders. To what extent breeding companies invest in additional testing and selection for specific MD resistance will depend on the performance profile of their strains. As with other diseases, to reduce MD losses worldwide under field conditions it would be helpful to validate, on pedigree chicks from commercial breeding programmes, the results obtained on experimental inbred lines in basic research and development of new types of vaccines.

Contribution to knowledge of underlying mechanisms and perspectives

Since the major histocompatibility complex (MHC) or B complex has been identified as a locus responsible for resistance (Hansen *et al.*, 1967), numerous experimental lines with specific MHC alleles have been developed in which three mechanisms of resistance have been identified.

The first is associated with at least one locus in the MHC. It has been shown that the B^{21} allele confers strong resistance; a few haplotypes (B^2, B^{14} and B^6) confer intermediate resistance and several others (B^1, B^3, B^5, B^{13}, B^{15}, B^{19} and B^{27}) confer susceptibility to MD (Longenecker and Mosmann, 1981). Three major types of genes have been identified in the chicken MHC (Guillemot *et al.*, 1988); they encode cell surface antigens with various tissue specificity and control the development of immune response. The MHC of the chicken is split into two independent genetic regions: the B and $Rfp\text{-}Y$ regions (Briles *et al.*, 1993); both of them encode class I and class II antigens and the B complex encodes the class IV antigens. The class I antigens that are expressed in the vast majority of the cells are involved in the presentation of endogenous peptides to CD8 T lymphocytes; when the peptide is of foreign origin (viral protein for example) the T lymphocytes kill the target cell. The class II

antigens present exogenous peptide to CD4 T lymphocytes, which activate cellular or humoral response through Th1 and probably the Th2 lymphocytes, respectively. The class IV antigens, first found expressed on erythrocytes, have no clear role at the moment. Briles *et al.* (1983) showed that, within the *B* complex, there was a gene located in the classI/class II region that is involved in resistance to MD. On the other hand *Rfp-Y* has little (if any) effect on the resistance (Wakenell *et al.*, 1996; Vallejo *et al.*, 1997; Lakshmanan and Lamont, 1998). Studies on the level of expression of class I and class II antigens at the surface of cells have revealed that the expression of class II antigens was equivalent in resistant and susceptible chickens. On the contrary, the resistant B^{21} chickens had fewer class I antigens at the cell surface (Kaufman *et al.*, 1995). The mechanism by which low class I antigen expression could account for resistance has not yet been elucidated. The more likely hypothesis at the moment is that resistant chickens develop a better natural killer (NK) cytotoxicity. NK cells belong to the category of T cytotoxic lymphocytes; they eliminate tumoral or infected cells. A complex balance between inhibitory and stimulatory receptors controls NK cytotoxicity (O Long and Wagtmann, 1997). In addition to their role in presentation of peptides to CD8 T lymphocytes, MHC class I antigens may bind to inhibitory receptors at the surface of NK cells, thus inhibiting NK cytotoxicity. Resistant chickens that express fewer class I antigens could thus develop a better NK response and eliminate the tumour or infected cells more efficiently. Indeed it has been shown that inoculation of MDV enhances NK activity (Sharma, 1981) and that chickens from the resistant N line have a better NK activity than chickens from the susceptible P line (Sharma and Okazaki, 1981). However, direct measurement of class I antigens at the cell surface cannot be used for selection because it requires flow cytometric analysis, which is too costly and time consuming. Identification of new markers involved in the regulation of MHC antigen expression should provide new tools to identify easily chickens that express low

levels of class I antigen but this will require much work and time, as this regulation is complex and most probably involves many genes.

The second mechanism of resistance was found in lines 6_1 and 7_2, which are, respectively, resistant and susceptible to MD (Stone *et al.*, 1970) and share the same MHC haplotype (B^2). In a backcross $(6_1 \times 7_2) \times 7_2$ population, Bumstead (1998) showed that resistance was associated with a locus on chromosome 1, equivalent to the region in mammals where genes that regulate the NK activity lie. Evaluation of the viral loading by quantitative PCR showed that viraemia was lower in the resistant line 6_1 (Bumstead *et al.*, 1997). As evaluation of the viraemia requires inoculation of the chickens in protected facilities, it could not be used as a routine test for selection.

A third mechanism has been identified outside MHC. The growth hormone (*GH*) gene is implicated in the control of numerous physiological parameters, including immune response. The GH was found to be synthesized by lymphocytes in culture and different immune cells express the GH receptor; it could have an immunostimulatory effect on primary lymphoid organs (Marsh, 1992). Polymorphism of the *GH* gene was thus investigated in two strains of White Leghorn chickens selected for resistance or susceptibility to MD: one *GH* allele was present with a higher frequency in the resistant strain (Kuhnlein *et al.*, 1997). This result indicates that *GH* could either be a marker gene of resistance or be directly involved in resistance, which is supported by very recent results showing that the product of the *GH* gene may interact *in vitro* with a viral protein expressed only in a virulent strain, and that *in vivo* the viral and GH proteins may be co-expressed in some MDV-infected cells (Liu *et al.,* 2001).

Outlook for contribution of molecular genetics

All these results should contribute to increased selection efficiency. In

experimental lines, the B^{21} haplotype is strongly associated with resistance. Little is known about the effect of MHC on resistance to MD in commercial lines. Few data indicate that B^{21} could be associated with resistance in broilers. Typing of MHC haplotypes in large populations is now possible due to the development of a PCR test using a polymorphic microsatellite located in the *B* complex (Zoorob *et al.*, 1998). The identification of the MHC haplotypes segregating in commercial lines and the evaluation of their role in resistance could contribute to an increase in the general level of resistance through selection of the haplotypes associated with resistance.

Positional and functional genomics, i.e. the two major areas of development of molecular genetics, will provide new markers. International collaboration has produced a map of the chicken genome where 2000 loci have been mapped (Schmid *et al.*, 2000). The markers from this map may be used to reveal the non-MHC loci associated with resistance. By using 127 markers it has been possible to identify seven loci that are associated with different MD traits (Yonash *et al.*, 1999).

Functional genomics allows simultaneous analysis of the level of expression of many genes (more than 1000 with DNA microarrays). This approach was used to identify genes that were induced following infection with MD virus (MDV). Microarrays containing more than 1000 cDNA from an activated T-cell library have been hybridized with whole mRNA from MDV-infected and non-infected fibroblasts. Twenty-two genes were shown to be up-regulated in infected fibroblasts (Morgan *et al.*, 2001). Using this technique in resistant and susceptible chickens will reveal new genes implicated in resistance, which should contribute to both a better understanding of resistance and the development of marker-assisted selection.

Even if these recent results still have to be confirmed in commercial populations, they open up large areas of research and may, in the longer term, have promising practical applications and provide new ways of understanding the mechanisms of resistance.

Resistance to Avian Leukosis Viruses

Avian retroviruses can cause a variety of transmissible benign and malignant neoplasms gathered under the denomination leukosis/sarcoma diseases (for a review see Payne and Fadly, 1997). These viruses are now termed avian leukosis virus (ALV)-related viruses and classified as γ retroviridae. They have similar physical and molecular characteristics and share a common group-specific antigen. ALV-related viruses that occur in chickens have been divided into six subgroups (A, B, C, D, E and J) on the basis of their host range in chicken embryo fibroblasts, interference patterns and viral envelope antigens borne by the viral envelope glycoproteins.

Resistance to infection is the first level of resistance to be recognized. It leads to a state of absolute resistance: genetically resistant chicks are resistant to infection and tumour induction by ALV-related viruses of the subgroups involved. A second line of resistance results in chicks susceptible to infection developing resistance to tumour development. This second level of resistance has been studied mainly with Rous sarcoma.

Resistance to virus infection

Infection by ALV-related viruses of subgroups A, B and C is controlled by three independent autosomal loci designated *TVA* (Tumour Virus A subgroup), *TVB* and *TVC*, respectively, reviewed by Crittenden (1991). The *TVB* locus also controls responses to subgroup D virus (Pani, 1975) and linkage occurs between *TVA* and *TVC* loci (Payne and Pani, 1971). Inheritance of cellular resistance to infection is of a simple Mendelian type. Alleles for susceptibility and resistance exist at each locus and the susceptibility alleles are dominant over the resistance alleles. Inheritance of resistance to subgroup E virus is more complex. Initial findings demonstrated involvement and interaction of genes at two autosomal loci (Payne *et al.*, 1971). It was later reported

that susceptibility alleles at the *TVB* locus are required for susceptibility to subgroup E virus and the actual existence of a separate *TVE* locus is debatable (Crittenden *et al.*, 1973).

Alleles for susceptibility are identical to cell-membrane surface receptors that interact with viral envelope glycoproteins and allow viral penetration and infection of the cell. The nature of such receptors has recently been identified. The ALV subgroup A receptor is related to the low-density lipoprotein receptor (Bates *et al.*, 1993). Subgroups B, D and E of ALV utilize tumour necrosis factor receptor (TNFR)-related proteins to infect susceptible chicken cells (Brojatsch *et al.*, 1996; Adkins *et al.*, 1997). The *CAR1* gene encoding a receptor specific for ALV subgroups B and D maps to the chicken *TVB* locus (Smith *et al.*, 1998). Genetic resistance to infection with subgroup J virus has not been identified in chickens, although a number of other avian species are resistant (Payne *et al.*, 1992). Moreover, a receptor for ALV subgroup J has not yet been identified.

In practice, selection for resistance to viral infection is difficult to achieve for the following reasons: (i) susceptibility and resistance to infection are subgroup specific; (ii) genotyping of unknown populations requires cumbersome testing; and (iii) resistance to the actual predominating subgroup J virus has not been identified. Indeed, mutant subgroup viruses are likely to arise that could overcome resistance from a single gene. There is obvious value in increasing resistance of infected birds to development of neoplasms.

Resistance to tumour development

Resistance to development of neoplasms has been studied experimentally mainly with Rous sarcoma. Inoculation of susceptible chickens with Rous sarcoma virus (RSV) rapidly leads to formation of a sarcoma (a tumour of connective tissue). The fate of the tumour varies considerably from one animal to another, which is expected to increase the efficiency of selection. Chickens that develop massive tumours that persist through the experimental period of observation and may die are classified as progressors, while chickens whose tumours grow but then decrease in size and may disappear completely, due to the development of an immune response, are classified as regressors.

Selection experiments and estimated genetic parameters

A preliminary study by Greenwood *et al.* (1948) showed that non-susceptibility to Rous sarcoma was heritable as the result of both resistance to infection and tumour development. Gyles *et al.* (1967, 1968) developed research on the regression of RSV-induced tumours: they observed highly significant differences in regression between a susceptible White Leghorn and a resistant jungle fowl and studied F_1 and F_2 crosses between both lines, which suggested genetic influence on tumour regression. Their results led them to conclude the existence of two mechanisms of resistance, the first impairing malignancy and the second impairing tumour growth. Gyles and Brown (1971) showed that the rate of Rous sarcoma regression could be increased by selection. The base population on which their selection experiments were performed was a cross between Leghorns and jungle fowl. Selection was based on both individual and family performance after viral inoculation at 6 weeks of age. In the sixth generation the percentage of regressors was 59.2%, vs. 14.3% in the first generation. Gyles *et al.* (1982) estimated the heritability of number of days to regression and death to be 0.38 and 0.26 in these lines, which is consistent with the efficacy of selection.

MHC genetic influence on regression of Rous sarcoma

The MHC B complex was the first major determinant identified in the control of RSV tumours. In the early work of Collins *et al.* (1977), the two inbred lines 6_1 (B^2/B^2) and

15_1 (B^5/B^5) were used to produce F_1 and F_2 generations. Segregant chickens of the F_2 generation inoculated into the wing-web with a Bryan strain of RSV of subgroup A at 6 weeks of age were scored for tumour size and showed the association of the B^2 haplotype with regression. Neither D nor I blood group loci genotypes nor sex significantly affected tumour growth, demonstrating the crucial role of the *B* complex in determining the outcome of RSV-induced tumours in these lines at least. In the same period, Schierman *et al.* (1977) investigated sarcoma regression, in a backcross between two partially congenic lines, G-B1 (B^{13}/B^{13}) and G-B2 (B^6/B^6). Rous sarcoma was induced by wing-web inoculation with the Schmidt-Ruppin strain of subgroup B. The results in the challenged backcross progeny, (G-B1 × G-B2) × G-B1, led to the assumption that a single, dominant gene linked to the MHC has a major effect on the development of RSV-induced sarcoma.

The *B* complex was later shown to control not only primary tumours induced by viral inoculation but also the tumour growth induced by subviral DNA constructs carrying the v-*src* oncogene without any viral replication gene. Intramuscular inoculation of v-*src* DNA allows investigation of tumour-specific resistance without any contribution of viral response (Fung *et al.*, 1983; Halpern *et al.*, 1990). Taylor *et al.* (1992) observed a significant difference in the growth of v-*src* primary tumours in two chicken lines congenic for *B* complex alleles.

As demonstrated by Collins *et al.* (1985), the development of metastases after a primary viral inoculation is partially controlled by MHC, depending on the genetic background of the chicken population. Taylor *et al.* (1992, 1994) observed regression of v-*src* primary tumours and more resistance to v-*src* tumour metastasis in congenic line 6.6-2(B^2B^2) compared with congenic line 6.15-5(B^5B^5).

Genetic organization of the MHC complex does not allow independent segregation of the B-F and B-L regions. However, a few recombinants between B-F/B-L and B-G regions have been produced by Hala *et al.* (1976) and Briles and Briles (1977). The use of these recombinants to study regression in Rous sarcoma has shown that the locus responsible for tumour regression lies within the B-F/B-L region or in a region closely linked to it (Collins and Briles, 1980; Plachy and Benda, 1981; Aeed *et al.*, 1993; Auclair *et al.*, 1995).

A phenomenon of allelic complementation between MHC haplotypes has been observed by different groups using crosses of inbred congenic lines and backcross generation progenies (Cutting *et al.*, 1981; Plachy, 1985; Senseney *et al.*, 2000). Interestingly, using a moderate dose of virus, Senseney *et al.* (2000) described increased regression of Rous sarcomas in $B^Q B^{17}$ chickens from the second backcross generation of line UCD001 ($B^Q B^Q$) inbred jungle fowl and line UCD003 ($B^{17}B^{17}$) compared with genotypes $B^Q B^Q$, $B^{17}B^{17}$. It should be noted that the B^Q haplotype has serological and electrophoretic similarity to the B^{21} haplotype (Miller *et al.*, 1984), which confers resistance to MD.

Non-MHC genetic control of sarcoma regression

The studies reported by Cutting *et al.* (1981) and Plachy (1985) detected not only MHC gene complementation as described above but also the effect of non-MHC-linked genes in the control of resistance to Rous sarcoma. The frequency of regressor chickens observed in the backcross matings and hybrids corresponded to the expected frequency of birds heterozygous for allelic genes at two independent loci. The effect of non-MHC genes has been shown to be critical for regression of Rous sarcoma (Brown *et al.*, 1984) using similar or identical MHC haplotypes in different genetic backgrounds. The relative influence of MHC and non-MHC genes was also evaluated by Gebriel and Nordskog (1983). Several loci have been shown to play a role in the fate of Rous sarcoma. Using Regional Poultry Research Laboratory chicken lines, Gilmour *et al.* (1983) showed the effect on regression of Rous sarcoma of two independent autosomal loci, *Ly-4* and *Th-1*,

which encode surface alloantigens on T lymphocytes. These results confirmed the report of Marks *et al.* (1979), who demonstrated the role of genes other than B and L blood groups in the same lines of chickens. LePage *et al.* (2000) examined the effect of segregating combinations of genes encoding blood group antigen systems A, C, D, E, H, I, P and L within B^2B^5 and B^5B^5 MHC backgrounds on regression of Rous sarcoma. The L system, or a locus linked to it, was shown to play a role in resistance to Rous sarcoma. The expression of endogenous viral genes is an additional genetic factor that has been shown to have an effect on the fate of Rous sarcoma in chickens. Plachy *et al.* (1985) found an association between expression of endogenous *gag* gene products and progression of Rous sarcoma.

Non-MHC genetic control of sarcoma regression explains why selection may be effective even within lines homozygous for *B* haplotypes. Selection by progeny testing within a White Leghorn B^{19}/B^{19} line led to the production of two divergent lines: one progressor and the other regressor (Dambrine *et al.*, 1986). Subsequent crosses between these two lines have demonstrated an association between the *ALVE1* endogenous locus, or a locus closely linked to it, and progression of Rous sarcoma (Thoraval *et al.*, 1999). The expression status of *ALVE1* remains to be determined in this chicken line, as it was initially described as a silent locus (Rovigatti and Astrin, 1983).

Other factors involved in the control of regression

It is an established fact that host age at inoculation influences the incidence of sarcoma regression and that the rate of regression increases with age. Other environmental factors may play a part in tumour growth, and reduction in tumour size has been reported with restricted feeding of chickens (Clark *et al.*, 1980). The strain of RSV used for inoculation has to be taken into consideration. Using different virus strains of subgroups B and C, McBride *et al.* (1981) observed that chickens of a given *B* complex genotype may be regressors to challenge

with one strain of virus but progressors to challenge with another strain belonging to the same viral subgroup. Regression of Rous sarcoma has been shown to be restricted to the virus subgroup used for inoculation and a significant host genotype/virus interaction was observed by Brown *et al.* (1984), thus confirming the data of Nordskog and Gebriel (1983).

Mechanisms underlying resistance to Rous sarcoma

ANTIGENS INVOLVED IN RESISTANCE. Two kinds of antigen may be involved in resistance to Rous sarcoma: viral proteins and v-*src* product- and transformation-associated antigens. A clear discrimination between these two types of response has been demonstrated by comparing development of Rous sarcoma following viral and v-*src* inoculation (Thoraval *et al.*, 1997). In the B^{19} White Leghorn progressors and regressors selected, two different responses were identified depending on the families used. One family of B^{19} chickens was resistant to tumour growth induced by both viral and v-*src* inoculation, thus indicating a response directed against v-*src* product- and transformation-associated antigens. On the other hand, another family of B^{19} chickens resistant to tumour growth after viral inoculation developed Rous sarcoma after intramuscular v-*src* inoculation, thus suggesting an antiviral response (Thoraval *et al.*, 1997).

INVOLVEMENT OF IMMUNE RESPONSES. Evidence that immune mechanisms have a role in the response against Rous sarcomas has been accumulating since the 1970s. The first line of evidence came from the observation of impairment of sarcoma regression after thymectomy (Cotter *et al.*, 1976b) or bursectomy (McBride *et al.*, 1978). The role of immune responses has also been demonstrated by the reduction of sarcoma growth after priming inoculations. Virus or tumour homogenate (Gyles *et al.*, 1977) and v-*src* inoculation (Wisner *et al.*, 1991; Plachy *et al.*, 1994) were shown to reduce development of Rous sarcoma. Vaccination with a low-oncogenic strain of RSV (Plachy *et al.*,

1995) also had a protective effect, thus demonstrating the involvement of viral antigens. Halpern *et al.* (1996) established major antigenicity of the v-*src* product and moderate but demonstrable antigenicity of the endogenous c-*src* product. Whitfill *et al.* (1986) demonstrated sarcoma remission in progressor chickens after transfer of histocompatible peritoneal macrophages and blood lymphocytes from regressors of RSV-induced tumours. Similarly, Plachy *et al.* (1994) showed a protective effect of immune cells from regressors of v-*src* DNA-induced tumours.

Several functional *in vitro* assays have been used to reveal the involvement of cell-mediated immunity in Rous sarcoma regression. An *in vitro* colony inhibition test (Sjogren and Jonsson, 1970), a leukocyte migration inhibition test (Cotter *et al.*, 1976a) and a Cr[51] microcytotoxicity assay (McGrail *et al.*, 1978) showed higher activity of lymphocytes from RSV-induced regressor chickens. Involvement of MHC-restricted cytotoxic T lymphocytes (CTLs) in regression of Rous sarcoma was demonstrated by Thacker *et al.* (1995) using target cells expressing MHC B-F cDNA inserted into a recombinant ALV vector. The mechanism of MHC-mediated recognition was explained by Kaufman *et al.* (1995), who showed recognition of more peptides from RSV by the regressor haplotype. Macrophage function has also been shown to be greater in regressor chickens than in progressors (Qureshi and Taylor, 1993). Cell-mediated immune responses are highly modulated by cytokines. Using a neutralizing monoclonal antibody to chicken type I interferon α in regressor chickens and recombinant chicken type I interferon α and interferon γ, Plachy *et al.* (1999) observed beneficial effects of both cytokines on sarcoma regression.

All these investigations illustrate the clear involvement of immune responses in resistance to Rous sarcoma, the complexity of the mechanisms that may be involved and the difficulty of discovering which factor plays a key role in the control of resistance.

Long-term perspectives

Rous sarcoma is a very valuable experimental model to investigate resistance to avian retrovirus-induced tumours, to improve knowledge on MHC recognition and to identify the role of other gene families in the control of tumour development. It should be noted that the influence of an MHC haplotype has been reported not only for Rous sarcoma but also for LL and MD (Bacon *et al.*, 1981). We have shown an association between *ALVE1* and progression of Rous sarcoma (Thoraval *et al.*, 1997). *ALVE1* has been mapped to the chicken chromosome 1 in a region close to the region containing the MD resistance gene described by Bumstead (1998). To improve breeders' chicken lines, further studies are needed to determine whether genetic markers are involved in the control of different kinds of tumour.

The practical applications of these experimental findings in poultry breeding need further investigations. As demonstrated by Hartmann (1997), the effects of the genetic systems first evaluated in experimental chicken lines must be verified in different genetic backgrounds.

Resistance to Salmonellosis

In the study of genetic resistance to *Salmonella*, the selection experiments took place before the research into differences between breeds or lines. The researchers first focused on resistance to acute disease, i.e. morbidity and mortality, as salmonellosis was at that time a significant source of animal and economic loss.

Resistance to disease

Selection experiments

The first selection experiments for increased resistance to salmonellosis in fowl took place many years ago. As early as 1928, Lambert and Knox cited prior works

that showed that selective breeding could enhance the resistance of mice to typhoid and of fowl to *Salmonella pullorum* (Roberts and Card, 1926, quoted by Lambert and Knox, 1928). They therefore initiated study of the genetics of resistance to *Salmonella gallinarum* by comparing the resistance of the offspring of 219 White Leghorns selected on resistance or susceptibility to oral infection. Neither age nor dose were clearly specified: animals weighed 'between 2 and 3 pounds' and the dose of *S. gallinarum* (SG), described as 'massive', was close to the lethal dose 50%, thereafter noted as LD_{50}, as it resulted in 47.7% mortality. Survivors, were further selected for fewest clinical reactions. Their offspring were infected intraperitoneally at 1 week of age with 1.2×10^7 bacteria. Selection resulted in increased resistance in the offspring, i.e. 47.7% less mortality. On the basis of these promising results, Lambert (1932) took these first two generations of selection and selected four more generations. Over the following generations, selection was based both on individual survival and family selection for lower mortality. At the fifth generation, the mortality rate was only 9.4% in the selected flock vs. 85% in the control flock. The kinetics of mortality also differed widely between the two stocks. According to the results of crosses between selected and unselected flocks, the influence of passive or acquired immunity appeared to be very small.

Estimation of genetic parameters

More recently, heritability of resistance to death was estimated by Beaumont *et al.* (1999) on 419 chicks from an egg-type line. Animals were inoculated intramuscularly at 1 day of age with a dose close to 10^3 colony forming units (cfu) and 32.46% of them died. Mortality (assessed as an all-or-none trait) was analysed using the threshold model (Gianola and Foulley, 1983) at 0.14 ± 0.10 and at 0.62 ± 0.16 from the sire and dam components, respectively, confirming that resistance to mortality was partly inherited. Discrepancy between these results might have resulted from maternal

or dominance effects (Ollivier, 1981) but, as chicks were the offspring of *Salmonella*-free flocks, the authors favoured the hypothesis of sampling variations between both components.

Janss and Bolder (2000) studied a meat-type outbred line previously selected for feed conversion. They inoculated 548 2-week-old chicks originating from 25 sires and 85 dams with 1.5×10^7 or 2×10^7 cfu *Salmonella enteritidis* PT4 intramuscularly. Mortality checked over 4 weeks was 29.2%. Heritability of mortality (i.e. resistance to mortality) was estimated at 0.12 ± 0.08, using the threshold model. Survival time was also investigated for the animals that survived and heritability was estimated at 0.06 ± 0.13. The correlation between the above two traits was estimated at -0.68 ± 1.05. If confirmed, this result suggests that selection for reduced mortality could increase the length of survival, and thus result in higher risk of contamination for both consumer and other animals.

Between-line variations

Many studies have shown that chicken lines differ considerably in resistance to salmonellosis. In particular, higher resistance of White Leghorns was noted as early as 1935 by Roberts and Card (quoted by Hutt and Scholes, 1941) and confirmed by the latter in a large comparison of breeds based on resistance to natural exposure or experimental inoculation with *S. pullorum* (SP) at 1 day of age or at 30 h. The authors suggested that this difference might be due to the effect of systematic testing of layers and culling of positive animals. This hypothesis was reinforced by DeVolt *et al.* (1941), who showed that selection of Single Comb Rhode Island Reds for progeny survival in the presence of natural infection by SP was effective. Moreover, in spite of the higher resistance of White Leghorns, such selection led to similar levels of survival in selected Rhode Island Reds and infected White Leghorn flocks where positive animals were systematically eradicated (i.e. about 30% instead of 8% in the most susceptible animals). Smith (1956) studied the

resistance of animals to oral inoculation with 5×10^7 *S. gallinarum*. In addition to the already known higher susceptibility of Leghorns, wide variations in resistance with age were noted. Much later, Aire and Ola Ojo (1974) observed that Nigerian cockerels showed better body condition and blood parameters and higher antibody titres after intramuscular inoculation than White Leghorns; however the resistance of the latter may have been modified by selection since Smith's study. An effect of MHC was observed by Cotter *et al.* (1998) but not by Bumstead and Barrow (1988).

Indirect selection criteria

All the above results show that selection for increased resistance to mortality can be effective in different breeds. However, it requires experimental infection that could be avoided through the use of indirect selection criteria. Hutt and Crawford (1960) compared the resistance of two lines divergently selected on body temperature. Day-old chicks were inoculated orally with a culture of SP. Mortality rates varied widely in the experiment but in all cases higher mortality was observed in the low-temperature line, showing the efficacy of this indirect criterion of selection. In order to study the possibility of genetic modification of a component of the immune response, Pevzner *et al.* (1981) studied SP antigen titre, another indirect criterion. They performed a selection experiment in a population segregating for the B^1, B^2 and B^{19} alleles of the B complex. A significant response was observed only in the B^1 subgroup, which were shown to be the lowest immune responders. The antibody response was highest in the third generation, with a 50% difference between the lines. Realized heritability varied from 0.22 to 0.07 in the last generation, while estimated heritabilities from the sire and dam components were 0.21 and 0.28 in the high-titre and low-titre lines, respectively, demonstrating that this criterion may be selected effectively. Kaiser *et al.* (1998) also observed a high heritability of antibody response 10 days after inoculation of *S. enteritidis*

vaccine. Correlation with bacterial burdens at 3 and 6 weeks estimated from the mean contamination of unvaccinated half-sibs was at −0.87 for caecal content ($P < 0.05$) but was at very low and at non-significant values for the spleen and liver. However, 4 weeks after inoculation of an *aro4* strain, heritability of antibody response seemed to be much lower (Beaumont *et al.*, 1999), possibly because the interval between inoculation and measurement of antibody titre was longer. More information is needed to appreciate the type of improvement that may be expected from this indirect selection criterion.

Long-term perspectives

In the longer term, selection for increased resistance to mortality will no doubt be facilitated by identification of the major genes underlying it. Bumstead and Barrow (1988) initiated a new series of studies on this topic, which is further described by Bumstead in Chapter 18. They first compared the resistance of inbred poultry lines, whose within-line variation is very low, and observed wide variations in LD_{50} after intramuscular inoculation of *Salmonella typhimurium* (from 0.84 ± 0.23 to 4.53 ± 0.20). Comparison of the resistance of pure lines and of a backcross between the most susceptible and resistant lines suggested the existence of a major dominant gene. Bumstead and Barrow (1993) observed that the pattern of resistance across inbred poultry lines was similar for different *Salmonella* serotypes (*S. gallinarum*, *S. pullorum* and *S. enteritidis*) differing considerably in virulence. This result suggests that there might be a general mechanism of resistance to all serotypes of *Salmonella* and Bumstead and Barrow (1988) had already reported that this gene showed 'a superficial resemblance' to the formerly named *Ity* gene (for resistance to immunity to *S. typhimurium*) known to be responsible for enhanced intracellular killing of bacteria by macrophages. The LPS mutation in mice also results in extreme susceptibility to the most virulent Gram-negative bacteria, including *Salmonella*, as

it renders animals naturally tolerant to otherwise lethal doses of bacterial lipopolysaccharide (LPS), an abundant component of the bacterial membrane of Gram-negative bacteria. Both genes were recently identified as *Nramp1* (natural resistance associated macrophage protein 1) (Vidal *et al.*, 1995) and *Tlr4* (toll-like receptor 4) by Poltorak *et al.* (1999) and Qureshi *et al.* (1999). Both were cloned in fowl by Hu *et al.* (1996) and Leveque *et al.* (2000). Their effects were estimated in 1-day-old chicks by Hu *et al.* (1997) on a cross between two inbred lines using the closely linked *TNC* (tenascin C) gene as a marker for *TLR4*. The effects of both explained 33% of the difference in mortality occurring during the first week after inoculation between these lines. Girard-Santosuosso *et al.* (2001) also observed a significant effect of *VIL1*, a gene closely linked to *NRAMP1*, on number of cfu in the spleen 3 days after inoculation in an outbred line and on older animals.

Resistance to carrier-state

The main concern with *Salmonella* nowadays is the risk of human infection, which most often results from asymptomatic infection and excretion, hereafter called carrier-state. Increased resistance to carrier-state will therefore probably be the ultimate goal of selection but very little is known about its genetic control. Investigating it involves investigating *Salmonella* contamination several weeks after inoculation. The studies on this new criterion were initiated in the 1990s.

Comparison of lines

Resistance to carrier-state was investigated through the comparison of four outbred lines whose LD_{50} after intramuscular inoculation at 1 day of age differed considerably (Guillot *et al.*, 1995). Both adult hens at the peak of laying and 1-week-old chicks were studied. The former were orally contaminated with 10^9 bacteria and slaughtered 4 weeks later; Protais *et al.* (1996) observed

significant differences in the percentages of contaminated systemic (spleen, ovary) and intestinal organs (intestines and caeca) but not in the percentages of contaminated liver. Chicks were orally inoculated at 1 week of age with 5×10^4 *Salmonella* bacteria, which were investigated at regular intervals up to 12 weeks after infection by Duchet-Suchaux *et al.* (1997). The authors observed significant and wide variations in both the rates and levels of caecal colonization whereas very few differences were observed for liver, spleen and ovary infection. All these results suggest that genetics could be involved in the control of this complex trait. This hypothesis was confirmed by estimated heritability.

Estimation of genetic parameters

Beaumont *et al.* (1999) inoculated a total of 304 hens and spleen and caeca were contaminated in 35.6% of them. Heritability of the risk of contamination (assessed as an all-or-none trait) from the sire and dam components was estimated at 0.47 ± 0.21 and 0.13 ± 0.26, respectively, for the spleen, and at 0.24 ± 0.15 and 0.23 ± 0.26 for the caeca (Beaumont *et al.*, 1999). The discrepancy between sire and dam estimates might have originated from dominance or maternal effects but also from sampling variations. Because the frequency of contamination was too low, it was not possible to estimate heritability of liver or ovary contamination. In chicks, heritability of the contamination level (i.e. of the number of cfu per organ) in the caeca was estimated by Berthelot *et al.* (1998) on 819 chicks. Whether contamination was assessed without or with pre-enrichment, *Salmonella* was detected in 53.1 and 67.6% of animals and heritability was estimated at 0.12 ± 0.12 and 0.20 ± 0.12, respectively, using the threshold model. The latter appears to be a more reliable measurement; moreover it corresponds better to the consumer risk. In the aforementioned study, Janss and Bolder (2000) also measured caecal carriage in those animals that survived 4 weeks after infection; it was assessed by the logarithm of the number of cfu and set at zero for those

animals that were free of *Salmonella* (24.5%). Heritability was estimated at 0.12 ± 0.08 and the genetic correlation with survival at 0.26 ± 0.55, which suggests that increasing resistance to mortality would increase the level of caecal carriage and emphasizes the value of direct selection for increased resistance to carrier-state and not to mortality. Preliminary results from Stern *et al.* (1991) suggested that selection for resistance to caecal colonization (at 1 week of age) could be efficient, as a difference in colonization level could be observed among the most susceptible and resistant offspring of the first generation of a divergent selection.

Long-term perspectives

All these results show that selection for increased resistance to *Salmonella* carrier-state is achievable. However, measurements must be performed in protected areas and are very expensive. Moreover, infected animals must be culled, which involves selecting breeders on their relatives' performance (sib selection). Using indirect criteria (such as immune response to vaccination) that do not require inoculation with pathogens might be possible but further investigations are needed, in particular to estimate genetic relationships between this indirect trait and resistance more effectively. To predict any undesirable effect of putative selection, it will also be important to estimate the genetic correlations between resistance and traits of economic significance, such as egg and meat production. This will permit prediction of the indirect effects of selection for increased resistance. The most promising research area consists of identifying marker gene(s) of resistance or the gene itself. This would be of great value, as the resistance of all candidates for selection could be assessed from a blood sample by molecular analysis (marker-assisted selection). However, very little is known to date about the effects of the genes involved in resistance to salmonellosis, such as *NRAMP1* or *TLR4*, on resistance to carrier-state. Moreover, the control of resistance to salmonellosis is probably only partly linked to the control

of resistance to *Salmonella* carrier-state. Further and extensive research is thus needed to identify all the genes involved in the control of resistance to carrier-state.

Resistance to *Escherichia coli*

Resistance to *Escherichia coli* has been widely studied, but the focus has been on the antibody response to vaccination in order to study the genetics of the immune response. There have been many comparisons of lines that have shown wide variations in resistance as well as many interactions with major genes and the environment. Marsteller *et al.* (1980) compared lines selected by Siegel (1962) for high (HW) or low (LW) body weight and observed significant interactions between antibody response, dwarf genotype and chicken line. Dunnington *et al.* (1986) compared offspring from the aforementioned lines as well as from a line selected for higher antibody response to sheep erythrocytes. The animals were inoculated at 12 or 26 days and reared at normal or low temperature after 9 days and a significant genotype–environment interaction was observed with age and temperature. Praharaj *et al.* (1986) also showed that this trait was influenced by heterosis, as could be expected for a general component of fitness.

An initial selection experiment for high and low immune responses was performed by Pitcovski *et al.* (1987). Selection in a base population of White Rocks was based on mean family antibody titre after vaccination with *E. coli* (EC) strain O:78 K:80 or Newcastle disease virus (NDV) of a mesogenic strain at 10 and 18 days, respectively. Two replicates were obtained with one and four generations. In both replicates, the antibody response developed earlier and to a higher level in the high-response line. Realized heritability of pooled antibody titres was 0.72 and 0.67, respectively, showing that the selection was efficient. Antibody titres to both antigens increased in the high-response line; in the second and third generations, the difference was greater for NDV than for

EC. Lower mortality after challenge was reported in the high-response line (7% vs. 23% in the low-response line) but lower body weight.

The selection experiment of Leitner et al. (1992) only dealt with antibody response to EC at 10 days of age. Two replicates were obtained and divergent lines selected. No indirect response on any other trait was observed. Wide differences in antibody response were observed, with a ratio of titres between the high- and low-response lines of 2.2, i.e. higher than reported by Pitcovski et al. (1987). However, average realized heritability was lower: 0.15 and 0.27 in the low- and high-response lines, respectively. The difference between lines may be due to the selection limit of a zero titre in the low-response line; Yonash et al. (1996) showed that the difference was also related to an interaction between the line and the interval between inoculation and measurement of antibody response. It is of note that higher antibody titres were observed in females. Differences in mortality after challenge with 10^9 cfu were seen only on vaccinated animals: mortality was thus significantly higher in the low lines, by 20% in one replicate ($P < 0.05$) and 13% in the other ($P = 0.08$). This selection resulted in earlier maturation of the immune system, which should be favourable to resistance to other diseases. Heller et al. (1992) reported a higher immune response to E. coli and NDV but no challenge was performed.

Resistance to Newcastle Disease

The first results concerning Newcastle disease were obtained by Godfrey (1952). They were based on observations of a natural outbreak of disease attributed to NDV as the symptoms closely resembled Newcastle disease and vaccinated animals did not exhibit any symptoms. Mortality varied from 2.6 to 13.4% but did not seem to correlate with laying intensity. Comparison of mortality between a cross or the pure lines used to produce this cross suggested the existence of maternal effects or, as

suggested by Francis and Kish (1955), maternal immunity. The latter observed differences in resistance between sire families. After a natural outbreak in their C and K lines, Cole and Hutt (1961) also reported both line and family variations in resistance to NDV vaccine, but the differences were fairly small as mortality rates varied from 0.79 to 7.15%. They concluded that selection should be possible. Gordon et al. (1971) used analysis of variance to estimate heritability of mortality rate on a total of 5071 chicks; as their estimates varied from 0.07 to 0.17 according to the year, they concluded that selection would not be effective. However, these results should be reviewed using methods adapted to an all-or-none trait and lower mortality rates (which amounted here to > 82%). Pitcovski et al. (1987) showed that antibody response at 18 days of age could be selected. Sacco et al. (1994) estimated heritability of primary and secondary antibody responses to NDV on a total of 931 turkeys originating from a line selected for higher 16-week body weight to be 0.38 ± 0.07 and 0.30 ± 0.06, respectively, whereas the genetic correlation between both was 0.49 ± 0.15 and the genetic correlation with antibody response to Pasteurella multocida vaccines was less than 0.31. The practical implications are limited: while it should be more efficient to select on the primary response, the economic interest is lower because it develops more slowly, to a lesser degree and persists for a shorter time than the secondary response, with which there is little correlation.

Resistance to Other Diseases

Very little information is available regarding the genetics of resistance to other diseases. In all cases it is limited to demonstration of the existence of differences between lines, which suggests the existence of genetic variability but does not prove it. Moreover, the extent of genetic variability has never been estimated. Very little information is available about resistance to avian

infectious bronchitis (Bumstead *et al.*, 1989; Otsuki *et al.*, 1990), *Campylobacter jejuni* (Stern *et al.*, 1990), egg drop syndrome (Picault *et al.*, 1982), infectious laryngo-tracheitis virus (Loudovaris *et al.*, 1991), *Mycobacterium avium* (Gross *et al.*, 1989), necrotic enteritis (Siegel *et al.*, 1993), or *Staphylococcus aureus* (Cotter and Taylor, 1991). More studies have been performed on resistance to infectious bursal disease and *P. multocida*.

Resistance to infectious bursal disease

Infectious bursal disease is a highly contagious disease of considerable economic importance. It results not only in morbidity and mortality but also in immuno-depression, which is in turn the cause of further diseases. Fadly and Bacon (1992) compared the responses of six congenic lines differing in the *B* haplotype. The birds were inoculated with 500 EID_{50} (embryo infectious dose 50%, i.e. the dose that contaminates 50% of embryos). After inoculation at 4 weeks of age (the age when most chicks are affected), no line differences in responses of lymphocytes to mitogens were observed but mortality rates ranged from 10 to 79%. *B* haplotype 15 seemed to confer increased resistance to mortality but not to immunodepression or severity of lesions. The authors therefore concluded that genetics was involved in resistance to mortality but no evidence could be found of its effect on immuno-depression (which is economically more important) or response to vaccination. Okoye and Aba-Adulugba (1998) observed that, under controlled rearing conditions, Nigerian breeds were more susceptible than commercial pullets and broilers, whereas the converse is often reported. They interpreted this difference as a confusion between the hardiness of local breeds under field conditions and higher resistance. Bumstead *et al.* (1993) investigated the genetics of resistance through the comparison of 11 inbred or partially inbred lines. Animals were inoculated intranasally

with a very virulent strain. Most mortality occurred 2–8 days after inoculation and wide variations were observed, as the mortality rate varied from 0 to 79.2%. More severe lesions were observed in the most susceptible lines. Crosses showed that resistance was partially dominant but the hypothesis of one major gene could not be tested with sufficient power, because of the low number of animals.

Resistance to *Pasteurella*

Studies of resistance to *Pasteurella* began with the observation by Saif *et al.* (1984) of differences in response by experimental turkey lines to a natural outbreak of fowl cholera due to *P. multocida*. Higher mortality rates (28.5 and 25%, respectively) were observed in two lines. One was selected for higher 16-week body weight (line F) but was not reared with the other lines, which restricted the validity of the comparison. In the second line (E), selected for higher egg production, the mortality rate was twice as high as that of a control light line (RBC1) reared in the same pens. Sharaf *et al.* (1988) therefore further compared lines E and RBC1 after experimental inoculation and also observed higher susceptibility of line E, with a mortality rate at 66.7 vs. 31.8%. In contrast, Sacco *et al.* (1991) did not observe significantly higher susceptibility of line E but the mortality rate in line F was 72.1% vs. the overall mortality rate of 51.2%. Further studies allowed Nestor *et al.* (1999) to conclude that there may be variability in resistance between commercial and experimental lines that could be related to the growth potential and to the shape of the growth curve. No estimated herita-bility or genetic correlation of resistance is yet available but, on a total of 931 turkeys from a line selected for higher 16-week body weight, Sacco *et al.* (1994) estimated heritability of primary and secondary antibody responses to *P. multocida* vaccines to be 0.46 ± 0.07 and 0.33 ± 0.07, respectively. As the genetic correlation between both was -0.14 ± 0.17, selecting for

increased responses did not seem to be very appropriate.

Conclusion

The above results show that selection for increased resistance is feasible. Most studies have focused on the diseases with the most deleterious consequences. However, appreciation of the degree of severity may change with time: for example, *Salmonella* used to be an important cause of morbidity and mortality of animals. In developed countries, it appears nowadays to be a zoonotic disease, which enhances the importance of carrier-state. The emergence of hypervirulent strains, as in MD, and of new pathogens such as J-leukosis should stimulate new areas of research. The results will contribute to reduction of veterinary costs and consumption of antibiotics; they will also be very important for the development of organic farming. Moreover, animals from a selection nucleus are no longer in contact with pathogens, meaning that no natural selection for increased resistance can occur. Resistance should therefore be taken into account in selection schemes to prevent any decrease in resistance.

The value of this approach will be strongly dependent on the extent to which results obtained with experimental lines may be extrapolated to commercial lines and on the magnitude of interactions between hosts, viral variants or bacterial strains and vaccines. Experiments will be needed to investigate this critical point and, in particular, factorial designs involving several strains of chickens and types of vaccination treatments.

Much will no doubt be learned from molecular genetics. Identifying the mutations underlying susceptibility and resistance will alleviate the need for experimental inoculation, which often precludes the possibility of practical applications of selection for increased resistance. However, this will take time, while classical selection may already be commenced and may

facilitate identification of the genes underlying resistance.

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21 Selection for Disease Resistance: Molecular Genetic Techniques

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Poultry meat (chicken, turkey, duck and specialty birds) and eggs provide one of the most important protein sources for the world. Worldwide poultry product consumption for 2000 is estimated to be 55 million tonnes, making poultry second only to pork (Roenigk, 1999). However, given the steady increase in poultry consumption and market share, and the favourable cost of poultry relative to other meats, poultry consumption should overtake pork consumption in the next 10–20 years (i.e. by about 2020) as the leading meat consumed in the world.

To meet the growing demand of consumers, besides breeding for improved genetic performance the poultry industry has become more consolidated and chicken rearing more concentrated to enhance efficiency. While this allows for more economical meat and egg production, it has the unfortunate consequence that disease outbreaks occur more frequently and with greater severity. Changes in animal husbandry (e.g. 'all in, all out' rearing), new vaccines etc. have helped to alleviate some of the problems, but improved or alternative control measures are still needed in the near future to meet existing and emerging disease problems.

Genetic resistance to disease is attractive as breeders have been exploiting genes for disease resistance for years, even with limited knowledge about the genetic or molecular basis of such phenotypes. When available, genetic resistance provides a reliable, economical and environmentally sound strategy for disease control. The advent of modern molecular genetics has raised the promise that genes conferring disease resistance can be identified and elite strains with high disease resistance selected based on molecular markers, which has been demonstrated in pigs, cattle and many plant species for growth and reproductive traits.

This chapter reviews the current methods used to identify resistance genes for specific diseases in chickens. Genetic resistance to the majority of diseases is usually complex or controlled by many genes as well as influenced by environmental conditions. Thus, the candidate gene approach and genome-wide quantitative trait loci (QTL) scans will be discussed in detail. Also shown is how the new techniques of DNA microarrays (global gene expression) and two-hybrid (protein interaction) screens can be integrated with genetic studies to reveal positional candidate genes for QTL. Marek's disease (MD) will be the primary example used to support when and how to implement specific techniques.

Getting Started

While obvious, the first requirement before initiating a search for disease resistance genes is to gather all the information that is

known about the disease in chickens and, if available, in other species. Heritability of the disease, correlation with production traits, the existence of characterized strains, the presence of a good disease model, assays to measure traits, defined disease resistance genes or pathways and other related information are all useful in developing a strategy, interpreting experimental results and designing new hypotheses and experiments. One recommended starting point is the review by Gavora (1990), which remains an excellent source of information for many poultry diseases. Also, several chapters in this book describe specific diseases.

In the case of MD, there is a fair amount of knowledge obtained from chicken studies. MD is a lymphoproliferative disease caused by the Marek's disease virus (MDV), an oncogenic avian herpesvirus, and a serious economic concern to the poultry industry. MD is most frequent in chickens at 12–24 weeks of age but younger chicks can also be afflicted. Any organ or tissue can be involved, though tissues of the nervous systems are most often afflicted. Clinical symptoms of MD vary but diseased chickens often show partial or total paralysis, blindness and visible tumours. Internal examinations normally reveal enlarged peripheral nerves and several lymphomas in visceral organs.

Chickens resistant to MD are those that fail to develop characteristic symptoms upon exposure to virulent MDV. Genetic differences in resistance to fowl paralysis, assumed to be MD, between different chicken lines have been reported for more than 60 years (Asmundson and Biely, 1932). The heritability of MD resistance within lines and line crosses has been reported in several studies, with estimates ranging from a low of 0.06 (Hartmann and Sanz, 1974) to a high of 1.00 (Ameli *et al.*, 1992). The large variation in heritability estimates may be due to the use of different chicken lines and MDV strains, dissimilar environmental conditions and inconsistencies in how MD was defined and measured. Desirable correlations between MD resistance and egg production or egg quality traits have been reported frequently.

Experimental lines with high or low MD incidence are available, as MD resistance or susceptibility can be selected. For example, progeny testing to select sire and dams resulted in the development of Cornell lines N and P, which after four generations of selection had 4 and 96% MD incidence, respectively; MD incidence in the unselected control stock was approximately 52% (Cole, 1968). Considerable interest has focused on the inbred lines 6 and 7. Initiated in 1939 by the Regional Poultry Research Laboratory (renamed the Avian Disease and Oncology Laboratory, or ADOL, in 1990), fewer than 3% of the line 6 chicks will succumb to MD when inoculated with the JM/102W strain of MDV at 1 day of age. In contrast, similar inoculations into line 7 chicks will result in greater than 85% mortality. As these lines are over 99% inbred, almost all the loci are homozygous, which greatly simplifies the genetic analysis of any cross using these two lines or their derivatives since two alleles, at most, will be segregating. Some commercial pure lines, and the sires used to reproduce these lines, have also been screened for MD incidence.

Resistance to MD, like most other diseases, is controlled by multiple genes or QTL. For example, the level of disease resistance as measured by mortality among F_1 siblings of a cross between lines 6 and 7 is intermediate to the parents: approximately 60% (Stone, 1975). The levels of resistance observed in an F_2 population, where the entire genome is segregating, encompass a large spectrum, indicating that more than one gene for resistance is involved. Further analysis of additional crosses suggests that only a few loci of sizeable effect can account for the majority of the resistance to MD (Stone, 1975).

The best understood mechanism for the involvement of genetic resistance to MD involves the major histocompatibility complex (MHC) or, as it is known in the chicken, the *B* complex. The MHC contains 19 tightly linked genes (Kaufman *et al.*, 1999) including three loci known as *B-F* (similar to mammalian Class I genes), *B-G* (class IV, unique to poultry) and *B-L* (similar to mammalian Class II genes), which control cell-surface

antigens. The *B-G* locus is expressed in erythrocytes, which enables convenient typing of blood groups. By measuring the frequency of specific blood types, it has been observed that certain *B* alleles can be associated with resistance or susceptibility. Chickens with the *B*21* allele have been found to be more resistant than those chickens with other *B* haplotypes (Briles *et al.*, 1977; Bacon, 1987; Bacon and Witter, 1992). Other studies have allowed for the relative ranking of the other *B* alleles: moderate resistance, *B*2*, *B*6*, *B*14*; susceptibility, *B*1*, *B*3*, *B*5*, *B*13*, *B*15*, *B*19*, *B*27* (Longenecker and Mosmann, 1981). However, the *B* haplotype effect is dependent on the genetic background, as shown by several studies (e.g. Bacon *et al.*, 1981; Hartman, 1989). Not surprisingly, the *B* haplotype also influences vaccinal immunity, as some haplotypes exhibit better protection with vaccines of one serotype than of a different serotype (Bacon and Witter, 1994a,b).

Cell-mediated immunity (CMI) has been associated with resistance to MD. Typically, CMI has been tested by measuring the ability of mitogens to activate normal peripheral T lymphocytes to undergo blast transformation and proliferation. Lymphocytes from line N chickens surprisingly respond less to mitogen stimulation than lymphocytes from line P chickens (Schat *et al.*, 1978; Lee and Bacon, 1983). Older chickens of other resistant lines have similar lower responses to mitogens (Schat *et al.*, 1978; Fredericksen and Gilmour, 1981). Since herpesviruses require activation of T lymphocytes for infection, Calnek (1985) suggested that the genetic resistance derived from line N is due not to a superior immune response but rather to a slower rate of CMI, which may limit the number of targets for infection in resistant chickens. This hypothesis is supported by the observation that the total and proportional number of infected T cells is greater in spleen cells from line P than from line N chickens (Calnek *et al.*, 1984). Using flow cytometry and immunohistology, Baigent and co-workers (1998, 1999) reported that the distribution of lymphocytes is different in lines 6 (MD resistant) and 7 (MD susceptible), with the susceptible animals exhibiting densely packed TCRαβ+ CD4+ cells around MDV-infected B cells. Finally, macrophages that may suppress T-cell mitogenic responses could play a role in resistance, as there are more macrophages in line N than in line P chickens (Higgins and Calnek, 1976; Lee *et al.*, 1978; Sharma, 1980).

Besides the MHC, other genetic factors are known to exist that have a major influence on resistance to MD. For example, lines 6 and 7 chickens share the same *B* haplotype, *B*2* (class I and II sequences are identical; Hunt and Fulton, 1998), yet differ greatly with respect to resistance to MD. The size of the primary lymphoid organs (thymus and spleen) and the number of lymphocytes in line 6 chickens are significantly smaller than those found in line 7 chickens (Fredericksen and Gilmour, 1981; Lee *et al.*, 1981). Spleen and thymus cells from line 6 chickens also absorb less MDV than do similar cells from line 7 chickens (Gallatin and Longenecker, 1979; Powell *et al.*, 1982). Thus, in contrast to MHC-controlled resistance, these early studies suggested that non-MHC genetic resistance may be related to the number of target cells. However, more recent immunocytological studies have challenged this hypothesis. Baigent and Davison (1999) showed that B lymphocytes, the initial target for MDV cytolytic infection, were more numerous in line 6 than in line 7 (Baigent *et al.*, 1998) but the number of cells that were positive for the MDV protein pp38 were more numerous in line 7 than in line 6. Combined with the dense packing of lymphocytes around MDV-infected cells observed in line 7, these results suggest that efficient replication and spread of MDV is the reason why line 7 is susceptible to MD compared with line 6.

Although chickens from lines 6 and 7 share the same *B* haplotype, differences in immunity seem to play a role in resistance. After infection with MDV, lymphocytes from line 6 but not line 7 chickens were able to reduce the number of viral plaques and exhibited cell-mediated cytotoxicity against MD tumour cells (Lee *et al.*, 1981). These results suggest that both cellular antiviral and antitumour responses play a role in the resistance of line 6 chickens. As

characterized by MHC-controlled resistance, CMI is also involved as older line 7 chickens have superior mitogenic responses in contrast to line 6 chickens; however, at very young ages, the relative levels of mitogen response are reversed (Fredericksen and Gilmour, 1981; Lee and Bacon, 1983). Thus, although the MHC may not always be involved, other genes influencing immune responses may be important for genetic resistance.

All of the information gathered would not have been possible if a good disease model were not established. MDV is highly cell associated; thus, isolates must be propagated in cell culture using chick kidney cells (CKC), chick embryo fibroblasts (CEF) or duck embryo fibroblasts (DEF). Stocks of inoculum can be maintained for prolonged periods of time by freezing MDV-infected cells in liquid nitrogen. Traditionally, the quantification of infectious material is determined by the production of visible plaques in cell culture. Chicks are usually infected by intra-abdominal inoculation of infectious material, though contact with infected birds can also readily transmit the disease. If possible, birds should be kept in secure facilities or chambers to minimize the influence of environmental factors. In addition, it is desirable to use chickens that are specific pathogen free (SPF). When the virus strain, the amount of inoculum, the age of the chick at challenge and the environment are controlled and clean birds are used, MD is fairly reproducible. Under these conditions, the disease incidence within a line or between individuals best reflects the genotype. Optimally, with genetic crosses, parental strains should have a large difference in disease incidence or related traits, with the F_1 progeny intermediate to the parents and F_2 or backcross (BC) progeny exhibiting a wide range.

Disease is a binary or threshold trait, which makes it more difficult to perform association studies with genetic markers. To overcome this limitation partially, it is highly desirable to define the disease as best as possible into components that are measurable on individuals. For MD, besides determining if a bird had the disease and identifying affected organs, many other traits that are highly associated with MD should be measured such as viraemia (MDV titre in peripheral blood lymphocytes (PBL) at a specified time) and length of survival (number of days until moribund). The repertoire of potential agents to screen will continue to grow, which may force a judicious selection of the assays. In our work (Vallejo *et al.*, 1998; Yonash *et al.*, 1999), we have developed two indices that incorporated several traits. The first was a tumour index that gave a 0 (no tumours) to 5 (multiple visceral plus multiple neural lesions) rating, depending on the severity of the disease. The MD index was a combined score of weighted values for viraemia, number of tissues with MD tumours, disease, length of survival and tumour index.

Finally, since a resource population can be generated and measured only once, it is wise to measure other traits that might indirectly influence the trait of interest. For example, the body weight of the bird might influence disease resistance, since a fixed challenge dose is given per animal. The sex of the animal may also play a role. Thus, these 'unrelated' traits should be recorded to perform covariate analysis and data adjustment, if necessary.

The ability to measure a large number of individual animals for separate disease and disease-related traits is the rate-limiting step for the vast majority of genetic studies. It cannot be overemphasized that great care and thought need to be spent on the conditions and parameters of the experiment.

Candidate Gene Approach

Following a review of the literature, some diseases may yield detailed information on the biological pathway, immunology or molecular basis of the trait in poultry or another organism. In such instances, one or more genes that might influence the trait can often be hypothesized based on this prior knowledge. If this is the case, it is a reasonable strategy to test each gene for association with the trait in a phenotyped population. This 'candidate gene' approach

has not been used extensively in poultry, most likely because of the paucity of characterized genes and immunological pathways for the majority of diseases. This limitation of biological information will hopefully diminish as more chicken genes are identified and their function revealed. Also, there is a growing number of genes that may be relevant from studies in model species, especially human and mouse.

As well as looking for candidate genes, a prudent initial approach is to examine the disease for an MHC effect, if this has not already been done. The MHC contains numerous genes encoding antigen processing and presenting molecules, as well as other immunologically related genes, which play a critical role in the regulation of the immune response. Therefore, it is not surprising that the MHC was one of the first regions to undergo extensive analyses. In addition to blood-typing reagents for many *B* haplotypes, the development of at least three sets of *B* congenic lines (inbred lines that vary only in MHC) have greatly simplified genetic studies to determine an MHC effect. Consequently, as well as MD, the MHC has been associated with genetic resistance to a number of diseases, e.g. coccidiosis (Lillehoj *et al.*, 1989; Caron *et al.*, 1997).

As discussed by Bumstead in Chapter 18, genetic resistance to salmonellosis is one of the few success stories of a pure candidate gene approach in chickens. Like MD, chicken lines vary in the degree of resistance, indicating a genetic component. In susceptible chickens, a systemic infection of the reticuloendothelial system occurs shortly after bacterial infection, with death soon after. To measure salmonellosis resistance of a line, chickens are challenged with a range of bacterial colony-forming units (cfu) to determine the mean lethal dose (LD_{50}) value. Based on this criterion, the inbred chicken lines 6, N and W1 can be classified as resistant and the lines 7, 15I and C as susceptible to challenge with *Salmonella* species (*S. typhimurium*, *S. gallinarum*, *S. pullorum* and *S. enteriditis*) (Bumstead and Barrow, 1993). Crosses between resistant and susceptible lines demonstrate that resistance is fully dominant and is not

associated with either sex or the MHC (Bumstead and Barrow, 1988).

In mice, genetic resistance to infection with *Salmonella* is controlled by two known major loci named *Nramp1* (natural resistance-associated macrophage protein 1) and *Lps* (responsiveness to lipopolysaccharide). The *Nramp1* gene in mice has been cloned and found to encode an integral membrane phosphoglycoprotein with structural similarity to ion channels and transporters (Cellier *et al.*, 1996; Vidal *et al.*, 1996). *NRAMP1*, the chicken homologue, which has also been identified, exhibits a high degree of sequence conservation and similarity in tissue expression compared with mouse *Nramp1*, suggesting that both NRAMP1 proteins have the same function (Hu *et al.*, 1996). Combined with other information that shows resemblance in salmonellosis between chicken and mouse, *NRAMP1* and *LPS* are good candidates for controlling genetic resistance.

To test the involvement of these two genes in chicken, resource populations were developed by matings between resistant and susceptible inbred chicken lines (Hu *et al.*, 1997). The proportion of animals surviving was determined over a 15-day period as a function of the *NRAMP1* and/or *LPS* genotype. Following analysis, a single nucleotide polymorphism (SNP) in the *NRAMP1* gene in line C together with a marker linked to *LPS* accounted for 33% of the differential resistance early in infection between the parental lines. This data suggests that *NRAMP1* and *LPS* control resistance to salmonellosis in chicken.

NRAMP1 and salmonellosis resistance can be used to highlight the relative strengths and weaknesses of the candidate gene approach. The greatest advantage lies in the simplicity of the method. Only a single gene is being directly tested to see if it can explain some or all of the variation observed in the disease trait measure. Thus, sophisticated methods or analyses are not required to determine association, which also makes it attractive for smaller research laboratories. Virtually any resource population that is segregating for the disease can be used. Actually, in stark contrast to the pedigreed,

multi-generational populations that are required for genome-wide QTL scans, it is preferable to use populations where multiple generations have reduced the amount of linkage disequilibrium (LD or the coinheritance of adjacent markers). This enhances the opportunity to use chickens derived from commercial lines, where experimental and field data on specific diseases may have been collected.

Disadvantages include the need to identify a polymorphism in the gene. This problem is even more challenging if only cDNA sequence or gene information from another species is known. In F_2 populations or other resource populations with significant LD, the candidate gene is acting more like a genetic marker. Thus, even if an association is found, it is impossible to distinguish between the gene or linked genes as the source of the phenotypic variation. Determination of allele frequencies is necessary to rule out possibilities such as genetic drift or an uneven sire contribution to the population as the cause rather than the candidate gene.

Genome-wide QTL Scans

In most situations, there will be insufficient information to test one or more candidate genes conferring genetic resistance for a particular disease. Even if one gene is revealed, there will be a desire to identify additional disease-resistance genes. In this case, the preferred method is to conduct a genome-wide scan for QTL, or regions in the genome that contain one or more contributing to the trait of interest. This technique is now possible due to the existence of molecular genetic maps (see Groenen and Crooijmans, Chapter 26). Genome-wide QTL scans are attractive because no assumptions are made as to what genes and gene effects underlie resistance to a disease and, theoretically, it should be possible to identify all the genes with moderate effect that confer genetic resistance. Success in poultry and other species has spurred more interest in this approach. This section will give a brief overview of QTL scans (see Van Arendonk and Bovenhuis, Chapter 24) and focus on new approaches to identifying or characterizing disease-resistance genes once a QTL has been identified.

Initial scan

The basic concept behind a QTL scan is fairly simple. A resource population is developed through matings to generate a number of pedigreed individuals which, following challenge or exposure to disease agents, segregate for disease and encompass a wide range of disease-related traits. Some or all of the individuals are genotyped using markers that are evenly spaced through the genome. Statistical analysis systematically scans each region to determine whether a particular marker or marker interval is in LD or associated with a disease-resistance gene.

The design of the resource population will often depend on whether lines or populations that differ for disease resistance are available. For both MD and salmonellosis, lines that are resistant or susceptible under specific challenge conditions exist. In these types of situations, it is desirable to use these lines to generate F_2 or BC populations, which produce significant LD including specific markers and disease-resistance gene alleles. Darvasi (1998) determined the power of QTL detection as a function of the number of progeny and mating design.

In commercial breeding programmes, moderately sized half-sib families that have been sib or progeny tested are sometimes available, or can be produced for specific diseases. While there has yet to be a report describing a QTL conferring disease resistance to poultry using this design, the potential exists and needs to be kept in mind as new technologies emerge. Large numbers of animals, the opportunity to use chickens from existing breeding programmes and the ability to access several generations enhance the attractiveness of this design.

Informative markers that will be genotyped should be no more than 40 cM apart to

detect the QTL, and there is little advantage in marker spacing below 10 cM (Darvasi *et al.*, 1993). At present, microsatellite markers (tandem repeats of 1 to 6 bases) are the preferred class of genetic markers, as they are the most abundant on the genetic map and have multiple alleles. However, the greater frequency of SNPs, the importance of these types of markers in the biomedical field and the fact that genotypes can be obtained without running gels suggest that SNPs will replace microsatellites in the near future. To reduce the amount of genotyping, selective genotyping of the phenotypic extremes (highly resistant or susceptible animals), which are the most informative, is often employed (Lander and Botstein, 1989). Selective DNA pooling takes this step further by pooling the DNA of the individuals comprising the resistant or susceptible extremes and screening for allele frequency differences (Darvasi and Soller, 1994). Selective DNA pooling has been demonstrated in dairy cattle, where very precise trait measurements are obtainable (Lipkin *et al.*, 1998; Mosig *et al.*, 2001). Our experiments using purified DNA and fresh or frozen blood indicate that allele frequency differences are accurately measured by selective DNA pooling; and in a retrospective study QTL for MD resistance were identified in experimental resource populations (Lipkin *et al.*, 2002). It will be interesting to see whether this method continues to be successful with other poultry diseases and resource populations where the trait measures may not be as accurate.

There is a variety of methods for detecting and mapping QTL. The simplest way is analysis of variance (ANOVA), which analyses a single marker at time. Besides its simplicity and the ability to add covariates (other factors) readily, our experience with actual data indicates that ANOVA is relatively robust in detecting QTL. Unfortunately, this method cannot locate QTL well and the size of effect will be underestimated in proportion to the genetic distance between the marker and the QTL. Interval mapping systematically scans adjacent pairs of markers, which overcomes the limitations of ANOVA (Lander and Botstein, 1989); the

main difficulties with interval mapping are the need for specialized software and the assumptions and constraints that the programme places. The regression method by Haley and Knott (1992) offers a viable alternative, which is also computationally easier to implement though user-friendly software is not available. Statistical significance for declaring a suggestive or significant QTL is somewhat controversial and two ways are commonly used. As described by Lander and Kruglyak (1995), one can estimate the significance levels for the resource population. The second method, called the permutation test, randomizes the phenotypic data while keeping the genotypic data intact. After 1000 or more iterations, significance levels are determined empirically (Churchill and Doerge, 1994).

Thus far, genome-wide scans have revealed 14 QTL for resistance to MD (Vallejo *et al.*, 1998; Yonash *et al.*, 1999), a different QTL for resistance to MD (Bumstead, 1998), one QTL (in addition to *NRAMP1* and *LPS*) for resistance to salmonellosis (Mariani *et al.*, 2001; see also Bumstead, Chapter 18), and one QTL for resistance to coccidiosis (Zhu *et al.*, 2003; see also Pinard-van der Laan *et al.*, Chapter 19). Also, three QTL accounting for general immune response were found to be associated with antibody response to Newcastle disease virus (NDV) and *Escherichia coli*, and resistance to *E. coli* (Yonash *et al.*, 2001; see also Lamont *et al.*, Chapter 22). In all cases, the resource populations were derived from experimental crosses between divergent lines or characterized strains to produce BC or F_2 progeny.

Reducing the confidence interval

The sobering reality is that even when QTL are identified, the precision of where they are located is relatively poor. This reflects a combination of the size of the resource population and the amount of variation that a QTL accounts for. Computer simulations demonstrate that the confidence interval for placing a QTL with moderate effect

detected in large resource populations is still 10–20 cM. Undoubtedly, in disease studies where the trait measures are not as accurate or biological constraints make them difficult to reproduce, the confidence interval may grow to 50 cM or the entire chromosome. So, regardless of whether the objective is to use marker-assisted selection, where only tightly linked markers are required or disease gene identification, it is necessary to fine-map the QTL. The ability to fine-map QTL (for disease resistance or any trait) and identify the causative gene is the greatest challenge currently confronting genomics research.

In fine-mapping, the effort is focused on a single QTL and not the entire genome. Thus, the goal is identical to mapping a simple Mendelian gene except that the other QTL, and the variation that they produce, must be accounted for or controlled. Selective phenotyping, recombinant progeny testing and advanced intercross line are methods demonstrated in model species and may be applicable to poultry. In all cases, one attempts to produce additional recombinants in the confidence interval, which are detected by the genetic markers, to resolve the location of the QTL. The methods differ in the way that the progeny are generated. Unfortunately, this requires the production of many individuals, multiple generations, or both.

Selective phenotyping essentially replicates the initial resource population to produce very large F_2 or BC populations. However, only individuals that are recombinant for markers that define the QTL interval are phenotyped. Theoretically, genotyping with markers within the region should refine the position of the QTL. Selective phenotyping has the advantage that this resource population can be used to verify the initial QTL scan. The disadvantage is that, under optimal conditions, very large numbers of progeny (e.g. 3840) are needed to resolve the QTL within a 5 cM interval, depending on the mode of inheritance (dominant, additive or recessive) and the size of the effect (Darvasi, 1998). Also, it may not be possible to reduce the confidence interval even with additional progeny. Furthermore, as many

disease challenges are initiated at day of hatch and measured shortly after, there may not be sufficient time to identify the individuals recombinant for the interval.

In recombinant progeny testing, one or more individuals heterozygous for markers in the interval, and presumably for the QTL, are identified from the initial resource population. These individuals are subsequently crossed to a parental strain to produce progeny segregating for the disease. As in selective phenotyping, genotyping with the markers in the interval will identify individuals recombinant for the interval and should refine the location of the QTL. Compared with selective phenotyping, recombinant progeny testing requires significantly fewer individuals to be produced and phenotyped (Darvasi, 1998). However, this strategy may be effective only for QTL with large and dominant effects.

Advanced intercross lines are produced by crossing two parental strains to generate an F_1, followed by multiple semi-random intercrossings (F_2, F_3, . . ., F_n) (Darvasi and Soller, 1995). This design reduces the amount of LD with each generation. Thus, a QTL with a confidence interval of 20 cM in an F_2 population is reduced to 4 cM in the F_{10} generation (Soller and Andersson, 1998). The primary advantage is that populations do not need to be produced until they are required for phenotyping. The disadvantage is that many generations (and much time) are needed.

When inbred lines are available, they should be the first choice as the genetics are greatly simplified and there are several proven methods for fine-mapping QTL. One of these methods is recombinant congenic strains (RCS). As proposed by Demant and Hart (1986), RCS are produced by limited backcrossing between two strains and subsequent brother–sister matings. The result is the creation of 15–20 strains, each of which carries a random ~12.5% (when two BC matings are used) of the donor genome in the genetic background of the recurrent parent. The major advantage of this approach is that the QTL that control the complex trait found in the donor strain are spread out among different RCS. In other words, the QTL that

controlled the trait in the donor line are genetically dissected and found individually or in pairs in different RCS. This greatly simplifies a trait by transforming it from a multigenic one to a series of single gene traits. Fine-mapping of QTL is readily achievable by mating an RCS that carries a donor-strain QTL to the recurrent parent line. RCS can also reveal epistatic interactions, as designed crosses involving two or more QTL can easily be produced.

To generate chicken strains that might help to elucidate the genetic basis for susceptibility to MD, the development of RCS was initiated in 1991 using the MD-susceptible line 7 as the donor genome in the MD-resistant line 6 genetic background. In 2000, the seventh generation of inbreeding by brother–sister matings was completed. Following challenge with MDV, three of the 19 6C.7 RCS are relatively susceptible to MD. Thus, we have created strains that are susceptible to MD even though the majority of the genome is derived from an MD-resistant background. This data suggests that QTL of major effect for MD susceptibility exist and can be fine-mapped. Also, as the genetic makeup of each line is known, phenotypic screening of the lines followed by segregation analysis gives clues as to the function of each QTL.

It may be interesting to note that, since lines 6 and 7 differ for a number of other traits, our 6C.7 RCS can be used to characterize and fine-map QTL for many other traits. These traits include: disease resistance to salmonellosis, avian leukosis and infectious bronchitis; immune response to mitogens, mixed lymphocytes and specific antigens; size of the bursa and thymus; fertility and hatchability; fat deposition; and social behaviour.

Molecular approaches for revealing positional candidate genes

Given the large effort that is required to fine-map a disease resistance QTL, it is not surprising that there is a need for alternative methods of identifying genes conferring genetic resistance besides positional cloning. Luckily, poultry geneticists can benefit from the new methods and vast amount of information that is being generated largely from the Human Genome Project.

One popular approach is to identify positional candidate genes from comparative mapping. As discussed by Burt (see Chapter 29), there is a surprising amount of conserved synteny (gene linkage) between the chicken and human genomes. By aligning the QTL with the corresponding region of the human genome, or any species rich in map information, often there are genes that could account for the phenotype. For example, in the case of MD, a QTL on chicken chromosome 1 is equivalent to a region on mouse chromosome 6 that contains the *CMV1* gene, a natural killer (NK) cell activation receptor (Brown *et al.*, 2001; Lee *et al.*, 2001). The power of the comparative positional candidate gene approach will increase as the chicken comparative map improves and the functions of more genes are defined.

Molecular approaches may offer the greatest potential for identifying disease-resistance genes. Rather than using genetics (DNA) to guide the search, molecular approaches screen RNA or proteins for targets that are in biological pathways. As only a small subset of these genes or proteins will have a genetic basis, the challenge is to integrate the new molecular approaches with genetics. Described below are two techniques that we are investigating to identify MD resistance genes.

DNA microarrays or chips measure the expression of thousands of genes in a parallel process (see Cogburn *et al.*, Chapter 31). Briefly, DNA of known or unknown genes is spotted at very high density at specific locations on a solid support to generate a microarray. In the 'classical' or most widely used form, mRNA is reverse transcribed and hybridized to the microarrays, followed by quantification of the amount of material bound by the spot. Thus, one is essentially performing a Northern blot, only multiplied several thousand-fold. As this gives a gene expression 'fingerprint', by comparing samples from two or more treatments it is possible to associate the

expression of particular genes with the phenotype. Since the original publication (Schena *et al.*, 1995), this technology has been highly touted and a number of papers have successfully demonstrated the ability of this procedure to provide insights on complex biological systems.

The first generation of DNA microarrays, which contain a limited number of cDNAs obtained from a splenic T-cell library, has been developed (Morgan *et al.*, 2001). These DNA microarrays were hybridized using RNAs obtained from peripheral blood lymphocytes of chicks from lines 6 and 7, uninfected (2 weeks old) and various times after infection with 2000 plaque-forming units (pfu) MDV strain JM/102W (Liu *et al.*, 2001a). Due to the large amount of mRNA needed for labelling, it was necessary to pool the lymphocytes from ten chicks. However, this pooling had the beneficial effect of reducing any individual variation that might have occurred between chicks within a treatment group. In the first experiment, 105 genes exhibited twofold or greater differences in gene expression between lines 6 and 7 (either uninfected or 8 days post challenge). In the second experiment (replication is especially essential for DNA microarrays), 25 of these genes were replicated.

While informative in identifying genes and associated pathways, DNA microarray data cannot distinguish between genes that have a genetic basis vs. those that are secondarily affected. Also, the number of differentially expressed genes can be quite large. More importantly, the level of gene expression variation that is biologically relevant has not been determined. Thus, to identify the genetic determinants of disease resistance, we mapped differentially expressed genes. In other words, our hypothesis is that genes that are differentially expressed and map to a QTL region are strong positional candidate genes. This strategy is supported by the fact that *GH1* (see below) and *SCYC1* were revealed. *SCYC1*, which encodes for lymphotactin, a cytokine that attracts CD4+ and CD8+ T cells, has previously been mapped to a region in GGA1 (Hughes and Bumstead, 2000), near an MD QTL.

Viral–chicken protein interactions may be another method of screening for disease-resistance genes. MDV, like all viruses, must use the host cell machinery to replicate. Some of the chicken genes encode proteins that interact with MDV proteins and a subset of these chicken genes may confer genetic resistance. Thus, it has been our goal to identify most, if not all, of the MDV–chicken protein interactions.

SORF2 demonstrates one success using a protein interaction approach. The RM1 MDV recombinant clone stimulated interest in SORF2. RM1 contains a lone reticuloendotheliosis virus (REV) long terminal repeat (LTR) immediately upstream of SORF2, which could account for the loss of oncogenicity (Jones *et al.*, 1996); the parental strain JM/102W is virulent. As SORF2 transcription was disrupted in RM1, we hypothesized that chicken proteins that interact with SORF2 may be involved in MD resistance. Protein interactions can be screened using a yeast two-hybrid assay (Fields and Song, 1989) or a number of growing two-hybrid systems (reviewed in Cagney *et al.*, 2000). Using SORF2 as our bait, we screened a chicken splenic cDNA library. Several clones for growth hormone (GH) were recovered (Liu *et al.*, 2001b). The specific interaction between SORF2 and GH was verified using several other assays. Integrating in genetics, we tested GH as a candidate gene in a commercial resource population. The analysis demonstrated that GH is associated with several MD-associated traits but only in chickens with the MHC *B*2/B*15* genotype.

The Future Looks Bright

Genomics research in human and model species has clearly demonstrated the power and rapid growth of the field. For example, in 1991 the Human Genome Project was in its infancy and now the human genome has been completely sequenced (Lander *et al.*, 2001; Venter *et al.*, 2001). By generating resources and methods that enable high throughput and parallel processing, and integrating multiple disciplines and

approaches, genomics is able to produce large amounts of data, which in turn produce great insights on gene identification, gene function, biological pathways and ultimately how entire genomes produce phenotypes.

In poultry, the available resources are about 5–10 years behind those for similar model species. Luckily, this gap is getting smaller as groups are hard at work generating more tools. In particular, the first-generation bacterial artificial chromosome (BAC) contig (physical) map will be released soon. This map will enable the generation of markers (microsatellites and SNPs) across the entire genome. Furthermore, the BAC contig map will help to refine the genetic map and the human–chicken comparative map, as well as providing an address for the vast number of expressed sequence tags (ESTs) that are being produced. It is highly likely that the chicken genome will be completely sequenced in the next 5–10 years, given the interest from the international community. The challenge for future scientists will be to incorporate this vast amount of information.

Technologies will also continue to be developed and refined that will be applicable to poultry. Proteomics and whole-cell assays are especially promising. While undoubtedly these technologies will have to be adapted in an economical manner for use in poultry, they will likely impact poultry research just as has been the case for PCR, automated DNA sequencing and DNA microarrays.

In conclusion, while the decades-old promise of disease gene identification and marker-assisted selection for disease resistance has not yet been met, the days are numbered. Already, many QTL and some genes for disease resistance have been revealed. New resources and technologies will make it much easier to genotype numerous individuals and collect molecular phenotypes. What has not occurred and will not be easy to accelerate is the ability to phenotype traits at the whole-animal level. Perhaps an even bigger hurdle may be the architecture of QTL, as studies have shown that the effects are due to closely linked genes (e.g.

Pasyukova *et al.*, 2000) or are only observed with certain epistatic interactions (e.g. Fijneman *et al.*, 1996). Despite these limitations, incorporation of new methods and integration with model species will enhance our ability to reveal gene function and biological pathways for disease resistance.

Note Added at Proof Stage

In 2002, the National Human Genome Research Institute (NHGRI) at the US National Institutes of Health (NIH) rated the chicken genome as a 'high priority' for whole genome sequencing. The Washington University in St Louis School of Medicine Genome Sequencing Center was granted permission to perform the work. It is anticipated that the sequencing portion of the effort will be completed by August 2003, followed by the assembly and annotation shortly thereafter.

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22 Selection for Disease Resistance: Direct Selection on the Immune Response

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Introduction

Rationale for direct selection for immune response

The overall goal in direct selection for immune response in poultry is the enhancement of animal health. Why, then, is selection for an immune response trait often preferable to direct selection for resistance to pathogens? There is a wide difference in the myriad of pathogenic organisms to which poultry may be exposed in their lifetime and, therefore, it is difficult to identify only one or a few infectious organisms that would serve as the appropriate selection targets. Great efforts are expended to keep breeding stock isolated from disease organisms, yet the commercial offspring may be shipped globally and exposed to numerous and varied pathogenic challenges as they are raised throughout the world under a variety of management conditions. Thus, the genetic enhancement of natural immunity mechanisms that may be broadly effective in conveying heightened resistance to whole classes of pathogenic organisms may

be an effective strategy that will protect birds in production under a wide variety of environmental conditions (Lamont, 1998).

There are very practical and economic reasons for pursuing enhancement of immunity via direct selection for immune response. The facilities and expertise needed to evaluate the response of birds to pathogenic challenge are quite limited in availability, expensive and labour intensive. It is important not to expose breeding stock to disease-causing organisms and so facilities and populations separate from the breeding stock must be used. In contrast, non-infectious antigens can be used to mimic and measure the response of the immune system as an estimate of the predicted immune activity level that would occur in an actual challenge situation. As consumers continue to voice concerns regarding the wholesomeness and safety of animal-derived foods, it is important to find effective methods to reduce chronic use of antibiotics and to enhance the effectiveness of vaccine protection by improving, via genetic selection, the innate ability of birds to respond to antigenic challenges.

Approaches to direct selection for immune response

Divergent selection for a single trait: antibody response

The two-way divergent selection design for a multi-determinant and non-pathogenic antigen such as sheep red blood cells (SRBC) is a direct and efficient way to create divergent populations. This model, first successfully applied in mice by Biozzi *et al.* (1979), led to large differences in magnitude of antibody (Ab) responses. This trait was under polygenic control and expected to be controlled by six to ten loci. Nowadays, this design is still under rich development and helps to gain insight in understanding the relationships between the different facets of immunity, production traits and identifying genetic markers (Puel and Mouton, 1996; Araujo *et al.*, 2000). The fruitful avenue of divergent selection for antibody reponse to SRBC has also been pursued in chickens and the results of the two major long-term selection experiments will be reviewed in this chapter. In addition to non-pathogenic antigens, the immune response to antigens that are related to pathogens, such as vaccine preparations, can also be used as a direct selection criterion. An example of long-term selection for vaccine response is given in this chapter.

Divergent selection for multiple traits of immune response

Because many facets of the immune system, including but not limited to antibody response, may be involved in mounting responses towards pathogens, selection for multiple different immune responses is relevant. Animals may be selected for an index of immune responses to improve general immune response. Another strategy consists of generating parallel lines of chickens, each selected for one criterion. The latter design allows maximum progress for each immune trait, which might be recombined later by crossing the lines. Examples of selection experiments using each approach are presented in this chapter.

Examples of direct selection

There are numerous examples in the scientific literature of direct selection for immune response in poultry. This chapter will review five of the largest, best characterized and longest-term direct selection experiments for immune response in poultry. The purpose is to illustrate the range of results that occur in such selection programmes and the type of information about correlated responses in immunity disease resistance and production traits, underlying immune mechanisms and associated genes that can be derived from such studies.

Selection for Single-antibody Response to Sheep Red Blood Cells in a White Leghorn Population

Very long-term, divergent selection experiments for antibody response to SRBC have been carried out at Virginia Tech (Blacksburg, Virginia, USA) using the Cornell Randombred White Leghorn as the base population (King *et al.*, 1959). Inoculations of 0.1 ml of a 0.25% suspension of SRBC were administered intravenously (brachial vein) to 41–51-day-old chicks. Antibody levels were determined by haemagglutination, with titres defined as the $\log_2$ of the reciprocal of the highest dilution with detectable haemagglutination. The trait under direct selection was total SRBC haemagglutinating antibody response (high, HA, or low, HA) 5 days after inooculation. Breeders were selected by truncation selection, based on individual phenotypes within each line.

Responses to selection

Responses to direct selection for SRBC antibody response occurred immediately, with significant differences in SRBC antibody between the two selected lines in the S_1 generation, and between both selected lines and the control line by the S_3 generation (Siegel and Gross, 1980). Realized heritabilities for 5-day antibody titres were

0.30 and 0.23 in the HA and LA lines, respectively. These values remained relatively consistent throughout the generations. Heterosis was generally low (Ubosi *et al.*, 1985b). In a set of diallele crosses, reciprocal effects were important only for female progeny, suggesting sex-linked effects on the Z chromosome (Boa-Amponsem *et al.*, 1997a).

Antigen dosage, antigen route, and chick age effects

There were significant line-by-SRBC dosage interactions. Low dosage (0.025%: Ubosi *et al.*, 1985a) accentuated the line effect and high dosage (2.5% or higher: Ubosi *et al.*, 1985a; Boa-Amponsem *et al.*, 1997b) masked the line effect. This masking was due to an increase in antibody titres in LA but not HA chicks. In a study of primary and secondary response (Boa-Amponsem *et al.*, 2000), patterns of antibody response differed by line, resulting in a line-by-dosage-by-day interaction. Secondary responses differed by line but a dosage effect was only present in the LA line.

The specific route of inoculation affected the antibody production (Boa-Amponsem *et al.*, 2001). With intravenous inoculation, the HA always had higher primary antibody response than the LA line. With intramuscular inoculation, there was line-by-dosage interaction. After booster (secondary) inoculations, responses were the same between lines. In a separate study, the frequency and the titre values of responders were lower in LA than HA chicks, after inoculation at a young age (7 days) (Ubosi *et al.*, 1985b; Boa-Amponsem *et al.*, 1998a).

Antibody kinetics

The antibody response kinetic pattern was measured in two generations, S_9 and S_{14} (Ubosi *et al.*, 1985a; Martin *et al.*, 1989). Antibody kinetic profiles to SRBC were unique in each selected line. Both peak and persistency of antibody titres were lower in LA than in HA chicks. After a second inoculation, the relative memory response was similar for both lines.

In the S_{24} generation, antibody kinetics were evaluated in hens over a period of 180 days (Yang *et al.*, 1999). The HA hens had a higher peak antibody and a greater persistence of titres. There was a positive correlation among titres measured at different times.

Correlated responses of production traits

A difference between the antibody-selected lines was seen in body weight as early as 7 days after hatch (Boa-Amposem *et al.*, 1998a). At 28 days of age, body weight differences diverged from 10% in the S_3 generation (Siegel and Gross, 1980) to 15% in the S_{14} generation (Martin *et al.*, 1990) to 20% in the S_{20} generation (Pinard-van der Laan *et al.*, 1998), with the LA line being heavier than the HA line. Sexual maturity (age at first egg) also changed as a correlate to antibody selection. The LA pullets matured earlier than HA pullets, 13 days earlier in the S_{10} (Siegel *et al.*, 1982), 22 days in the S_{14} (Martin *et al.*, 1990) and 30 days in the S_{20} generation (Pinard-van der Laan *et al.*, 1998).

The HA and LA lines did not differ for feed efficiency (Gross and Siegel, 1981; Ubosi *et al.*, 1985c). However, line-by-diet effects occurred for treatments such as feeding of deoxycorticosterone (Gross and Siegel, 1981), vitamin E (Yang *et al.*, 2000) and different diet regimens (Dunnington *et al.*, 1996a; Boa-Amponsem *et al.*, 1998a, 1999). These types of interactions of genotype with environment must be considered in commercial poultry applications.

In the S_9 generation, HA chicks had smaller thymuses and larger bursas than LA chicks, relative to body weight, but there were no differences in relative liver and spleen weights (Ubosi *et al.*, 1985c). These relationships persisted in the S_{23} generation, with the exception of spleens (Boa-Amponsem *et al.*, 1998b).

Correlated responses to infections

Because the ultimate objective of selection for immune response is to enhance resistance to disease, the responses of the HA and LA lines to parasites, bacteria and viruses were measured. In offspring of S_3 parents, HA chicks had higher Newcastle disease virus antibody and were more resistant to mites, *Mycoplasma gallisepticum*, *Eimeria necatrix* and a splenomeglia virus, but were more susceptible than LA chicks to two bacterial infections, *Escherichia coli* and *Staphylococcus aureus* (Gross *et al.*, 1980; Dunnington *et al.*, 1991). The greater resistance of the HA line to viral and parasitic agents was confirmed in subsequent generations (S_{10} to S_{12}) to *Eimeria tenella* in both natural and controlled exposures (Martin *et al.*, 1986) and in natural exposure to Marek's disease (Dunnington *et al.*, 1986).

The wide range of infectious agents to which the HA lines had greater resistance illustrates the value of correlated changes in immunity that occurred with the direct selection for SRBC antibody response. However, the greater susceptibility of the same lines to two bacterial pathogens makes it clear that defence properties against different categories of infectious organisms may fall under different genetic regulation, and that not all correlated responses will be in the same favourable direction.

Correlated responses in blood group systems

The blood groups systems A, B, C, D, E, H, I and L were typed in S_{10} generation progeny and their parents (Dunnington *et al.*, 1984). Both lines were homozygous for allele L^2 and had similar allelic frequencies for I^2 and I^4. The selection lines differed in allelic frequencies for the other systems, with systems A, B, C and H being at or near fixation. The *B* (MHC) system, known to influence many immune traits, was of special interest.

Line LA was almost fixed for B^{13} while line HA had a very high frequency of B^{21}.

The proportion of B^{13} in line LA remained very high, being 99% in the S_{10} and 98% in the S_{13} generation (Martin *et al.*, 1990). During the same period, the B^{21} allele in line HA increased from 80 to 99%.

Satellite population: replicated selection for divergent SRBC antibody titres

To investigate the role of the *B* system further and alleviate the confounding that had occurred with antibody selection, a satellite population was established. The base population was formed by an F_1 cross of HA and LA birds, then backcrossing for three generations to each parental line to produce sublines that were 93% of either HA or LA background, but segregating for B^{13} and B^{21}. After six generations of relaxed selection, the HA background line became the base for a replicated selection experiment for divergent antibody response, using the same procedure as in the previous selection experiment. In this experiment, however, all parents in the base population were MHC-heterozygous $B^{13}B^{21}$ and the lines were replicated. The replicated high and low lines are named H1, H2, L1 and L2, respectively.

In agreement with the preceding experiment, there was rapid and significant divergence in SRBC antibody between the H and L lines. Gene frequency changes showed a positive association of B^{21} with higher titres in line H1 and no association in line H2, and suggested an association between B^{13} and low antibody titres in line L2 and the opposite in line L1 (Dunnington *et al.*, 1996b). A five-generation pooled analysis suggests that the B^{21} homozygous state is associated with the highest antibody titres and the B^{13} homozygote with the lowest. The genetic association between the *B* system and antibody production to SRBC is complex.

Selection for Single-antibody Response to Sheep Red Blood Cells in a Medium-heavy Brown Laying Population

Divergent selection for antibody response to SRBC

From 1980, chickens were selected for high (H) and low (L) antibody titre at 5 days after an immunization at 35 days of age with SRBC, from a base population of ISA Warren chickens. A control (C) line was maintained by random mating (Pinard *et al.*, 1992). There were about 300 chicks each in the H and L lines and 250 chicks in the C line per generation. After three generations, there were significant differences in antibody response between the lines, and the genetic difference between the high and low lines reached 5.1 phenotypic standard deviations in the 18th generation (Bovenhuis *et al.*, 2002). Genetic parameters were estimated twice. First, after nine generations, the heritability of antibody titre was estimated by restricted maximum likelihood fitting a mixed animal model (DFREML; Meyer, 1989) to be, over lines, 0.31 (Pinard *et al.*, 1992). After 18 generations, and using Markov Chain Monte Carlo methods, a lower value of 0.18 was found (Bovenhuis *et al.*, 2002). This difference could be due to a decrease in additive genetic variance in the later generations. Although a continuing increase of antibody titre is observed for the H line, it tended to plateau in the L line after the 12th generation. This emphasizes the difficulty in discriminating among '0 Ab-titre' animals in the L line, where the biological threshold may not yet be reached.

Sex effects are often reported on immune response and disease traits but not always in a consistent way. In this selection study, Bovenhuis *et al.* (2002) estimated a genetic correlation of 0.92 between male and female antibody titres, a value not significantly different from 1.0.

Antibody response to SRBC and major histocompatibility complex

The first genes studied as markers of immunity in the lines were the major histocompatibility complex (MHC). Four different serotypes (B^{14}, B^{19}, B^{21} and B^{24}-like) were identified and further characterized for *B-G* and *B-F* (Pinard and Hepkema, 1993). After nine generations, all MHC types were still present in the C line, but alleles showed divergent frequency distributions in the H and L lines. The B^{21}-like was becoming most frequent in the H line and absent in the L line, whereas the B^{14}-like was predominant in the L line and rare in the H line (Pinard *et al.*, 1993a). When estimated across lines, significant effects of MHC on SRBC antibody titre were found, as well as interactions with genetic background. To refine the analysis, an F_2 cross from the H and L lines allowed equal distribution of all haplotype combinations in a genetic random background (Pinard and van der Zijpp, 1993). A higher antibody response associated with the B^{19}-like and B^{21}-like haplotypes and a lower antibody response with the B^{14} and B^{24}-like ones were confirmed. The MHC genotypes, however, explained only 2.5% of the phenotypic variation, and the remaining genotype 31%, which indicates the need to seek other genetic markers, as described below.

Correlated responses for immune traits and disease resistance

Selection for high or low antibody titres to SRBC has also resulted in divergent antibody responses to other antigens (Parmentier *et al.*, 1994, 1996). This correlated change reflects the overall capacity of the H or L line animals to respond to antigenic challenges. The relative ranking in antibody titres for responses measured to

T-cell-dependent soluble (or 'protein') antigens (such as keyhole limpet haemacyanin, trinitrophenol, bovine serum albumin, SRBC and vaccines; F11 (*E. coli*), Newcastle disease virus, infectious bronchitis virus and infectious bursal disease virus) is consistently H > C > L. In contrast, no such difference exists in antibody responses to partly T-cell independent antigens (e.g. *Brucella abortus*, *Salmonella* H antigens). The *in vitro* T-cell reactivity response to the mitogen concanavalin A is lower in the H line than in the L line. Phagocyte activity, however, was not different in the selected lines (Kreukniet *et al.*, 1994). In contrast, in Biozzi mice there was a clear difference between the H and L antibody-selected lines. Other differences in immune traits are that the H line has more CD4^{+} T cells and B cells, whereas the L line has more CD8^{+} T cells and TCR-1 (γ/δ) T cells (Parmentier *et al.*, 1995).

Differences in disease resistance were studied at three time points. In the ninth generation, lines were compared for their resistance to Marek's disease (Pinard *et al.*, 1993b). Birds from the L line were more susceptible (70% mortality) than those from the C line (43%), but the H line was not more resistant (41%) than the C line. No significant differences in resistance to *Eimeria* spp. were found between the H and L lines in the 14th and 17th generations (Parmentier *et al.*, 2001).

Correlated responses for production traits (body weight and egg production)

Body weight of H and L line chickens differed significantly at the 14th generation (at ages of 5 and 17 weeks), with the L line being at least 10% heavier than H line chicks (Parmentier *et al.*, 1996). A similar difference was observed in the 20th generation. The increase in the requirements of maintenance in both H and L lines, as compared with the C line, is apparently not directly related to the amount of antibody produced. The exact physiological mechanism underlying the negative correlation

between body weight gain and antibody levels line is unknown (Mashaly *et al.*, 2000). Both for body weight and growth the ranking is L > C > H line. Hens of the H line mature earlier, starting to lay their first egg 1 week earlier than L line hens.

Mapping of genes for immune traits and disease resistance

At the 18th generation, a reciprocal intercross was made between the H and L selection lines to identify genomic regions and, ultimately, genes controlling the type and magnitude of immune responses in chicken. A total of 700 second-generation animals (G_2) were genotyped with 180 microsatellite markers. Phenotypic measurements including primary and secondary antibody response to SRBC (the selection criterion), *E. coli*, KLH–TNP and *Mycobacterium butyrium*, and immune-mediated innate and acquired resistance parameters in a controlled Newcastle disease virus (NDV) infection, were collected. The genotypic and phenotypic information, therefore, can serve as the basis to identify, by linkage analysis, the chromosomal regions and genes involved in the regulation of innate and acquired disease resistance in these animals.

Selection for Single-antibody Response to *Escherichia coli* Vaccine in a Heavy Meat-type Population

The productive lifespan of broilers is very limited because of their young market age, and it was hypothesized that disease resistance of broilers depends mainly on their early antibody response. As early as the 1970s, significant genetic variation in antibody response to *E. coli* (EC) and NDV vaccination of 4-week-old broilers was revealed (Soller *et al.*, 1981). Therefore, divergent selection experiments for early antibody responses to bacterial vaccine antigens were initiated.

Long-term selection for response to *E. coli* vaccine

In 1987, a long-term selection experiment for antibody response to EC vaccine was initiated from a commercial broiler dam-line (Anak, Israel) base population. Results from a previous short-term selection experiment conducted from the same base population, on an index of EC and NDV vaccine antibodies (Pitcovski *et al.*, 1987a,b), helped to refine the procedures used in the long-term experiment. Maximal variation in antibody titres was exhibited by chicks vaccinated at 10 days of age and bled for antibody determination 10 days after vaccination (Leitner *et al.*, 1992); hence this procedure was used in the first five selection cycles. Antibody titres were determined by ELISA (Leitner *et al.*, 1990). Each selection (high or low antibody) was conducted in two replicated sublines, and a control unselected line was also maintained. In each generation about 100 chicks per subline (total 500) were evaluated for antibody response and the breeders were selected by truncation selection (ten males and 30 females per line).

Response to selection

Estimates of heritability of EC antibody averaged 0.15 and 0.27 in the low- and high-Ab lines, respectively, and realized heritability averaged 0.27 (Leitner *et al.*, 1992). In the S_3 generation, antibodies were detected in chicks of the high-Ab line vaccinated as early as 2 days of age, while in the low-Ab line, antibodies were not detected until chicks were vaccinated at 4 days of age or later.

In S_3 and S_4, antibody titres were significantly higher in the high line than low line chicks after immunization with NDV and SRBC, and the lines similarly differed in serum levels of beta and gamma globulins, in lymphocyte mitogenic response and in phagocytic activity (Heller *et al.*, 1992). After EC vaccination and challenge with pathogenic EC, mortality of low-Ab line chicks was two times higher than high-Ab chicks (Leitner

et al., 1992). Without EC vaccination, mortality following EC challenge was similar between selected lines.

Antibody dynamics

To investigate evidence from the S_5 generation of differences in antibody dynamics between the selected lines (Yonash *et al.*, 1996), high-Ab, low-Ab and high × low F_1 chicks were produced from S_5 parents (Yonash *et al.*, 2000b). Each chick was vaccinated at 8, 10 or 12 days of age, and its antibody titre was determined five times (6, 8, 10, 12 and 14 days after vaccination). At each age-by-sampling combination, the highest and lowest titres were exhibited by the high-Ab and low-Ab line chicks, respectively, with the divergence increasing with age. The high × low chicks exhibited intermediate mean antibody at all ages, suggesting an additive nature of the genetic divergence.

To increase the divergence in early antibody response further, titres at 8 or 12 days after vaccination were used as the criterion of selection for high-Ab or low-Ab lines, respectively, in the subsequent generations. The two sublines within each direction of selection were merged. The unselected control line was terminated and the commercial dam-line from which the selection was initiated was thereafter used as a control.

The dynamics of antibody response was further evaluated in S_9 (Yonash *et al.*, 1996). High-Ab line chicks were vaccinated at 8 or 10 days of age, low-Ab line chicks at 10 or 12 days of age, and control chicks were vaccinated at one of the three ages. Antibody titre was determined twice for each chick, at 8 or 10 days after vaccination. Divergence between the selected lines in antibody titres resulted mainly from a large difference in rate of antibody accumulation and from a difference in age at which the chick became capable of response (Yonash *et al.*, 1996). The highest estimate of antibody heritability (0.43) was similar in both high-Ab and low-Ab lines, but it was obtained for titres at 18 and 22 days of age, respectively (Yonash *et al.*, 1996). This result indicated

that both lines maintained genetic variation for early antibody response, but at different vaccination age-by-sampling combinations.

Response to *E. coli* challenge

Chicks were first infected with infectious bronchitis virus (IBV) injection and then intratracheally with pathogenic EC (Yunis *et al.*, 2002a). High mortality (8–20%) occurred in the slow-growing selected lines and their cross, and much higher mortality (approximately 40%) occurred among the control broilers, which were 38% heavier. Within-line antibody levels were higher in chicks with colibacillosis, suggesting that these antibodies were produced as a result of the infection but were not protective.

The effect of genetic divergence in antibody response on viability of broilers was also studied under field conditions (Yunis *et al.*, 2000). The experimental populations consisted of six groups: high-Ab and low-Ab lines, commercial dam-line as a control (CC) and three crosses: high–low, high–CC and low–CC. The high-Ab and low-Ab groups exhibited the highest and lowest antibody titres, respectively (Yunis *et al.*, 2000). Mean antibody of the CC group equalled the average of the selected lines and each of the three crosses exhibited mid-parent antibody titres, indicating additive genetic control. The fast-growing group (CC) showed the highest mortality, and the slow-growing antibody-selected lines and the crosses between them showed the lowest. Despite the similar mean body weight of the high–CC and low–CC crosses, mortality was one-third higher in the latter, suggesting that high antibody production reduces mortality only in broilers that grow faster than the selected lines.

Correlated responses in antibodies

In early generations of the long-term selection experiment, lines selected for high or low antibody response to EC similarly diverged in their antibody response to NDV and to SRBC. In a later generation, the high-Ab line exhibited higher titres to inactivated infectious bursa disease virus (Pitcovski *et al.*, 2001). Data from the on-farm study with the selected lines, the control line and their crosses (Yunis *et al.*, 2002b) yielded estimates of highly positive genetic correlations between antibody responses to natural EC exposure and to the infectious bursal disease virus and NDV vaccines. The six genetic groups ranked similarly by mean antibody to all three antigens, indicating a common genetic control of early antibody response to all these antigens. These results suggest that selection for high-Ab response of young broilers to a controlled immunization with a single antigen can improve their antibody response to vaccination against other pathogens.

Mapping of genes for immune traits and disease resistance

The lines selected for antibody response to *E. coli* vaccine were used to generate F_2 and backcross (BC_1) resource populations for the purpose of identifying genes and chromosomal regions controlling the antibody production. The likely candidate gene region of the MHC was evaluated for multiple classes of genes. Evidence was found for associations of *Tap2*, class II and class IV MHC genes being associated with antibody response to *E. coli* (Yonash *et al.*, 1999, 2000a). These genes, however, explained only a part of the genetic variation and thus additional studies sought the location of other polygenes controlling antibody response. A microsatellite linkage-disequilibrium scan with a limited starting coverage of the genome identified regions on chromosome 2 and linkage groups 31 and 34 as being associated with antibody response traits in broilers (Yonash *et al.*, 2001).

Index Selection for Multi-trait Immune Response in a White Leghorn Population

Animals and index selection

The base population consisted of about 500 Ottawa Strain 7 chicks (Gowe and Fairfull, 1980). Four lines were formed by selecting the high- and low-responding siblings to establish the H and L lines, respectively, with ten different sires used for each replicate (1 or 2). All progeny (approximately 500) were tested in each generation. Chickens with the highest (or lowest) ranking breeding values within each sire family within each high (or low) immune-response line were chosen as breeders, with avoidance of sib mating. Ten sires and 40 dams were used as breeders in each of the four lines in each generation.

Antibody response was measured using commercial ELISA kits on samples collected at 3 weeks after vaccination from birds given *M. gallisepticum* (MG bacterin) (MG) and *Pasteurella multocida* bacterin (PM) vaccines at 6 weeks of age. Cell-mediated immune response was measured by using response (PHA) to phytohaemagglutinin (PHA-P) injection in the wing web (van der Zijpp, 1983; Lamont and Smyth, 1984) at 10–12 weeks of age. Reticuloendothelial clearance, an estimate of phagocytic ability, was measured as clearance of an intravenous injection of colloidal carbon (Glick *et al.*, 1964; Lamont, 1986) as modified by Cheng *et al.* (1991).

A selection index was used to rank the chickens for immunocompetence within each family in each generation. A balanced gain in all three facets of immune response was chosen as the selection goal, because no estimates of economic values of these traits were available. Genetic covariances were set to zero because no estimates of covariances for these traits in chickens were available and estimates for similar traits in other species were small (Biozzi *et al.*, 1979). The parameters used to calculate index weights were adjusted in each generation to reflect added information. Heritabilities, genetic variances and phenotypic variances and covariances were estimated in each generation from information of all previous and the present generations.

Heritabilities and genetic correlations of MG, PM, PHA and carbon clearance assay (CCA), and response to selection

Detailed evaluation of quantitative genetic parameters was conducted in selection generations 1 and 2 (Cheng *et al.*, 1991) and generations 5, 6 and 7 (Kean *et al.*, 1994b). Heritability estimates of the immunological traits ranged from 0.06 to 0.53. Genetic correlations among immunological traits were generally negative and insignificant (Cheng *et al.*, 1991). Heritability estimates for antibody response were the highest of all traits at 0.21 to 0.31. These (MG and PM vaccine antibodies) are in a range similar to that found for response to other commercial vaccines, such as *Salmonella enteritidis* (Kaiser *et al.*, 1998). The genetic correlation (−0.86) between PHA and CCA was significant, which is in agreement with a study on a different population (Cheng and Lamont, 1988).

In generations 5 to 7, heritability estimates pooled across three generations (two cycles of selection) were, respectively, 0.16 and 0.09 for the index, 0.31 and 0.08 for MG, 0.21 and −0.02 for PM, 0.06 and 0.05 for CCA, and 0.08 and 0.12 for PHA (Kean *et al.*, 1994b). Phenotypic correlations among traits were generally small and several estimates were negative. Estimates of genetic correlation varied widely.

In evaluation of generations 5, 6 and 7, significant differences between selection directions were found for the overall index in generation 7, for MG in generations 5 and 7, for PM in generation 7 and for PHA in generation 5. Significant differences were present between replicates in antibody response to MG in generations 5, 6 and 7, the overall index in generations 5 and 6, in PHA in generations 6 and 7, and in PM in generation 6.

Correlated responses in growth and reproductive traits and antibody production

Very few consistent differences related to selection occurred in juvenile and adult body weights, age of first egg, 32-week egg weight, rate of egg production, or mortality (Kean *et al.*, 1994b).

Chickens hatched from S_9 were tested for antibody responses to SRBC and *B. abortus* antigen (Nelson *et al.*, 1995). All birds were injected at 4 and 9 weeks of age, and blood samples were taken at 6, 8 and 10 days after immunization. For secondary SRBC responses, the titre of the high index lines was still rising at 10 days, whereas the low-line titre had reached a peak at 8 days after immunization. For *B. abortus* responses, high and low lines differed after secondary immunization, with the high lines always higher than the low lines, and the differences decreasing as time after immunization increased.

To evaluate the kinetics of response to the specific selection vaccines (MG and PM), chicks of S_{10} were immunized at 6 weeks of age with both vaccines, and serum antibody levels were analysed at 1, 2, 3, 4, 5, 12 and 21 weeks after vaccination. Antibody response curves were approximated from the actual antibody data by using non-linear regression and new parameters, the curve maximum (y_{max}) and the time of the maximum (t_{max}), were calculated (Weigend *et al.*, 1997). The high index lines had significantly higher response than low lines to both vaccines, with large differences in the response kinetics (Fig. 22.1). There was a significant positive correlation between the response to the two antigens in both lines at 0 and 21 weeks after vaccination but the y_{max} and t_{max} to the two vaccines were not correlated. The results of this study indicate that the kinetics and persistence of antibody reaction have different genetic regulation in response to each vaccine.

Correlated response to Marek's disease virus

The base population of this selection experiment had a natural outbreak of Marek's disease (MD) resulting in 40% mortality in the herpes virus turkey (HVT) vaccinated
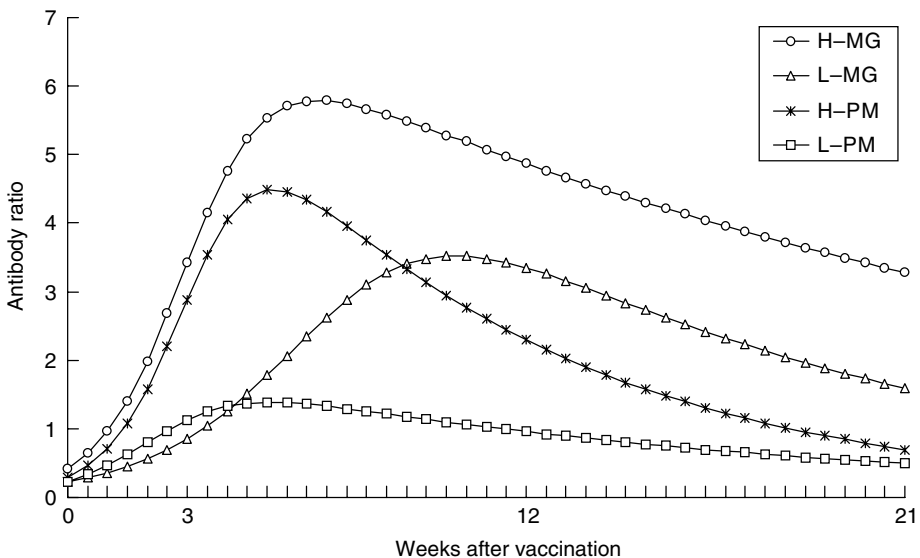
Fig. 22.1. Average antibody response curves to *Mycoplasma gallisepticum* (MG) and *Pasteurella multocida* (PM) vaccine, approximated to the antibody ratios measured several times during a period of 21 weeks after vaccination, of chicken lines selected for high (H) and low (L) multi-trait immune response. (Reproduced with permission from *Poultry Science* 76, 1248–1255.)

chicks. The starting material for selection, therefore, was strongly naturally selected for survival to MD virus exposure. Additional correlated changes in Marek's resistance, as a result of several index selection generations, were studied. Chicks were challenged with one of three MD virus strains (Lamont *et al.*, 1996). In unvaccinated chicks, the mean percentages of MD were 12, 50 and 64% after JM/102, Md5 and RB-1B virus strain challenge, respectively, with no differences due to genetic selection. There was no difference in vaccine protection to Md5 challenge between high and low index-selected lines. With RB-1B challenge, however, the low index lines had a significantly greater vaccine protection index (92.6) than the high-response index lines (72.0). The ability to express detectable genetic differences in vaccine-induced immunity to MD in these selected lines may be virus-strain specific. Alternatively, because the Md5 and RB-1B challenges were administered at 2 and 20 days, respectively, it may require an interval between the vaccination and challenge that is sufficient to develop protective immune mechanisms.

The MHC and endogenous viral genes

After four cycles of index selection, MHC (*B* system) frequencies were studied using a serological approach (Kean *et al.*, 1994a). Frequencies of B^{21} were greater in the low index lines and the B^{12} and B^{19} frequencies were greater in the high lines. A gene substitution model showed that the B^2 haplotype was positively associated with MG, B^{21} was positively associated with the multi-trait index and B^{13} had a negative effect on both MG and the index.

Later, DNA analysis was undertaken to determine which chromosomal regions exhibited significant frequency differences associated with immune-response selection. The class IV (or *B-G* region) genes were studied in the S_{10} generation (Weigend and Lamont, 1999) by hybridization with an MHC class IV (*B-G*) probe (Miller *et al.*,

1991). Significant differences between selection directions, pooled over replicates, were found for six restriction fragments, but only one band frequency difference was consistent between the two replicates. The class II (*B-L*) region genes, which have a key role in antigen presentation (Kaufman and Lamont, 1996), were studied by hybridization with an MHC class II (*B-L*) probe (Xu *et al.*, 1989). Only one band showed a significant difference in frequency associated with selection direction, in the pooled replicates (Weigend and Lamont, 1999). With an *Rfp-Y* region probe (Briles *et al.*, 1993; Miller *et al.*, 1994) only one of three polymorphic bands had significantly different frequency between index-selected lines, and that was in one replicate only (Lakshmanan and Lamont, 1998). These results suggest that selection for multi-trait immune response did alter the frequency of specific MHC alleles and also indicate the value of replicated lines in genetic selection experiments.

In offspring of the S_2 generation, birds were evaluated for endogenous viral (*ev*) gene sequences. The *ev*6 and *ev*9 differed in frequency between selected lines, and both *ev* genes were in lower frequency in the lines selected for high index of immunoresponsiveness (Lamont *et al.*, 1992).

Parallel Selection for Multi-trait Immune Response in a White Leghorn Population

Selection design and results

From an unselected base population of White Leghorn chickens, three selected lines were generated using the following selection criteria: one line (ND3) was selected for high antibody response to NDV HB1 vaccine at 3 weeks after vaccination at 3.5 weeks of age; one line (PHA) was selected for high cell-mediated immune response, using the wing-web delayed-type hypersensitivity (DTH) response to phytohaemagglutinin, at 9 weeks of age; and one line (CC) was selected for high phagocytic activity, measured as clearance of carbon at 12 weeks of age. A control line was

maintained by random mating. At the beginning of the selection, the dwarf gene was present in the line but was further eliminated because it was found to have a negative effect on the immune responses (Pinard and Monvoisin, 1994).

All birds were measured for the three immune traits. Selection started in 1994 and was performed every year, measuring about 800 animals per generation, i.e. 200 birds per line. The results on six generations of selection and a total of 5048 birds measured are presented (Fig. 22.2).

Selection was successful for the three immune traits for both phenotypic and genetic responses. Figure 22.2 shows the significant responses obtained in each line for the immune trait for which the birds of that line were selected, expressed as the mean of breeding values estimated with an animal model.

Genetic parameters for immune traits and correlations between immune traits

Heritabilities and correlations for immune traits were estimated on data and relationships from the six generations, using an animal model and the VCE software

(Groeneveld, 1997). Heritabilities estimated for ND3, PHA and CC were moderate but all were significant (0.35, 0.13 and 0.15, respectively).

Genetic independence between the three immune traits was shown in two different ways. Because all birds were evaluated for all three traits, it was possible to estimate genetic correlations between traits and also to analyse whether selecting for one immune trait had modified the level of response for another immune trait (indirect selection). In both instances, neither significant correlated responses nor significant correlations between any pair of the three immune traits were found.

These results, together with the selection responses, show clearly that one can modulate different facets of immunoresponsiveness through genetic selection. Additional studies are required to refine the selection procedure and to determine relationships with resistance to disease.

Immune traits and the MHC: MHC type frequency and MHC effects

The three immune traits under selection in this experiment are likely polygenic,
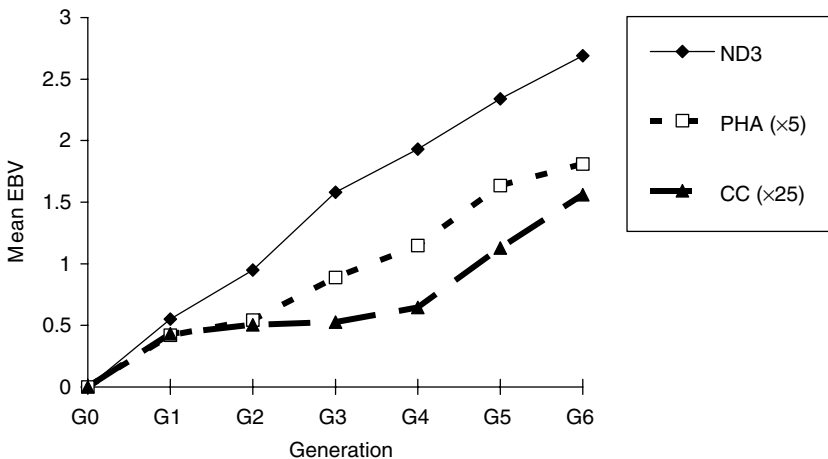
Fig. 22.2. Responses to selection for ND3 (antibody response) in the ND3 line, PHA (cellular response) in the PHA line and CC (phagocytic activity) in the CC line. Estimated breeding values (EBV) for ND3, PHA and CC were estimated with the PEST software (Groeneveld, 1990) by applying an animal model.

with many immune response genes being unknown. However, some candidate genes of frequent importance in immunity, such as the MHC, were investigated for their associations with immunoresponsiveness of the birds of this experiment.

Birds were all MHC typed since the base population (G_0) and six different *B-G* haplotypes were found by serology and RFLP analysis: B^{15}, B^{19}, B^{21}, B^{121}, B^{34} and B^{124}. These were associated with five *B-F* haplotypes, the B^{21} and B^{121} containing a single B^{21} *B-F* type. In the base population, all haplotype combinations were found and heterozygosity level was high (78%). The effect of selection on the MHC type frequency was evaluated over the generations in the different lines. Significant changes in MHC haplotype frequencies occurred in the selected lines, especially in the ND3 line. Changes may be suggestive for a direct or linked effect of MHC genes on the selected immune traits but should never be taken as proof of effect. In this experiment, changes in MHC haplotype frequency could be explained by their effect on selected trait in the ND3 line, but not clearly in the other lines.

It was therefore of relevance to analyse whether effects of MHC haplotypes on the three different immune traits were comparable. Effects of MHC were estimated using an animal model on all the generations, thus taking into account family effects. The MHC effects clearly varied among the different immune traits (Table 22.1). For example, the B^{34} haplotype was associated with a higher antibody and phagocytic responses, whereas B^{124} conferred a higher antibody and cellular responses.

Immune Traits and Body Traits

It is intuitive, if not yet fully understood, that an animal is a whole organism and that different physiological functions may be competitive for its limited resources during its lifetime. Selecting for increased immune responses may have induced broad physiological changes. Indeed, some changes were noticed in this experiment. For example, birds selected for higher cellular response (PHA line) showed higher values than did the other lines for body weight at 9 weeks and 17 weeks of age, as well as for body temperature and haematocrit values. In addition, genetic correlations were negative between body weights at both ages and antibody response, whereas 9-week body weight was positively associated with phagocytic activity and was negatively associated with cellular response. Negative relations between antibody response and body weight had been reported previously (Martin *et al.*, 1990; Parmentier *et al.*, 1998). In sum, these results indicate that there may be a delicate balance of optimum immunoresponsiveness and productivity to be achieved.

Table 22.1. Effect of MHC haplotypes on antibody response (ND3), cellular response (PHA) and phagocytic activity (CC).

Immune trait					
ND3		PHA		CC	
Haplotype*	Estimate	Haplotype	Estimate	Haplotype	Estimate
34	0.58[a]	124	0.062[a]	34	0.013[a]
21	0.54[a]	15	0.044[ab]	19	0.005[ab]
124	0.49[a]	19	0.034[ab]	21	0.005[ab]
15	0.20[b]	34	0.024[ab]	15	0.002[ab]
19	0[c]	21	0[b]	124	0[b]

*Haplotypes are ranked according to decreasing effect on each immune trait.
[a,b]Estimates with different superscripts differ ($P < 0.01$) among MHC haplotypes for a given trait.

Conclusions

Selection lines for immune response

Design and analysis of selected lines

Many design and analysis choices contribute to the validity of the results and conclusions generated from the study of selected lines. Because of the high phenotypic variation that exists in many immune response traits, the population under study must be sufficiently large to yield reliable results. Founder effects should be taken into account in the design, selection and analysis of such studies to ensure that the conclusions drawn are not simply based on the genetic contributions of a few individuals to the establishment of the initial generations of the divergently selected lines. Although the total population size of each generation is usually a limiting factor in most selection experiments, there is much scientific value to be derived from the use of control lines or replicated lines or, ideally, both (Pinard-van der Laan *et al.*, 1998). A control line provides the information needed to correct for the large impact of environmental factors on immune traits and to improve the precision of estimation of genetic parameters of the selected traits. It also serves as the reference point for observed changes in gene and marker allele frequency differences and in phenotypes of the selected lines, which is especially helpful in the instance of immune traits reaching asymptotes. The availability of replicated lines allows a clearer differentiation between those responses that occurred as a result of the divergent selection procedure and those that were caused by founder effects or genetic drift. This information is crucial for the effective application of the results of such studies into other populations.

The analysis of divergently selected populations is complicated by the population structure, in that the background genes of the selected lines cannot be considered to be randomly distributed. The appropriate statistical models to estimate genetic parameters and breeding values, therefore, take into account the relationships between individuals over generations of selection (e.g. Groeneveld, 1990, 1997; Groeneveld and Kovak, 1990). The non-random distribution of the background genes in selected lines also dictates the need for appropriate statistical methods in the estimation of effects of gene and markers putatively linked to the immune response traits (Kennedy *et al.*, 1992).

Selected line use for identifying QTL and genes controlling immune traits

The combination of molecular typing and classification of chickens on the basis of distinct immune parameters and resistance to various pathogens will provide insight into the identity and the contribution of specific genes to the type and magnitude of immune responses and disease resistance. This type of genomic analysis will help to unravel the gene–gene interactions, with respect both to immune responses in general and to relations between 'health' and 'production' traits.

Because many genes control immune traits and the MHC genes generally explain only a small part of the genetic variability (Pinard and van der Zijpp, 1993), immune-response selected lines are a material of choice for identifying other genetic markers of immune response and disease resistance. As the first stage, evidence of allele frequency differences between the phenotypically distinct, divergently selected lines is sought. This frequency difference suggests the linkage of the identified markers with the actual genes or QTL controlling the phenotypic traits. This first stage must not be taken as proof of such linkage, however, because many other factors such as genetic drift and founder effect can also contribute to the marker frequency differences between lines. Definitive proof of marker–QTL linkage should be sought by testing the suggestive markers in appropriately designed resource populations, such as backcross or intercross populations (Lynch and Walsh, 1998; Patterson, 1998). Such studies have been successfully performed in the mouse (Puel *et al.*, 1998) and are in early stages in the chicken (Yonash *et al.*, 2001).

Correlated responses to direct selection for immune response

Correlations with other immune traits

In examining the pattern of responses that occurred over the several major selection experiments reported in this chapter, it is clear that there are certain consistencies that exist among these separate experiments. One consistency that emerges immediately is that direct selection for immune response is quite successful in altering the phenotype under direct selection, especially for the antibody response phenotypes. There is also evidence for the generality of the altered response mechanisms; that is, divergent selection for antibody response for one antigen often shows the same correlated changes for other antigens. Quite fortuitously for achieving the ultimate goal of enhanced disease resistance, there are many instances of beneficial changes in disease resistance associated with selection for higher antibody response. It is, however, not universal that high-antibody selected lines are more resistant to disease, which may be a function of the specific pathology induced by different types of infection. Thus, application of direct selection for immune response must be applied in a practical setting only with the knowledge that some correlated changes may be undesirable, and the correlations should be well characterized before widespread alteration of commercial breeding populations begins.

Another generally consistent observation is that immune response mechanisms of different types (e.g. antibody production, cell-mediated immunity, phagocytic activity) show very low, no, or even negative correlations. This indicates the relative independence of the genetic control of each of these traits and suggests that each of these mechanisms may be independently evaluated and changed in breeding populations. Given the usual three-way and four-way crosses of commercial poultry, the independence of the different categories of immune response traits opens the door to improve different traits in different grandparent lines separately and intensively, and

receive the benefits of all improvements in the crossbred commercial birds. For this approach to be effective, the detailed nature of the inheritance of the individual traits, additive and dominance effects and gene interactions must be characterized.

One gene complex, the MHC, was confirmed in the selection studies as having a small yet consistent effect on the immune traits. This is not unexpected, given the gene-rich nature of the MHC region and the already defined role of many of the MHC genes in antigen presentation and immune response (Kaufman and Lamont, 1996). Evidence was again presented for the beneficial role of one specific allele: B^{21}. Because the role of the MHC explains only a small percentage of the variation in the immune traits, however, it is clear that much investigation yet remains to determine the many other polygenes that also contribute to the genetic control of immune function.

Correlations with production traits

The ultimate fate of information about physiological markers of immune response, or associated molecular markers, will depend on the manner in which they can be readily integrated into the complex, and very effective, selection programmes already utilized by the poultry industry. Ideally, the immunity selection will enhance or at least not compromise the ongoing selection for other production parameters (such as egg production or growth rate) in the commercial populations. Although immune trait selection had no or small effects on many traits, one major negative correlation frequently occurred that will be of major importance in the application of immunity selection. There appears to be a strong negative correlation between antibody production and body weight, and this result is consistent across many types of genetics. This negative correlation is likely explained by the 'limited resources' model. In this model, each individual animal is considered as a unit in which the different physiological systems, such as protein deposition in muscle and protein secretion in antibodies, compete for the limited available nutrient

resources (Pinard-van der Laan *et al.*, 1998). To overcome this negative correlation effectively, a better understanding is needed of the nutrient requirements of an activated immune system and of the individual genes that contribute to the shared and separate mechanisms controlling protein synthesis in muscle tissue and lymphocytes.

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23 Genetic Markers: Prospects and Applications in Genetic Analysis

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Introduction

Genetic variation broadly representing any of the many types of DNA sequence variations is the quintessential prerequisite of any genetic study. Variation in the DNA sequence is referred to as polymorphism. Specific subtypes of polymorphisms are usually caused by base-pair substitutions, insertions or translocations, and tandem repeats or short regions of deletion. Polymorphism leads to genetic variation, and different forms of the same gene that occur at a locus are known as alleles. Genes code for proteins, and genes that differ in their DNA sequences may cause differences in the protein they encode. Most of these genetic variations do not affect gene function but some polymorphisms may cause modification of proteins that are involved in the architecture of traits of economic importance.

Genetic variation within families, within species and between populations is assessed by genetic markers. Thus genetic markers are the basic tool for geneticists. Genetic markers may be operationally defined as a phenotypically recognizable genetic trait that can be used to identify a genetic locus, a linkage group, or a recombination event. The three properties that define a genetic marker are: locus specificity, polymorphism and ease of genotyping.

The quality of a genetic marker is measured by the level of heterozygosity (H) and the polymorphic information content (PIC).

Heterozygosity of a locus is defined as the probability that an individual is heterozygous for that locus for a population. H is given as:

$$H = 1 - \sum_{i=1}^{l} p_i^2$$

where p_i is the frequency of ith allele among a total of l alleles. Population geneticists usually describe a locus as polymorphic when p is at least 1% in a given population. Ott (1992) classified a genetic marker with heterozygosity of higher than 70% as highly polymorphic.

PIC (Botstein *et al.*, 1980) describes the probability that, for a given progeny, one could identify which homologue of a given parent was transmitted to the progeny, the other parent being genotyped as well. PIC is also the product of the heterozygosity of the parents and informativeness of the offspring.

$$PIC = 1 - \sum_{i=1}^{l} p_i^2 - 2 \sum_{i=1}^{l} \sum_{j=i+1}^{l} p_i^2 p_j^2$$

When the number of alleles is small, the level of heterozygosity is usually greater than PIC. However, with a large number of alleles, PIC is approximately equal to heterozygosity. The genetic interpretation of a genetic marker depends on the sequence

complexity of the genome and the kind of variation the marker identifies (Liu, 1998). O'Brien (1991) categorized genetic markers into two types: (i) markers derived from genes of known function as Type I; and (ii) markers derived from anonymous DNA segments as Type II. Dodgson *et al.* (1997) further classified DNA-based markers into fingerprint markers (minisatellites, random amplified polymorphic DNA, amplified fragment length polymorphism) and clone/sequence-based markers (restriction fragment length polymorphism, microsatellites, sequence tag sites, expressed sequence tags, single-strand conformation polymorphism and single nucleotide polymorphism). Apparently, most fingerprint markers are Type II whereas the clone/sequence-based markers are Type I. The rapid development and application of most genetic markers have come to depend on the PCR.

PCR uses the biochemistry of DNA replication to amplify specific sequences of DNA (Saiki *et al.*, 1985; Mullis *et al.*, 1986). Unlike DNA cloning, the specific DNA fragment can be amplified entirely *in vitro*, and PCR dramatically reduces the time involved in obtaining DNA for analysis. It allows the molecular analysis of very small amounts of DNA. Single-copy sequences from single sperm can be amplified (Cui *et al.*, 1989). The ease, time factor and small amounts of template DNA needed are the main reasons that PCR has revolutionized molecular analysis.

PCR uses a variety of thermostable DNA polymerases that are not inactivated by the heat denaturation step. Specific sequences are targeted for amplification by using two oligonucleotide primers complementary to the DNA sequence of the opposite strands flanking the region to be amplified. By repeated temperature cycling, denaturation of the target template, annealing of the oligonucleotide primers, and elongation from the primers by DNA polymerase, a specific sequence defined by the two PCR primers is amplified exponentially. The exponentially amplified DNA fragment becomes the major product of the PCR reaction and can be used for a variety of applications. In theory, the copy number of the amplified fragment doubles each cycle, but this expectation is never met. Premature termination of elongation, incomplete denaturation, incomplete primer annealing, or chemical breakdown of the PCR product will limit the amplification.

PCR works because the oligonucleotide primers needed to initiate DNA synthesis can anneal to specific sequences with a high affinity and specificity. Primer sequences can be designed to amplify single-copy sequences found in complex genomes. This necessary attribute of PCR is also one of its weaknesses. In order to amplify a specific sequence, prior knowledge of the DNA sequence is needed to design the oligonucleotide PCR primers. PCR is especially suited to genetic typing, because once the DNA sequence has been obtained, PCR tests can be designed to type DNA polymorphisms in large segregating populations rapidly and efficiently.

There are many books on PCR, and it is essential that researchers understand primer design and the effects of altering PCR conditions if they are attempting to amplify new templates on a routine basis. A handy reference that is often overlooked in basic PCR strategy guides is Weissensteiner and Lanchbury (1996). These authors describe the use of the PCR reaction additive betaine to improve the amplification efficiency of problem templates.

Restriction Fragment Length Polymorphism (RFLP)

Prior to the advent of DNA cloning and Southern blot analysis, markers were confined to protein polymorphisms detected by gel electrophoresis or serology. DNA cloning and Southern blotting have made it possible to detect polymorphism in genomic DNA. RFLP results from changes in the DNA sequence (usually base substitution) that create or destroy restriction endonuclease cleavage site (Jeffreys, 1985). Identification of RFLP requires gel electrophoresis to separate DNA fragments of different sizes or lengths from DNA subjected to digestion with endonuclease (restriction

enzymes). This is followed by transfer of the fragments to a nylon membrane (Southern blot), which are then hybridized by a radioactively labelled single-copy DNA probe and visualized after exposure to an X-ray film.

PCR-RFLP can identify polymorphisms from known sequences when oligonucleotide primers are designed to amplify a DNA fragment using PCR methodology and thereafter subjecting the amplified product to restriction enzyme digestion and gel electrophoresis. The majority of single nucleotide polymorphisms (SNP) do not affect sequences recognized by available restriction enzymes. The sequence of the oligonucleotide primers used in the PCR amplification can often be altered to generate a sequence recognized by a restriction enzyme that incorporates a known polymorphism. The altered sequence of the PCR product of one allele can then be cut with a restriction enzyme (Friedman *et al.*, 1991; Petty *et al.*, 1991; Schwartz *et al.*, 1991). PCR primers can be labelled for fluorescent detection of the DNA fragments or standard gel electrophoresis and ethidium bromide staining can be used.

A marker based on the presence or absence of a restriction endonuclease cleavage site will have only two alleles (biallelic), and its usefulness as a genetic marker is limited by its low heterozygosity. In a large random mating population, the maximum frequency of heterozygotes from a biallelic marker is 50%. Development of RFLP markers can be time consuming and can involve screening several expensive restriction enzymes and/or probes before a suitable polymorphism is detected.

Despite the limiting heterozygosity that can be obtained with RFLPs, they still provide useful co-dominant markers (Jeffreys, 1979) and have been used to construct linkage maps in humans (Botstein *et al.*, 1980; Donis-Keller *et al.*, 1987). RFLPs have been extensively used to search for markers linked to disease loci whose gene products are not known, e.g. cystic fibrosis (Tsui *et al.*, 1982) and Huntington's disease (Gusella and Wexler, 1983). In poultry, RFLP markers have been part of the markers used to develop the chicken genetic maps

(Bumstead and Palyga, 1992; Levin *et al.*, 1994a; Groenen *et al.*, 2000) and for MHC typing (Zheng *et al.*, 1999; Weigend *et al.*, 2001). RFLPs have also been the subject of several trait association studies (Fotouhi *et al.*, 1993; Feng *et al.*, 1997, 1998; Kuhnlein *et al.*, 1997; Aggrey *et al.*, 1998; Li *et al.*, 1998a; Nagaraja *et al.*, 2000).

Variable Number of Tandem Repeats (VNTR) Markers

Minisatellites and DNA fingerprinting

Using RFLP in genetic analyses can be limiting because of its biallelic nature. Critical individuals that are homozygous for the RFLP marker makes them uninformative for pedigree analysis. Such limitations can be ameliorated by developing multi-allelic locus markers. A multi-allelic marker system, in contrast to RFLP, can be highly informative in linkage analysis. The discovery of hypervariable (minisatellite) regions (HVR) in the genome (Wyman and White, 1980) led to the demonstration of multi-allelic loci in the human genome. HVR consist of simple tandem repeat regions of DNA or minisatellites. Hypervariability at these minisatellites results from changes in the number of repeats, presumed to be driven either by unequal recombination between misaligned minisatellites or by slippage at replication forks leading to the gain or loss of repeat units (Jeffreys, 1979). Hypervariable minisatellites of relatively short (11–60 bp) oligonucleotide sequence that give rise to a relatively high frequency of alleles (due to variation in the number of repeats) but represent *only a single locus* have been designated variable number of tandem repeats (VNTR) loci (Nakamura *et al.*, 1987). Jeffreys *et al.* (1985a) demonstrated that a DNA probe based on a set of tandem repeats can be detected by hybridizing to genomic DNA, a number of loci containing tandem repeats of similar sequences. The restriction fragment length pattern revealed by the sum of VNTR loci constitute a 'genetic fingerprint'

(Nakamura *et al.*, 1987). The DNA finger-print pattern is unique to an individual (Jeffreys *et al.*, 1985b) and is inherited in a Mendelian manner; the patterns have been used reliably in parentship and sibship identification studies (Jeffreys *et al.*, 1985a).

Despite the multi-allelic advantage of minisatellites, there are some major problems associated with their use as markers. They are not uniformly distributed throughout the genome, as they reside primarily in heterochromatic regions near telomeres and centromeres. In addition, they are difficult to sub-clone for detailed analysis (Dodgson *et al.*, 1997). Nevertheless, DNA fingerprinting has been applied to poultry to measure inbreeding and genetic distances between breeding populations (Kuhnlein *et al.*, 1989, 1990) and has also been the subject of quantitative trait loci (QTL) studies in chickens (Hillel *et al.*, 1989; Plotsky *et al.*, 1990; Haberfeld and Hillel, 1991; Haberfeld *et al.*, 1991; Kuhnlein *et al.*, 1991; Dunnington *et al.*, 1992; Zhu *et al.*, 1996).

Microsatellites

Microsatellite markers, also known as simple sequence repeats (SSRs), short tandem repeats (STRs), or simple sequence length polymorphisms (SSLPs), are a subclass of highly repetitive short DNA sequences in all eukaryotic genomes and to a limited extent in prokaryotes (Tautz and Renz, 1984). They consist of stretches of repeated short nucleotide motifs of 1–6 bp and are ubiquitous in eukaryotic genomes (Tautz and Renz, 1984; Weber and May, 1989). Microsatellites are multi-allelic, highly polymorphic because of the variable number of repeats, and are co-dominant markers (Tautz, 1989; Dodgson *et al.*, 1997). Since they are usually less than 100 bp long and embedded in DNA with unique sequence, they can be amplified *in vitro* using PCR methodology.

Although microsatellites are stable, there is evidence suggesting that they may show mutational bias and heterozygote instability (Amos *et al.*, 1996; Primmer *et al.*,

1996). Mutation events in microsatellites are biased towards alleles with a large number of repeats (Weber and Wong, 1993). While the tandem repeats of varying length occur stochastically in the genome, it is conceivable that some intrinsic property that results in a net gain of repeat units with time may be required for the evolution of extremely long microsatellites (Primmer *et al.*, 1996).

The polymorphic nature of microsatellites makes them ideal markers for constructing high-resolution genetic maps (Hearne *et al.*, 1992; Cheng *et al.*, 1995; Groenen *et al.*, 2000) in order to identify QTL (Vallejo *et al.*, 1998; Van Kaam *et al.*, 1998). Microsatellite markers have been applied in individual identification studies (Longmire *et al.*, 1993), in forensic science (Gill *et al.*, 1985), in parentage testing (Primmer *et al.*, 1995) and in medical diagnostics (Campuzano *et al.*, 1996).

Microsatellites can be identified in two ways: (i) by searching through the EMBL and GenBank DNA databases for repeat sequences (Love *et al.*, 1990); and (ii) by generating genomic libraries in sequencing vectors by ligation with frequently cutting restriction enzymes (e.g. *Hae*III, *Alu*I or *Rsa*I), then screening for putative microsatellites by hybridizing with simple repeat probes ((GT)n, (GA)n, or (TC)n) and sequencing the positive clones (Cornall *et al.*, 1991). Oligonucleotide primers for PCR are then designed from DNA sequences flanking the repeats to provide amplified products of approximatedly 100 bp. Scoring of repeats has consisted of resolving PCR-amplified alleles by non-denaturing gel electrophoresis and ethidium bromide staining (Singer *et al.*, 1990). Enhanced detection sensitivity has also been demonstrated by using silver staining (Smale *et al.*, 1990). Other scoring methods include utilizing 6% denaturing polyacrylamide sequencing gels and visualizing by either autoradiography or automated sequencing machines, using either radioisotope or fluorescent labels, where the PCR primer is end-labelled, or labelled deoxynucleotides are incorporated during elongation (Ottonello *et al.*, 1987). Detecting microsatellite repeats on denaturing

polyacrylamide gel is time consuming and labour intensive, and not suitable for large throughput of samples. Recent techniques that are suitable for automation have emerged. Fluorescent labelling of PCR primers (Carrano *et al.*, 1989) coupled with gel scanning has improved resolution and lends itself to automation; however, it is still dependent on the use of polyacrylamide gels and scanning instrumentation. A ligase-assisted spacer addition (LASA) method based on ELISA format has been developed (Brockhurst *et al.*, 2001). The LASA method eliminates the use of electrophoresis and lends itself to automation, which is a necessity for high throughput of large population samples.

Sequence Tagged Sites (STSs) and Expressed Sequence Tags

STSs were proposed by Olson *et al.* (1989) as a common language to translate all types of mapping landmarks. An STS is a short (200–500 bp) DNA sequence that has a single occurrence in the genome and whose base sequence is known. Each STS is characterized by a unique pair of primers and a corresponding PCR product. STSs can be used to correlate the linkage map with the physical map. Clones containing the STS can be used to orient DNA sequence and physical mapping data. Genetic maps based on STSs have been constructed for bovines (Barendse *et al.*, 1994) and humans (Hudson *et al.*, 1995). Hudson *et al.* (1995) demonstrated that the success rate for STSs derived from the last 200 bp of 3′-untranslated regions (UTRs) of cDNA was similar to that for STSs derived from random genomic DNA, consistent with the idea that introns rarely occur near the ends of 3′-UTRs. STSs derived from the 3′-UTR have the advantage of being part of expressed genes. Polymorphic microsatellites can consequently be derived from STSs (Beckmann and Soller, 1990; Khatib *et al.*, 1993) and can provide utility markers for genome mapping.

An expressed sequence tag (EST) is a segment of a sequence from a cDNA clone that corresponds to an mRNA (Adams *et al.*, 1991). ESTs are created by partially sequencing randomly chosen transcripts that have been converted into cDNA.

ESTs are, therefore, STSs derived from cDNA clones. Random automated ESTs have been valuable because they presumably serve as large numbers of previously unknown genes expressed in any given cell or tissue. ESTs represent real functional genes and are more useful as genetic markers than non-functional or anonymous markers. Unlike other genetic markers, ESTs are stored in databases (GenBank, dbEST and EMBL). Automated 'single-pass' sequencing usually results in approximately 3% error or base ambiguity rate and contamination with vector sequence has been a problem. Adams *et al.* (1991) recommended an EST of at least 150 bp with less than 3% ambiguous base calls to be the minimum criteria for submission into a database. These databases have been used successfully to identify novel gene homologues. Many gene families (e.g. chemokine) have been identified through the use of EST databases (Boguski *et al.*, 1993). Several chicken and turkey ESTs have been developed, mapped and deposited in databases (Bumstead *et al.*, 1994; Ruyter-Spira *et al.*, 1996; Spike *et al.*, 1996; Li *et al.*, 1998b; Smith *et al.*, 2001). Currently, there two publicly available chicken EST databases (udgenome.ags.udel.edu/chickest/chick.htm; genetics.hpi.uni-hamburg.de).

Mapping ESTs from selective cDNA libraries can also be used to add candidate genes that affect traits of economic importance to the chicken genome map (Bumstead *et al.*, 1994). ESTs have also been used for comparative mapping among different species of the same genus (Smith *et al.*, 2000, 2001) or for divergent individuals within the same species (Jones *et al.*, 1998).

Random Amplified Polymorphic DNA (RAPD)

RAPD or arbitrarily primed PCR is a PCR technique that does not require prior knowledge of the DNA sequence. RAPD

polymorphisms can be used to construct genome linkage maps quickly (Welsh and McClelland, 1990; Williams *et al.*, 1990). Gene mapping using RAPD markers has several advantages over other methods: (i) an arbitrary set of primers can be utilized, (ii) no preliminary work, such as isolation of cloned DNA probes, filter preparations or nucleotide sequencing, is required; and (iii) each RAPD marker is the equivalent of an STS (Williams *et al.*, 1990). The major problem with the technique has been reproducibility of the results. McClelland and Welsh (1994a,b) reviewed this technique and provided reasonable suggestions for making this technique more reliable. RAPD usually utilizes a single oligonucleotide primer (10 nucleotides or greater in length) that is allowed to anneal and elongate at multiple arbitrary target sites in the genome by manipulating the PCR conditions so that the annealing and elongation is less specific. This is usually accomplished by raising the Mg^{2+} concentration and lowering the annealing temperature so that mismatched primer binding and elongation can occur in the PCR reaction. The arbitrary priming requires the chance association of primer annealing sites in the proper orientation to generate a PCR product. Multiple different-sized fragments are amplified in each reaction creating an anonymous 'fingerprint' pattern when the fragments are separated by electrophoresis. DNA sequence changes within and surrounding the primer annealing site can result in differential amplification (Okimoto *et al.*, 1997). The polymorphic DNA fragments generated are nearly always dominant genetic markers and are limited in their utility because of this. Multiple polymorphism can be detected with each primer or primer combination.

A wide range of different lengths and types of sequences of primers are used (McClelland and Welsh, 1994a,b) to generate RAPD polymorphisms. Primers based on repetitive elements such as microsatellites (Zietkiewicz *et al.*, 1994; Weising *et al.*, 1995) or transposon-like sequences (Levin *et al.*, 1994b) appear to be more dependent on arbitrary priming than amplification

of those specific sequences (Weising *et al.*, 1995; Okimoto *et al.*, 1997). Other techniques have been applied in RAPD analysis such as restriction digest of the template before PCR (Levin *et al.*, 1993, 1994b), and Southern blot hybridization of RAPD fingerprints with specific probes (Richardson *et al.*, 1995) to generate more polymorphisms with the same primer sets, but these additions do not appear to be used routinely.

The RAPD technique can be used to place a relatively large number of markers on a linkage map quickly and is useful in targeting markers to specific portions of the genome (Reiter *et al.*, 1992). Levin *et al.* (1993) placed 13 RAPD markers on the chicken Z chromosome. The number of RAPD markers and CR1 repetitive element-derived primer markers (Levin *et al.*, 1994b) that have an arbitrary priming component (Okimoto *et al.*, 1997) accounted for 112 markers on the East Lansing chicken linkage map (Groenen *et al.*, 2000).

RAPD fragments can be DNA sequenced and turned into SNP markers (Okimoto *et al.*, 1997) and RAPD technology has the potential to place very large numbers of markers on a linkage map, but the problems of reproducibility and the time needed to convert them to more useful markers seems to have limited their application in poultry genome linkage mapping.

RAPD polymorphisms have been used to fingerprint various chicken lines as a means of differentiating them and estimating genetic distance and diversity (Plotsky *et al.*, 1995; Zhang *et al.*, 1995; Semenova *et al.*, 1996; Smith *et al.*, 1996; Wei *et al.*, 1997; Romanov and Weigend, 2001a; Weigend and Romanov, 2001). RAPD has been used for the same purpose for other species such as turkeys (Smith *et al.*, 1996), guinea fowl (Sharma *et al.*, 1998), quail (Sharma *et al.*, 2000a) and ducks (Dolmatova *et al.*, 2000).

There is an RAPD technique called differential display that detects gene expression differences among transcripts (Mathieu-Daude *et al.*, 1999) but its main use is detection of transcript differences and not necessarily genetic polymorphisms.

Amplified Fragment Length Polymorphism (AFLP)

AFLP is a technique similar to RAPD. Prior knowledge of the sequence is not needed and the technique is purported to be more reliable than RAPD applied to the same organism (Barker *et al.*, 1999; Bagley *et al.*, 2001). A comprehensive review of the technique can be found in Savelkoul *et al.*, 1999. The principles behind the methodology of AFLP (a combination of PCR-RFLP) for specific allele amplification was first proposed by Yunis *et al.* (1991) for typing of human leukocyte antigen (HLA)-DR alleles (HLA class II antigen has three alleles, DR, DP and DQ). The protocol of Yunis *et al.* (1991) was improved by limiting the number of restriction endonucleases (Danzé *et al.*, 1995). AFLP as a DNA fingerprinting technique is based on the amplification of subsets of genomic restriction fragments using PCR (Vos *et al.*, 1995). AFLP technology is a registered trademark of Keygene NV. DNA quality and preparation are important to this technique. Genomic DNA is digested with two restriction enzymes, one a 6-bp cutter and the other a 4-bp cutter that cuts the DNA more frequently than the 6-bp cutter. This digested DNA is then ligated to DNA adaptors as it is being restriction digested. The adaptors are designed to alter the sequence of the restriction site so that the enzyme will no longer recognize the genomic DNA-adaptor hybrid molecule, so that any genomic-to-genomic ligations will be recut by the restriction enzyme, but the genomic DNA-adaptor hybrid will be stable. The product of this restriction digest and ligation is used as a template for PCR involving primers specific to sequences of the adaptors with extensions of 1–3 nucleotides (nt) on the 3′ end of the oligonucleotide primers. This selective extension is supposed to reduce the number of fragments that match the primer sequence. Only restriction fragments in which the nucleotides flanking the restriction site match the selective extension will be amplified. The selective nucleotides have the function of reducing the number of potential amplified fragments

(Liu, 1998). There is a perfect match until the extension and then one out of four fragments will match with a 1-nt extension; and if both primers have a 3-nt extension, 1×0.25^{-6} or 1 in 4096 fragments will have a perfect match. It is recommended that the primer corresponding to the less frequent cutting restriction enzyme be labelled for fragment identification.

AFLP is simple, rapid and inexpensive to develop and, as a result, a large number of genetic markers can be typed quickly. The main disadvantage of AFLP is its biallelic nature compared with microsatellite markers that are multi-allelic. However, because of the relatively low frequency of microsatellites in the avian genome compared with the mammalian genome (Primmer *et al.*, 1997), AFLP markers have been utilized to enhance the density of linkage maps of chickens (Herbergs *et al.*, 1999; Knorr *et al.*, 1999; Groenen *et al.*, 2000). For high-throughput genotype determination, AFLP band conversion into STSs is necessary and some procedures have been developed to this end (Knorr *et al.*, 2001; Meksem *et al.*, 2001; Myburg *et al.*, 2001; Yang *et al.*, 2001). AFLP markers are scored as dominant markers (dominant homozygote indistinguishable from heterozygote) that have limited applications in mapping of QTL and marker-assisted breeding. However, quantitative assessment of band intensity of AFLP markers could eliminate this problem and allow them to be utilized in the same manner as co-dominant markers (Piepho and Koch, 2000; Jansen *et al.*, 2001; Piepho, 2001).

Single-strand Conformation Polymorphism (SSCP)

PCR-SSCP (Orita *et al.*, 1989a,b) depends on the difference in secondary structure conformation of single-stranded polymorphic DNA sequences. The alternative secondary structures can be resolved by differential mobility in non-denaturing electrophoretic gels. Single-nucleotide polymorphisms present in fragments of several hundred bp can be detected with this method (Yao *et al.*,

1996). It is a simple procedure that involves PCR amplification, denaturing the DNA into its two complementary strands, and electrophoresing this product under conditions that will allow secondary structure formation. Single-stranded DNA mobility is sequence dependent because of varying degrees of intra-strand base pairing and the resulting looping and compaction. Precise knowledge of the sequence variation is not necessary. The original method required radioactivity, but non-radioactive methods using ethidium bromide or silver staining for DNA detection have been developed (Hongyo *et al.*, 1993; Oto *et al.*, 1993). An extension of SSCP, multi-temperature single-strand conformation polymorphism (MSSCP), has been developed (Kaczanowski *et al.*, 2001). With MSSCP, the gel temperature is increased during electrophoresis and this increases the sensitivity of mutation detection. The limitation is that the size of the DNA fragments that can be tested is relatively short, 200 bp with a detection of from 80 to 90% of the mutations (Nollau and Wagener, 1997). In addition, several gel running conditions should be tried to produce consistent secondary structure differences between polymorphic DNA sequences. Analysis of larger fragments can be attempted, but the number of mutations that can be detected decreases as the length of the PCR product increases. SSCP has an advantage over RFLP analysis, in that it can detect DNA polymorphisms and point mutations at a variety of positions in DNA fragments (Orita *et al.*, 1989a,b). SSCP can be used to detect polymorphisms as an initial screen and it can be used to identify specific polymorphisms for allele identification or genetic mapping purposes.

SSCP methodology has been used extensively to detect mutations in human genes as well as genes in domestic animals (Lagziel *et al.*, 1996; Roberts *et al.*, 1996; Yao *et al.*, 1996). Morisson *et al.* (1998) and J. Smith *et al.* (2000) used SSCP to place a number of fluorescence *in situ* hybridization (FISH) mapped cloned sequences on the chicken genetic linkage map. Sequence variation detected in the chicken extracelluar fatty acid binding protein detected by SSCP

was found to be associated with abdominal fat (Wang *et al.*, 2001). There are several reports on using SSCP to map single genes (e.g. Hughes and Bumstead, 2000; Ge *et al.*, 2001).

There are two techniques similar to SSCP that are not widely used for poultry genetics, but that do have extensive use in other organisms: heteroduplex analysis (HET) and denaturing gradient gel electrophoresis (DGGE). Nollau and Wagener (1997) gave brief overviews of these techniques. DGGE has advantages in the number of polymorphisms detected and accuracy of detection over SSCP, but requires a gradient of urea or formamide in the acrylamide gel and heteroduplex formation to achieve the highest sensitivity. In the HET analysis, PCR product heteroduplexes are formed by denaturation and reannealing with a test sequence and the heteroduplex DNA is run on a non-denaturing gel. Mismatched heteroduplexes will often have altered mobility on the electrophoretic gel. The DGGE analysis is dependent on the differential stability of the double-stranded DNA molecule in relation to the sequence. Less stable base pairs disassociate first and the most stable last. Heteroduplexes formed between two polymorphic sequences can be easily detected by the altered migration of the DNA due to the altered denaturation properties of the mismatched heteroduplex relative to the matched strands.

PCR Amplification of Specific Alleles (PASA)

This technique is known by several names: PASA (Bottema *et al.*, 1993), amplification refractory mutation system (ARMS) (Newton *et al.*, 1989) or allele-specific PCR (ASPCR) (Wu *et al.*, 1989). ASPCR primers are designed so that the polymorphic sequence is at the 3′ end of the primer. For one allele the primer is a perfect match, but for the alternative allele the primer has the mismatched 3′ end. Under certain PCR reaction conditions (minimal deoxynucleotide triphosphate concentrations and often

lower $MgCl_2$ and *Taq* polymerase concentrations), if the primer matches the genomic sequence efficient elongation occurs and a PCR product is formed, but if there is a 3′ primer mismatch there is no efficient elongation from the primer and no or very little PCR product is formed. This gives a plus or minus PCR result. Longer PCR fragments over 500 bp produce cleaner results (less non-specific amplification) than short PCR fragments. Reaction conditions and PCR annealing temperatures have to be optimized for each primer set.

There is a variation of this technique called PCR amplification of multiple specific alleles (PAMSA) (Dutton and Sommer, 1991), which is a co-dominant marker type and can detect two alleles in the same PCR reaction mix. Two allele-specific primers are used in the same PCR reaction mix in combination with a common primer. The allele-specific primers differ from each other by length with an oligonucleotide tail added to one of the allele-specific primers. In this way the PCR products of the two different allele-specific primers can be distinguished by size. Short PAMSA products can more easily be distinguished from each other by electrophoresis, due to the limit on the length of the oligonucleotide tail. Primer sequence modifications can allow the accurate use of shorter PAMSA fragments (Okimoto and Dodgson, 1996). PAMSA has the advantage in that it is not a plus/minus result, and that at least one PCR product amplifies in every reaction, but the short fragments are separated on polyacrylamide gels or Metaphor™ agarose or Synergel™ agarose gels. The common primer can be fluorescently labelled and the PAMSA products scored on an automated sequencing unit. It sometimes takes more effort to adjust the PCR conditions to achieve allele-specific amplification. The ability to design PASA or PAMSA primers is dependent on the sequence surrounding the polymorphism. Lack of sequence complexity can make primer design impractical.

Fifty-two markers have been placed on the chicken linkage map using these techniques (Smith and Cheng, 1998; Suchyta *et al.*, 2001) and they can be routinely used to type relatively large populations for specific alleles or mitochondrial haplotypes (Okimoto *et al.*, 1999).

Single-nucleotide Polymorphism (SNP)

SNPs (pronounced 'snips') are single base-pair variations in DNA. SNP is the most common source of genetic variation in the human genome, accounting for about 90% of sequence differences (Collins *et al.*, 1998) at an overall frequency of about one per 1000 bases (Taillon-Miller *et al.*, 1998). SNP is a broad term used to describe any form of single base-pair change in a population. Narrower classes of SNPs include the following.

- *Anonymous SNP*: SNPs that have no known effect on gene function. This type is thought to be the most common type of SNPs and is of great value in mapping, linkage disequilibrium and genome diversity studies.
- *cSNP*: SNPs in the coding regions of a gene, i.e. located within protein coding sequence, which may interfere with gene function by the alteration of the amino acid composition.
- *Candidate SNP*: SNPs thought to have putative functional effect.
- *rSNP*: SNPs in the regulatory region of a gene. The regulatory region governs gene expression, thus rSNPs, though rare, are potentially as valuable as cSNPs.
- *pSNP*: when phenotype is changed as a result of altered protein function or expression as a consequence of altered amino acid, cSNP or an rSNP may be labelled a pSNP.
- *Synonymous SNP*: when a base pair change occurs in a cSNP, but the cSNP still codes for the same amino acid.

SNPs detected in 3′-UTR ESTs have been shown to have a lower polymorphism rate than random genomic sequence consistent with greater constraint in genic sequences (Wang *et al.*, 1998). In systematic studies of SNPs in sets of human genes, about half of cSNPs cause missense

mutations in the corresponding proteins while half are silent (Cargill *et al.*, 1999; Halushka *et al.*, 1999).

Unlike micro- and minisatellites with multiple alleles, SNPs have only two alleles (biallelic). The level of heterozygosity can be increased by combining alleles of different SNPs into haplotypes. It is crucial to consider many linked markers simultaneously in linkage studies when SNP markers are used, because of its lower polymorphism (Kruglyak, 1997). Over a million human SNPs have been catalogued (snp.cshl.org/data). SNP marker discovery in chickens grossly lags behind that of humans. SNPs from ESTs have been reported by Li *et al.* (1998), Ruyter-Spira *et al.* (1998) and Smith *et al.* (2001). The average density of SNPs in the human genome is about 1 kb^{-1}. The abundance of SNP in the genome makes it a powerful tool for genetic studies. SNPs can serve as genetic markers for genes of low penetrance for linkage studies in families, linkage disequilibrium in populations, and association and comparative genomic studies.

The current status of the field of SNP analysis is largely at the level of developing automated technologies and improving existing tools, in order to upgrade the collection resources that researchers may use for genome studies (Brooks *et al.*, 2001). Appropriate automation depends on the method. However, the choice of method depends on the scale and objective of the project. There are various methods for high-throughput SNP genotyping.

The first element is the interrogating of an SNP. This is a sequence of molecular, physical and chemical procedures for distinguishing alleles of an SNP (Gut, 2001). The procedures for interrogating an SNP are as follows.

1. Complementary oligonucleotide sequences are hybridized to target sequences derived from genomic DNA in order to distinguish alleles. Molecular beacons, allele-specific oligonucleotide probes and fluorescent dye/quencher systems are used to visualize the results of the hybridizations (Tyagi *et al.*, 1998).

2. Enzymatic modification after hybridization is often used to distinguish alleles and increase fidelity of allele detection. Among the enzymatic methods are ARMS and kinetic PCR (Newton *et al.*, 1989; Germer *et al.*, 2000), 5′ nuclease assay (Holland *et al.*, 1991), primer extension (Syvänen, 1999), oligonucleotide ligation assay (OLA) (Barany, 1991), flap endonuclease (Harrington and Lieber, 1994) and pyrosequencing (Ronaghi *et al.*, 1999).

The second element in SNP is the analysis or measurement of the allele-specific products (Gut, 2001). The commonly used methods include the following.

1. *Gel-based analysis.* Sequencing using gel analysis has been used for SNP genotyping. Hybridization (Shuber *et al.*, 1997), primer extension (Pastinen *et al.*, 1996) and OLA (Grossman *et al.*, 1994)-based SNP genotyping on gels have been demonstrated.

2. *Fluorescent reader-based analysis.* Ligation reaction with oligonucleotides linked to fluorescent chromophores is a sensitive analytical method (Chen *et al.*, 1998, 1999).

3. *Array-based analysis.* DNA microarray (chips) is a series of oligonucleotides chemically attached to a non-porous solid support, usually a glass slide. Hybridizing an unknown sequence to a known sequence on the DNA chip identifies complementary single nucleotide change in the unknown sequence (Shumaker *et al.*, 1996; Wang *et al.*, 1998). Khanna *et al.* (1999) utilized microarrays to detect allele-specific products generated by OLA.

4. *Mass spectrometry-based analysis.* Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) (Little *et al.*, 1997; Kwok, 1998) has been used as a tool to distinguish between genotypes based on the mass of the variant DNA sequence. This method has been utilized for high-throughput SNP genotyping (Bray *et al.*, 2001). Analysis of both single and multiplex PCR and mini-sequencing reactions can be performed using MALDI-TOF instruments. Because multiple polymorphisms can be detected in a single

reaction, MALDI-TOF analysis can be used to genotype multiple markers in an individual.

Technologies that are useful for the analysis of multiplex genotyping in one individual should be extended to include large numbers of individuals. Future developments should be directed towards automation of the sample preparation steps, reducing reaction volumes, increasing sensitivity and reducing the overall cost of genotyping.

Applications of Genetic Markers

Genetics is the study of variation, and genetic variations can be studied at several levels (genic, individual, family and population). The selection of suitable genetic markers for genetic analysis is dependent on the questions intended to be answered. An ideal marker would be multi-allelic, highly polymorphic, widely available, inexpensive to genotype and evenly distributed in the genome, but no one marker fits all of these characteristics. Each marker has both genetic and technical features. The genetic features include the distribution of the marker in the genome, biallelic or multi-allelic, the level of polymorphism, the level of heterozygosity, mutation rate, and the degree of dominance. The technical features include the degree of automation of the genotyping process: ease, accuracy and the cost of genotyping. The choice of a genetic marker would depend on the objective of the researcher and usually involves a trade-off between the genetic and technical features of the markers of choice.

The inheritance pattern exhibited by a marker and the amount of genetic variation it can detect may limit its usage. When the question at hand is unique individual identification, the choice of genetic markers would depend on its properties (nature of inheritance, single- or multi-locus and allele frequency). Genetic markers that have been used in individual identification studies include VNTR, RFLP, RAPD and AFLP. Co-dominant markers enable the investigator to visualize both alleles at a single locus simultaneously, and are preferable to dominant markers such as RAPD and AFLP. RAPDs and AFLPs are relatively easy to generate and efficient but limited by the amount of variation they can detect, because they exhibit dominant inheritance.

Genetic markers have applications in quantifying variation in and among populations. Several studies have been undertaken in poultry, plants and other animal populations with the goal of assessing genetic variability to optimize sampling strategies and conserve genetic resources. Measurement of genetic diversity with genetic markers is of relevance in assessing extinction risks in populations (Vanhala *et al.*, 1998; Zhou and Lamont, 1999). The standing levels of genetic diversity in populations contribute to long-term sustainability. RAPDs, AFLP and microsatellites have been used to eliminate redundancy in germplasms. Both single- and multi-locus markers have been used to evaluate genetic relationships among breeds and strains (Smith *et al.*, 1996; Mohd-Azmi *et al.*, 2000; Romanov and Weigend, 2001b). Genetic markers can be used to assess genetic drift and shifts in populations. Mating system parameters, including avoidance of inbreeding, and changes in mating systems in disturbed populations can be tracked with genetic markers (Kuhnlein *et al.*, 1989, Grunder, *et al.*, 1994). Genetic markers offer a good tool for maintaining genetic diversity. Maintenance of genetic resources for utilization has come to rely on genetic markers. Selection (artificial or natural), as well as genetic drift in populations, can be revealed by genetic markers (Sharma *et al.*, 2000b, 2001).

Poultry improvement exploits genetic variability within a population. The importance of genetic variation in understanding the genetic architecture of traits of economic importance is exemplified by the effort to develop a high-resolution linkage map of the chicken genome and numerous QTL studies. Genetic markers can be used to track subsets of sequence variants that pertain to function and ultimately assign genotype to phenotype in individuals. Many factors will make achieving this goal difficult, including the

large volume of data arising from the possibility that many polymorphisms contribute small effects to a single phenotype (Hartman *et al.*, 2001). Cataloguing sequences that affect phenotypes would enable geneticists to go beyond studying single genes to the studying of arrays of genes and how they interact in the architecture of traits. The power of EST and SNP marker technologies to provide associations between large numbers of genes involved in the genetic architecture of complex traits provides a better understanding of complex pathways of traits.

Effective utilization of genetic markers requires an interface of population and quantitative genetics with the emerging field of bioinformatics. In conclusion, genetic markers serve as the basic tool for the study of genetics. The choice of marker should be balanced between relative cost, health hazards, ease of development, efficiency of use and the level of genetic variation appropriate for the genetic question of interest.

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24 Designs and Methods to Detect QTL for Production Traits Based on Mapped Genetic Markers

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Introduction

Genetic improvement through artificial selection has been an important contributor to the enormous advances in productivity that have been achieved since the 1950s in plant and animal species that are of agricultural importance. Most of the traits that are selected for are complex quantitative traits, which means that they are controlled by several genes, along with environmental factors, and that the underlying genes have quantitative effects on the phenotype. So far, most selection has been on the basis of the observable phenotype, which represents the collective effect of all genes and the environment.

For many years, people have recognized the need for genetic evaluation of animals (e.g. Lush, 1931; Hazel, 1943; Henderson, 1949) and today best linear unbiased prediction (BLUP) has become the most widely accepted method for genetic evaluation of domestic livestock. Since Henderson (1949) first published the method for genetic evaluation, it has evolved from application to sire models in the early years to animal models and multivariate analysis more recently. These methods optimally combine phenotypic and pedigree information collected in a population. The application

of these methods has resulted in big changes in the performance of poultry. Besides selection within lines, the development of superior crossbreds through the combination of several improved lines or breeds plays an important role in poultry breeding.

The selection programmes were conducted without any knowledge of the genetic architecture of the selected trait. Dense linkage maps consisting of polymorphic marker loci are available for most livestock species and this is an important tool to unravel the genetic nature of quantitative traits in animals by identifying genes or chromosomal regions that affect the trait – so-called quantitative trait loci (QTL) (Groenen *et al.*, 2000; Anderson, 2001; see also Groenen and Crooijmans, Chapter 26).

Molecular genetic techniques can be used to collect more detailed genotypic information on individuals as well as information on which alleles or chromosomal segments are transmitted from parent to offspring. The purpose of this chapter is to provide background on the design and analysis of QTL mapping experiments in poultry. Mapping of QTL opens new opportunities for genetic evaluation and selection, as will be briefly described here and in other chapters of this book.

Phenotype/Genotype Relationship

The quantitative genetic approach to selection is based on knowledge of population genetic parameters for the traits of interest, such as heritabilities, genetic variances and genetic correlations. These parameters can be estimated using statistical analysis of phenotypic data from pedigrees (see Swaczkowski, Chapter 11). However, the genetic architecture of the trait itself is treated as a black box, with no knowledge of the number of genes that affect the trait, let alone of the effects of each gene or their locations in the genome. More specifically, quantitative genetic theory is based on Fisher's infinitesimal genetic model (Bulmer, 1980), in which the trait is assumed to be determined by an infinite number of genes, each with an infinitesimally small effect. Despite the obvious flaws of the infinitesimal model, the tremendous rates of genetic improvement that have been achieved attest to the usefulness of the quantitative genetic approach.

The prediction of an animal's breeding value is based on phenotypes of the animal itself and/or those of its relatives. When only observations on the trait of interest are considered, the contribution of observations on relatives to an animal's breeding value depends on the additive genetic relationship, i.e. the proportion of genes shared in common by descent, and the heritability of the trait. BLUP methods, by which estimates of fixed effects and predictions of breeding values are obtained simultaneously, require that the inverse of the numerator relationship matrix between animals (**A**), which is generally very large, be known. Henderson (1976) described a method for writing the inverse of **A** directly from pedigree records and inbreeding coefficients. This has enabled the use of improved methods for estimation of genetic parameters and prediction of the breeding value of animals. The concept of the numerator relationship matrix has been extended to the gametic relationship matrix (**G**), where paternal and maternal gametes of an animal are considered separately (Smith and Allaire, 1985; Tier and Sölkner, 1993). The gametic relationship matrix has been used for constructing the relationship matrix due to dominance effects (Schaeffer et al., 1989; Smith and Mäki-Tanilla, 1990) and for the analysis of gametic imprinting effects (Gibson et al., 1988; Schaeffer et al., 1989; Tier and Sölkner, 1993). In building the relationship matrix or its inverse at either the animal or gametic level, no knowledge of the actual contribution of a parent to its offspring is used. Instead, use is made of Wright's (1922) inbreeding coefficients and the coefficients of relationship between animals.

Traits

Observed scale

Many of the characters of interest to animal breeders are of a quantitative nature. On the other hand, there are other economically important traits that show a discrete phenotypic distribution, sometimes with as few as two classes. Many characters associated with reproduction and diseases are measured in a limited number of discrete classes. Lush et al. (1948), as one of the pioneers in genetic analysis of all-or-none traits in an animal breeding context, analysed mortality data in poultry. The essential difficulty posed by all-or-none traits, which these authors recognized and tried to accommodate, is that means and variances of the traits are correlated on the observed scale (McQuirk, 1989). In more recent analyses, the discrete phenotype is represented as a function of the animal's liability, which is modelled as a function of fixed effects and genetic and environmental effects (Gianola and Foulley, 1983). The underlying distribution of the genetic contribution is usually normal or logit of form. The remainder of this chapter will concentrate on phenotypes, which are measured on a continuous scale.

Importance

Poultry breeding made a substantial contribution to the increase of production volume and production efficiency per bird during the second half of the 20th century (Albers and Groot, 1998). In order to maintain an acceptable rate of progress, it is important that new tools are developed for effective selection on new traits such as leg weakness, cardio-respiratory problems, absorption problems, feather-pecking behaviour and disease resistance. These traits have in common that accurate recording of phenotypes on a large scale is difficult and that information on selection candidates is often not available prior to selection.

Selection for improved disease resistance in poultry is highly desirable for animal welfare, but also because reducing the losses due to disease is an excellent way of improving production efficiency. Selection for disease resistance is difficult to include in breeding programmes, because of the lack of accurate records on disease incidence in breeding flocks and the management practices that aim at minimizing exposure to pathogens. As a result there is considerable interest in finding genetic markers or diagnostic tests that can be applied to improve breeding programmes.

Dissecting the Genome

Molecular genetic techniques have made it possible to identify differences between individuals at the DNA level. In particular, the development of microsatellite markers as an abundant source of polymorphic and convenient markers has boosted the generation of linkage maps in the most important livestock species. These maps provide the basis for detection and exploitation of genes segregating at loci that have an effect on important traits. These are referred to as QTL. The principle of QTL mapping is not new; it was used by Sax (1923) to map a QTL associated with seed size by linkage

to a pigment locus in the bean *Phaseolus vulgaris*. The main practical limitation to implementing QTL mapping was the lack of a large number of evenly spaced Mendelian marker loci in most species. Two technical advances have opened the door for detailed characterization of the genetic architecture of quantitative traits: the discovery of abundant polymorphic neutral molecular markers and the development of sophisticated statistical methods for mapping QTL (MacKay, 2001).

QTL Mapping Methods

The principle of QTL mapping is simple. All that is required is two inbred strains in which different alleles at loci affecting the trait of interest are fixed, and a polymorphic molecular marker linkage map. In order to map the QTL, the inbred traits are mated to produce an F_1 population, which is subsequently used to produce a mapping population of backcross or F_2 individuals. The phenotype and multi-locus genotype of each individual in the mapping population are determined. In its simplest form, QTL mapping involves going through the genome, one marker at a time, dividing the individuals into marker genotype classes, and doing a statistical test to determine whether there is a significant difference in phenotype between the marker genotype classes. If there is such a difference, then the QTL is linked to the marker. This procedure, as described, underestimates the effect of the QTL by an amount that is proportional to the distance of the QTL from the marker locus, but this problem is readily overcome by mapping the QTL relative to two flanking markers (interval mapping).

If it is assumed that the inbred lines are fixed for alternative marker and QTL alleles, there are a number of important features of this design (Soller, 1991).

- All individuals in the F_1 are heterozygous for the marker as well as for the QTL.

- There is complete linkage disequilibrium between the marker and the QTL in the F_1.
- There are only two marker alleles and two QTL alleles in the population under study, each allele with a frequency of 0.5.
- All individuals in the F_1 have the same genotype and the same linkage phase.

A design based on inbred lines is frequently used in laboratory animals (e.g. mice) and plants. For poultry and other farm animals, however, inbred lines are seldom available. In some experiments, crosses between lines with extreme phenotypes have been used instead. In addition, rearing large numbers of F_1 and F_2 animals is only practical for some farm animals (e.g. chickens) but not for others (e.g. cattle), due to the long generation interval and the costs of such an experiment (Bovenhuis *et al.*, 1997). As an alternative to a designed experiment involving crosses between lines or breeds, existing outcross populations can be used. In the latter approach the structure of breeding populations is exploited to map genes that are segregating within these elite populations. There are some important differences between analysing data from a cross between inbred lines and an outcross population.

- Only a fraction of the (grand)parents will be heterozygous for the marker as well as for the QTL.
- (Grand)parents might have different linkage phases and therefore observations in the mapping population cannot simply be pooled across families: marker effects need to be analysed within families.
- QTL might have more than two alleles in the population and allele frequencies are unknown.
- Linkage phase between marker alleles is unknown and needs to be inferred from the mapping population.

An experiment involving a cross between divergent populations is primarily focused on finding QTL that explain the variance between populations, whereas an experiment within one outcross population will reveal QTL that explain the genetic variation within a population. It is still an open question whether QTL segregating in crosses between divergent lines also contribute to the genetic variance within breeds. De Koning *et al.* (1999) demonstrated that a cross between divergent lines can be used for the detection of QTL that explain between- and within-population genetic differences. A combination of both approaches is expected to provide information that is more informative for the dissection of the genome. Using divergent lines increases the probability that F_1 parents are heterozygous for the QTL and thereby increases the power of an experiment. At the same time, it will shift the emphasis towards finding genes that explain the differences between breeds. Emphasis on detecting genes that explain differences within a population offers the opportunity of marker-assisted selection within the breed. The choice of the population(s) involved in a genome mapping experiment depends largely on the goal of the experiment. When the emphasis is on finding genes that are involved in certain characteristics (i.e. genetic variation between lines), a cross between divergent lines is to be preferred. A single population or a cross between slightly divergent lines is to be preferred when the emphasis is on explaining within-population genetic differences or implementation of marker-assisted selection schemes.

In a number of poultry genome mapping experiments, a cross between different lines is used. In several cases, crosses between very divergent breeds have been used, such as the cross between Red Jungle Fowl and White Leghorn in Sweden (Andersson, personal communication) or the Sasumadorri and White Plymouth Rock cross in Japan (Tatsuda and Fujinaka, 2001). In other cases the lines used were less divergent, such as the cross between lines divergently selected from a common base (Yonash *et al.*, 2001; Bovenhuis *et al.*, 2002) or the cross between commercial breeds used in Wageningen (Van Kaam *et al.*, 1998; Groenen *et al.*, 2001).

Principles of QTL Mapping

Inbred lines

To illustrate the principles of QTL mapping, let us first consider a cross between inbred lines in which the mapping population is genotyped for marker (with alleles *M1* and *M2*), which is linked to QTL (with alleles *Q1* and *Q2*). Inbred line 1 is homozygous for *M1* and *Q1* while inbred line 2 is homozygous for *M2* and *Q2*. These two lines are crossed to produce an F_1 population. Based on the F_1, two types of design can be used for detecting QTL: the F_2 design and the backcross design. In a backcross design, F_1 individuals are crossed with one of the parental lines. Two types of backcross design are possible but consider a backcross with line 1. All F_1 individuals are double heterozygous and the *M1* and *Q1* alleles are on one chromosome and the *M2* and *Q2* alleles are on the other chromosome. An F_1 individual can produce four types of gametes: *M1Q1*, *M1Q2*, *M2Q1* and *M2Q2*. The *M1Q1* and *M2Q2* gametes are the parental types, i.e. this combination of alleles also occurred in one of the parents. Meiotic recombination generated the combination of alleles (*M1Q2* and *M2Q1*) which were not present in the either of the parents and these gametes are referred to as recombinant. For genes close together on the same chromosome, the physical linkage of parental allele combinations makes independent assortment impossible and hence produces recombination frequencies significantly lower than 50%.

Table 24.1 gives the frequency of the gametes produced by an F_1 individual and the corresponding genotypes and expected phenotypic values in the offspring. The expected difference in average phenotype ($\bar{y}$) between individuals in the backcross carrying the *M1M1* and *M1M2* marker genotype is equal to:

$$\bar{y}_{M1M1} - \bar{y}_{M1M2} = (1 - 2\theta)(a + d)$$

This equation illustrates that the expected difference depends on the recombination rate between *M* and *Q* (θ), the additive gene effect (*a*) and the dominance deviation (*d*) at the QTL.

An F_2 population is generated by crossing F_1 individuals. In the F_2 population, three marker genotypes can be distinguished: *M1M1*, *M1M2* and *M2M2*. The expected phenotypic difference between F_2 individuals carrying the *M1M1* and the *M2M2* genotype is equal to:

$$\bar{y}_{M1M1} - \bar{y}_{M2M2} = 2(1 - 2\theta)a$$

Compared with the backcross design, this contrast for a given recombination rate (θ) is only influenced by the additive gene effect at the QTL. Information on the size of the dominance deviation (*d*) can be obtained by including information on F_2 offspring with the *M1M2* genotype.

Outbred populations

Linkage disequilibrium in an outbred population between a genetic marker and a QTL can be found within families. This is due to the cosegregation of the marker and the QTL. The principle underlying the detection of a QTL in an outbred population will be illustrated for a two-generation design,

Table 24.1. Illustration of QTL mapping in a backcross population: frequency of gametes produced by F_1 parent and the resulting genotype and expected phenotype in the offspring.[a]

Gamete F_1 parent	Type	Frequency	Genotype offspring	Phenotype offspring
M1Q1	Parental	½ (1 − θ)	*M1M1Q1Q1*	μ + *a*
M1Q2	Recombinant	½ θ	*M1M1Q1Q2*	μ + *d*
M2Q1	Recombinant	½ θ	*M1M2Q1Q1*	μ + *a*
M2Q2	Parental	½ (1 − θ)	*M1M2Q1Q2*	μ + *d*

[a]θ is the recombination rate between *M* and *Q*, μ is the population mean, *a* is the additive effect at the QTL and *d* is the dominance effect at the QTL.

where sires have a large number of progeny and where the analysis is performed within half-sib families. The basic idea of the design is to trace marker alleles from the sire to his offspring and to determine whether offspring that have inherited alternative marker alleles from the sire differ with respect to the quantitative trait. When assuming a population with two marker alleles and two QTL alleles, in total ten different marker–QTL genotype combinations can be distinguished (Table 24.2).

Out of the ten possible sire genotypes in Table 24.2, only two are informative for the detection of the QTL, namely *M1Q1/M2Q2* and *M1Q2/M2Q1*. For these two types of sires, the transmission of the chromosomal segment can be traced from sire to offspring (i.e. the sire is heterozygous for the marker) and a difference in the quantitative trait is expected when the QTL is linked to the marker (i.e. the sire is heterozygous for the QTL). For the *M1M1* offspring it is certain that they inherited the *M1* allele from the sire and similarly for the *M2M2* offspring it is certain that they inherited the *M2* allele from the sire. For the *M1M2* offspring, however, it is not known which allele came from the sire and which from the dam. The expected difference in the quantitative trait for the *M1Q1/M2Q2* sire between *M1M1* and *M2M2* offspring is equal to:

$$\bar{y}_{M1M1} - \bar{y}_{M1M2} = [1 - 2\theta] \times [a + (q - p)d]$$

where $\bar{y}$ is the mean phenotype, θ is the recombination rate between *M* and *Q*, *a* is the additive gene effect and *d* is the

dominance deviation at the QTL. The expected difference also depends on the allele frequency of *Q1* (*p*) and *Q2* (*q*) in the population. If it is assumed that the dominance deviation (*d*) is equal to zero, the expected difference between the groups of offspring inheriting a different marker allele from the sire reduces to $(1 - 2\theta)a$. This contrast is independent of the population QTL allele frequencies and is half the contrast observed for the cross between inbred lines.

- For an *M1Q2/M2Q1* sire, the expected difference between *M1M1* and *M2M2* offspring is equal to $-[1 - 2\theta][a + (q - p)d]$. The absolute size of the expected difference is the same as for *M1Q1/M2Q2* of sires but the sign differs.
- For sires homozygous at the QTL, no difference is expected between offspring inheriting alternative marker alleles. The proportion of sires homozygous at the QTL depends on the frequency of the QTL alleles and the number of QTL alleles. With only two QTL alleles, the proportion of sires with a homozygous QTL genotype is at least 50%. This minimum proportion reduces when more than two alleles are segregating at the QTL.

Design of Experiments

QTL mapping experiments involve many animals to be genotyped and performance tested (Soller and Genizi, 1978). Consequently, experimental designs need to be optimized to minimize costs of data collection and genotyping. Three different types of designs can be distinguished: (i) cross between divergent lines; (ii) within-family analysis where families could be full-sib or half-sib families; and (iii) full pedigree analysis exploiting all relations in a population.

Weller *et al.* (1990) computed the powers of experiments for balanced two- and three-generation half-sib designs for outbred populations. They quantified the influence on the power of size of QTL effect, heritability, family size and number

Table 24.2. All possible marker–QTL genotype combinations for a marker with two alleles (*M1* and *M2*) and a QTL with two alleles (*Q1* and *Q2*). The alleles on one chromosome are written adjacently and are separated from those on the other chromosome by a slash.

Marker geno- type	QTL genotype		
	Q1Q1	*Q1Q2*	*Q2Q2*
M1M1	*M1Q1/M1Q1*	*M1Q1/M1Q2*	*M1Q2/M1Q2*
M1M2	*M1Q1/M2Q1*	*M1Q1/M2Q2*	*M1Q2/M2Q2*
		M1Q2/M2Q1	
M2M2	*M2Q1/M2Q1*	*M1Q1/M1Q1*	*M2Q2/M2Q2*

of half-sib families. They focused on dairy cattle breeding in which the half-sib family structure is predominant. In poultry and pigs, however, full-sib structures are also feasible. Van der Beek *et al.* (1995) evaluated the powers of experiments with a full-sib structure. Some of their results are summarized here.

Inferences about the presence of a QTL linked to a marker are based on the marker contrast. The marker contrast is expected to be zero if no QTL is linked to the marker or if a parent is homozygous for the linked QTL. The marker contrast is expected to be non-zero if a QTL is linked to the marker and the parent is heterozygous for the linked QTL. The square of the marker contrast divided by the square of the standard error of the marker contrast is used to compute a test statistic. When it is assumed that the phenotypic variance of the trait is known, this test statistic is a χ^2 statistic. Under the null hypothesis of no linked QTL, the statistic is a central χ^2 distribution. The null hypothesis is rejected when the statistic is larger than threshold T, the $(1 - \alpha)$ percentile of the central χ^2 distribution where α is the type I error rate. The power of a QTL mapping experiment is equal to the probability that the null hypothesis of no linked QTL is rejected, i.e. the probability that the test-statistic is larger than threshold T. The power of an experiment can be calculated (Weller *et al.*, 1990) as:

$$\text{power} = \sum_{x=0}^{n_p} P(x) \times P\left[\chi^2(\text{NC}(x), n_p) > T\right]$$

where x is the number of parents that are heterozygous for the QTL, n_p is the number of parents for which a marker contrast is computed, $P(x)$ is the binomial probability that x out of n_p parents are heterozygous for the QTL, $\chi^2(\text{NC}(x), n_p)$ is a non-central χ^2 variable with n_p degrees of freedom and with a non-centrality parameter $\text{NC}(x)$, $\text{NC}(x)$ is the non-centrality parameter for the distribution under the alternative hypothesis given that x parents are heterozygous for the QTL and $P[\chi^2(\text{NC}(x), n_p) > T]$ is the probability that the non-central χ^2 variable exceeds the threshold T. The non-centrality of the distribution depends on the

expectation for the marker contrast, standard error of the marker contrast and the number of parents that are heterozygous for the linked QTL.

These parameters depend on the design of the experiment, as shown by Van der Beek *et al.* (1995), who looked at two- and three-generation designs. In both designs, marker genotypes are determined on parents and their offspring. In a half-sib design, marker genotypes are obtained for the sire and the half-sib offspring but not for the mates. In a full-sib design, marker genotypes are obtained for both parents and their full-sib offspring. In a two-generation design, the phenotypes are recorded on the offspring generation, whereas in a three-generation design each offspring is mated to produce grand-offspring. Phenotypes but not marker genotypes are recorded on these grand-offspring.

Table 24.3 presents the powers of two-generation experiments with full-sib and half-sib family structures for various numbers of families, various numbers of

Table 24.3. Power of experiments with a two-generation half-sib (HS2) or two-generation full-sib (FS2) family structure for a QTL explaining 1% of the phenotypic variance and with a heterozygosity of 50%, for various number of families (N_{fam}), various number of offspring per family (N_{off}) and two heritabilities ($h^2 = 0.1$ and $h^2 = 0.4$). (Source: Van der Beek *et al.*, 1995.)

		$h^2 = 0.1$		$h^2 = 0.4$	
N_{fam}	N_{off}	HS2	FS2	HS2	FS2
5	50	0.02	0.02	0.02	0.02
	100	0.03	0.04	0.03	0.04
	200	0.05	0.09	0.06	0.11
	400	0.13	0.24	0.15	0.31
	800	0.34	0.59	0.38	0.69
10	50	0.02	0.03	0.02	0.03
	100	0.04	0.05	0.04	0.07
	200	0.08	0.15	0.09	0.19
	400	0.23	0.43	0.26	0.54
	800	0.57	0.86	0.62	0.92
20	50	0.03	0.03	0.03	0.04
	100	0.05	0.09	0.06	0.11
	200	0.14	0.27	0.16	0.36
	400	0.42	0.72	0.47	0.83
	800	0.85	0.99	0.88	0.99

offspring per family and for two heritabilities of the observed trait. The results refer to a situation where interval mapping is used to detect a QTL that explains 1% of the phenotypic variance, that has a heterozygosity of 50% and that is located at the midpoint of a 20 cM interval flanked by two informative markers. An experiment with a full-sib family structure and n families has about the same power as an experiment with a half-sib family structure and $2n$ families for all values of n (Table 24.3). For a heritability of 0.1, an experiment with five full-sib families and 800 offspring per family had a power of 0.59 and an experiment with ten half-sib families and 800 offspring per family had a power of 0.57. The efficiency of an experiment with a full-sib family compared with a half-sib family structure was close to 2: 2.06 for $h^2 = 0.1$ and 2.25 for $h^2 = 0.4$. The power of an experiment with a two-generation family structure increased with increasing the number of families and with increasing the number of offspring per family (Table 24.3). Increasing the number of offspring per family was more efficient than increasing the number of families, e.g. for $h^2 = 0.1$, an experiment with ten full-sib families and 200 offspring per family had a power of 0.43, whereas an experiment with 20 full-sib families and 100 offspring per family had a power of 0.27.

The power of an experiment with a two-generation family structure increased with increasing heritability if the effect of the QTL, expressed in phenotypic standard deviation units, remained constant (Table 24.3). For an experiment with five half-sib families and 800 offspring per family, the power was 0.34 for $h^2 = 0.1$ and 0.38 for $h^2 = 0.4$. The effect of h^2 on the power is slightly larger for a full-sib than for a half-sib family structure.

Powers of experiments with a three-generation family structure are given in Table 24.4 for three different family structures, namely HS3, FS3 and FSHS. In the three-generation half-sib structure (HS3), a sire has several half-sib offspring and each half-sib offspring is mated to several unrelated animals to produce one half-sib grand-offspring per mate per half-sib

Table 24.4. Power of experiments with a three-generation family structure (HS3, FS3 and FSHS) for trait with heritability of 0.10 and a QTL explaining 1% of the phenotypic variance and with a heterozygosity of 50%, for various number of families (N_{fam}), various number of offspring per family (N_{off}) and various number of grand-offspring per offspring (N_{goff}). (Source: Van der Beek *et al.*, 1995.)

N_{fam}	N_{off}	N_{goff}	HS3	FS3	FSHS
5	25	10	0.02	0.02	0.02
		50	0.04	0.04	0.11
		100	0.06	0.06	0.22
	50	10	0.03	0.03	0.04
		50	0.10	0.11	0.30
		100	0.15	0.15	0.55
	100	10	0.06	0.08	0.11
		50	0.26	0.31	0.67
		100	0.37	0.41	0.88
10	25	10	0.02	0.02	0.03
		50	0.06	0.07	0.18
		100	0.09	0.09	0.39
	50	10	0.04	0.05	0.06
		50	0.17	0.19	0.53
		100	0.26	0.27	0.82
	100	10	0.09	0.13	0.18
		50	0.45	0.54	0.91
		100	0.62	0.68	0.99

offspring. Weller *et al.* (1990) named a design with this family structure the 'granddaughter design'. In the three-generation full-sib family structure (FS3), a pair of parents has several full-sib offspring. Each full-sib offspring is mated to one unrelated and ungenotyped animal to produce several full-sib grand-offspring per full-sib offspring. In the last family structure (FSHS), a pair of parents has several full-sib offspring as in FS3 but in FSHS each full-sib offspring is mated not to one but to several ungenotyped animals to produce one half-sib grand-offspring per mate per full-sib offspring. The power of an experiment with an HS3 family structure was similar to the power of an experiment with an FS3 family structure if the number of families (N_{fam}), number of offspring per family (N_{off}) and the number of grand-offspring per offspring (N_{goff}) were equal. For a heritability of 0.1, the power for

an FS3 family structure was 1 to 1.2 times the power for an HS3 family structure. For a heritability of 0.4, the power of an FS3 family structure was 0.85 to 1 times the power for an HS3 family structure (Van der Beek *et al.*, 1995). With full-sib offspring, two marker contrasts can be computed per family, while with half-sib offspring only one marker contrast can be computed. A family structure with full-sib grand-offspring, however, was less efficient than a family structure with half-sib grand-offspring. This resulted from the fact that the standard error of the marker contrast was larger when full-sib instead of half-sib grand-offspring were used.

The power of an experiment with an FSHS structure was higher than the power of an experiment with either an HS3 or an FS3 family structure. For a heritability of 0.1, the power of an experiment with an FSHS family structure was 1 to 4 times the power of an experiment with an HS3 or FS3 family structure (Table 24.4). This difference increased with an increase in heritability (Van der Beek *et al.*, 1995). The power of an experiment with a three-generation family structure increased with increasing the number of families and with increasing the number of offspring per family (Table 24.4). As with two-generation experiments, the power increased more by doubling the number of offspring per family than by doubling the number of families. The power increased more by doubling the number of offspring than by doubling the number of grand-offspring per offspring.

Factors influencing the power of the design

The results in Tables 24.3 and 24.4 show that family structure is an important factor in designing QTL mapping experiments. For a two-generation experiment, more offspring are required for a certain power than for a three-generation experiment. Consequently, more animals had to be typed for marker loci for a two-generation experiment than for a three-generation experiment. Fewer offspring per two-generation

experiment, however, were required than grand-offspring per three-generation experiment. This implies that fewer trait values have to be obtained for a two-generation experiment than for a three-generation experiment. For example, an experiment with ten two-generation full-sib families with 800 offspring per family had a power of 0.86, whereas a similar power (0.91) could be obtained with ten families in an FSHS design, with 100 full-sib offspring per family and 50 half-sib grand-offspring per full-sib offspring. The number of animals to be genotyped in the two-generation experiment is eight times the number in the three-generation design, whereas the number of phenotypes is 0.16 times the number of phenotypes in the three-generation experiment. In deciding on a design, the costs of typing the markers and the costs of recording trait values have to be considered as well as the time required to collect all the information. The costs of recording trait values are low if the trait is routinely collected for management or breeding purposes, such as milk production in dairy cattle. Furthermore, the family structure of the commercial dairy cattle population enables experiments with a three-generation half-sib family structure (e.g. Georges *et al.*, 1995; Spelman *et al.*, 1996). A three-generation design opens opportunities when interest lies in multiple trait and where each trait needs a specific environment. In the Wageningen poultry experiment, for example, groups of third-generation offspring were kept under different environmental conditions to measure individual feed intake (individual housing), carcass quality (group housing) and ascites (lower ambient temperature).

The variance explained by the QTL is important and depends on a^2 and heterozygosity of the QTL. The effect of doubling a^2 on power is the same as the effect of doubling the number of families (N_{fam}). As the effect of doubling N_{fam} is given in Tables 24.3 and 24.4, the effect of doubling a^2 can also be derived from those tables. Heterozygosity at the QTL was assumed to be 0.5. For a QTL with two alleles this is the maximum heterozygosity in a population that is in genetic

equilibrium. For a lower heterozygosity at the QTL, more families are needed for equal power. The relation between heterozygosity at the QTL and required number of families is about linear (Weller *et al.*, 1990). In the above power calculations, one outbred population was used; the efficiency of an experiment with a cross between divergent populations is higher (Soller, 1991). Genes with large effects are expected to segregate at higher frequencies in a cross than within an outbred population.

Selective genotyping

The number of marker genotypings in QTL mapping experiments is considerable, especially for the two-generation design. Selective genotyping may be a useful alternative to decrease the number of marker genotypings. The principle underlying selective genotyping (i.e. the genotyping of only those individuals that are extreme for a quantitative trait) was first described by Lebowitz *et al.* (1987). For crosses between inbred lines, Darvasi and Soller (1992) derived approximate formulae for the marker contrast and variance after selective genotyping has been applied. Selective genotyping in an outbred population should be applied within families. Bovenhuis and Spelman (2000) derived formulae for the marker contrast after selective genotyping in outbred populations. Darvasi and Soller (1992) and Bovenhuis and Spelman (2000) showed that selective genotyping can result in a considerable reduction of the number of genotypings for a given power. Selectively genotyping the 20% highest and 20% lowest animals resulted in a small reduction of the power compared with genotyping all animals.

Selective genotyping is often argued to have a major limitation if experiments are aimed at studying many traits. In such cases, selective genotyping could be applied to each of the traits, which would imply that most of the population needs to be genotyped and, thus, no reduction in genotyping can be obtained. In an experiment, one has to

choose to which trait selective genotyping should be applied. The highest power of detecting a QTL affecting a trait is achieved if selective genotyping is applied to the trait itself. However, the power of detecting a QTL affecting the trait is only slightly reduced if selective genotyping is applied to a highly correlated trait. The power is considerably reduced if selective genotyping is applied to traits with a correlation close to zero with the trait of interest. Bovenhuis and Spelman (2000) demonstrated that the power to detect a QTL affecting a correlated trait was at least as great as with random genotyping. In practice, this implies that selective genotyping should be applied to the trait of greatest interest, which might not be an optimal strategy for all traits.

Most QTL mapping experiments are not very large and will significantly detect genes with large effects, but for most QTL the evidence will not be conclusive. Those suggestive QTL need to be verified in further experiments (confirmation studies). A potentially interesting way of verifying marker–QTL associations is by using selective genotyping.

The high costs of screening large populations for marker allele frequencies can be markedly reduced by use of selective DNA pooling (Darvasi and Soller, 1994; Lipkin *et al.*, 1998). The marker contrast is determined by differences in allele frequency in pooled DNA samples of individuals taken from the extreme high and low tails of the phenotypic distribution of the population. Estimation of allele frequencies in pooled DNA samples is based on a linear relation between the allele frequency in the group of individuals making up the pool and the final allele band intensity after genotyping the pool, as determined by densitometry. In a retrospective study, Lipkin *et al.* (2002) successfully applied DNA pooling to an F_2 resource population created to map QTL affecting Marek's disease resistance. They observed very close correspondence between differences in allele frequency obtained on pools and differences obtained with individual genotyping. Their results support the proposition that selective DNA pooling can be combined with the large

half-sib families routinely produced in commercial genetic improvement programmes in layer and broiler chickens to provide high-power mapping of QTL affecting major production traits.

Choice of design

A QTL mapping experiment should be carefully designed. It is not possible to make a general statement on the design that is to be preferred. The experimenter needs to make a decision on the population(s) to be included in the QTL mapping experiment. This choice influences the probability that parents are heterozygous for QTL affecting the trait but it also influences the extent to which results can be applied in a commercial population. The heterozygosity at the QTL can be increased by using a divergent cross. However, it is still an open question to what extent these QTL also explain genetic variation within lines.

A QTL mapping experiment is expensive and the gains of optimizing the design may be high. Tables 24.3 and 24.4 demonstrate that more animals had to be typed for marker loci for a two-generation experiment than for a three-generation experiment. However, the number of animals to be phenotyped was larger in the three-generation design. In addition, the three-generation design enables the collection of information on a range of phenotypes and thereby offers the opportunity for information on interaction phenotypes. For example, production traits can be recorded for one group of offspring in a disease-free environment, while disease-resistance traits can be recorded for another group of offspring in a challenge experiment.

When the costs of recording phenotypes are low compared with the costs of genotyping, a three-generation design will result in lower total costs than a two-generation design. The costs of recording phenotypes also play an important role in deciding on the use of selective genotyping.

Methods of Analysis

Genetic mapping of a trait comes down to finding those chromosomal regions that tend to be shared among relatives performing well and tend to differ between the latter and poorly performing relatives. Conceptually, this mapping amounts to the following steps: scanning the entire genome with a dense collection of genetic markers; calculating an appropriate linkage statistic at each position along the genome; and identifying the regions in which the statistic shows a significant deviation from what would be expected under independent assortment (Lander and Kruglyak, 1995). A practical approach for estimating the position of a QTL is to perform a genome-wide scan for QTL using linkage analysis methods. This would result in a region of, say, 20 cM, that most likely contains the QTL. Next, this region is covered with additional markers, and linkage disequilibrium mapping methods can be used to narrow the position of the QTL. Finally, comparative mapping and/or positional candidate cloning may be used to identify the QTL (Anderson, 2001). Grisart *et al.* (2002) successfully identified a gene on bovine chromosome 14 in a QTL region that was initially identified using linkage analysis.

Different approaches can be used to analyse data from a QTL mapping study (Bovenhuis *et al.*, 1997; Hoeschele *et al.*, 1997; Doerge, 2002). This chapter will consider four different methods: variance analysis; regression; mixture models; and random effects models. Methods based on a variance component approach are described in another chapter. The first three methods are generally used for the analysis of an experiment with a two- or three-generation design in which transmission of marker alleles is traced from parent(s) to offspring. Methods are categorized based on the statistical-genetic model (number of QTL alleles, number of genetic effects) underlying the analysis and the information used in the analysis. For some of the models

different parameter estimation procedures can be used, e.g. maximum likelihood or Markov chain Monte Carlo; consequently, categorizing on the basis of parameter estimation procedure might be misleading (Bovenhuis *et al.*, 1997).

Analysis of variance using a single marker

Traditionally, detection of QTL has been performed by contrasting the phenotypes for marker genotype effects (e.g. Soller *et al.*, 1976). In this type of analysis (for example, using *t*-test, ANOVA and simple linear regression statistics), markers are analysed one at a time to assess the segregation of a phenotype with respect to a marker genotype. Typically, the null hypothesis tested is that the mean of the trait value is independent of the genotype at a particular marker. The null hypothesis is rejected when the test statistic is larger than a crucial value, and the implication is that a QTL is linked to the marker under investigation. The marker genotype effects can be tested using an *F*-test, as is common in analyses of variance. For an outcross population, significant marker effects at the population level are only expected if there is a linkage disequilibrium between the QTL and the genetic marker. Neimann-Sorensen and Robertson (1961) noted that for an outcross population, analysis of marker genotype effects should be performed within sire families. This can be done by contrasting progeny within a family that have inherited alternative parental alleles. This method of analysis yields estimates of marker allele substitution effects. However, the analysis does not provide any information about the location of the QTL, i.e. the model cannot distinguish between a tightly linked QTL with a small effect and a loosely linked QTL with a large effect. Another disadvantage of the method is that some of the progeny cannot be assigned to one of the two parental alleles. These animals have to be excluded from the analysis, resulting in reduced power.

Regression methods

Single-marker analysis investigates individual markers independently, and without reference to their position or order. When markers are placed in genetic (linear) map order, so that the relationships between markers are understood, the additional genetic information gained from knowing these relationships provides the necessary setting to address confounding between QTL effect and location. In addition to supplying the structure in which to search for QTL, the estimated genetic map benefits the estimation of missing marker information by using the surrounding marker genotypes to infer knowledge of the missing marker genotypes.

Haley and Knott (1992) and Martinez and Curnow (1992) independently introduced a regression method of analysis that is capable of utilizing information from flanking markers. When using information from a single marker only, regression is equivalent to analysis of variance. Instead of regression on marker genotypes, regression is performed on the probability of having a QTL genotype, given the genotypes for the flanking markers.

Line cross analysis

Under the line cross model, it is assumed that the two founder populations, although they may share alleles at the marker loci, are fixed for alternative alleles at the QTL affecting the trait of interest. The principles involved for an F_2 resource population are described here.

The analysis of an F_2 population can be considered in two stages. In the first stage, at locations throughout the genome, the probability of an F_2 offspring being each of three possible QTL genotypes (QQ, Qq and qq) is calculated at 1 cM intervals along the genome conditionally on the marker genotypes. In the second stage, these probabilities are used in a least-squares framework to investigate the genetic model underlying the trait of interest. Following Falconer and

Mackay (1996) and denoting the effect of *QQ* as *a*, the effect of *Qq* as *d* and the effect of *qq* as −*a*, the phenotype of an offspring at a given position in the genome can be written as a linear model in terms of the additive and the dominance contributions at a QTL:

$$y_i = \mu + c_{ai}a + c_{di}d + e_{ij}$$

where μ is the mean, c_{ai} is the coefficient for the additive component for individual *i* at the given position that, denoting the probability of an individual being genotype *XX* as prob(*XX*), is equal to prob(*QQ*) − prob(*qq*), c_{di} is the coefficient for the dominance component for individual *i* at the given position, which is equal to prob(*Qq*), and e_{ij} is the residual effect. The calculation of these probabilities and the QTL effects are described by Haley *et al.* (1994).

The method developed by Haley *et al.* (1994) is suitable for crosses where the lines may be segregating at marker loci but can be assumed to be fixed for alternative alleles at the major QTL affecting the traits under analysis (e.g. crosses between divergent selection lines or breeds with different selection histories). The simultaneous use of multiple markers from a linkage group increases the sensitivity of the test statistic, and thus the power for the detection of QTL, compared with the use of single markers or markers flanking an interval. Use of multiple markers can also remove the bias in the estimated position and effect of a QTL that may result when different markers in a linkage group vary in their heterozygosity in the F_1 and are considered only singly or a pair at a time.

Half-sib and full-sib models for outcross populations

A multi-marker approach for interval mapping in a paternal half-sib design was described by Knott *et al.* (1996). In this design, the relationship between the sire and its offspring is used and all additional relationships between and within half-sib groups are ignored. For each offspring, the probability of inheriting the sire's first gamete of a chromosome is calculated at 1 cM intervals conditional on the most-linkage phase of the sire and the marker

genotypes of the offspring and its parent(s). A QTL with a gene substitution effect is fitted at 1 cM intervals along the chromosome:

$$y_{ij} = \mu + s_i + b_i x_{ij} + e_{ij}$$

where μ is the overall mean, s_i is the average effect of half-sib family *i*, b_i is allele substitution effect for the QTL in half-sib family *i*, x_{ij} is the conditional probability for individual *j* of inheriting the first parental allele and e_{ij} is the residual effect. The regression is nested within half-sib families because the assignment of the first gamete is random and not all sires are heterozygous for the QTL. Furthermore, the linkage phase between a marker and a QTL can differ between families. The number of QTL alleles is only constrained by the number of families. The test statistic is calculated as an *F* ratio for every map position within and across families. For details on the calculation of the test statistic, see Spelman *et al.* (1996).

The above method was extended to full-sib family structure by Van Kaam *et al.* (1998). In that case, marker information on both parents and their full-sib offspring is used to trace the transmission of QTL alleles from both parents to their offspring and an allele substitution effect is calculated for both parents.

Combination of approaches

De Koning *et al.* (1999) applied linkage analysis on Meishan crossbred pigs under two genetic models: (i) an outbred line cross model, where the founder lines were assumed to be fixed for different QTL alleles; and (ii) a within-family model, where a unique allele substitution effect was fitted within every paternal half-sib family. They demonstrated that the combined use of both models provides additional insight into the genes involved and their effects, compared with the use of one of these approaches. The latter is because animals within each divergent breed may also exhibit genetic variation for the QTL. The QTL effects estimated under a line cross model are biased downwards when QTL alleles are not fixed in the parental

lines, whereas a half-sib analysis is able to detect genes, albeit at a lower power, when alleles are segregating in one or both breeds.

Mixture model analysis

Weller (1986; Weller *et al.*, 1990) developed mixture model methods to detect QTL using single marker information in crosses between inbred lines and for outcross populations. Solutions are obtained by maximizing the likelihood function. In calculating an individual's QTL genotype probability, information from marker genotype as well as information from its phenotype is used. The mixture model approach suggested by Weller (1986) for crosses between inbred lines has been extended to take into account information from flanking markers (Lander and Botstein, 1989). A mixture model approach for the mapping of QTL in outbred populations was presented by Jansen *et al.* (1998). This method can be applied to situations in which information about the genotype of an individual is incomplete.

Random effects model

A QTL mapping experiment in an outbred population is typically based on genotypes for two generations of animals (parents and their offspring) and on phenotypes measured on animals of the second generation or their offspring. In most statistical analyses, the first-generation animals (parents) are assumed to be unrelated; the second-generation animals (offspring) are assumed to be related only through one common parent (usually the sire) in a half-sib analysis or through both parents in a full-sib analysis. This assumption does not hold for most commercial livestock populations, in which many additional genetic relationships are present. For example, in a dairy cattle population, bull dams may have multiple sons tested in the population, or bull

dams are related to grandsires. In these situations, a full pedigree analysis, accounting for all relationships, is more appropriate. Such an analysis cannot be performed by traditional analysis methods such as linear regression or maximum likelihood interval mapping, because these methods assume unrelated grandsire families and only two generations of genotyped individuals. Bink and Van Arendonk (1999) developed a Bayesian approach for a full pedigree analysis with incomplete marker data. Simulation results for a typical dairy cattle population indicated a significant increase in power of QTL detection when all relationships were included in the analysis.

In the genetic model, allelic effects at the QTL in an outbred population may be represented by normally distributed random effects, where covariances between allelic effects depend on the identity by descent probabilities that are derived from marker information (Fernando and Grossman, 1989; Van Arendonk *et al.*, 1994). Normality of QTL effects is a robust assumption to allow segregation of many alleles in the population and to allow changes in allelic effects over generations. Changes in allelic effects might originate from mutations, interactions between genes, dominance, and interaction with the environment. As an alternative to this so-called infinite-allele QTL approach, a single biallelic QTL can be modelled in which individuals are assigned to QTL genotypes based on information from flanking markers (Hoeschele *et al.*, 1997). This approach can be extended to situations with more alleles. When molecular genetic information on the mutations causing the allelic differences increases, it becomes conceptually more appealing to use a finite-allelic model for the QTL. However, even in cases where the actual number of alleles is known, this does not imply that the number of effects to be modelled is finite. The effects of a single mutation on the phenotype might differ depending on the genetic background (e.g. family) in which it occurs.

For more information on these types of models, see Yi and Xu, Chapter 25.

Correcting phenotypes

In the analysis of a two-generation design, the least-squares framework can be extended to incorporate fixed effects and covariates in the model. In a three-generation design, a two-step procedure is mostly used. In the first step, the trait values on the third-generation animals are corrected for fixed effects and covariates and subsequently accumulated for each second-generation animal. In the second step, the adjusted and accumulated trait values are used in the interval regression procedure. The number of offspring may differ between second-generation animals. This difference influences the residual variance of the average trait values and should be included in subsequent analysis. Van Kaam *et al.* (1998) described the weights in a regression method for a three-generation full-sib design.

Effects to be estimated

QTL mapping studies also provide a better insight into the mode of inheritance of traits. So far, the emphasis has been on QTL on the autosomes that show Mendelian inheritance (additive effects and dominance deviation). Recently, a few QTL have been described in livestock species that exhibit non-Mendelian inheritance. Cockett *et al.* (1996) found that the callipyge gene, which results in muscular hypertrophy in sheep, shows a non-Mendelian inheritance pattern, referred to as polar over-dominance, where only heterozygous individuals having inherited the callipyge mutation from their sire express the double-muscling phenotype.

Genomic imprinting is commonly regarded as a rare phenomenon and consequently is ignored in most studies. Nezer *et al.* (1999) and Jeon *et al.* (1999) identified an imprinted gene near the IGF-2 locus on porcine chromosome 2 that affects muscle mass and fat deposition. De Koning *et al.* (2000) indicated that genomic imprinting might be a more common phenomenon than

previously thought. They detected five QTL, of which four were subject to imprinting. For at least two of these regions, imprinting had not been reported before in pigs, and the known homologies to humans and mice did not reveal obvious positional imprinted candidates.

For detection of imprinting in a QTL analysis, it is essential that parental origin of marker alleles can be derived for the offspring (De Koning *et al.*, 2000). This requirement excludes studies based on F_2 crosses or a single backcross between inbred lines, as commonly used in mice and rats. These model species have contributed enormously to the current understanding of genetic variation. The inability to detect imprinting in the most commonly used mapping designs has certainly contributed to the current feeling that imprinting is a rare phenomenon.

Multiple QTL Analysis

Currently, single-QTL methods are still widely used in plant, animal and human genetics, but they are intrinsically inappropriate for complex traits affected by multiple QTL. In experimental plant applications, multiple-QTL models are now used more and more frequently; background QTL are taken into account by including them (via linked markers) as cofactors in the model (proposed by Jansen, 1992; see Jansen, 1996, for a review). This can be done in plants because complete marker maps are available for many plant species and also because experimental plant populations, e.g. F_2 or backcross, are easier to deal with from the analytical point of view. In animal and human applications, the effects of background QTL are often modelled by a single variance component term. In general populations, a marker can be segregating in some families whereas the QTL is not and vice versa. Then a marker linked to a putative QTL cannot be used as the cofactor in the (expectation or mixture) model, as also indicated by e.g. Spelman *et al.* (1996). In such cases, the QTL really should be included instead of the marker as cofactor

in the model, although the putative QTL can be put close to or even on top of a marker. Eventually dense marker maps may become available in human and animal applications, and, with cofactors for background QTL, it may not be necessary to include other parameters for genetic background control. Modelling via cofactors will also make it possible to explain differences in variances between families originating from segregation of different sets of genes and therefore residual variance may be assumed to be homogeneous; this cannot be achieved by a model term for polygenic background effect.

Selective DNA Pooling

DNA markers are now widely used for the detection and mapping of QTL. Selective genotyping – the marker assay of only individuals with the more extreme phenotypes for a quantitative trait – can provide considerable savings in genotyping costs while retaining most of the statistical power for detection of QTL affecting the trait on which the selection is based (Lebowitz *et al.*, 1987; Bovenhuis and Spelman, 2000). For single-trait studies it will almost never be useful to genotype more than 50% of the population (the high and low tail). However, linear model estimates of the QTL effect, for which individuals without genotypic information are excluded from the analysis, will be biased by the selective genotyping (Darvasi and Soller, 1992).

A mixture model approach for the mapping of QTL in outbred populations was presented by Jansen *et al.* (1998). This method can be applied to situations in which information about the genotype of an individual is incomplete. Incomplete information might be caused by the impossibility of tracing the inheritance of an allele at a locus in an individual, unknown linkage phases between loci, unknown QTL genotype and unknown genotypes for markers. The method can, therefore, be applied to selectively

genotyped data, but no information is available on the properties of the estimates.

A model can be defined to describe the relationship between phenotype and 'known' genotype. Since the genotype is in reality unknown, the possible genotype configurations that arise from this uncertainty then become the components of a mixture and this can be handled by an expectation maximization (EM) algorithm that yields maximum likelihood estimates of the model parameters (Jansen *et al.*, 1998). A simulation study is used to investigate the performance of this mixture model approach when selective genotyping is employed within a half-sib family structure (Johnson *et al.*, 1999). This approach was also compared with the multi-marker regression method (Knott *et al.*, 1994).

The major cost in the detection of QTL with the aid of genetic markers is that due to DNA collection and typing. Selective genotyping can provide considerable cost savings, particularly in those populations where recording of phenotypes is done on a routine basis, with little loss in accuracy of detection of QTL. Linear model regression estimates of allelic effects, such as those obtained from a multi-marker regression method, are biased upwards when selection is used to genotype only those individuals that are extreme for the quantitative trait. This is due to the positive correlation between residual effects and the QTL effect in the pooled tails population that magnifies the allelic effect.

On the basis of simulation work (Johnson *et al.*, 1999), the mixture model method would appear to yield estimates of gene substitution effects that are not biased by selective genotyping. This is the case not only for the primary trait on which selection is based but also for a correlated trait, provided that the latter is analysed jointly with the primary trait. Presumably critical to this result is the fact that the Monte Carlo (MC) EM method, when sampling missing marker genotypes, takes into account not only known marker information but also all phenotypic observations for the trait.

Linkage Disequilibrium Mapping

The quest for genetic loci corresponding to QTL will ultimately arrive at a point where the QTL is mapped to a small chromosomal region, in which all genes and predicted genes are known. Which of these loci is associated with the QTL? Having determined the genetic locus corresponding to the QTL, linkage disequilirium mapping can be used to determine what molecular polymorphisms – single nucleotide polymorphisms (SNPs), small insertions/deletions or large insertions – define a functional QTL allele. Linkage disequilibrium is a measure of the correlation of allele frequencies at two polymorphic loci. Population genetic theory predicts that a molecular marker locus will be in linkage disequilibrium with a molecular variant affecting the quantitative trait (the quantitative trait nucleotide, or QTN) only if they are very tightly linked – provided that the population demography satisfies the theoretical assumptions.

The linkage disequilibrium between a single marker and a QTL can be measured by estimating the effect of the marker on the quantitative trait in a regression analysis. This approach is extended to multiple marker loci by estimating the effect of marker haplotypes on the quantitative trait. The marker haplotypes that have identical marker alleles in a region surrounding the QTL are expected to show similar haplotype effects, since the identical markers indicate that the region is identity-by-descent (IBD) and thus the haplotypes are expected to carry similar QTL alleles. In statistical terms, similar haplotype effects imply that the covariance between the haplotype effects is high. Whether two marker haplotypes have identical alleles in a region surrounding the QTL depends on the position of the QTL; hence, the covariance between the haplotype effects depends on the position of the QTL. Meuwissen and Goddard (2000) developed a method for the estimation of the QTL position using the linkage disequilibrium between several closely linked markers and the QTL.

Statistical Significance

Regardless of the method used to estimate and locate single or multiple QTL, once the test statistics are calculated the likelihood of the event is assessed. The statistical basis of these comparisons relies on model assumptions, the most common of which requires the quantitative trait values to be normally distributed (Doerge, 2002). If in fact these assumptions hold, many wonderful statistical properties follow and valid conclusions can be drawn from a range of powerful statistical tests. In reality, however, the distribution of the trait values is not normal, and needs to be considered as a mixture of (normal) distributions. Violating the normality assumption has an impact on the distribution of the statistic used to test for a QTL, which makes standard statistical procedures potentially inaccurate.

One approach to obtaining the distribution (or behaviour, in the long term) of the test statistic is to use a computer simulation to produce the data (Lander and Botstein, 1989; Jansen, 1994). Thousands of data sets, taken from the same statistical model, are simulated and analysed in order to represent the statistical distribution of the particular test statistic. From this distribution, one chooses the level of statistical significance or threshold above which results are considered statistically significant (valid). This approach assumes that the true model is used to simulate the data, which is rarely the case. Non-parametric resampling methods (Lander and Kruglyak, 1995) provided a useful alternative to simulation-based thresholds. In a large number of studies, the permutation resampling (Churchill and Doerge, 1994) is used as a means of randomizing the phenotypic (trait) data for the purpose of evaluating any test statistic under a null hypothesis that tests for a QTL. For a discussion of this and alternative methods, see Doerge (2002).

Mapping of QTL is plagued by the statistical problem of multiple tests. If only one test is done, 5% is conventionally accepted as a significance threshold. That

is, the result of a statistical test is deemed 'significant' if its probability of occurring by chance alone is 5% or less. However, if many tests are done on the same data, as occurs when associations between multiple markers and the quantitative trait are considered, 5% of the associations are expected to be significant by chance. So, the significance threshold for the entire experiment must be adjusted downwards to correct for the number of independent tests.

Chromosome-wise significance thresholds, which account for multiple testing on a single chromosome, can be determined empirically by permutation resampling. Lander and Kruglyak (1995) distinguished two significance thresholds: suggestive and significant linkage. The first level was suggestive linkage, where one false positive is expected in a genome scan. For claiming significant linkage a 5% genome-wide significance level can be applied which can be calculated from the empirically determined chromosome-wide significance level using a Bonferroni correction.

In comparing results from different QTL experiments, most attention is focused on significant results. However, it is important to pay attention to the significance thresholds that have been applied.

Molecular Tools

Molecular genetic analyses of quantitative traits lead to the identification of two broadly different types of genetic loci that can be used to enhance genetic improvement programmes: (i) causal mutations; and (ii) presumed non-functional genetic markers that are linked to QTL (indirect markers). Causal mutations for quantitative traits are hard to find and difficult to prove and few examples are available. By contrast, non-functional or anonymous polymorphisms are abundant across the genome and their linkage with QTL can be established by evidence of empirical associations of marker genotypes with trait phenotype. Two approaches are used to identify indirect markers: (i) directed searches using candidate-gene approaches in unstructured populations; and (ii) genome-wide searches in specialized populations, such as F_2 crosses. Because candidate-gene markers focus on polymorphisms in a gene that are postulated to affect the trait, they are often tightly linked to the QTL. A candidate-gene marker can occasionally represent the functional variant itself, although this is difficult to prove. Genome scans, conversely, can only identify regions of chromosomes that affect the trait. The length of these regions is typically 10–20 cM, but the exact position and number of QTL in the region is unknown.

The candidate gene approach can be very powerful and can detect loci even with small effects, provided that the candidate gene represents a true causative gene. However, there are often many candidate genes and a major trait locus might fail to be identified simply because of the gaps in knowledge about gene function (Anderson, 2001). Candidate gene tests must also be interpreted with caution as spurious results can occur because of linkage disequilibrium to linked or non-linked causative genes or because the significance thresholds have not been adjusted properly when testing multiple candidate genes. A genome scan will always find the map location of a trait locus with a major effect, provided that an accurate genetic model has been postulated, a reasonable sample size has been used and the marker set provides full genome coverage. A genome scan will fail to detect trait loci with smaller effects if they do not reach the stringent significance thresholds that must be applied when doing a large number of tests in a full genome scan.

High-resolution mapping of trait loci can be carried out by IBD mapping (Anderson, 2001), which involves the collection of DNA samples from individuals that have inherited a certain allele at a trait locus from a common ancestor. The samples are screened with genetic markers to detect the minimum chromosomal region IBD among the individuals. A recent study of dairy cattle populations revealed fairly strong linkage disequilibrium for loosely linked loci and even weak linkage disequilibrium between

loci on different chromosomes (Farnir *et al.*, 2000). Linkage disequilibrium in farm animal populations is generated by genetic drift, the effects of which are more marked because of the limited number of breeding animals, migration and population stratification. The presence of linkage disequilibrium increases the probability of finding true associations between genetic markers and trait loci but also increases the risk of spurious associations in candidate gene studies. For example, admixture (interpopulation gene flow) between populations that have different gene frequencies at the marker and different values of the trait will cause spurious linkage disequilibrium, even between unlinked loci.

Relation to Marker-assisted Genetic Evaluation

Molecular genetic information can be used to enhance several breeding strategies through what is broadly referred to as marker-assisted selection (MAS). For an overview of different strategies for MAS in the improvement of agricultural populations, we refer to Dekkers and Hospital (2002). Genetic evaluation plays an important role in breeding programmes. The remainder of this section will discuss strategies to utilize QTL information in genetic evaluation schemes.

Genotypes at marker loci give information on transmission of genes from parents to offspring and that information can be used in predicting the individual's additive genetic value at linked QTL (Fernando and Grossman, 1989; Van Arendonk *et al.*, 1994). A mixed linear model exists for simultaneous evaluation of fixed effects, genetic effects at the QTL and the additive polygenic effect. These methods are efficient when marker data are complete but computations become intractable when the proportion of ungenotyped individuals increases. At present, the high costs for marker genotyping prohibit the routine genotyping of large numbers of animals. Bink *et al.* (1998a) presented an approach for marker-assisted genetic evaluation allowing incomplete information on a single marker that is linked to the QTL. The efficiency of this procedure can be greatly improved by absorbing the genetic effects of ungenotyped offspring in a reduced animal model (Bink *et al.*, 1998b).

Marker-assisted genetic evaluation will first be introduced in the nucleus of the population, i.e. the group of animals with high genetic potential that are the candidate parents for the next generation. For poultry the nucleus might be a closed population, whereas for dairy cattle the nucleus has an open character. In an open nucleus system, parents of nucleus individuals may be present in the commercial population. Procedures to incorporate information from these parents (without including them explicitly in the genetic evaluation) need to be developed, similar to procedures that incorporate foreign information in national genetic evaluation of dairy cattle.

In prediction of genetic effects at marked QTL, there may be a desire to assume that there are accurate estimates for QTL location and QTL variance. This would greatly reduce computational requirements. Initially the number of animals within a breeding population with observed marker genotypes will probably be too small for accurate estimation of QTL parameters (Spelman and van Arendonk, 1997). In that case, parameter estimates obtained from a QTL mapping experiment seem most appropriate. The amount of information within the nucleus will increase over time and this enables estimation of the genetic parameters from the current breeding population and increase the accuracy of genetic evaluations. Information on multiple QTL can be combined into a single relationship matrix where each QTL is weighed according to its genetic variance. The weighting will differ between traits, which opens attractive opportunities for selection particularly in situations with antagonistic relationships between traits.

Some Results

Khatib (1994) studied the association of 28 microsatellite markers with juvenile growth rate in a cross between White Leghorn layer females and a single White Rock broiler male. He found 11 markers that were significantly associated with growth rate, but sizes of effects were not given. In a genome-wide scan with 437 microsatellite markers, Van Kaam et al. (1999a) reported four QTL that exceeded the significance threshold in a feed efficiency experiment. Microsatellite genotypes were determined on ten full-sib families with on average 45 offspring per family. Feed efficiency was recorded on over 2000 grand-offspring. The QTL analysis was undertaken by modelling the segregation from generation one to generation two, using a full-sib across-family regression interval mapping approach. Genome-wise significance thresholds were derived using permutation test and a Bonferroni correction. The most significant QTL was located on chromosome 1 and had a 4% genome-wise significance for feed intake between 23 and 48 days. Furthermore, this QTL exceeded suggestive linkage for growth between 23 and 48 days and body weight at 48 days. A second QTL was located on linkage group WAU26 and showed suggestive linkage for feed intake between 23 and 48 days. The same full-sib families were used to produce another set of 2000 grand-offspring to map QTL affecting carcass traits (Van Kaam et al., 1999b). This experiment revealed a suggestive QTL affecting carcass percentage on chromosome 1 and a suggestive QTL affecting meat colour on chromosome 2.

Tatsuda and Fujinaka (2001) identified two QTL affecting body weight at 13 and 16 weeks in an F_2 cross between the indigenous Satsumadori and White Plymouth Rock chickens. The QTL were mapped to chromosomes 1 and 2 and the positions were in agreement with earlier studies.

In another study of Marek's disease (MD), searching a smaller resource population of egg-type chickens with fewer markers yielded 14 different genomic regions (QTL) associated with the disease (Yonash et al., 1999). The successful detection of QTL in this small population was probably due to the unique population derived from two inbred parental lines that differed in susceptibility to MD.

A population comprising F_2 and BC families, derived from a cross between two meat-type (broiler) lines that were divergently selected for early immune response, exhibited large genetic variation that facilitated the successful detection of RFLP markers linked to the selected trait (Yonash et al., 2001). However, because of the outbred nature of the parental lines, the necessary linkage disequilibrium was obtained only within families (Hillel, 1997). These studies utilized a unique resource population, derived from a cross between two divergent lines, to search for linkages between microsatellite DNA markers and QTL associated with immune response in young broilers.

QTL on sex chromosome

Most of the emphasis in QTL mapping has been directed to autosomal chromosomes, but there might also be QTL located on the Z chromosome. These QTL might explain the observed difference in mean and variance of growth between males and females. Furthermore, Tixier-Boichard et al. (1995) found a genetic correlation between adult male and female body weight of 0.71. Hagger (1994) reported a genetic correlation of 0.84 between adult male and female body weights. These findings suggest that male and female body weights are genetically different traits, which might be due to genes located on the sex chromosome. In mice, a large QTL was detected on the sex chromosome affecting body weight (Rance et al., 1997a,b). The estimated effect of the QTL was approximately 20% of the mean body weight in males and females at 10 weeks of age. On the sex chromosome of pigs, QTL were detected affecting backfat thickness and intramuscular fat content

(Harlizius *et al.*, 2000). These findings suggest that, in other species, genes affecting growth traits might also be detected on the sex chromosome. Detection of genes located on the Z chromosome differs from the detection of genes located on autosomal chromosomes. Hamoen *et al.* (2001) scanned the chicken Z chromosome for genes affecting growth traits and feathering. For this purpose parents and full-sib offspring were genotyped for 17 markers on the Z chromosome. Phenotypic data were only available for grand-offspring. Only the segregation of male chromosomes provides information on the presence of genes; therefore, a paternal half-sib interval mapping approach was used. The feathering gene was detected significantly and was located between markers ADL0022 and MCW0331. No significant indications were found for the presence of QTL affecting growth traits on the Z chromosome.

Utilizations

Phenotypic and genotypic information will be available for the selection of parents for the next generation. As pointed out by Lush (1933), there is no single desired genotype, i.e. the desired genotype cannot be produced by a single generation of selection. Combining genetic material from different individuals over a number of generations produces the desired genotype. Over generations, the selection scheme capitalizes on recombination and Mendelian sampling in bringing together a better combination of alleles. For longer-term progress, it is essential that sufficient genetic variation be maintained.

Woolliams *et al.* (1999) showed that rate of genetic gain is equal to the product of the Mendelian sampling term and the long-term genetic contributions summed over ancestors. This relationship explicitly shows that sustained genetic gain can only be achieved if animals with a non-zero long-term genetic contribution have an above-average Mendelian sampling term. This underlines the fact that selection programmes need to aim at newly arising variation. Rates of inbreeding are proportional to the sum of squared long-term genetic contributions (Wray and Thompson, 1990).

When choosing between breeding schemes, one has to find a balance between short-term and intermediate/long-term response. In general, increased short-term response is accompanied by decreased long-term response. Long-term response can be safeguarded by maintaining genetic variation, i.e. by constraining the rate of inbreeding. It can be inferred from the genetic contributions theory that, given a restriction on the rate of inbreeding, genetic gain is maximized when the long-term contributions of ancestors increase linearly with their Mendelian sampling term once their breeding value is above a certain threshold (Woolliams and Bijma, 2000). This theoretical upper boundary is generally unattainable since it is not possible either to measure the true breeding value without error or to set genetic contributions exactly to their desired values. However, using a dynamic selection procedure, genetic gain can effectively be maximized while restricting the rate of inbreeding (Meuwissen, 1997; Grundy *et al.*, 1998). With the same rate of inbreeding, the dynamic rule obtained up to 44% more genetic gain than simple BLUP selection.

Optimizing the longer-term genetic gain results in putting a higher weight on the information from the selection candidate or its offspring, i.e. the Mendelian sampling term of the individual. Molecular genetic data plays an important role here because it provides information on genetic material that has been transmitted by the parent to its offspring. Molecular genetic information offers the opportunity for better exploitation of phenotypic information but collecting phenotypic information remains important in order to identify the genetically unique individuals.

For the practice of animal breeding, identification of QTL that are subject to imprinting has several implications (De Koning *et al.*, 2000). Imprinting calls for a revision of methods for genetic evaluation that currently ignore non-Mendelian expression. The net result of gametic imprinting

is a reduction of the expected phenotypic covariance between parents and offspring relative to that between sibs. Identification of imprinted loci opens new perspectives for crossbreeding, which is common practice in pig and poultry breeding. Imprinted genes could further accommodate differentiation between sire and dam lines.

Prospects

Genetic improvement programmes for poultry can be enhanced by the use of molecular genetic information in crossbreeding and selection programmes. The prospects for MAS are greatest for traits that are difficult to improve through conventional means, because of low heritability or the difficulty and expense of recording phenotypes. Selection using linked markers can be effective and does not require identification of functional mutations, although some level of fine mapping is required. Detection of functional mutation will improve the efficiency of selection and will increase understanding of quantitative genetic variation and the relationships between traits. The number of tools for studying quantitative genetic variation is expected to increase in the coming years.

Bolivar *et al.* (2001) explored the use of knockout/congenic mouse strains for isolating and mapping QTL. Because most knockout strains have been bred to be B6.129 congenic strains, they can be used to test for QTL in the targeted chromosomal area as long as there is a genetic difference between B6 and 129. Bolivar *et al.* (2001) tested a number of knockout/congenic strains in a series of behavioral tests in which mouse performance has a significant genetic component.

It seems only a matter of time before initiatives will be taken to sequence the genomes of farm animals. In combination with bioinformatic analysis, this will be an important step towards unravelling the molecular basis for a variety of phenotypic traits.

Advances in technology are providing tools that allow the simultaneous quantification of both gene expression and protein expression. Specifically, gene-expression arrays have become more accessible in recent years, and are providing new and abundant levels of information on genome-wide patterns of transcription. It is expected that gene-expression and protein arrays will provide valuable information towards understanding gene function.

How many genes?

How many QTL affect variation in a quantitative trait? This simple question is not easy to answer. The number of QTL mapped in any one experiment is always a minimum number. It may be obvious, but is often forgotten, that QTL can only be mapped if there are allelic differences between the two parent strains used to construct the mapping population. To the extent that these strains are a limited sample of the existing genetic variation, it should not be surprising if different QTL are found in different studies. Designs that use parent strains derived from divergent artificial selection experiments will, however, contain a more representative fraction of segregating variation than will two random inbred lines. Furthermore, an axiom of QTL mapping is that the harder one looks, the more QTL one finds. There are two reasons why the number of QTL is expected to increase with the sample size (number of backcross or F_2 individuals). Firstly, the lower limit for the magnitude of QTL effect that is detectable in any mapping experiment is set by the sample size, and increasing the sample size allows mapping of QTL with smaller effects. Secondly, the precision of mapping depends on the ability to separate linked QTL by recombination. In general, the larger the sample size, the more recombinant events, and the more QTL that are detectable, given a sufficiently dense marker map.

Progress towards understanding the basis of quantitative genetic variation is likely to come from studying allelic

variation at specific QTL (Barton and Keightley, 2002). Cloning of QTL that cause phenotypic differences between selected populations should become more common, aided by the revolution in genomic technology, and should lead to the elucidation of the nature of such QTL. Achieving a satisfactory understanding of variation within populations is likely to be much more challenging. The information that we are seeking is the distribution of sizes of allelic effects at individual QTL and their frequencies within the population in question, as well as information on their effects on fitness. Highly replicated experiments that isolate QTL by fine-scale mapping could yield information on the allelic variants that segregate at specific regions of the genome or even specific loci, along with their frequencies within a population. Association studies should also reveal whether allelic variants repeatedly correlate with phenotypic variation for specific traits. Gaining such information will require very large experiments, larger perhaps than have previously been contemplated, but essential if we are to know the causes of variation among organisms – including our own species.

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25 Designs and Methods to Detect QTL for Production Traits Based on Random Genetic Models

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Introduction

Most production traits in poultry are quantitative in nature. Quantitative traits are defined as traits that have a continuous phenotypic distribution (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Variations of these traits are often controlled by the segregation of multiple loci, called quantitative trait loci (QTL). Therefore, quantitative traits are often synonymously called polygenic traits. Another characteristic of quantitative traits is that environmental factors can play a large role in determining the phenotypic variance. The polygenic nature of quantitative traits and their ability to be modified by environment make the study of their genetic basis more difficult than for monogenic traits. Traditional methods of quantitative genetics that use only the phenotypic and pedigree information cannot separate the effects of individual loci but examine the collective effect of all QTL. With the rapid development of molecular technology, large numbers of molecular markers (DNA variants) can be generated with ease. Most molecular markers are functionally neutral, but they normally obey the law of Mendelian inheritance. Therefore, the relative positions of the markers along the genome

(called the marker map) can be reconstructed using observed recombinant events. The joint segregating patterns of markers, in conjunction with phenotypic and pedigree information, provide additional information about the genetic basis of quantitative traits, including the number and chromosomal locations of QTL, the mode of gene action and the sizes (effects) of individual QTL. A complete description of the properties of QTL is called the genetic architecture. Study of the genetic architecture of quantitative traits using molecular markers is called QTL mapping.

There are two primary types of data and designs used for mapping QTL: data derived from line crosses that include backcrosses, F_2 or more derived generations; and pedigree-based data collected from outbred populations. For QTL mapping purpose, crosses between inbred lines have the fewest complications, such as the number of QTL alleles, and the linkage phases of the parental markers are known. Statistical methods of QTL mapping are well developed for line-cross designs, mainly including the least-square method (e.g. Haley and Knott, 1992), the iterative re-weighted least-square method (e.g. Xu, 1995, 1998), the maximum likelihood (ML) method (e.g. Lander and Botstein, 1989; Jansen, 1993; Zeng, 1993;

Kao *et al.*, 1999) and the Bayesian method (e.g. Satagopan *et al.*, 1996; Satagopan and Yandell, 1996; Sillanpää and Arjas, 1998; Stephens and Fisch, 1998). These methods test the effects of gene substitution and therefore are referred to as the fixed model approach (Xu and Atchley, 1995).

The random model approach is more appropriate when data are sampled from outbred populations (Xu and Atchley, 1995). Under the random model approach, variance of QTL segregation rather than the effect of allelic substitution is estimated (Haseman and Elston, 1972; Goldgar, 1990; Schork, 1993; Fulker and Cardon, 1994; Xu and Atchley, 1995). Estimating genetic variances and covariances is the first step in understanding the genetic mechanisms of quantitative traits. It is also the basis for developing efficient selection programmes, because selection acts by consuming existing genetic variances. In classical quantitative genetics, genetic variances are estimated by the phenotypic resemblance between relatives (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Parallel to estimating genetic variances in classical quantitative genetics, molecular techniques allow the further partitioning of the overall genetic variance into components corresponding to particular points or chromosomal segments of the genome (Goldgar, 1990; Schork, 1993; Kruglyak and Lander, 1995; Xu and Atchley, 1995; Xu, 1996).

QTL analysis in outbred populations is more difficult than that in inbred line crosses. The number of alleles at the segregating QTL is unknown in outbred populations. The marker linkage phases may be unknown or only partially known. The markers may be non-informative or ungenotyped. Inbreeding loops may exist in the pedigrees. All of these complications necessitate sophisticated statistical methods for QTL mapping in outbred populations. This chapter reviews such statistical methods and starts with the experimental designs for random model approaches to QTL mapping. The statistical and genetic model is then described in detail. The definition of the meiosis indicator, which is used to specify the path of gene flow in pedigrees, is defined

and the two methods of QTL mapping under random models (maximum likelihood method based on identity-by-descent-based variance component model, and Bayesian methods) are discussed. Finally, some extensions to multiple heterogeneous base populations, correlated founders and combining population association analysis with linkage analysis are outlined.

The Experimental Designs

The foundation for QTL mapping is linkage disequilibrium, as it creates marker–trait association between QTL and linked segregating marker loci (Lynch and Walsh, 1998). Therefore, the first step in QTL mapping is to produce a mapping population with linkage disequilibrium. Line crossing is a common experimental design for this purpose in crop plants and some laboratory animals. All F_1 offspring in crosses between inbred lines are genetically identical and show complete linkage disequilibrium for genes differing between lines. Using F_1 parents, a number of segregating populations, such as backcross, F_2 and more derived generations, can be generated for mapping.

Inbred lines are usually generated through many generations of selfings or matings between close relatives. As a result, it is not feasible to obtain inbred lines in natural populations, simply because of biological, temporal or economic limitations. For this reason, mapping QTL in such outbred populations should be based on existing material. However, outbred populations do not usually show strong disequilibrium; therefore, samples with linkage disequilibrium required for QTL mapping have to be selected. As with inbred lines, a variety of designs have been proposed for obtaining such samples. A common practice is to cross two outbred populations and mimic the inbred line-crossing process. Such crosses can sometimes generate the required amount of disequilibrium. This approach may be practical for plants but is not applicable for most animals, because of their low fertility. In addition, a few parents may not be a good representation of the population from which

the parents are sampled. Results obtained from these crosses may not represent the population variation but largely reflect the genetic sampling error. A reasonable design for outbred populations is to collect a number of relatives derived from many founders as the mapping population. Linkage disequilibrium is always expected in such mapping populations, even if the whole population from which the founders are sampled is in linkage equilibrium.

Any mapping population can be viewed as a *pedigree*. A pedigree is a specification of the genealogical relationships among a set of individuals. A convenient form of this specification is to identify the sire and the dam of each individual. Individuals at the top of the pedigree, whose parents are not included in the pedigree, are the *founders* of the pedigree. In contrast, a *non-founder* is defined as an individual with both parents included. Individuals without offspring are referred to as *final individuals*. Traditionally, founders are assumed to be non-inbred and unrelated. In animal populations, the simplest pedigrees may be full-sib families and half-sib families, but there are also various complex pedigrees where the founders and the offspring are mated consecutively. Clearly, the gene pool of a pedigree is wholly determined by the alleles carried by the founders, which are referred as founder alleles. Any alleles in the pedigree can be traced back to one of the founder alleles.

There are two strategies for pedigree analysis: the fixed model and the random model approaches. Which model should be chosen? It should be decided whether the founders chosen to form the pedigree are a *random sample* from the base population. If they are, the use of the random model approach should be considered, provided that there is an interest in understanding the genetic properties of the base population. If the parents of the mapping population are not randomly sampled and there is no desire to understand the base population, but only the mapping population, then the fixed model is more appropriate. Under the random model approach, the interest is in making a statistical inference about the base population. In this case, usually the interest

is in the variances of the genetic effects in the base population. Therefore, the estimated QTL variances reflect the actual genetic variation existing in the population where the experimental units are sampled. On the other hand, if there are only a few founders who are not randomly sampled from a large reference population, the interest may be only in the values of the actual allelic effects and the dominance effects for the founders in hand. The fixed model approach is very common in designed line-crossing experiments, e.g. F_2 and backcross (BC) designs, where the average effect of allelic substitution is the parameter of interest.

Similar to multiple-cross designs in inbred lines, mating designs derived from multiple heterogeneous base populations are expected to increase the statistical power of QTL mapping because the QTL responsible can be detected to either within-population or between-population genetic variations. In such mating designs, a sample is randomly selected from each heterogeneous population. The founders from each population are mated with founders from other populations with an equal chance, to form an outbred F_1 population. The matings of the F_1 are completely random so that the alleles of the multiple heterogeneous populations are well integrated into the hybrid population. The F_2 or advanced generations can be taken as the mapping populations. In this chapter, QTL mapping is first discussed in pedigrees with founders coming from one base population. Later in the chapter the methods are extended to the case where the founders are sampled from multiple heterogeneous populations.

The Genetic Model

Assume that the mapping population consists of n individuals with arbitrary pedigree relationships, and that among the n individuals there are m founders and $(n-m)$ non-founders. It is first assumed that the founders are non-inbred and are unrelated. (Cases where the founders are inbred and genetically related to each other are considered later.) Let y_i represent the

observed value of a production trait for the ith individual. When the trait is controlled by multiple genes acting independently, y_i can be described by the following linear model:

$$y_i = \mathbf{X}_i\boldsymbol{\beta} + \sum_{j=1}^{l}(u_{ji}^p + u_{ji}^m + v_{ji}) + e_i \qquad (1)$$

where $\mathbf{X}_i$ is a known design matrix for a vector of non-genetic effects $\boldsymbol{\beta}$ (including the overall mean), l is the number of QTL on all chromosomes, u_{ji}^p and u_{ji}^m are the paternal and maternal allelic effects for the ith individual at the jth QTL, $u_{ji} = u_{ji}^p + u_{ji}^m$ is then the additive effects for the ith individual at the jth QTL, v_{ji} is the dominance effect for the ith individual at the jth QTL, and e_i is the residual (environmental) effect.

Denote $\mathbf{a}_j$ as a $2m \times 1$ vector for the effects of the founder alleles (m founders each with two alleles) and $\mathbf{d}_j$ as a vector of interaction effects (dominance effects) between all possible pairs of the $2m$ founder alleles at the jth QTL. The dimension of $\mathbf{d}_j$ is $m(2m + 1)$. The dimension of $\mathbf{d}_j$ can be reduced greatly in some mating designs where it is impossible for some founder alleles to be combined in any descendant. Hereafter the dimensions of $\mathbf{a}_j$ and $\mathbf{d}_j$ are denoted as k_a and k_d, respectively. Since each allele in the descendants can be traced back to one of the founder alleles, the allelic effects, u_{ji}^p and u_{ji}^m, and the dominance effect v_{ji} for the ith individual at the jth QTL take an element of $\mathbf{a}_j$ and $\mathbf{d}_j$, respectively. Therefore, the QTL effects of all individuals can be expressed as linear functions of the allelic effects and their interactions in the founders, i.e. $u_{ji}^p = \mathbf{Z}_{ji}^p\mathbf{a}_j$, $u_{ji}^m = \mathbf{Z}_{ji}^m\mathbf{a}_j$ and $v_{ji} = \mathbf{W}_{ji}\mathbf{d}_j$, leading to:

$$y_i = \mathbf{X}_i\boldsymbol{\beta} + \sum_{j=1}^{l}(\mathbf{Z}_{ji}^p + \mathbf{Z}_{ji}^m)\mathbf{a}_j + \sum_{j=1}^{l}\mathbf{W}_{ji}\mathbf{d}_j + e_i \qquad (2)$$

where each of $\mathbf{Z}_{ji}^p$, $\mathbf{Z}_{ji}^m$ and $\mathbf{W}_{ji}$ has one element taking one and all other elements taking zero. $\mathbf{W}_{ji}$ is wholly determined by $\mathbf{Z}_{ji}^p$ and $\mathbf{Z}_{ji}^m$. For example, if the paternal allele of individual i is traced back to allele 3 of the founders and the maternal allele is traced back to allele 10 of the founders, then the third element of $\mathbf{Z}_{ji}^p$ is 1 and all other

elements are 0, and the tenth element of $\mathbf{Z}_{ji}^m$ is 1 and all other elements are 0.

In matrix notation, models (1) and (2) can be expressed as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \sum_{j=1}^{l}(\mathbf{Z}_j^p + \mathbf{Z}_j^m)\mathbf{a}_j + \sum_{j=1}^{l}\mathbf{W}_j\mathbf{d}_j + \mathbf{e}$$
$$(3)$$

In the random QTL effects model approach, the QTL allelic and dominance effects are random variables. It is usually assumed that the means of $\mathbf{a}_j$ and $\mathbf{d}_j$ are zero and the covariance matrices are $\mathbf{I}_{k_a}\sigma_{a_j}^2$ and $\mathbf{I}_{k_d}\sigma_{d_j}^2$, where $\mathbf{I}_k$ is a $k \times k$ identity matrix, and $\sigma_{a_j}^2$ and $\sigma_{d_j}^2$ are the allelic and dominance variances, respectively, at the jth QTL. Note that here it is assumed that all founders are unrelated, and thus the covariance matrices of $\mathbf{a}_j$ and $\mathbf{d}_j$ are diagonal. (The situation where the founders may be related is discussed later.) In this chapter, the vector of the non-genetic effects $\boldsymbol{\beta}$ is treated as fixed effects. The vector of the environmental effects $\mathbf{e}$ is assumed to follow an $\mathrm{N}(\mathbf{0}, \mathbf{I}_n\sigma_e^2)$ distribution.

Equation (3) appears to be a typical mixed model. However, there are two major differences between this QTL model and a conventional mixed model. Firstly, the number of QTL controlling a quantitative trait is generally unknown, resulting in an unknown dimension of the model. Secondly, the design matrices $\mathbf{Z}_j^p$, $\mathbf{Z}_j^m$ and $\mathbf{W}_j$, called the allelic inheritance matrices and the dominance design matrix, are hidden because the allelic inheritances of a QTL are not observable. The dominance design matrix $\mathbf{W}_j$ is a function of $\mathbf{Z}_j^p$ and $\mathbf{Z}_j^m$. The distributions of $\mathbf{Z}_j^p$ and $\mathbf{Z}_j^m$ depend on the QTL location, the pedigree structure and the marker information. Inferring these distributions is the key to genetic mapping in pedigrees. The next section will introduce an efficient method to derive $\mathbf{Z}_j^p$, $\mathbf{Z}_j^m$ and thus $\mathbf{W}_j$.

The primary aim of QTL mapping is to detect the number of QTL and estimate the chromosomal positions and the genetic variances of the identified QTL. Only those QTL with enough contributions to the phenotypic variation of the trait can be detected. Therefore, the effects of the

undetected QTL are absorbed into the residual error. In the random model approach, the founders of the pedigree are randomly sampled from a base population and the interest is in detecting the segregating QTL in the base population. Therefore, the parameters of interest are the allelic and dominance variances $\sigma^2_{a_j}$ and $\sigma^2_{d_j}$, while the allelic and dominance effects are treated as missing values.

Paths of Gene Flow and Meiosis Indicators

A prerequisite for statistical analysis of model (3) is to infer the allelic inheritance matrices $\mathbf{Z}^p_j$ and $\mathbf{Z}^m_j$ ($j = 1, 2, \ldots, l$). The allelic inheritance matrices are determined by the unobserved paths of gene flows. Therefore, it is necessary to specify the

origin of each allele in the pedigree and identity-by-descent (IBD) patterns between any two alleles. Figure 25.1 shows a simple pedigree with four founders and six non-founders. The ordered genotype (paternal followed by maternal) is assumed to be known and is given under each member of the pedigree (Fig. 25.1a). This pedigree can be redrawn by showing the allelic form of the founders and the paths of gene flows (Fig. 25.1b), called the descent graph (Sobel and Lange, 1996). The descent graph is a graphical representation of the allelic inheritance. In Fig. 25.1b, the pedigree is ordered in such a way that every individual is preceded in the listing by its parents. From the descent graph, the allelic inheritance matrices for the locus under consideration can easily be constructed. For example, the paternal allele and maternal allele for the ninth individual come from the second and
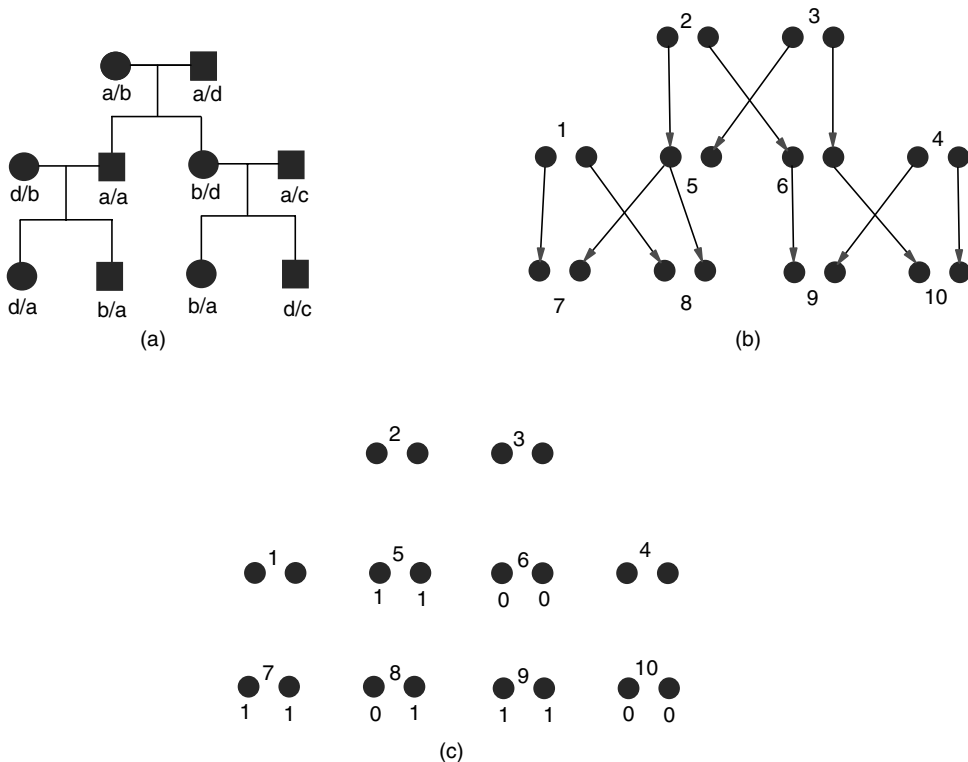
Fig. 25.1. A simple pedigree with four founders and six non-founders: (a) conventional representation; (b) genetic descent graph; (c) meiosis indicators.

the third founder alleles, respectively. Therefore, the second element of $\mathbf{Z}_{j8}^p$ is 1 and all other elements are 0; and the third element of $\mathbf{Z}_{j8}^m$ is 1 and all other elements are 0. Clearly, the two alleles carried by this individual are not IBD because they are not copies of the same founder gene.

The patterns of gene flow or the descent graphs are determined by the Mendelian segregation events in the pedigree. The segregation of genes can be fully specified by meiosis indicators, or called segregation indicators (Thompson, 1994, 1996). The meiosis indicator is denoted by s_{ji}^p for the paternal allele and s_{ji}^m for the maternal allele, respectively, for the ith individual at the jth locus. These indicator variables are defined as $s_{ji}^p = 1$ if the paternal allele of individual j inherits the paternal allele of its father, and $s_{ji}^p = 0$ otherwise. Similarly, $s_{ji}^m = 1$ if the maternal allele of individual j inherits the paternal allele of its mother, and $s_{ji}^m = 0$ otherwise. The meiosis indicators of all non-founders determine the pattern of gene flow in a pedigree. Figure 25.1c gives an example, where meiosis indicators are shown under each non-founder (paternal followed by maternal). It can be seen that Figs 25.1b and 25.1c are completely equivalent in the context of genetics.

For complicated mating designs, it is not convenient to infer the allelic inheritance matrices $\mathbf{Z}_j^p$ and $\mathbf{Z}_j^m$ ($j = 1, 2, \ldots, l$) directly. Here, the meiosis indicators are used to derive them indirectly by a recursive process, as follows. Consider a pedigree with m founders and the $2m$ founder alleles are labelled from 1 to $2m$. Note that alleles $2k - 1$ and $2k$ are the two alleles of the kth founder. Assume that matrices $\mathbf{Z}_j^p$ and $\mathbf{Z}_j^m$ have already been built up to the first $(i - 1)$th rows and the ith row is ready to be built. If the ith individual is a founder, say the kth founder, then the $(2k - 1)$th element of $\mathbf{Z}_{ji}^p$ and the $(2k)$th element of $\mathbf{Z}_{ji}^m$ are 1. If the ith individual is not a founder but the progeny of individuals i_f (father) and i_m (mother), then:

$$\mathbf{Z}_{ji}^p = s_{ji}^p \mathbf{Z}_{ji_f}^p + (1 - s_{ji}^p)\mathbf{Z}_{ji_f}^m, \text{ and}$$
$$\mathbf{Z}_{ji}^m = s_{ji}^m \mathbf{Z}_{ji_m}^m + (1 - s_{ji}^m)\mathbf{Z}_{ji_m}^m \qquad (4)$$

where $\mathbf{Z}_{ji_f}^p$, $\mathbf{Z}_{ji_f}^m$, $\mathbf{Z}_{ji_m}^p$ and $\mathbf{Z}_{ji_m}^m$ have been previously built because $i_1 \leq i - 1$ and $i_2 \leq i - 1$.

The definition of meiosis indicator is suitable for both marker loci and QTL. The space of the meiosis indicators at a marker locus is constrained by the observed marker genotypes, and the probability distribution depends on the marker information and the pedigree structure. The probability distribution of the meiosis indicators for QTL can be derived from the linked markers and the observed phenotypes. The algorithms for sampling meiosis indicators for markers and QTL are discussed later. Here, two basic properties for meiosis indicators, which are useful in developing the algorithms, are mentioned as follows. The meiosis indicators for all individuals at the same locus are *a priori* independent, and $p(s_{ji}^p = 0) = p(s_{ji}^p = 1) = p(s_{ji}^m = 0) = p(s_{ji}^m = 1) = 1/2$. From the genetic linkage law, the indicators at loci on the same chromosome are dependent. The pattern of dependence depends on the process of meiosis. However, under assumptions of absence of genetic interference in meiosis, the meiosis indicators of an individual at loci on the same chromosome form a first-order Markov process. Therefore, the indicator at a locus depends only on that of the flanking loci.

Maximum Likelihood Methods Under the Variance Component Models

The variance component methods have been widely used in quantitative genetics. The variance component approach to linkage analysis was originally developed by Haseman and Elston (1972) via single marker sib-pair analysis and extended by Fulker and Cardon (1994) for interval mapping and Fulker *et al.* (1995) for multiple point mapping. A maximum likelihood approach of the IBD-based variance component analysis was introduced by Goldgar (1990) and further investigated by others (e.g. Schork, 1993; Amos, 1994; Fulker and Cardon, 1994; Xu and Atchley, 1995). This approach has been extended to pedigrees of

arbitrary complexity (Almasy and Blangero, 1998; George *et al.*, 2001). The IBD-based variance component analysis has been recognized as a very powerful statistical method for QTL mapping in outbred populations, such as humans and animals. With this method all possible biological relationships are simultaneously used to dissect the genetic architecture of a quantitative trait. The method requires fewer assumptions than other methods with regard to the genetic model underlying the expression of the trait in question. For instance, knowledge of the actual genetic mechanism of the trait, such as the number of alleles per locus and the allelic frequencies, is not required (Goldgar, 1990; Schork, 1993; Amos, 1994; Fulker and Cardon, 1994; Xu and Atchley, 1995; Almasy and Blangero, 1998). Conventionally, the method decomposes the overall genetic variance into several variance components, one being due to the segregation of a putative QTL at the position being tested and the other due to the effect of a polygenic term (the collective effects of all other quantitative loci affecting the trait). The key to separating the contribution of a putative QTL from that of the polygene is the differentiated proportion of alleles IBD shared by relatives at the QTL and the polygene. The IBD proportion varies from one locus to another, which provides the capability of separating individual QTL and locating QTL on the chromosome.

Basic principles of the variance component method

Given the number of QTL, the allelic inheritance and dominance design matrices, model (3) is a standard mixed model. As in the customary mixed model, the random effects represented by $\mathbf{a}_j$, $\mathbf{d}_j$ and $\mathbf{e}$ ($j = 1, 2, \ldots, l$) have the following properties:

$$E(\mathbf{a}_j) = E(\mathbf{d}_j) = \mathbf{0},$$

$$Cov(\mathbf{a}_j, \mathbf{a}_{j'}) = \begin{cases} \mathbf{I}_{k_a}\sigma^2_{a_j} & \text{if } j = j' \\ 0 & \text{if } j \neq j' \end{cases},$$

$$Cov(\mathbf{d}_j, \mathbf{d}_{j'}) = \begin{cases} \mathbf{I}_{k_d}\sigma^2_{d_j} & \text{if } j = j' \\ 0 & \text{if } j \neq j' \end{cases},$$

$$Cov(\mathbf{a}_j, \mathbf{d}_{j'}) = 0, E(\mathbf{e}) = 0, Var(\mathbf{e}) = \mathbf{I}_n\sigma^2_e,$$
and
$$Cov(\mathbf{a}_j, \mathbf{e}) = Cov(\mathbf{d}_j, \mathbf{e}) = 0 \qquad (5)$$

Using these assumptions, model (3) leads to:

$$E(\mathbf{y}) = \mathbf{X}\boldsymbol{\beta} \qquad (6)$$

and

$$Var(\mathbf{y}) = \mathbf{V} = \sum_{j=1}^{l} \boldsymbol{\Pi}_j \sigma^2_{a_j} + \sum_{j=1}^{l} \boldsymbol{\Delta}_j \sigma^2_{a_j} + \mathbf{I}_n \sigma^2_e \qquad (7)$$

where $\boldsymbol{\Pi}_j = (\mathbf{Z}^p_j + \mathbf{Z}^m_j)(\mathbf{Z}^p_j + \mathbf{Z}^m_j)^T$, $\boldsymbol{\Delta}_j = \mathbf{W}_j\mathbf{W}_j^T$, and the superscript T stands for matrix transposition.

The iith elements, denoted by $\pi_{j,ii}$, and $\theta_{j,ii}$, in $\boldsymbol{\Pi}_j$ and $\boldsymbol{\Delta}_j$ can be expressed as:

$$\pi_{j,ii'} = (\mathbf{Z}^p_{ji} + \mathbf{Z}^m_{ji})(\mathbf{Z}^p_{ji'} + \mathbf{Z}^p_{ji'})^T$$
$$= \begin{cases} 2 + 2\mathbf{Z}^p_{ji}\mathbf{Z}^{m\,T}_{ji} & \text{for } i = i' \\ \mathbf{Z}^p_{ji}\mathbf{Z}^{p\,T}_{ji'} + \mathbf{Z}^m_{ji}\mathbf{Z}^{m\,T}_{ji'} + \mathbf{Z}^p_{ji}\mathbf{Z}^{m\,T}_{ji'} + \\ \quad \mathbf{Z}^m_{ji}\mathbf{Z}^{p\,T}_{ji'} & \text{for } i \neq i' \end{cases} \qquad (8)$$

and

$$\theta_{j,ii'} = \begin{cases} 1 & \text{for } i = i' \\ \mathbf{W}_{ji}\mathbf{W}^T_{ji'}, & \text{for } i \neq i' \end{cases} \qquad (9)$$

respectively. According to the definitions of vectors $\mathbf{Z}_{ji}$, the product term $\mathbf{Z}^p_{ji}\mathbf{Z}^{m\,T}_{ji}$ takes 1 or 0, depending on whether the two alleles at the jth locus carried by the ith individual are IBD ($\mathbf{Z}^p_{ji} = \mathbf{Z}^m_{ji}$) or not ($\mathbf{Z}^p_{ji} \neq \mathbf{Z}^m_{ji}$). Therefore, the expectation of $\mathbf{Z}^p_{ji}\mathbf{Z}^{m\,T}_{ji}$, i.e. $E(\mathbf{Z}^p_{ji}\mathbf{Z}^{m\,T}_{ji})$, is the inbreeding coefficient for individual i at the jth QTL. For different individuals, $\mathbf{Z}^p_{ji}\mathbf{Z}^{p\,T}_{ji'}$, $\mathbf{Z}^m_{ji}\mathbf{Z}^{m\,T}_{ji'}$, $\mathbf{Z}^p_{ji}\mathbf{Z}^{m\,T}_{ji'}$ and $\mathbf{Z}^m_{ji}\mathbf{Z}^{p\,T}_{ji'}$ also take 1 or 0, indicating whether the two paternal genes are IBD, the two maternal genes are IBD, the paternal gene of individual i and the maternal gene individual i' are IBD, or the maternal gene of individual i and the paternal gene individual i' are IBD, respectively. Similarly, $\mathbf{W}_{ji}\mathbf{W}^T_{ji'}$ takes one of the two values 1 and 0. If individuals i and i' share two IBD alleles ($\mathbf{W}_{ji} = \mathbf{W}_{ji'}$), then $\mathbf{W}_{ji}\mathbf{W}^T_{ji'} = 1$; otherwise, $\mathbf{W}_{ji}\mathbf{W}^T_{ji'} = 0$.

The maximum likelihood method requires that the number of QTL and the IBD

matrices are known, *a priori*. In fact, one of the main purposes of QTL mapping is to find the number of QTL. In addition, it may be difficult for the maximum likelihood method to handle too many unknown parameters. The usual treatment is to decompose the total genetic effect into the effect of a single QTL and the sum of the effects of the remaining QTL. The model is written as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + (\mathbf{Z}_j^p + \mathbf{Z}_j^m)\mathbf{a}_j + \mathbf{W}_j\mathbf{d}_j + \mathbf{A} + \mathbf{D} + \mathbf{e} \tag{10}$$

where $\mathbf{A} = \sum_{j' \neq j}^{l} (\mathbf{Z}_{j'}^p + \mathbf{Z}_{j'}^m)\mathbf{a}_{j'}$ is the sum of the additive effects of the remaining QTL (additive polygenic effect) and $\mathbf{D} = \sum_{j' \neq j}^{l} \mathbf{W}_{j'}\mathbf{d}_{j'}$ is the sum of the dominance effects of the remaining QTL (dominance polygenic effect). Accordingly, the total additive variance is partitioned into variance explained by the jth QTL and the variance contributed collectively by all other loci. The latter is called the additive polygenic variance and denoted by $\sigma_A^2 = \sum_{j' \neq j}^{l} \sigma_{a_{j'}}^2$. Similarly, the dominance variance can be partitioned into $\sigma_{d_j}^2$ and $\sigma_D^2 = \sum_{j' \neq j}^{l} \sigma_{d_{j'}}^2$. In the ML method, both the marker information and the pedigree structure are used to infer matrices $\mathbf{Z}_j^p$, $\mathbf{Z}_j^m$ and $\mathbf{W}_j$, but use only the pedigree structure to infer $\mathbf{Z}_{j'}^p$, $\mathbf{Z}_{j'}^m$ and $\mathbf{W}_{j'}$ ($j' \neq j$). It has been noticed that the distributions of allelic inheritance and dominance design matrices are the same for any pair of loci if marker information is not considered. Because of this, the genetic variances at a specific locus and the polygenic variances can be separated.

Under model (10), the expectation of the phenotypic values $\mathbf{y}$ is the same as that in equation (6), but the variance–covariance matrix of $\mathbf{y}$ becomes:

$$\text{Var}(\mathbf{y}) = \mathbf{V} = \boldsymbol{\Pi}_j\sigma_{a_j}^2 + \boldsymbol{\Delta}_j\sigma_{d_j}^2 + \boldsymbol{\Pi}\sigma_A^2 + \boldsymbol{\Delta}\sigma_D^2 + \mathbf{I}\sigma_e^2 \tag{11}$$

where $\boldsymbol{\Pi}_j$ and $\boldsymbol{\Delta}_j$ are the IBD matrices for the jth QTL; $\boldsymbol{\Pi}$ and $\boldsymbol{\Delta}$ are the IBD matrices for the polygenic components, which depend only on the pedigree structure. With this

model, the list of unknown parameters of interest becomes $\boldsymbol{\theta} = \{\boldsymbol{\beta}, \sigma_e^2, \sigma_{a_j}^2, \sigma_{d_j}^2, \sigma_A^2, \sigma_D^2\}$.

As previously explained, matrices $\boldsymbol{\Pi}_j$ and $\boldsymbol{\Delta}_j$ represent the patterns of gene IBD of the pedigree, and are thus called IBD matrices. Without marker information, the probability distributions for the IBD values at any QTL are completely determined by pedigree relationships. In this situation, the genetic variances of different QTL cannot be separated. In fact, the corresponding IBD measurements at the locus level depend not only on the pedigree relationships but also on the actual genotype of each individual at the locus of interest. Conditional on pedigrees and marker information, these locus-specific IBDs are still variables. For example, consider the genotypic configurations of progenies from mating type $A_1A_2 \times A_3A_4$. There are four possible types of progeny, each with an equal frequency. The four possible genotypes are A_1A_3, A_1A_4, A_2A_3 and A_2A_4. If two sibs are sampled from this family, ignoring the order of sampling, there are ten possible sib-pairs. Suppose a pair of sibs is observed with genotypic configuration A_1A_3-A_1A_3, it is known immediately that they have received exactly the same alleles from their parents and thus $\pi_{j,ii'} = 1$. The two sibs behave like identical twins for this locus. If A_1A_3-A_2A_4 is observed, it is known that they do not share any IBD alleles, and thus behave like two unrelated individuals. The variation of the IBD values across loci provides a theoretical basis to distinguish different QTL and thus the key to the variance component method of QTL mapping.

Likelihood function and likelihood ratio test statistic

Given the fact that the IBD matrices are random variables, the full likelihood function should take into consideration the joint distribution of the IBD matrices. The full likelihood function for model (10) is expressed as:

$$L(\boldsymbol{\theta}|\mathbf{y}) = \sum_{\boldsymbol{\Pi}_j, \boldsymbol{\Delta}_j} L(\boldsymbol{\theta}|\mathbf{y}, \boldsymbol{\Pi}_j, \boldsymbol{\Delta}_j, \boldsymbol{\Pi}, \boldsymbol{\Delta})p(\boldsymbol{\Pi}_j, \boldsymbol{\Delta}_j)$$

$$= \sum_{\mathbf{z}_j^p, \mathbf{z}_j^m} L(\boldsymbol{\theta}|\mathbf{y}, \Pi_j(\mathbf{Z}_j^p, \mathbf{Z}_j^m), \Delta_j(\mathbf{W}_j), \Pi, \Delta)$$
$$p(\mathbf{Z}_j^p, \mathbf{Z}_j^m)$$

$$= \sum_{\mathbf{S}_{j\cdot}} L(\boldsymbol{\theta}|\mathbf{y}, \Pi_j(\mathbf{S}_{j\cdot}), \Delta_j(\mathbf{S}_{j\cdot}), \Pi, \Delta) p(\mathbf{S}_{j\cdot})$$

$$(12)$$

where $\boldsymbol{\theta} = \{\boldsymbol{\beta}, \sigma_e^2, \sigma_{a_j}^2, \sigma_{d_j}^2, \sigma_A^2, \sigma_D^2\}$ represents the parameters of interest given the number of QTL (the locations of QTL are not listed here) and $\mathbf{S}_{j\cdot} = \{s_{ji}^p, s_{ji}^m\}_{i=m+1}^n$ denotes the meiosis indicators for all non-founders at the jth QTL. The second and third alternative expressions of the above equation are due to the fact that the IBD matrices can be expressed as functions of the allelic inheritance matrices and functions of the meiosis indicators. Under the assumption that $\mathbf{y}$ is multivariate normal, the conditional likelihood function has the following form (see equation 13 at bottom of page),

where $\mathbf{V}$ is given in equation (11). $L(\boldsymbol{\theta}|\mathbf{y}, \Pi_j$ $(\mathbf{Z}_j^p, \mathbf{Z}_j^m), \Delta_j(\mathbf{W}_j), \Pi, \Delta)$ and $L(\boldsymbol{\theta}|\mathbf{y}, \Pi_j(\mathbf{S}_{j\cdot}), \Delta_j$ $(\mathbf{S}_{j\cdot}), \Pi, \Delta)$ have the same form as $L(\boldsymbol{\theta}|\mathbf{y}, \Pi_j, \Delta_j, \Pi, \Delta)$.

Algorithms that are based on the full likelihood functions, i.e. equation (12), are referred to as the 'distribution methods'. The distribution methods involve the summations over the spaces of the corresponding variables. The summations will quickly become computationally prohibitive as the size of pedigree increases. Therefore, the full likelihood methods are impractical for animal populations where the sizes are usually large. The usual practice is to construct the likelihood function with $\{\Pi_j\}$ and $\{\Delta_j\}$ replaced by their expectations $\{E(\Pi_j)\}$ and $\{E(\Delta_j)\}$. Therefore, the approximate likelihood function is expressed as (see equation 14 at bottom of page), where $E(\mathbf{V}) = E(\Pi_j)\sigma_{a_j}^2 + E(\Delta_j)\sigma_{d_j}^2 + \Pi\sigma_A^2 + \Delta\sigma_D^2 + \mathbf{I}_n\sigma_e^2$. The method is referred to as

the 'expectation method'. Statistically, the expectation method is approximate but has been discovered to lose little in statistical power (Fulker and Cherny, 1996; Gessler and Xu, 1996). In addition, the expectation method is computationally much more efficient than the distribution method (Xu, 1996). Because of this, only the expectation method is discussed in this section. The expectations of the IBD matrices rely on the locations of QTL, marker information and pedigree relationships. The calculation of the expectations will be discussed in the next subsection.

In the variance component approach of QTL mapping, the parameters of interest are $\sigma_{a_j}^2$ and $\sigma_{d_j}^2$; the remaining elements in $\boldsymbol{\theta}$ are called nuisance parameters. For each chromosomal position, parameters can be estimated using ML methods. There are a variety of algorithms available for solving ML estimations of variance component models (Searle *et al.*, 1992).

The two parameters of interest can be tested for significance by fitting the model with all parameters and obtaining the natural log of the likelihood of the data, $\ln(L_1)$, and refitting without those two parameters, obtaining $\ln(L_0)$, the log of the likelihood under the null hypothesis. The test statistic,

$$\lambda = -2[\ln(L_0) - \ln(L_1)] \qquad (15)$$

is asymptotically distributed as a χ^2 with degrees of freedom equal to the number of parameters tested. If there is a QTL at a particular chromosome position, the IBD matrices at that position will match Π_j and Δ_j and the test statistic will show a peak at that position. The chromosome position is allowed to vary so that the whole chromosome is searched from one end to the other for evidence of QTL.

$$L(\boldsymbol{\theta}|\mathbf{y}, \Pi_j, \Delta_j, \Pi, \Delta) = \frac{1}{(2\pi)^{n/2}|\mathbf{V}|^{1/2}} \exp(-\tfrac{1}{2}(\mathbf{y} - \mathbf{X}\boldsymbol{\beta})^T \mathbf{V}^{-1}(\mathbf{y} - \mathbf{X}\boldsymbol{\beta})) \qquad (13)$$

$$L(\boldsymbol{\theta}|\mathbf{y}, E(\Pi_j), E(\Delta_j), \Pi, \Delta) = \frac{1}{(2\pi)^{n/2}|E(\mathbf{V})|^{1/2}} \exp(-\tfrac{1}{2}(\mathbf{y} - \mathbf{X}\boldsymbol{\beta})^T E(\mathbf{V})^{-1}(\mathbf{y} - \mathbf{X}\boldsymbol{\beta})) \qquad (14)$$

Inference of the IBD matrices

The IBD matrices of a QTL cannot be observed and must be inferred from markers linked to the putative QTL. There will be some uncertainty associated with the statistical inference of IBD. What can be obtained is the conditional distributions of the IBD variables, given marker information. When QTL mapping is conducted, the conditional expectations of the IBD variables are used in place of the true IBDs. In outbred populations considered here, marker information is often incomplete. Unknown linkage phases, non-informative markers and missing marker genotypes complicate the calculation of IBD matrices. Several methods for inferring IBD matrices from observed marker data have been developed. The methods suitable for simple pedigrees such as full-sib families or half-sib families will be discussed first and then the methods for complex pedigrees will be outlined.

Inferring IBD matrices in full-sib families

In outbred populations, markers may be partially informative. In such situations, two flanking markers may not extract the maximum amount of information about the segregation of a putative QTL. Because of this, markers outside the interval can provide additional information. In these situations, a desirable procedure is to use all markers simultaneously, a procedure called multipoint mapping (Fulker et al., 1995; Kruglyak and Lander, 1995). A number of multipoint methods have been proposed for calculating the distributions of IBD in full-sib families (e.g. Fulker et al., 1995; Kruglyak and Lander, 1995; Olson, 1995; Xu and Gessler, 1998). Kruglyak and Lander (1995) developed a multipoint method using a hidden Markov model approach. Their method uses all markers to predict the conditional distribution of the IBD value of a QTL. Although it may be the most efficient method with regard to extracting the maximum amount of information from markers, its computational load scales exponentially with family size and for practical purposes this limits the algorithm to

situations with fewer than ten sibs. Fulker et al. (1995), Olson (1995) and Xu and Gessler (1998) also espoused a multipoint approach, although they used the estimated IBD values of all markers to infer the expectation of the IBD at each putative QTL. The multipoint method of Fulker et al. (1995) takes a multiple regression approach to estimate the IBD value of a putative QTL from the IBD values of the markers, which is an extension of the interval method of Fulker and Cardon (1994). Instead of deriving the conditional distributions of IBD matrices of a putative QTL, the methods of Olson (1995) and Xu and Gessler (1998) both employ the hidden Markov model to estimate the IBD states of a putative QTL, using the probability distributions of IBD values at marker loci. These methods can handle an arbitrary number of sibs and are computationally much faster than the multipoint method of Kruglyak and Lander (1995), though they are slightly less efficient. In this chapter, the method of Xu and Gessler (1998) is discussed in detail.

Consider a full-sib family where the parents are non-inbred and unrelated. For sibs i and i', the IBD values at the jth QTL can be expressed as:

$$\pi_{j,ii'} = [s_{ji}^p s_{ji'}^p + (1-s_{ji}^p)(1-s_{ji'}^p)] + [s_{ji}^m s_{ji'}^m + (1-s_{ji}^m)(1-s_{ji'}^m)]$$
$$= \tfrac{1}{2}(\pi_{j,ii'}^p + \pi_{j,ii'}^m) \qquad (16)$$

and

$$\delta_{j,ii'} = [s_{ji}^p s_{ji'}^p + (1-s_{ji}^p)(1-s_{ji'}^p)]$$
$$[s_{ji}^m s_{ji'}^m + (1-s_{ji}^m)(1-s_{ji'}^m)]$$
$$= \pi_{j,ii'}^p \pi_{j,ii'}^m \qquad (17)$$

where s_{ji}^p and s_{ji}^m ($s_{ji'}^p$ and $s_{ji'}^m$) are meiosis indicators as defined above $\pi_{j,ii'}^p = s_{ji}^p s_{ji'}^p + (1-s_{ji}^p)(1-s_{ji'}^p)$ takes 1 or 0, representing whether the paternal alleles of the sibs i and i' are IBD or not. The definition of $\pi_{j,ii'}^m$ is similar to that of $\pi_{j,ii'}^p$.

Assuming that there are K ordered markers on the chromosome of interest, the IBD values of these markers are denoted by $\pi_{k,ii'}$ and $\delta_{k,ii'}$ ($k = 1, 2, \ldots, K$). It is assumed that the QTL is located between markers k and $k + 1$ for $K - 1 \geq k \geq 1$. It has been mentioned that,

under the assumptions of absence of genetic interference in meiosis, the sequence of the meiosis indicators on the same chromosome form a first-order Markov chain. Therefore, the sequences $\{\pi_{1,ii'}^{p} \ldots \pi_{k,ii'}^{p}, \pi_{j,ii'}^{p}, \pi_{k+1,ii'}^{p} \ldots \pi_{K,ii'}^{p}\}$, $\{\pi_{1,ii'}^{m} \ldots \pi_{k,ii'}^{m} \pi_{j,ii'}^{m} \pi_{k+1,ii'}^{m} \cdots \pi_{K,ii'}^{m}\}$ and $\{\pi_{1,ii'} \ldots \pi_{k,ii'} \pi_{j,ii'} \pi_{k+1,ii'} \cdots \pi_{K,ii'}\}$ are also first-order Markov chains. The IBD values can be estimated by inferring the meiosis indicators s_{ji}^{p} and s_{ji}^{m} ($s_{ji'}^{p}$ and $s_{ji'}^{m}$), or $\pi_{j,ii'}^{p}$ and $\pi_{j,ii'}^{m}$, or directly $\pi_{j,ii'}$. Xu and Gessler (1998) developed a method to infer the distribution of $\pi_{j,ii'}^{p}$ or $\pi_{j,ii'}^{m}$. Their method can be easily modified to $\pi_{j,ii'}$ and $\delta_{j,ii'}$ as follows.

Note that $\pi_{j,ii'}$ takes one of the four states $\{0+0, 0+1, 1+0, 1+1\}$. The four states represent that sibs i and i' share no alleles, share the paternal but not the maternal alleles, share the maternal but not the paternal alleles, or share both alleles IBD, respectively. Similarly, $\delta_{j,ii'}$ takes one of the two values $\{1, 0\}$. If individuals i and i' share both alleles IBD, $\delta_{j,ii'} = 1$, otherwise $\delta_{j,ii'} = 0$. The transition probability matrix between $\pi_{k,ii'}$ and $\pi_{l,ii'}$ is:

$$T_{kl} = \begin{bmatrix} \Psi^2 & \Psi(1-\Psi) & (1-\Psi)\Psi & (1-\Psi)^2 \\ \Psi(1-\Psi) & \Psi^2 & (1-\Psi)^2 & (1-\Psi)\Psi \\ (1-\Psi)\Psi & (1-\Psi)^2 & \Psi^2 & \Psi(1-\Psi) \\ (1-\Psi)^2 & (1-\Psi)\Psi & \Psi(1-\Psi) & \Psi^2 \end{bmatrix} \tag{18}$$

where $\Psi = r_{kl}^2 + (1 - r_{kl})^2$ and r_{kl} is the recombination fraction between loci k and l.

The conditional probability of IBD state of QTL given the observed marker information $\mathbf{M}$ is:

$$\Pr(\pi_{j,ii'}|\mathbf{M}) = \frac{\Pr(\pi_{j,ii'})\Pr(\mathbf{M}|\pi_{j,ii'})}{\sum_{\pi_{j,ii'}} \Pr(\pi_{j,ii'})\Pr(\mathbf{M}|\pi_{j,ii'})} \tag{19}$$

where $\Pr(\pi_{ii'})$ is the prior distribution of the IBD state. The prior probability is the unconditional distribution, which equals $1/4$ for each of the four possible IBD states.

To calculate $\Pr(\pi_{j,ii'}|\mathbf{M})$, it is necessary to derive the conditional probability $\Pr(\mathbf{M}|\pi_{j,ii'})$. Let $\Pr(\pi_{k,ii'}|\mathbf{M})$ be the conditional distribution for the kth marker given the observed marker genotypes $\mathbf{M}$. Then the conditional probability $\Pr(\mathbf{M}|\pi_{j,ii'})$ can be expressed as:

$$\Pr(\mathbf{M}|\pi_{j,ii'}) = \sum_{\pi_{1,ii'}} \ldots \sum_{\pi_{k,ii'}} \Pr(\pi_{1,ii'}|\mathbf{M}) \ldots$$
$$\Pr(\pi_{K,ii'}|\mathbf{M})\Pr(\pi_{1,ii'}, \ldots, \pi_{K,ii'}|\pi_{j,ii'}) \tag{20}$$

Since the sequence $\{\pi_{1,ii'} \ldots \pi_{k,ii'} \pi_{j,ii'} \pi_{k+1,ii'} \ldots \pi_{K,ii'}\}$ is a Markov chain, the conditional probability $\Pr(\pi_{1,ii'}, \ldots, \pi_{K,ii'}|\pi_{j,ii'})$ can be written as:

$$\Pr(\pi_{j,ii'}, \ldots, \pi_{K,ii'}|\pi_{j,ii'}) = \Pr(\pi_{1,ii'}|\pi_{2,ii'})$$
$$\ldots \Pr(\pi_{k,ii'}|\pi_{k-1,ii'})\Pr(\pi_{j,ii'}|\pi_{k,ii'}) \times$$
$$\Pr(\pi_{k+1,ii'}|\pi_{j,ii'}) \ldots \Pr(\pi_{K,ii'}|\pi_{K-1,ii'}) \tag{21}$$

Substituting equations (21) and (20) into equation (19) and using matrix notation gives (see equation 22 at bottom of page),

where $\mathbf{1} = (1 \quad 1 \quad 1 \quad 1)^{\mathrm{T}}$, $\mathbf{D}_k = \mathrm{diag}\{\Pr(\pi_{k,ii'} = 0+0) \quad \Pr(\pi_{k,ii'} = 0+1) \quad \Pr(\pi_{k,ii'} = 1+0) \quad \Pr(\pi_{k,ii'} = 1+1)\}$, $k = 1, \ldots, M$, $\mathbf{T}_{kl}$ is the transition matrix between $\pi_{ii'}^{k}$ and $\pi_{ii'}^{l}$, and:

$$\mathbf{D} = \begin{cases} \mathrm{diag}(1\,0\,0\,0) \text{ for } \pi_{j,ii'} = 0+0 \\ \mathrm{diag}(0\,1\,0\,0) \text{ for } \pi_{j,ii'} = 0+1 \\ \mathrm{diag}(0\,0\,1\,0) \text{ for } \pi_{j,ii'} = 1+0 \\ \mathrm{diag}(0\,0\,0\,1) \text{ for } \pi_{j,ii'} = 1+1 \end{cases}$$

Since $\delta_{j,ii'} = 1$ if $\pi_{j,ii'} = 1$, and $\delta_{j,ii'} = 0$ otherwise, at the time when the probability distribution of $\pi_{j,ii'}$ is calculated, that of $\delta_{j,ii'}$ is also generated as a by-product. The conditional expectations of $\pi_{j,ii'}$ and $\delta_{j,ii'}$ are calculated as:

$$E(\pi_{j,ii'}|\mathbf{M}) = \Pr(\pi_{j,ii'} = 1+1|\mathbf{M}) +$$
$$\tfrac{1}{2}[\Pr(\pi_{j,ii'} = 0+1|\mathbf{M}) +$$
$$\Pr(\pi_{j,ii'} = 1+0|\mathbf{M})] \tag{23}$$

and

$$E(\delta_{j,ii'}|\mathbf{M}) = \Pr(\pi_{j,ii'} = 1+1|\mathbf{M}) \tag{24}$$

The additional work left to the practitioners is to calculate the probabilities of IBD states of an individual marker given the observed genotypes of this marker, i.e. $\Pr(\pi_{k,ii'}|\mathbf{M})$. If a

$$\Pr(\pi_{j,ii'}|\mathbf{M}) = \frac{\mathbf{1}^{\mathrm{T}}\mathbf{D}_1\mathbf{T}_{12}\mathbf{D}_2 \ldots \mathbf{D}_k\mathbf{T}_{kj}\mathbf{D}\mathbf{T}_{j(k+1)}\mathbf{D}_{k+1} \ldots \mathbf{D}_{K-1}\mathbf{T}_{(K-1)K}\mathbf{D}_K\mathbf{1}}{\sum_{\pi_{j,ii'}} \mathbf{1}^{\mathrm{T}}\mathbf{D}_1\mathbf{T}_{12}\mathbf{D}_2 \ldots \mathbf{D}_k\mathbf{T}_{kj}\mathbf{D}\mathbf{T}_{j(k+1)}\mathbf{D}_{k+1} \ldots \mathbf{D}_{K-1}\mathbf{T}_{(K-1)K}\mathbf{D}_K\mathbf{1}} \tag{22}$$

marker is fully informative, the IBD states of each marker shared by sibs are observed (known). Otherwise, the probabilities of IBD states can be inferred based on the observed marker genotypes. Computing marker IBD probabilities is an easy task in full-sib families.

Inferring IBD matrices in complex pedigrees

For complex pedigrees, the calculation of IBD matrices is complicated by the fact that the pattern of gene flow at a putative QTL or a partially informative marker is difficult to derive. The genotypes, and thus the IBD matrices at a putative QTL, are not observed and must be inferred from linked marker loci. Given the availability of full marker information, the expectation of IBD matrix Π_j can be estimated by using recursive algorithms (e.g. Fernando and Grossman, 1989; Van Arendonk *et al.*, 1994; Wang *et al.*, 1995) or correlation-based algorithms (e.g. Amos, 1994; Almasy and Blangero, 1998). These methods also provide a basis for the more real situations where marker information is incomplete.

The recursive algorithms were previously developed to calculate QTL's gametic IBD matrix using a single fully informative marker linked to the QTL (Fernando and Grossman, 1989; Van Arendonk *et al.*, 1994; Wang *et al.*, 1995), which is similar to the method used to construct the numerator relationship matrix (e.g. Henderson, 1976).

Extensions to multiple fully genotyped markers were made by Grignola *et al.* (1996). Given the QTL's gametic IBD matrix, matrix Π_j can be obtained since a simple linear relationship exits between the gametic relationship matrix and the IBD matrix Π_j (Wang *et al.*, 1998). Alternatively, the IBD matrix Π_j can also be constructed recursively as described below.

The recursive algorithms are based on the recursive equation (4). Assume that the matrix $\Pi_j = (\mathbf{Z}_j^p + \mathbf{Z}_j^m)(\mathbf{Z}_j^p + \mathbf{Z}_j^m)^T$ has already been built up to the first $(i-1)$th rows and columns, giving $E(\mathbf{Z}_{ji_1}^p \mathbf{Z}_{ji_2}^{p\ T})$, $E(\mathbf{Z}_{ji_1}^p \mathbf{Z}_{ji_2}^{m\ T})$, $E(\mathbf{Z}_{ji_1}^m \mathbf{Z}_{ji_2}^{p\ T})$, and $E(\mathbf{Z}_{ji_1}^m \mathbf{Z}_{ji_2}^{m\ T})$, $i_1 = 1, \ldots, i-1$; $i_2 = 1, \ldots, i-1$. Next to build the ith row and column it is necessary to calculate $E(\mathbf{Z}_{ji}^p + \mathbf{Z}_{ji}^m)(\mathbf{Z}_{ji}^p + \mathbf{Z}_{ji}^m)^T$ and $E(\mathbf{Z}_{ji'}^p + \mathbf{Z}_{ji'}^m)(\mathbf{Z}_{ji}^p + \mathbf{Z}_{ji}^m)^T$, $i' = 1, \ldots, i-1$. The father and the mother of individual i are donated as i_f and i_m, respectively. Then, according to the recursive equation (4) (see equation 25 at bottom of page).

Since $i_f \le i-1$, $i_m \le i-1$ and $i' \le i-1$, the terms $E(\mathbf{Z}_{ji_f}^p \mathbf{Z}_{ji_m}^{p\ T})$, $E(\mathbf{Z}_{ji'}^p + \mathbf{Z}_{ji'}^m)(\mathbf{Z}_{ji_f}^p + \mathbf{Z}_{ji_m}^p)^T$ etc. are known at this moment. The probability distribution of the segregation indicators at a QTL can be derived from that of the flanking markers (see equation 26 at bottom of page).

where $\mathbf{s}_{ji} = (s_{ji}^p, s_{ji}^m)$, $\mathbf{s}_{ji}^L$ and $\mathbf{s}_{ji}^R$ are the meiosis indicators of the left flanking locus and the right flanking locus of the jth QTL for the ith individual, respectively; $\mathbf{1} = (1\ 1\ 1\ 1)^T$,

$$E(\mathbf{Z}_{ji}^p \mathbf{Z}_{ji}^{mT}) = E(\mathbf{Z}_{ji_f}^p \mathbf{Z}_{ji_m}^{p\ T}) \Pr(s_{ji}^p = 1, s_{ji}^m = 1) + E(\mathbf{Z}_{ji_f}^p \mathbf{Z}_{ji_m}^{m\ T}) \Pr(s_{ji}^p = 1, s_{ji}^m = 0)$$
$$+ E(\mathbf{Z}_{ji_f}^m \mathbf{Z}_{ji_m}^{p\ T}) \Pr(s_{ji}^p = 0, s_{ji}^m = 1) + E(\mathbf{Z}_{ji_f}^m \mathbf{Z}_{ji_m}^{m\ T}) \Pr(s_{ji}^p = 0, s_{ji}^m = 0)$$

and

$$E(\mathbf{Z}_{ji'}^p + \mathbf{Z}_{ji'}^m)(\mathbf{Z}_{ji}^p + \mathbf{Z}_{ji}^m)^T = E(\mathbf{Z}_{ji'}^p + \mathbf{Z}_{ji'}^m)(\mathbf{Z}_{ji_f}^p + \mathbf{Z}_{ji_m}^p)^T \Pr(s_{ji}^p = 1, s_{ji}^m = 1)$$
$$+ E(\mathbf{Z}_{ji'}^p + \mathbf{Z}_{ji'}^m)(\mathbf{Z}_{ji_f}^p + \mathbf{Z}_{ji_m}^m)^T \Pr(s_{ji}^p = 1, s_{ji}^m = 0)$$
$$+ E(\mathbf{Z}_{ji'}^p + \mathbf{Z}_{ji'}^m)(\mathbf{Z}_{ji_f}^m + \mathbf{Z}_{ji_m}^p)^T \Pr(s_{ji}^p = 0, s_{ji}^m = 1)$$
$$+ E(\mathbf{Z}_{ji'}^p + \mathbf{Z}_{ji'}^m)(\mathbf{Z}_{ji_f}^m + \mathbf{Z}_{ji_m}^m)^T \Pr(s_{ji}^p = 0, s_{ji}^m = 0) \tag{25}$$

$$\Pr(\mathbf{s}_{ji} | \mathbf{s}_{ji}^L, \mathbf{s}_{ji}^R) = \frac{\Pr(\mathbf{s}_{ji}^L) \Pr(\mathbf{s}_{ji} | \mathbf{s}_{ji}^L) \Pr(\mathbf{s}_{ji}^R | \mathbf{s}_{ji})}{\sum_{\mathbf{s}_{ji}} \Pr(\mathbf{s}_{ji}^L) \Pr(\mathbf{s}_{ji} | \mathbf{s}_{ji}^L) \Pr(\mathbf{s}_{ji}^R | \mathbf{s}_{ji})} = \frac{\mathbf{1}^T \mathbf{D}_L \mathbf{T}_L \mathbf{D} \mathbf{T}_R \mathbf{D}_R \mathbf{1}}{\sum_{\mathbf{s}_{ji}} \mathbf{1}^T \mathbf{D}_L \mathbf{T}_L \mathbf{D} \mathbf{T}_R \mathbf{D}_R \mathbf{1}} \tag{26}$$

$$D_L = \begin{cases} \text{diag}(1\,0\,0\,0) \text{ if } \mathbf{s}_{ji}^L = (1\,1) \\ \text{diag}(0\,1\,0\,0) \text{ if } \mathbf{s}_{ji}^L = (1\,0) \\ \text{diag}(0\,0\,1\,0) \text{ if } \mathbf{s}_{ji}^L = (0\,1) \\ \text{diag}(0\,0\,0\,1) \text{ if } \mathbf{s}_{ji}^L = (0\,0) \end{cases},$$

$$D_R = \begin{cases} \text{diag}(1\,0\,0\,0) \text{ if } \mathbf{s}_{ji}^R = (1\,1) \\ \text{diag}(0\,1\,0\,0) \text{ if } \mathbf{s}_{ji}^R = (1\,0) \\ \text{diag}(0\,0\,1\,0) \text{ if } \mathbf{s}_{ji}^R = (0\,1) \\ \text{diag}(0\,0\,0\,1) \text{ if } \mathbf{s}_{ji}^R = (0\,0) \end{cases},$$

$$D = \begin{cases} \text{diag}(1\,0\,0\,0) \text{ if } \mathbf{s}_{ji} = (1\,1) \\ \text{diag}(0\,1\,0\,0) \text{ if } \mathbf{s}_{ji} = (1\,0) \\ \text{diag}(0\,0\,1\,0) \text{ if } \mathbf{s}_{ji} = (0\,1) \\ \text{diag}(0\,0\,0\,1) \text{ if } \mathbf{s}_{ji} = (0\,0) \end{cases},$$

$$T_L = \begin{pmatrix} (1-r_L)^2 & r_L(1-r_L) & (1-r_L)r_L & r_L^2 \\ r_L(1-r_L) & (1-r_L)^2 & r_L^2 & r_L(1-r_L) \\ (1-r_L)r_L & r_L^2 & r_L(1-r_L) & (1-r_L)r_L \\ r_L^2 & r_L(1-r_L) & (1-r_L)r_L & (1-r_L)^2 \end{pmatrix},$$

$$T_R = \begin{pmatrix} (1-r_R)^2 & r_R(1-r_R) & (1-r_R)r_R & r_R^2 \\ r_R(1-r_R) & (1-r_R)^2 & r_R^2 & r_R(1-r_R) \\ (1-r_R)r_R & r_R^2 & r_R(1-r_R) & (1-r_R)r_R \\ r_R^2 & r_R(1-r_R) & (1-r_R)r_R & (1-r_R)^2 \end{pmatrix}$$

r_L (r_R) are the recombination rates between the QTL and the left (right) flanking marker.

If marker information is not available, the distribution $\Pr(\mathbf{s}_{ji})$ takes its prior distribution, i.e. $\Pr(\mathbf{s}_{ji} = (1\ 1)) = \Pr(\mathbf{s}_{ji} = (0\ 1)) = \Pr(\mathbf{s}_{ji} = (0\ 0)) = \frac{1}{4}$. This is a situation we have derived for the IBD matrix of the additive polygenic component (DeBoer and Hoeschele, 1993). The above recursive algorithm is also suitable for calculating the IBD matrix at a fully genotyped marker (Davis *et al.*, 1996).

In complex pedigree analysis, an alternative approach for estimating the IBD matrices Π_j is the correlation-based method. Correlation-based methods have been widely applied to human genetic mapping, and implemented in the popular software SOLAR. Amos (1994) showed that the expectation of Π_j is a function of the estimated IBD matrix for a genetic marker and a matrix of correlations between the proportions of genes IBD at the marker and at the putative QTL. The correlation relationships of Amos (1994) can be expressed in matrix notation (Almasy and Blangero, 1998):

$$\mathrm{E}(\Pi_j) = \Pi + B(r,\theta) \otimes (\Pi_m - \Pi) \qquad (27)$$

where Π_m is the estimated IBD matrix for a linked marker, Π is the IBD matrix for the additive polygenic component, θ is the recombination rate between marker locus m and the QTL j, r denotes the rth kinship relationship, $B(r,\theta)$ is the correlation matrix between the proportion of alleles shared IBD at the marker and the QTL, and $\otimes$ represents the Hadamard product. Almasy and Blangero (1998) used the expectation method of Fulker *et al.* (1995) to extend equation (27) to use all linked markers.

Both recursive algorithms and correlation-based methods are efficient ways of calculating the IBD matrices given the availability of full marker information. In the correlation-based method, the IBD matrices of linked markers are needed. However, this requirement is not directly obtained for complex pedigrees with missing marker information. Almasy and Blangero (1998) suggest that Monte Carlo methods be used to impute marker genotypes for individuals not typed in a pedigree conditional on all other markers and pedigree information. With the recursive algorithm, we need the distributions for marker meiosis indicators. Wang *et al.* (1995) and Grignola *et al.* (1996) used Monte Carlo techniques to sample the genotypes of missing markers, though their methods have not used the meiosis indicators. Where there are many unobserved individuals on a pedigree, especially for multi-allelic loci, the space of meiosis indicators is much smaller than the space of genotypes. It is much easier to implement the Markov chain Monte Carlo (MCMC) algorithm in the space of the meiosis indicators than that of the genotypes (Thompson, 1994, 1996; Sobel and Lange, 1996). George *et al.* (2001) employed the meiosis indicators to estimate the IBD matrices in a variance component approach. In fact, the conditional expectation of the IBD matrix for a putative QTL can be expressed as a function of the meiosis indicators:

$$\mathrm{E}(\Pi_j|\mathbf{M}) = \sum_{\mathbf{S} \in \theta} \mathrm{E}(\Pi_j|\mathbf{S}) \Pr(\mathbf{S}|\mathbf{M}) \qquad (28)$$

where $\mathbf{S} = \{s_{ki}^p, s_{ki}^m\}_{i=m+1,k+1}^{n,K}$ is a set of meiosis indicators for markers linked to the jth

QTL, $\boldsymbol{\theta}$ is the space of the meiosis indicators matching the observed marker data, $E(\boldsymbol{\Pi}_j|\mathbf{S})$ is the expectation of the IBD matrix for the jth QTL conditional on $\mathbf{S}$, and $Pr(\mathbf{S}|\mathbf{M})$ is the conditional probability of the meiosis indicators $\mathbf{S}$ given the observed marker data $\mathbf{M}$. To evaluate equation (28), a Markov chain is run on the meiosis indicators or descent graphs $\mathbf{S}$. This chain has equilibrium distribution $Pr(\mathbf{S}|\mathbf{M})$. If a sequence of segregation indicators $\mathbf{S}^{(1)}, \ldots, \mathbf{S}^{(T)}$ is generated by running the chain, then the sample average

$$\frac{1}{T}\sum_{t=1}^{T} E(\boldsymbol{\Pi}_j|\mathbf{S}^{(t)}) \qquad (29)$$

will approximate $E(\boldsymbol{\Pi}_j|\mathbf{M})$ well for T sufficiently large. Computation of $E(\boldsymbol{\Pi}_j|\mathbf{S}^{(t)})$ can be achieved by using recursive algorithms or correlation-based algorithms described above because the descent graphs at marker loci have been visualized at this moment. There is a variety of methods proposed to sample the meiosis indicators at marker loci conditional on observed marker data and these will be discussed in detail below.

Bayesian Analysis for Mapping QTL Under the Random Effect Models

Basic principles of Bayesian analysis

ML deals with data and parameters. The likelihood is proportional to the probability (density) of the data given the parameters, which are fixed but unknown. In Bayesian analysis, however, everything is treated as a variable, and it deals essentially with observables (known quantities) and unobservables (unknown quantities). The observables are similar to the data in ML and the unobservables are similar to (but not exactly the same as) the parameters in ML. In the Bayesian context, parameters are treated as unknown variables with a prior distribution. The purpose of the Bayesian analysis is to infer the distribution of an unknown parameter conditional on the observables, called the posterior distribution. If the observables are denoted by a vector $\mathbf{y}$, the data vector, and unobservables by a vector θ, the parameter vector, including missing values, then the joint posterior distribution of the unobservables is:

$$p(\theta|y) = \frac{p(\theta, y)}{p(y)} = \frac{p(\theta)p(y|\theta)}{p(y)} =$$
$$\frac{p(\theta)p(y|\theta)}{\int p(\theta)p(y|\theta)d\theta} \propto p(\theta)p(y|\theta) \quad (30)$$

where $p(*)$ is a generic expression for probability density, $p(\mathbf{y}|\theta)$ is the likelihood function, $p(\theta)$ is the prior probability distribution of the unobservables and $p(y)$ is the marginal distribution of the observables, the so-called marginal likelihood of the model. Because the denominator does not depend on the parameters θ, an equivalent form omits the factor $p(y)$, yielding the unnormalized posterior distribution.

For most problems, there are more than one unobservable. If the vector θ is partitioned into $\theta = [\theta_i \ \theta_{-i}]$, where θ_i is a single element of the unobservables and θ_{-i} is the rest of the unobservables that exclude θ_i, then the marginal posterior distribution of θ_i is expressed by:

$$p(\theta_i|\mathbf{y}) = \int p(\theta_i \ \theta_{-i}|\mathbf{y})d\theta_{-i} \propto$$
$$\int p(\mathbf{y}|\theta_i \ \theta_{-i})p(\theta_i \ \theta_{-i})d\theta_{-i} \quad (31)$$

The summary statistics of the marginal posterior distribution, e.g. the means, the modes or the medians, are considered Bayesian estimates of parameter θ_i (Gelman $et\ al.$, 1995; Carlin and Louis, 1998). An interval estimate can be obtained simply by examining the posterior distribution.

Despite the simple-looking forms of equations (30) and (31), the challenging aspects of Bayesian analysis are twofold: (i) the development of a model, $p(\theta)\ p(y|\theta)$, which must effectively capture the key features of the underlying scientific problem; and (ii) the necessary computations to summarize the posterior distributions. Note that computations required for completing a Bayesian inference are the integrations (sums for discrete variable) over all unknowns in the joint distribution to obtain the marginal likelihood and over all but those of interest to remove nuisance parameters. In most situations, the marginal posterior distribution

does not have an explicit form, and thus it is difficult to evaluate analytically the parameters of interest. However, MCMC algorithms can be used to simulate the sample from the joint posterior distribution $p(\theta|\mathbf{y})$. The posterior sample contains all information about the parameters of interest. Ideally we might report the empirical distribution for each parameter, i.e. the histogram of the posterior sample. We can also calculate various numerical summary statistics of the posterior distribution, e.g. the mean, median and mode(s) of the posterior distribution.

The idea of MCMC algorithms is to simulate a random walk in the space of the unknowns, which converges to a stationary distribution that is the joint posterior distribution $p(\theta|\mathbf{y})$. The MCMC algorithms are usually carried out in an alternating conditional sampling. In other words, each of the sampling cycles through all elements of unobservables θ represents the drawing of each unobservable conditional on the current values of all other unobservables. The algorithms start from an initial point $\theta^{(0)}$ and proceed to update each of θ in turn. To update θ_i, sample from the conditional posterior distribution $P(\theta_i|\theta_{-i}, \mathbf{y})$, where θ_{-i} represents the vector of the current values of all other unknowns. When all the parameters are updated once, one cycle of iteration is finished. After a large number of iterations, say L_0, the Markov chain $\{\theta^{(1)} \theta^{(2)} \ldots \theta^{(L_0)}\}$ will reach a stationary distribution, where $\theta^{(k)}$ is vector θ evaluated at the kth iteration. After L_0, the Markovian process continues for another large number of iterations, say L_1, forming a stationary Markov chain $\{\theta^{(L_0+1)}\theta^{(L_0+2)} \ldots \theta^{(L_0+L_1)}\}$. These θ vectors are considered as a sample from $P(\theta|\mathbf{y})$, the joint posterior distribution of vector θ. When looking at only θ_i and ignoring θ_{-i} in the simulated sample, the simulated values of θ_i can be considered to be a sample from $P(\theta_i|\mathbf{y})$, the marginal posterior distribution of θ_i. Because there is a realized sample of θ_i, the mean of the sample is an empirical Bayesian estimate (posterior mean) of θ_i. Of course, the observations of the sample are correlated and the correlation should be taken into account when

the variance–covariance matrix of θ is calculated.

The next consideration is how to sample θ_i from $P(\theta_i|\theta_{-i}, \mathbf{y})$. Various approaches have been suggested to conduct MCMC. The Metropolis–Hastings (M–H) algorithm is a general term for a family of Markov chain simulation methods that are useful for drawing samples from Bayesian posterior distributions (Hastings, 1970). The Metropolis algorithm and the Gibbs sampler are two commonly used special cases of the M–H algorithm (Metropolis *et al.*, 1953; Geman and Geman, 1984). The M–H algorithm is a general acceptance–rejection approach, and works as follows.

1. Sample a candidate point θ_i^* from a proposal or candidate generating distribution, $q(\theta_i^*; \theta_i)$.

2. Calculate the importance ratio or the acceptance ratio, $\alpha = \dfrac{p(\theta_i^*|\theta_{-i}, \mathbf{y}) / q(\theta_i^*; \theta_i)}{p(\theta_i|\theta_{-i}, \mathbf{y}) / q(\theta_i; \theta_i^*)}$.

3. The candidate point θ_i^* is accepted with probability $\min(1, \alpha)$.

If $P(\theta_i|\theta_{-i}, \mathbf{y})$ has a standard form, which can be sampled from easily, then it is possible to sample from this conditional posterior distribution, or set $q(\theta_i^*; \theta_i) = P(\theta_i^*|\theta_{-i}, \mathbf{y})$. The method is then called Gibbs sampler (Geman and Geman, 1984). In the Metropolis algorithm, the proposal distribution is chosen to be symmetric, i.e. $q(\theta_i; \theta_i^*) = q(\theta_i^*; \theta_i)$, so that the acceptance ratio is:

$$\alpha = \frac{p(\theta_i^*|\theta_{-i}, \mathbf{y})}{p(\theta_i|\theta_{-i}, \mathbf{y})}$$

The reversible jump MCMC is an extension of the M–H sampler, permitting posterior samples to be collected from posterior distributions with varying dimensions (Green, 1995). With the Bayesian analysis of QTL mapping, the number of QTL can be treated as an unobservable, which naturally leads to consideration of the problem within the general framework of variable dimensional parameter estimation. Suppose there are two models, say model 1 and model 2, with dimensions n_1 and n_2, and parameters $\boldsymbol{\theta}^{(1)}$ and $\boldsymbol{\theta}^{(2)}$, respectively. A transition from model 1 to model 2 can be implemented by

drawing a vector of random variables $\mathbf{u}^{(1)}$ of length m_1, and then setting $\boldsymbol{\theta}^{(2)}$ to be an invertible deterministic function of $\boldsymbol{\theta}^{(1)}$ and $\mathbf{u}^{(1)}$. Similarly, to switch back, $\mathbf{u}^{(2)}$ of length m_2 will be generated and $\boldsymbol{\theta}^{(1)}$ set to some function of $\boldsymbol{\theta}^{(2)}$ and $\mathbf{u}^{(2)}$. The length of $\mathbf{u}^{(1)}$ and $\mathbf{u}^{(2)}$ must satisfy $n_1 + m_1 = n_2 + m_2$. Then the acceptance probability of the move from model 1 to model 2 is (Green, 1995) (see equation 32 at bottom of page).

where $p(\mathbf{y}\,|\,\boldsymbol{\theta}^{(k)})$ and $p(\boldsymbol{\theta}^{(k)})$ ($k = 1, 2$) are the likelihood function and the prior density, respectively, $j(\boldsymbol{\theta}^{(1)};\ \boldsymbol{\theta}^{(2)})$ and $j(\boldsymbol{\theta}^{(2)};\ \boldsymbol{\theta}^{(1)})$ are the probabilities of the moves from model 1 to model 2 and from model 2 to model 1, respectively, $q_k(\mathbf{u}^{(k)})$ is the proposal density for the $\mathbf{u}^{(k)}$ ($k = 1, 2$), and the last term in the ratio above is the Jacobian of the transformation from $(\boldsymbol{\theta}^{(1)}, \mathbf{u}^{(1)})$ to $(\boldsymbol{\theta}^{(2)}, \mathbf{u}^{(2)})$. In practice, the move from model 1 to model 2 will often be set up so that m_1 or m_2 is zero, depending on $n_1 > n_2$ or $n_1 < n_2$. For example, with $m_2 = 0$, the acceptance probability becomes (see equation 33 at bottom of page).

Green (1995) showed that, if moves are accepted with probabilities given by (32) or (33), then the chain satisfies detailed balance and, if irreducible, has the required distribution. For each move type, it is necessary to specify the probability $j(\boldsymbol{\theta}^{(1)};\ \boldsymbol{\theta}^{(2)})$ and $j(\boldsymbol{\theta}^{(2)};\ \boldsymbol{\theta}^{(1)})$ of attempting a move of that type. It is also necessary to specify the corresponding proposal distribution $q_k(\mathbf{u}^{(k)})$ when the move involves an increase in dimensionality of the parameter space. Therefore, even though this is a particular implementation of the reversible jump algorithm, it is still extremely flexible.

The key focus of QTL mapping is on making inferences about the number of QTL, their locations and effects. There are a number of advantages in arriving at inferential statements by using a Bayesian approach over the traditional methods. In the Bayesian approach, use can be made of prior information the parameters. In a situation where no prior knowledge is available, a flat prior (uniform over the whole range of the parameter space) can be chosen. Because the Bayesian approach provides the posterior distribution of the parameters, the posterior variances and confidence intervals of estimated parameters are obtained automatically. The significance test is also embedded in the posterior distribution of the parameters. Therefore, it is not necessary to obtain the critical value for a test statistic to declare significance. Bayesian analysis is preferable on the ground of convenience and flexibility to the use of full pedigrees and mapping multiple QTL, though it is computationally very demanding. Bayesian mapping fully takes into account the uncertainties associated with all unknowns in the QTL mapping problem, including the number and locations of QTL, effects of identified QTL, and the genotypes of markers and QTL. With the reversible jump MCMC, the number of QTL can be treated as a random variable, and its posterior distribution can be obtained. One can even calculate the posterior probability that some particular chromosomal region contains at least one QTL. Finally, the Bayesian method has the inherent flexibility introduced by its incorporation of multiple levels of randomness and the resultant ability to combine information from different sources. Therefore, the Bayesian approach could be extended to allow complicated models for complicated data structures.

The ideas of Bayesian QTL mapping were initially introduced by Hoeschele and Van Randen (1993a,b). The implementation through MCMC algorithm has been tested

$$\min\left\{1,\ \frac{p(\mathbf{y}|\boldsymbol{\theta}^{(2)})p(\boldsymbol{\theta}^{(2)})j(\boldsymbol{\theta}^{(2)};\ \boldsymbol{\theta}^{(1)})q_2(\mathbf{u}^{(2)})}{p(\mathbf{y}|\boldsymbol{\theta}^{(1)})p(\boldsymbol{\theta}^{(1)})j(\boldsymbol{\theta}^{(1)};\ \boldsymbol{\theta}^{(2)})q_1(\mathbf{u}^{(1)})}\left|\frac{\partial(\boldsymbol{\theta}^{(2)}, \mathbf{u}^{(2)})}{\partial(\boldsymbol{\theta}^{(1)}, \mathbf{u}^{(1)})}\right|\right\} \tag{32}$$

$$\min\left\{1,\ \frac{p(\mathbf{y}|\boldsymbol{\theta}^{(2)})p(\boldsymbol{\theta}^{(2)})j(\boldsymbol{\theta}^{(2)};\ \boldsymbol{\theta}^{(1)})}{p(\mathbf{y}|\boldsymbol{\theta}^{(1)})p(\boldsymbol{\theta}^{(1)})j(\boldsymbol{\theta}^{(1)};\ \boldsymbol{\theta}^{(2)})q_1(\mathbf{u}^{(1)})}\left|\frac{\partial\boldsymbol{\theta}^{(2)}}{\partial(\boldsymbol{\theta}^{(1)}, \mathbf{u}^{(1)})}\right|\right\} \tag{33}$$

in line-crossing experiments (e.g. F_2, back-crosses) (Satagopan and Yandell, 1996; Satagopan *et al.*, 1996; Sillanpää and Arjas, 1998; Stephens and Fisch, 1998) and for general pedigree data (Taller and Hoeschele, 1996; Uimari *et al.*, 1996; Heath, 1997; Uimari and Hoeschele, 1997; Bink *et al.*, 2000; Lee and Thomas, 2000). The works for line-crossing data always use the fixed model approach. The works for pedigree data usually use the random model, but most often assume a biallelic model. The biallelic QTL model greatly simplifies the statistical analysis. Although the biallelic model is proper in most situations, it can potentially fail if the actual number of polymorphic alleles is not two. Bink *et al.* (2000), Xu and Yi (2000) and Yi and Xu (2001) extended the Bayesian method to a multi-allelic QTL model, where allelic effects are assumed to be normally distributed. Yi and Xu (2000) developed a proximate Bayesian method based on an IBD-based variance component approach. This method does not need knowledge of the number of alleles.

Bayesian mapping under the identity-by-descent-based variance component model

Joint posterior distribution and prior distributions

In Bayesian framework, each item in the model is first classified into one of two categories: known quantities (observables) and unknown quantities (unobservables). The observables are the phenotypic values $\mathbf{y} = \{y_i\}_{i=1}^n$, the covariate data $\mathbf{X}$ and the marker data $\mathbf{M}$. The locations of markers on chromosomes are known *a priori*. The unknown quantities include parameters of interest and missing values. The parameters of interest are the number of QTL l, the

locations of QTL $\boldsymbol{\lambda} = \{\lambda_j\}_{j=1}^l$ and the model effects $\boldsymbol{\theta} = (\boldsymbol{\beta}, \sigma_{a_1}^2, \ldots, \sigma_{a_l}^2, \sigma_{d_1}^2, \ldots, \sigma_{d_l}^2, \sigma_e^2)$, where λ_j denotes the distance of the jth QTL from one end of the chromosome in which the QTL resides. The missing values include the additive and dominance effects of QTL, and the QTL IBD matrices $\boldsymbol{\Pi} = \{\boldsymbol{\Pi}_j\}_{j=1}^l$ and $\boldsymbol{\Delta} = \{\boldsymbol{\Delta}_j\}_{j=1}^l$. With the IBD-based variance component approach, the additive and dominance effects of QTL have been integrated out in the likelihood function and therefore do not have to be generated in the MCMC process.

In the Bayesian framework, the unknowns in the model are considered to be drawn from appropriate prior distributions. The joint posterior distribution of the unknowns $\{l, \boldsymbol{\lambda}, \boldsymbol{\theta}, \boldsymbol{\Pi}, \boldsymbol{\Delta}\}$, given the observables $\{\mathbf{y}, \mathbf{X}, \mathbf{M}\}$ and prior distribution of $\{l, \boldsymbol{\lambda}, \boldsymbol{\theta}, \boldsymbol{\Pi}, \boldsymbol{\Delta}\}$, is (see equation 34 at bottom of page).

The first term in equation (34) is the conditional distribution of the phenotypic data given all unknowns, which is usually called the likelihood function, and has the following form (see equation 35 at bottom of page)

where $\mathbf{V}$ is the covariance matrix of the phenotypic values $\mathbf{y}$ and defined in equation (7). The second and the third terms, $p(\boldsymbol{\Pi}|l, \boldsymbol{\lambda}, \mathbf{M})$ and $p(\boldsymbol{\Delta}|l, \boldsymbol{\lambda}, \mathbf{M})$, are the distributions of the IBD matrices conditional on the marker information, which depend on the locations of QTL, the marker data and the pedigree information. The last term in equation (34) is the joint prior distribution of l, $\boldsymbol{\lambda}$ and $\boldsymbol{\theta}$, the parameters of interest.

Assuming prior independence of the parameters, the joint prior distribution $p(l, \boldsymbol{\lambda}, \boldsymbol{\theta})$ can be factorized into the following products (see equation 36 at bottom of page).

The prior on the number of QTL is chosen to be Poisson with a predetermined Poisson mean μ, or uniform between 1

$$p(l, \boldsymbol{\lambda}, \boldsymbol{\theta}, \boldsymbol{\Pi}, \boldsymbol{\Delta}\,|\,\mathbf{y}, \mathbf{X}, \mathbf{M}) \propto p(\mathbf{y}\,|\,l, \boldsymbol{\lambda}, \boldsymbol{\theta}, \boldsymbol{\Pi}, \boldsymbol{\Delta})p(\boldsymbol{\Pi}\,|\,l, \boldsymbol{\lambda}, \mathbf{M})p(\boldsymbol{\Delta}\,|\,l, \boldsymbol{\lambda}, \mathbf{M})p(l, \boldsymbol{\lambda}, \boldsymbol{\theta}) \tag{34}$$

$$f(\mathbf{y}\,|\,l, \boldsymbol{\lambda}, \boldsymbol{\theta}, \mathbf{X}, \boldsymbol{\Pi}, \boldsymbol{\Delta}) = (2\pi)^{-n/2}\,|\mathbf{V}|^{-1/2}\exp\{-\tfrac{1}{2}(\mathbf{y}-\mathbf{X}\boldsymbol{\beta})^{\mathrm{T}}\mathbf{V}^{-1}(\mathbf{y}-\mathbf{X}\boldsymbol{\beta})\} \tag{35}$$

$$p(l, \boldsymbol{\lambda}, \boldsymbol{\theta}) = p(l)p(\boldsymbol{\beta})p(\sigma_e^2)\prod_{j=1}^{l}[p(\lambda_j)p(\sigma_{a_j}^2)p(\sigma_{d_j}^2)] \tag{36}$$

and a prespecified integer $l_{\max}$. Mean μ or maximum integer $l_{\max}$ is chosen to reflect the belief that there is a small number of QTL, which can be separated from the polygenic background. The QTL positions have a joint prior of $p(\boldsymbol{\lambda}) = \prod_{q=1}^{l} p(\lambda_q)$, where each $p(\lambda_q)$ is uniform across the whole genome when no information is available regarding the locations. The prior distributions of $\boldsymbol{\beta}$, σ_e^2, $\sigma_{a_j}^2$ and $\sigma_{d_j}^2$ are assumed to be uniforms on predefined intervals, although other priors can be used. The lower and upper bounders for all variance components are usually set to be zero and the phenotypic variance present in the data, respectively.

The MCMC algorithm

The marginal posterior distributions for all unknowns are evaluated by using MCMC algorithms. There are two methods to update the QTL IBD matrices. One method is to replace all the elements of the IBD matrices by the corresponding conditional expectations given marker information and the locations of the QTL. This treatment does not take advantage of the conditional distribution of the IBD matrices but will significantly shorten the Markov chain and thus speed up the computation. The other method is the full Bayesian treatment in which the conditional distributions of the IBD matrices are taken into account, which is much more complicated than the former method. Here the conditional expectations are used, i.e. replacing $\{\boldsymbol{\Pi}_j\}$ and $\{\boldsymbol{\Delta}_j\}$ by their conditional expectations denoted as $\{\hat{\boldsymbol{\Pi}}_j\}$ and $\{\hat{\boldsymbol{\Delta}}_j\}$.

The proposed MCMC algorithm starts from an initial point $(l^{(0)}, \boldsymbol{\lambda}^{(0)}, \boldsymbol{\theta}^{(0)}, \hat{\boldsymbol{\Pi}}^{(0)}, \hat{\boldsymbol{\Delta}}^{(0)})$ and proceeds to update each of the unknowns in turn. The starting values for $(l^{(0)}, \boldsymbol{\lambda}^{(0)}, \boldsymbol{\theta}^{(0)})$ can be selected randomly from the corresponding priors. For the selected initial QTL position, the corresponding expectations of the IBD matrices, $\hat{\boldsymbol{\Pi}}^{(0)}$ and $\hat{\boldsymbol{\Delta}}^{(0)}$, are then calculated using the methods described earlier. Updating $\boldsymbol{\theta}$ and $\boldsymbol{\lambda}$ are implemented using the Metropolis

algorithm. Updating the QTL number l requires a change in the dimension of the model and thus needs a reversible jump step. More specifically, given the current state of $(l, \boldsymbol{\lambda}, \boldsymbol{\theta}, \hat{\boldsymbol{\Pi}}, \hat{\boldsymbol{\Delta}})$, the MCMC proceeds as follows.

UPDATING THE MODEL PARAMETERS $\boldsymbol{\theta}$. All elements of $\boldsymbol{\theta}$ are updated simultaneously. New proposals for the elements of $\boldsymbol{\theta}$ are sampled from the symmetric uniform densities around their previous values. The proposals are accepted simultaneously with probability $\min(1, r)$, where:

$$r = \frac{p(\mathbf{y}|l, \boldsymbol{\lambda}, \boldsymbol{\theta}^*, \hat{\boldsymbol{\Pi}}, \hat{\boldsymbol{\Delta}})}{p(\mathbf{y}|l, \boldsymbol{\lambda}, \boldsymbol{\theta}, \hat{\boldsymbol{\Pi}}, \hat{\boldsymbol{\Delta}})} \qquad (37)$$

where $\boldsymbol{\theta}^*$ represents the proposed $\boldsymbol{\theta}$.

UPDATING THE QTL LOCATIONS $\boldsymbol{\lambda}$ AND THE EXPECTATIONS OF THE IBD MATRICES $\hat{\boldsymbol{\Pi}}$ AND $\hat{\boldsymbol{\Delta}}$. The order of QTL are not fixed when updating the QTL locations. Elements of $\boldsymbol{\lambda}$ are updated one at a time using the Metropolis algorithm. For the jth QTL, a proposal λ_j^* is sampled from a uniform distribution on the interval $[\lambda_j - d, \lambda_j + d]$, where d is the tuning parameter. The expectations of the IBD matrices for new location λ_j^*, denoted by $\hat{\boldsymbol{\Pi}}_j^*$ and $\hat{\boldsymbol{\Delta}}_j^*$, are then calculated according to the new position λ_j^*, the observed marker information and pedigree structure using the methods discussed earlier. The proposal is accepted with probability $\min(1, r)$, where:

$$r = \frac{p(\mathbf{y}|l, \boldsymbol{\theta}, \mathbf{X}, \hat{\boldsymbol{\Pi}}_{-j}, \hat{\boldsymbol{\Pi}}_j^*, \hat{\boldsymbol{\Delta}}_{-j}, \hat{\boldsymbol{\Delta}}_j^*)}{p(\mathbf{y}|l, \boldsymbol{\theta}, \mathbf{X}, \hat{\boldsymbol{\Pi}}, \hat{\boldsymbol{\Delta}})} \qquad (38)$$

where $\hat{\boldsymbol{\Pi}}_{-j} = \{\hat{\boldsymbol{\Pi}}_{j'}\}_{j' \neq j}^{l}$, $\hat{\boldsymbol{\Delta}}_{-j} = \{\hat{\boldsymbol{\Delta}}_{j'}\}_{j' \neq j}^{l}$.

UPDATING THE NUMBER OF QTL. Let $\vartheta = (\boldsymbol{\lambda}, \hat{\boldsymbol{\Pi}}, \hat{\boldsymbol{\Delta}}, \boldsymbol{\theta})$. Updating the number of QTL involves a change in the dimension of ϑ and thus needs a reversible jump step. Instead of drawing a QTL number randomly, the reversible jump step is usually facilitated by proposing to increase or decrease the number of QTL by 1. There are two ways to implement this reversible jump step: either pick a new location completely at random over the entire genome (or drop an existing QTL randomly) or split an existing QTL (or merge two

existing QTL) (Heath, 1997). Here only the first type of proposal is discussed to update the number of QTL. At each iteration, a random choice is made between attempting to add a QTL to the model or delete a QTL from the model, with predetermined probabilities $q(l+1; l)$ and $q(l-1; l) = 1 - q(l+1; l)$, respectively. Of course, $q(l+1; l) = 0$ if $l = l_{max}$ and $q(l-1; l) = 0$ if $l = 0$, otherwise $q(l+1; l) = 0.5$, for $0 < l < l_{max}$, is chosen although alternative values can be used as well.

If it is proposed to add a new QTL, a new position λ_{l+1}, new additive and dominance variances $\sigma^2_{a_{l+1}}$ and $\sigma^2_{d_{l+1}}$ and new IBD matrices $\hat{\mathbf{\Pi}}_{l+1}$ and $\hat{\mathbf{\Delta}}_{l+1}$ need to be generated for the new QTL. According to the reversible jump algorithm, the new value for ϑ is a deterministic function of the old value for ϑ and $\mathbf{u} = \lambda_{l+1}, \hat{\mathbf{\Pi}}_{l+1}, \hat{\mathbf{\Delta}}_{l+1}, \sigma^2_{a_{l+1}}, \sigma^2_{d_{l+1}})$, i.e. $\vartheta^* = h(\vartheta, \mathbf{u})$. The proposal distribution for $\mathbf{u} = \lambda_{l+1}, \hat{\mathbf{\Pi}}_{l+1}, \hat{\mathbf{\Delta}}_{l+1}, \sigma^2_{a_{l+1}}, \sigma^2_{d_{l+1}})$ is denoted as $q(\mathbf{u})$. The proposal is accepted with probability min(1, r), where (see equation 39 at bottom of page).

If the proposal is accepted, a QTL is added to the model, and the new QTL location, the new IBD matrices and the new additive and dominance variances are all accepted simultaneously; otherwise, the number of QTL remains unchanged.

Deleting a QTL simply takes the reverse process of adding a QTL. It is assumed that it is proposed to delete the jth existing QTL. Then denote $\mathbf{u} = \lambda_j, \hat{\mathbf{\Pi}}_j, \hat{\mathbf{\Delta}}_j, \sigma^2_{a_j}, \sigma^2_{d_j})$, and the new value for ϑ is created from the old value for ϑ by applying the inverse transformation and discarding u, i.e. $(\vartheta, \mathbf{u}) = h^{-1}(\vartheta^*)$. The probability of accepting the deletion is min(1, r), where (see equation 40 at bottom of page).

In practice, is is necessary to specify the move probabilities $j(x)$, the proposal distributions $q(\mathbf{u})$ and the function h, and

calculate the Jacobian. The move probabilities to add a QTL equal the predefined probabilities, i.e. $j(l+1; l, \vartheta) = q(l+1; l)$, $j(l; l-1; \vartheta) = q(l; l-1)$. If each one of the QTL has an equal chance to be removed, then $j(l; l+1, \vartheta^*) = (1/(l+1))q(l+1; l)$ and $j(l; l-1, \vartheta) = (1/l)q(l-1; l)$, where $1/(l+1)$ is the probability that the $(l+1)$th QTL is selected in equation (39), and $1/l$ is the probability that the jth QTL is selected in equation (40). The simplest way of generating $\mathbf{u}$ is to propose the location, the additive and dominance variances from the corresponding priors. In such a situation, the contributions from the prior and the proposal distributions for $\mathbf{u}$ to the acceptance rate can be cancelled out. The function h is usually chosen to be an identity transformation, i.e. $\vartheta^* = (\vartheta, \mathbf{u})$, which results in the Jacobian being 1. In the above specification, the equations (39) and (40) can be simplified as:

$$r = \frac{p(\mathbf{y}|l+1, \vartheta^*, \mathbf{X})p(l+1)q(l; l+1)}{p(\mathbf{y}|l, \vartheta, \mathbf{X})p(l)(1+l)q(l+1; l)} \quad (41)$$

and

$$r = \frac{p(\mathbf{y}|l-1, \vartheta^*, \mathbf{X})p(l-1)lq(l; l-1)}{p(\mathbf{y}|l, \vartheta, \mathbf{X})p(l)q(l-1; l)} \quad (42)$$

respectively.

Full Bayesian analysis of QTL mapping

Joint posterior distribution and prior distributions

The full Bayesian analysis takes the distributions of allelic inheritance matrices, allelic effects and dominance effects into consideration. These missing values are visualized in the MCMC process. The implementation of full Bayesian analysis for QTL mapping greatly depends on the assumption about the number of alleles

$$r = \frac{p(\mathbf{y}|l+1, \vartheta^*, \mathbf{X})p(l+1, \vartheta^*)j(l; l+1, \vartheta^*)}{p(\mathbf{y}|l, \vartheta, \mathbf{X})p(l, \vartheta)j(l+1; l, \vartheta)q(\mathbf{u})} \left| \frac{\partial \vartheta^*}{\partial(\vartheta, \mathbf{u})} \right| \quad (39)$$

$$r = \frac{p(\mathbf{y}|l-1, \vartheta^*, \mathbf{X})p(l-1, \vartheta^*)j(l; l-1, \vartheta^*)q(\mathbf{u})}{p(\mathbf{y}|l, \vartheta, \mathbf{X})p(l, \vartheta)j(l-1; l, \vartheta)} \left| \frac{\partial(\vartheta^*, \mathbf{u})}{\partial \vartheta} \right| \quad (40)$$

at QTL. In principle, the number of alleles can be treated as unknown, but this adds another level of complexity to the analysis. The common practice is to assume either a biallelic QTL model (e.g. Heath, 1997; Uimari and Hoeschele, 1997; Lee and Thomas, 2000) or a normal-effects QTL model (e.g. Xu and Yi, 2000; Yi and Xu, 2001). The latter is discussed here.

The observables in model (3) include the phenotypic values $\mathbf{y} = \{y_i\}_{i=1}^n$, the covariate $\mathbf{X}$ and the marker data $\mathbf{M}$. The marker data include the locations of markers on the chromosomes and the observed (possibly incomplete) marker genotypes. The observed marker genotypes in some individuals may not be fully informative and the patterns of allelic inheritance of such markers may also be unknown. The parameters of interest include the number of QTL l, the QTL locations $\boldsymbol{\lambda} = \{\lambda_j\}_{j=1}^l$, the QTL additive variances $\mathbf{V}_a = \{\sigma_{a_j}^2\}_{j=1}^l$, the QTL dominance variances $\mathbf{V}_d = \{\sigma_{d_j}^2\}_{j=1}^l$, the non-genetic effect $\boldsymbol{\beta}$, and the residual variance σ_e^2. The missing data include the complete marker genotype matrix, the QTL allelic inheritance matrices $\mathbf{Z}^p = \{\mathbf{Z}_j^p\}_{j=1}^l$ and $\mathbf{Z}^m = \{\mathbf{Z}_j^m\}_{j=1}^l$, the QTL allelic effects $\mathbf{a} = \{\mathbf{a}_j\}_{j=1}^l$, and the QTL dominance effects $\mathbf{d} = \{\mathbf{d}_j\}_{j=1}^l$. The QTL dominance design matrices are suppressed in the list of unknowns because they are completely determined by the QTL allelic inheritance matrices. As mentioned earlier, it is difficult to derive the distributions of QTL allelic inheritance matrices; instead, the meiosis indicators can be used to build the allelic inheritance matrices. Therefore, the allelic inheritance matrices are replaced by the corresponding meiosis indicators in the list of missing data. Similarly, the complete marker genotypes are specified by the meiosis indicators at the markers. Suppose that all K markers and l QTL are ordered $1, \ldots, K + l$ along the chromosomes. Denote the meiosis indicators for the markers

and the QTL by $\mathbf{S} = \{s_{ik}^p, s_{ik}^m\}_{i=m+1, k=1}^{n, K+l}$, where $s_{ik}^p(s_{ik}^m)$ is the meiosis indicator of the paternal (maternal) allele at the kth marker or QTL for the ith individual. The joint posterior distribution of all unobservables $\boldsymbol{\theta} = \{l, \boldsymbol{\lambda}, \mathbf{a}, \mathbf{d}, \mathbf{V}_a, \mathbf{V}_d, \mathbf{S}, \boldsymbol{\beta}, \sigma_e^2\}$ given the observables $\{\mathbf{y}, \mathbf{X}, \mathbf{M}\}$ and prior information for unobservables can be expressed as (see equation 43 at bottom of page).

The likelihood function in the above equation, $p(\mathbf{y}, \mathbf{X}, \mathbf{M} | \boldsymbol{\theta})$, can be factorized into:

$$p(\mathbf{y}, \mathbf{X}, \mathbf{M} | \boldsymbol{\theta}) = p(\mathbf{y} | \boldsymbol{\theta}, \mathbf{X}) \cdot p(\mathbf{M} | \boldsymbol{\theta}) \cdot p(\mathbf{X} | \boldsymbol{\theta}) \tag{44}$$

The penetrance function $p(\mathbf{y} | \boldsymbol{\theta}, \mathbf{X})$ depends on the distribution of $\mathbf{y}$. For normally distributed traits, it has the following form:

$$p(\mathbf{y} | \boldsymbol{\theta}, \mathbf{X}) = (2\pi\sigma_e^2)^{-\frac{n}{2}} \times \exp\left\{-\frac{1}{2\sigma_e^2}\mathbf{R}^T\mathbf{R}\right\} \tag{45}$$

where $\mathbf{R} = \mathbf{y} - \mathbf{Xb} - \sum_{j=1}^l (\mathbf{Z}_j^p + \mathbf{Z}_j^m)\mathbf{a}_j - \sum_{j=1}^l \mathbf{W}_j\mathbf{d}_j$. Note that the matrices $\mathbf{Z}_j^p$, $\mathbf{Z}_j^m$ and $\mathbf{W}_j$ are determined by the meiosis indicators of the jth QTL. The conditional distribution of the observed marker data $\mathbf{M}$, $p(\mathbf{M} | \boldsymbol{\theta})$, only depends on the marker segregation indicators, and takes the value of 1 if the marker segregation indicators are compatible with $\mathbf{M}$ and 0 otherwise. The distribution of $\mathbf{X}$ provides no information about the inference on $\boldsymbol{\theta}$, and thus can be ignored in the analysis (Gelman *et al.*, 1995).

The prior distributions for the number and the locations are set to be the same as those in the IBD-based variance component model described earlier. The prior for non-genetic effects $\boldsymbol{\beta}$ is generally assigned to be a multivariate normal distribution, i.e.:

$$\boldsymbol{\beta} \sim N(\boldsymbol{\beta}_0, \mathbf{B}_0) \tag{46}$$

where $\boldsymbol{\beta}_0$ and $\mathbf{B}_0$ are known prior mean and variance for $\boldsymbol{\beta}$. When no prior knowledge

$$p(\boldsymbol{\theta} | \mathbf{y}, \mathbf{X}, \mathbf{M}) \propto p(\mathbf{y}, \mathbf{X}, \mathbf{M} | \boldsymbol{\theta})\, p(l) \prod_{j=1}^l \{p(\lambda_j)p(\mathbf{a}_j | \sigma_{a_j}^2)p(\mathbf{d}_j | \sigma_{d_j}^2)p(\sigma_{a_j}^2)p(\sigma_{d_j}^2)\} \times$$
$$p(\boldsymbol{\beta})p(\sigma_e^2)p(\mathbf{S} | l, \boldsymbol{\lambda}) \tag{43}$$

is available, a flat or uniform prior distribution, i.e. p(**β**) ∝ constant, is usually used, which is a specific case of the normal prior where all elements of $\mathbf{B}_0$ converge to infinite.

The priors for the QTL additive and dominance effects are assumed to be independent normal so that:

$$\mathbf{a}_j \sim \mathrm{N}_{k_a}(0, \mathrm{I}_{k_a}\sigma^2_{a_j}) \qquad (47)$$

and

$$\mathbf{d}_j \sim \mathbf{N}_{k_d}(0, \mathrm{I}_{k_d}\sigma^2_{d_j}), \, j = 1, 2, \ldots, l \qquad (48)$$

Note that these priors reflect the assumptions that the founders are non-inbred and genetically unrelated. The additive and dominance effects at different loci are assumed to be mutually independent *a priori* and also independent of non-genetic effects **β** and the residual error **e**.

For the convenience of conjugacy, independent scaled inverted χ^2 distributions are used as priors for all variance components, so that:

$$\sigma^2_e \sim \mathrm{Inv} - \chi^2(v_e, s^2_e) \qquad (49)$$

$$\sigma^2_{a_j} \sim \mathrm{Inv} - \chi^2(v_{a_j}, s^2_{a_j}) \qquad (50)$$

and

$$\sigma^2_{d_j} \sim \mathrm{Inv} - \chi^2(v_{d_j}, s^2_{d_j}), \, j = 1, 2, \ldots, l \quad (51)$$

where $(v_e, v_{a_j}$ and v_{d_j} $(j = 1, 2, \ldots, l)$ are the 'degree of belief' parameters, and $s^2_e, s^2_{a_j}$ and $s^2_{d_j}$ $(j = 1, 2, \ldots, l)$ can be thought of as prior values of the appropriate variances. The flat or uniform priors for all variance components, i.e. $\sigma^2_e \sim$ constant, $\sigma^2_{a_j} \sim$ constant and $\sigma^2_{d_j} \sim$ constant, can be obtained by setting $v_e = v_{a_j} = v_{d_j} = 0$ and $s^2_e = s^2_{a_j} = s^2_{d_j} = -2$.

Since the segregation indicators are independent, the prior probability of the marker and the QTL segregation indicators can be factorized into the product of all individuals, i.e.:

$$p(\mathbf{S}|l, \lambda) = \prod_{i=m+1}^{n} p(\mathbf{S}_{\cdot i}|l, \lambda) \qquad (52)$$

where $\mathbf{S}_{\cdot i} = \{s^p_{ji}, s^m_{ji}\}^{K+l}_{j=1}$ represents the segregation indicators for the ith individual at all marker loci and QTL. Under assumption of no interference in meiosis, $\mathbf{S}_{\cdot i}$ forms a first-order Markov chain, so that:

$$p(\mathbf{S}_{\cdot i}|l, \lambda) = p(\mathbf{s}_{1i}) \prod_{j=2}^{K+1} p(\mathbf{s}_{ji}|\mathbf{s}_{(j-1)i}, \lambda) \quad (53)$$

where $\mathbf{s}_{ji} = \{s^p_{ji}, s^m_{ji}\}$.

The MCMC algorithm

In the full Bayesian analysis, the MCMC algorithm consists of the following steps: (i) updating the fixed effects **β** and residual variance σ^2_e; (ii) updating the QTL allelic effects $\mathbf{a} = \{\mathbf{a}_j\}^l_{j=1}$ and dominance effects $\mathbf{d} = \{\mathbf{d}_j\}^l_{j=1}$; (iii) updating the QTL allelic variances $\mathbf{V}_a = \{\sigma^2_{a_j}\}^l_{j=1}$ and dominance variances $\mathbf{V}_d = \{\sigma^2_{d_j}\}^l_{j=1}$; (iv) updating the marker and QTL meiosis indicators; (v) updating the QTL locations $\lambda = \{\lambda_j\}^l_{j=1}$; and (vi) updating the number of QTL: birth of a QTL (adding one new QTL to the model) or death of a QTL (removing one existing QTL from the model). The proposed algorithm starts from an initial point and proceeds to update each of the unknowns in turn. One complete pass over these six update steps defines a cycle of iteration. Updating steps (i)–(v) is conventional and involves no change in the dimension of the variable vector. The Gibbs sampler or the M–H algorithms can be applied to implement steps (i)–(v). Step (vi) involves change in the number of QTL and thus the dimension of the model. A reversible jump step is needed to change the number of QTL.

UPDATING THE NON-GENETIC EFFECTS, RESIDUAL VARIANCE, ALLELIC EFFECTS AND VARIANCES, AND DOMINANCE EFFECTS AND VARIANCES. Under the prior specifications, the fully conditional posterior distributions for these parameters have standard forms so that the Gibbs sampler can be applied to update these parameters. The fully conditional density of each of the unknowns is obtained by regarding all other parameters as known. Thus:

$$\boldsymbol{\beta}|\mathbf{y}, \mathbf{X}, \boldsymbol{\theta}_{-\beta} \sim \mathrm{N}(\tilde{\boldsymbol{\beta}}, \mathbf{V}_\beta) \qquad (54)$$

where $\tilde{\boldsymbol{\beta}} = [\mathbf{X}^T\mathbf{X}\sigma^{-2}_e + \mathbf{B}_0^{-1}]^{-1}[\mathbf{X}^T\sigma^{-2}_e(\mathbf{y} - \sum_{j=1}^{l}(\mathbf{Z}^p_j + \mathbf{Z}^m_j)\mathbf{a}_j - \sum_{j=1}^{l}\mathbf{W}_j\mathbf{d}_j) + \mathbf{B}_0^{-1}\boldsymbol{\beta}_0]$ and $\mathbf{V}_\beta = (\mathbf{X}^T\mathbf{X}\sigma^{-2}_e + \mathbf{B}_0^{-1})^{-1}$.

The fully conditional posterior density of the residual variance σ_e^2 is in the scaled inverted χ^2 form:

$$\sigma_e^2 | \mathbf{y}, \mathbf{X} \sim \text{Inv} - \chi^2(v_e + n, \frac{v_e s_e^2 + \mathbf{R}^T \mathbf{R}}{v_e + n}) \quad (55)$$

where $\quad \mathbf{R} = \mathbf{y} - \mathbf{X}\boldsymbol{\beta} - \sum\limits_{j=1}^{l} (\mathbf{Z}_j^p + \mathbf{Z}_j^m)\mathbf{a}_j - \sum\limits_{j=1}^{l} \mathbf{W}_j \mathbf{d}_j$.

The fully conditional posterior distribution of each additive and dominance effect is multivariate normal:

$$\mathbf{a}_j | \mathbf{y}, \mathbf{X}, \boldsymbol{\theta}_{-a_j} \sim \text{N}(\tilde{\mathbf{a}}_j, \mathbf{V}_{a_j}) \quad (56)$$

and

$$\mathbf{d}_j | \mathbf{y}, \mathbf{X}, \boldsymbol{\theta}_{-d_j} \sim \text{N}(\tilde{\mathbf{d}}_j, \mathbf{V}_{d_j}), j = 1, 2, \ldots, l \quad (57)$$

where $\boldsymbol{\theta}_{-a_j}$ and $\boldsymbol{\theta}_{-d_j}$ represent all elements of $\boldsymbol{\theta}$ except $\mathbf{a}_j$ and $\mathbf{d}_j$, respectively, (see unnumbered equation at bottom of page).

Clearly, sampling from $p(\mathbf{a}_j | \mathbf{y}, \mathbf{X}, \boldsymbol{\theta}_{-a_j})$ and $p(\mathbf{d}_j | \mathbf{y}, \mathbf{X}, \boldsymbol{\theta}_{-d_j})$ involves the inversion of matrices. The dimensions of these matrices depend on the number of the founders. It is computationally unfeasible in complicated designs with many founders. However, a scalar version of the Gibbs sampler can be used to sample the elements of a_j and d_j

alternatively, so inversion of matrices is not needed (Wang *et al.*, 1994). Let $\mathbf{a}_j = (a_{j1}, a_{j2}, \ldots, a_{jk_a})^T$, $\mathbf{d}_j = (d_{j1}, d_{j2}, \ldots, d_{jk_d})^T$, b_k be the kth element of the vector $(\mathbf{Z}_j^p + \mathbf{Z}_j^m)^T[\mathbf{y} - \mathbf{X}\boldsymbol{\beta} - \sum\limits_{j' \neq j}^{l} (\mathbf{Z}_{j'}^p + \mathbf{Z}_{j'}^m)\mathbf{a}_{j'} - \sum\limits_{j'=j}^{l} \mathbf{W}_{j'} \mathbf{d}_{j'}]$, $c_{kk'}$ the kk'th element of the matrix $(\mathbf{Z}_j^p + \mathbf{Z}_j^m)^T(\mathbf{Z}_j^p + \mathbf{Z}_j^m) + \mathbf{I}_{k_a} \frac{\sigma_e^2}{\sigma_{a_j}^2}$, h_k be the kth element of the vector $\mathbf{W}_j^T[\mathbf{y} - \mathbf{X}\boldsymbol{\beta} - \sum\limits_{j'=1}^{l} (\mathbf{Z}_j^p + \mathbf{Z}_j^m)\mathbf{a}_{j'} - \sum\limits_{j' \neq j}^{l} \mathbf{W}_{j'} \mathbf{d}_{j'}]$, and $f_{kk'}$ the kk'th element of the matrix $\mathbf{W}_j^T \mathbf{W}_j + \mathbf{I}_{k_d} \frac{\sigma_e^2}{\sigma_{d_j}^2}$. The fully conditional posterior distributions of a_{jk} and d_{jk} are (see equations 58 and 59 at bottom of page),

respectively, where $\boldsymbol{\theta}_{-a_{jk}}$ and $\boldsymbol{\theta}_{-d_{jk}}$ represent all elements of $\boldsymbol{\theta}$ except a_{jk} and d_{jk}.

The fully conditional posterior distribution for each additive and dominance variance is in the scaled inverted χ^2 form (see equations 60 at bottom of page and 61 at bottom of next page),

where $\boldsymbol{\theta}_{-\sigma_{a_j}^2}$ and $\boldsymbol{\theta}_{-\sigma_{d_j}^2}$ represent all elements of $\boldsymbol{\theta}$ except $\sigma_{a_j}^2$ and $\sigma_{d_j}^2$, respectively.

$$\tilde{\mathbf{a}}_j = [(\mathbf{Z}_j^p + \mathbf{Z}_j^m)^T(\mathbf{Z}_j^p + \mathbf{Z}_j^m) + \mathbf{I}_{k_a} \frac{\sigma_e^2}{\sigma_{a_j}^2}]^{-1}(\mathbf{Z}_j^p + \mathbf{Z}_j^m)^T[\mathbf{y} - \mathbf{X}\boldsymbol{\beta} - \sum\limits_{j' \neq j}^{l} (\mathbf{Z}_{j'}^p + \mathbf{Z}_{j'}^m)\mathbf{a}_{j'} - \sum\limits_{j'=j}^{l} \mathbf{W}_{j'} \mathbf{d}_{j'}],$$

$$\mathbf{V}_{a_j} = [(\mathbf{Z}_j^p + \mathbf{Z}_j^m)^T(\mathbf{Z}_j^p + \mathbf{Z}_j^m) + \mathbf{I}_{k_a} \frac{\sigma_e^2}{\sigma_{a_j}^2}]^{-1}\sigma_e^2,$$

$$\tilde{\mathbf{d}}_j = (\mathbf{W}_j^T \mathbf{W}_j + \mathbf{I}_{k_d} \frac{\sigma_e^2}{\sigma_{d_j}^2})^{-1}\mathbf{W}_j^T[\mathbf{y} - \mathbf{X}\boldsymbol{\beta} - \sum\limits_{j' \neq j}^{l} (\mathbf{Z}_j^p + \mathbf{Z}_j^m)\mathbf{a}_{j'} - \sum\limits_{j'=j}^{l} \mathbf{W}_{j'} \mathbf{d}_{j'}],$$

$$\text{and } \mathbf{V}_{d_j} = (\mathbf{W}_j^T \mathbf{W}_j + \mathbf{I}_{k_d} \frac{\sigma_e^2}{\sigma_{d_j}^2})^{-1}\sigma_e^2.$$

$$a_{jk} | \mathbf{y}, \mathbf{X}, \boldsymbol{\theta}_{-a_{jk}} \sim \text{N}((b_k - \sum\limits_{k' \neq k}^{k_a} c_{kk'} a_{jk'}) / c_{kk}, \sigma_e^2 / c_{kk}), k = 1, 2, \ldots, k_a \quad (58)$$

and

$$\mathbf{d}_{jk} | \mathbf{y}, \mathbf{X}, \boldsymbol{\theta}_{-d_{jk}} \sim \text{N}((h_k - \sum\limits_{k' \neq k}^{k_d} f_{kk'} a_{jk'}) / f_{kk}, \sigma_e^2 / f_{kk}), k = 1, 2, \ldots, k_d \quad (59)$$

$$\sigma_{a_j}^2 | \mathbf{y}, \mathbf{X}, \boldsymbol{\theta}_{\sigma_{a_j}^2} \sim \text{Inv} - \chi^2(v_{a_j} + k_a, \frac{v_{a_j} s_{a_j}^2 + \mathbf{a}_j^T \mathbf{a}_j}{v_{a_j} + k_a}) \quad (60)$$

UPDATING MARKER AND QTL MEIOSIS INDICATORS. The space of meiosis indicators at marker loci is determined by observed marker data. If the marker data is fully informative, the descent graph is unique and there is no need to update. In most situations, however, there may exist partially informative and/or missing markers so that the observed data correspond to a variety of descent graphs in large complicated pedigrees. What is wanted is to generate realizations of such descent graphs using MCMC simulations. The joint posterior distribution for all marker meiosis indicators is form-simple, or $p(\mathbf{S}|\mathbf{M})$. Meiosis indicators are *a priori* independent over all individuals at a marker locus. However, these indicators become dependent when conditioning on the observed marker phenotypes, because changing the meiosis indicators of an individual may lead to a corresponding change for the meiosis indicators of descendants. In a complex pedigree, therefore, updating meiosis indicators is a complicated issue.

Sobel and Lange (1996) developed a Metropolis algorithm to sample meiosis indicators, called the descent graph sampler. Suppose that the current state of meiosis indicators is denoted by $\mathbf{S}$, and a proposal from a proposal distribution $q(\mathbf{S}^*;\mathbf{S})$ is $\mathbf{S}^*$, then the acceptance ratio in M–H algorithm is (see equation 62 at bottom of page).

To implement the above M–H algorithm, the first requirement is to devise transition rules for moving between descent graphs. Thompson (1994, 1996) suggested a simple move that works as follows. A meiosis indicator is selected at random from the set of meiosis indicators over all markers and individuals; this process is completed by selecting a marker and a meiosis at random. Suppose that the selected meiosis indicator is that of the paternal allele for the ith individual at the kth marker or s_{ik}^p, a change from $s_{ik}^p = s$ to $s_{ik}^p = 1 - s$ is proposed. Due to the randomness of selecting the meiosis indicator, it can be shown that the proposal distribution is symmetric, or $q(\mathbf{S}^*;\mathbf{S}) = q(\mathbf{S};\mathbf{S}^*)$ (Sobel and Lange, 1996). Under the assumption of no genetic interference in meiosis, the acceptance ratio (62) reduces to (see equation 63 at bottom of page),

where $\mathbf{S}_{k\cdot}\{s_{ki}^p, s_{ki}^m\}_{i=m+1}^n$ and $\mathbf{M}_{k\cdot}\{\mathbf{M}_{ki}\}_{i=1}^n$ represent the meiosis indicators and the observed marker data at the kth marker for all individuals. The terms $p(s_{ki}^p|s_{(k-1)i}^p, s_{(k+1)i}^p)$ and $p(s_{ki}^{p*}|s_{(k-1)i}^{p*}, s_{(k+1)i}^{p*})$ can easily be calculated from genetic linkage laws. Efficient algorithms for calculating $p(\mathbf{M}_{k\cdot}|\mathbf{S}_{k\cdot})$ and $p(\mathbf{M}_{k\cdot}|\mathbf{S}_{k\cdot}^*)$ have been given by Sobel and Lange (1996) and Thompson (2001). This proposal only changes the recombinant/non-recombinant status in the two intervals adjoining marker k. Sobel and Lange (1996) and Lange (1997) devised several other transition rules to permit bigger moving steps and thus faster mixing of the Markov chain. Furthermore, a combination of these transition rules per step of the chain also guarantees irreducibility of the Markov chain, provided that recombination ratios between adjacent loci are strictly positive.

The meiosis indicators at QTL can be updated similarly. Unlike marker loci where the observed data put constraints on the number of legal descent graphs, all descent graphs at a QTL are legal because QTL genotypes are unobservable. However, the probability of a descent graph at a putative

$$\sigma_{d_j}^2 \Big| \mathbf{y}, \mathbf{X}, \boldsymbol{\theta}_{\sigma_{d_j}^2} \sim \text{Inv} - \chi^2(v_{d_j} + k_d, \frac{v_{d_j} s_{d_j}^2 + \mathbf{d}_j^T \mathbf{d}_j}{v_{d_j} + k_d}), j = 1, 2, \ldots, l \tag{61}$$

$$\alpha = \frac{p(\mathbf{S}^*|\mathbf{M})/q(\mathbf{S}^*;\mathbf{S})}{p(\mathbf{S}|\mathbf{M})/q(\mathbf{S};\mathbf{S}^*)} = \frac{p(\mathbf{M}|\mathbf{S}^*)p(\mathbf{S}^*)/q(\mathbf{S}^*;\mathbf{S})}{p(\mathbf{M}|\mathbf{S})p(\mathbf{S})/q(\mathbf{S};\mathbf{S}^*)} \tag{62}$$

$$\alpha = \frac{p(\mathbf{M}|\mathbf{S}^*)p(\mathbf{S}^*)}{p(\mathbf{M}|\mathbf{S})p(\mathbf{S})} = \frac{p(\mathbf{M}_{k\cdot}|\mathbf{S}_{k\cdot}^*)p(s_{ki}^{p*}|s_{(k-1)i}^p, s_{(k+1)i}^p)}{p(\mathbf{M}_{k\cdot}|\mathbf{S}_{k\cdot})p(s_{ki}^p|s_{(k-1)i}^p, s_{(k+1)i}^p)} \tag{63}$$

QTL is determined by the trait values and the descent graphs of the flanking markers. The acceptance ratio can be expressed as (see equation 64 at bottom of page),

where $\mathbf{S}_{(-k).} = \{s_{ji}\}_{j \neq k, i = m+1}^{l, n}$ represents all QTL meiosis indicators except those at the kth QTL; $p(\mathbf{y} \mid \mathbf{S})$ and $p(\mathbf{y} \mid \mathbf{S}_k^*, \mathbf{S}_{(-k).})$ are calculated using equation (45).

Although the descent graph sampler described above is not restricted to particular pedigree structures and has been shown to be useful for markers with a multitude of missing data (Sobel and Lange, 1996), it is a single-site M–H algorithm. A major difficulty with the MCMC method is to ensure proper mixing of the samples, and hence efficient Monte Carlo estimates. For complex pedigrees, single-site updating methods are not effective. Recently, a variety of joint-updating schemes have been developed. There are two kinds: updating jointly all meiosis indicators at a single locus, called M-sampler, or joint updating of meiosis indicators for all loci in a single meiosis, called L-sampler. For some pedigrees, these two kinds of sampler can be combined to produce more efficient samplers (ML-sampler).

The M-samplers are whole-locus sampling schemes and implemented on a locus-by-locus basis. If locus k under consideration is a marker locus, then the fully conditional distribution of $\mathbf{S}_{k.}$ given the observed marker data $\mathbf{M}$ and all other meiosis indicators $\mathbf{S}_{(-k).} = \{s_{ji}\}_{j \neq k, i = m+1}^{K+l, n}$ is:

$$p(\mathbf{S}_{k.} \mid \mathbf{S}_{(-k).}, \mathbf{M}) = p(\mathbf{S}_{k.} \mid \mathbf{S}_{(k-1).}, \mathbf{S}_{(k+1).}, \mathbf{M}_{k.})$$
(65)

That is, the distribution depends only on current values of the meiosis indicators of the flanking loci $\mathbf{S}_{(k-1).}$ and $\mathbf{S}_{(k+1).}$ and the observed marker data at $\mathbf{M}_{k.}$. If the locus is a putative QTL, then the fully conditional distribution of $\mathbf{S}_{k.}$ given the trait values $\mathbf{y}$ and all other meiosis indicators $\mathbf{S}_{(-k).}$ is:

$$p(\mathbf{S}_{k.} \mid \mathbf{S}_{(-k).}, \mathbf{y}) = p(\mathbf{S}_{k.} \mid \mathbf{S}_{(k-1).}, \mathbf{S}_{(k+1).}, \mathbf{y}) \,(66)$$

Sampling from $p(\mathbf{S}_{k.} \mid \mathbf{S}_{(-k).}, \mathbf{M})$ or $p(\mathbf{S}_{k.} \mid \mathbf{S}_{(-k).}, \mathbf{y})$ is complicated for general pedigrees.

The M-sampler is a great improvement over single-site methods. However, when there are multiple tightly linked marker loci, mixing can be poor. An alternative form of block-updating is to update jointly the meiosis indicators for markers in a given meiosis $\mathbf{S}_{.i}$. The L-sampler is a whole-meiosis Gibbs sampler for $\mathbf{S}_{.i}$, and can be implemented in a meiosis-by-meiosis basis (Thompson and Heath, 1999; Thompson, 2001). To update jointly the meiosis indicators of meiosis i, it is necessary to compute the following fully conditional distribution:

$$p(\mathbf{S}_{.i} \mid \{\mathbf{S}_{.i'}, i' \neq i\}, \mathbf{M})$$
(67)

Thompson and Heath (1999) developed an algorithm to update the meiosis indicators sequentially along the chromosome, which is similar to single-locus pedigree peeling (Elston and Stewart, 1971). It has been shown that, even for very tight linkage, the L-sampler can perform well. Furthermore, implementation of the M-sampler is almost unaffected by the complexity of the pedigree (Thompson and Heath, 1999).

UPDATING THE LOCATIONS OF QTL. The QTL locations λ are updated on a locus-by-locus basis. It is noted that the distribution of the meiosis indicators at a QTL is highly dependent on the location of the QTL. Therefore, when the location of a QTL is proposed, the meiosis indicators at this QTL should be redrawn. This updating scheme can greatly improve the mixing of the MCMC. However, the joint conditional posterior distribution for the location and the meiosis indicators at the jth QTL has a nonstandard form, i.e. (see equation 68 at bottom of page),

where $\boldsymbol{\theta}_{-(\lambda_j, s_{j.})}$ represents all elements of $\boldsymbol{\theta}$ except λ_j and $\mathbf{S}_{j.}$, $\mathbf{S}_{j.}^L$ and $\mathbf{S}_{j.}^R$ are the meiosis

$$\alpha = \frac{p(\mathbf{y} \mid \mathbf{S}^*)p(\mathbf{S}^*)}{p(\mathbf{y} \mid \mathbf{S})p(\mathbf{S})} = \frac{p(\mathbf{y} \mid \mathbf{S}_{k.}^*, \mathbf{S}_{(-k).})p(s_{ki}^{p*} \mid s_{(k-1)i}^p, s_{(k+1)i}^p)}{p(\mathbf{y} \mid \mathbf{S})p(s_{ki}^p \mid s_{(k-1)i}^p, s_{(k+1)i}^p)}$$
(64)

$$p(\lambda_j, \mathbf{S}_{j.} \mid \mathbf{y}, \mathbf{X}, \boldsymbol{\theta}_{-(\lambda_j, s_{j.})}) \propto p(\mathbf{y} \mid \mathbf{X}, \boldsymbol{\theta})p(\lambda_j)p(\mathbf{S}_{j.} \mid \mathbf{S}_{j.}^L, \mathbf{S}_{j.}^R, \lambda_j)$$
(68)

indicators for the left and the right flanking loci of the jth QTL, respectively. M–H algorithm is used to draw samples from this distribution. Let us denote the proposal distributions for λ_j^* and $\mathbf{S}_{j\cdot}^*$ by $q(\lambda_j^*; \lambda_j)$ and $q(\mathbf{S}_{j\cdot}^*; \lambda_j^*)$, respectively. The proposals λ_j^* and $\mathbf{S}_{j\cdot}^*$ are then accepted simultaneously with probability min$\{1, r\}$, where (see equation 69 at bottom of page).

The acceptance probability depends on the proposal distributions for the location and the meiosis indicators. New location λ_j^* is generally sampled uniformly from the interval $[\lambda_j - d, \lambda_j + d]$, where d is a predetermined tuning parameter, i.e. $q(\lambda_j^*; \lambda_j) = \tfrac{1}{2}d$ if $\lambda_j^* \in [\lambda_j - d, \ \lambda_j + d]$, and $q(\lambda_j^*; \lambda_j) = 0$. The highest efficiency can be reached if $\mathbf{S}_{j\cdot}^*$ is generated from its fully conditional posterior distribution, i.e. $q(\mathbf{S}_{j\cdot}^*; \lambda_j^*) = p(\mathbf{S}_{j\cdot}^* | \mathbf{y}, \mathbf{X}, \lambda_j^*, \boldsymbol{\theta}_{-(\lambda_j, s_{j\cdot})})$. Under this sampling strategy, the acceptance ratio r has the following form:

$$r = \frac{p(\mathbf{y} | \mathbf{X}, \boldsymbol{\theta}_{-(\lambda_j, s_{j\cdot})}, \lambda_j^*)}{p(\mathbf{y} | \mathbf{X}, \boldsymbol{\theta}_{-(\lambda_j, s_{j\cdot})}, \lambda_j)} \qquad (70)$$

Note that the meiosis indicators at the jth QTL, $S_{j\cdot}$ and $\mathbf{S}_{j\cdot}^*$, have been integrated out in equation (70). Therefore, the acceptance probability is independent of $\mathbf{S}_{j\cdot}$ and $\mathbf{S}_{j\cdot}^*$.

The above sampling strategy requires that $p(\mathbf{S}_{j\cdot}^* | \mathbf{y}, \mathbf{X}, \lambda_j^*, \boldsymbol{\theta}_{-(\lambda_j, s_{j\cdot})})$ is easily sampled. In simple mating designs (e.g. full-sib families) it is easy to sample from this fully conditional posterior distribution, since the meiosis indicators are independent among sibs. For peelable pedigrees, peeling algorithms can be used to sample from $p(\mathbf{S}_{j\cdot}^* | \mathbf{y}, \mathbf{X}, \lambda_j^*, \boldsymbol{\theta}_{-(\lambda_j, s_{j\cdot})})$, although they are computationally intense. In other situations,

Yi and Xu (2001) proposed sampling the meiosis indicators for the ith individual ($i \geq m + 1$) from the following distribution:

$$p(s_{ji}^* | y_i, \mathbf{X}_i, \boldsymbol{\theta}_{-(\lambda_j, s_{j\cdot})}, \lambda_j^*, s_{j(m+1)}^*, \ldots, s_{j(i-1)}^*) \tag*{(71)}$$

This conditional distribution is discrete, and is easily sampled (Yi and Xu, 2001). Therefore, the proposal distribution for $\mathbf{S}_{j\cdot}^*$ is (see equation 72 at bottom of page).

Evidently, this sampling scheme is applicable to any pedigree structures.

UPDATING QTL NUMBER. A birth step requires generation of the location λ_{l+1}, allelic variance $\sigma_{a_{l+1}}^2$, dominance variance $\sigma_{d_{l+1}}^2$, founder allelic effects $\mathbf{a}_{l+1}$, dominance effects between all possible pairs of the founder alleles $\mathbf{d}_{l+1}$, and meiosis indicators $\mathbf{S}_{(l+1)\cdot}$, for the new QTL. The new location λ_{l+1} and the variances $\sigma_{a_{l+1}}^2$ and $\sigma_{d_{l+1}}^2$ are usually sampled from the corresponding prior distributions. There are two ways of generating the allelic effects, dominance effects and the meiosis indicators. The allelic effects $\mathbf{a}_{l+1}$ and the dominance effects $\mathbf{d}_{l+1}$ can be simulated from the distributions $p(\mathbf{a}_{l+1} | \sigma_{a_{l+1}}^2)$ and $p(\mathbf{d}_{l+1} | \sigma_{d_{l+1}}^2)$, respectively. The meiosis indicators for all non-founders are jointly generated using the method of updating QTL meiosis indicators described above. New inheritance and dominance design matrices $\mathbf{Z}_{l+1}^P$, $\mathbf{Z}_{l+1}^m$ and $\mathbf{W}_{l+1}$ are then calculated using recursive equation (4). If the proposal distribution for $\mathbf{S}_{(l+1)\cdot}$ is denoted as $q(\mathbf{S}_{(l+1)\cdot})$, the proposal is then accepted with probability (see equation 73 at bottom of page).

$$r = \frac{p(\mathbf{y} | \mathbf{X}, \boldsymbol{\theta}_{-(\lambda_j, s_{j\cdot})}, \lambda_j^*, \mathbf{S}_{j\cdot}^*) p(\lambda_j^*) p(\mathbf{S}_{j\cdot}^* | \mathbf{S}_{j\cdot}^{*L}, \mathbf{S}_{j\cdot}^{*R}, \lambda_j^*) q(\lambda_j; \lambda_j^*) q(\mathbf{S}_{j\cdot}; \lambda_j)}{p(\mathbf{y} | \mathbf{X}, \boldsymbol{\theta}_{-(\lambda_j, s_{j\cdot})}, \lambda_j, \mathbf{S}_{j\cdot}) p(\lambda_j) p(\mathbf{S}_{j\cdot} | \mathbf{S}_{j\cdot}^L, \mathbf{S}_{j\cdot}^R, \lambda_j) q(\lambda_j^*; \lambda_j) q(\mathbf{S}_{j\cdot}^*; \lambda_j^*)} \qquad (69)$$

$$q(\mathbf{S}_{j\cdot}^*) = \prod_{i=m+1}^{n} p(s_{ji}^* | y_i, \mathbf{X}_i, \boldsymbol{\theta}_{-(\lambda_j, s_{j\cdot})}, \lambda_j^*, s_{j(m+1)}^*, \ldots, s_{j(i-1)}^*) \qquad (72)$$

$$\min\left\{ 1, \frac{p(\mathbf{y} | \boldsymbol{\theta}^*)}{p(\mathbf{y} | \boldsymbol{\theta})} \cdot \frac{p(l+1) \cdot p(\mathbf{S}_{(l+1)\cdot} | \lambda_{l+1}, \mathbf{S}_{(l+1)\cdot}^L, \mathbf{S}_{(l+1)\cdot}^R)}{p(l)} \cdot \frac{\dfrac{q(l; l+1)}{l+1}}{q(l+1; l) \cdot q(\mathbf{S}_{(l+1)\cdot})} \right\} \qquad (73)$$

where $\boldsymbol{\theta}^* = (\boldsymbol{\theta}, \lambda_{l+1}, \mathbf{a}_{l+1}, \mathbf{d}_{l+1}, Z_{l+1}^p, Z_{l+1}^m)$ with l in $\boldsymbol{\theta}$ replaced by $(l+1)$; $\mathbf{S}_{(l+1)\cdot}^L$ ($\mathbf{S}_{(l+1)\cdot}^R$) denotes the meiosis indicators for all pedigree members at the left (right) flanking locus of the location λ_{l+1}.

The death step is somewhat simpler. A random choice is made among the existing QTL, and the chosen QTL is then proposed to be deleted from the model. If the jth existing QTL is proposed to be deleted, the acceptance probability for the deletion is (see equation 74 at bottom of page),

where $\boldsymbol{\theta}^*$ means all elements of $\boldsymbol{\theta}$ except the items corresponding to the jth QTL. Other terms of this equation are defined similarly as in equation (73).

With the algorithm described above, the contributions from the priors and the proposal distributions for λ_{l+1}, $\sigma_{a_{l+1}}^2$, $\sigma_{d_{l+1}}^2$, $\mathbf{a}_{l+1}$, and $\mathbf{d}_{l+1}$ to the acceptance rate have been cancelled out in the acceptance ratio. The efficiency is mainly determined by the proposal distribution for the meiosis indicators. Alternatively, the meiosis indicators can be generated from $p(\mathbf{S}_{(l+1)\cdot}|\lambda_{l+1}, \mathbf{S}_{(l+1)\cdot}^L, \mathbf{S}_{(l+1)\cdot}^R)$ and the allelic and dominance effects from a fully conditional posterior distribution $q(\mathbf{a}_{l+1}, \mathbf{d}_{l+1}) = p(\mathbf{a}_{l+1}, \mathbf{d}_{l+1}|\mathbf{y}, \mathbf{X}, \boldsymbol{\theta}, \lambda_{l+1}, \sigma_{a_{l+1}}^2, \sigma_{d_{l+1}}^2, \mathbf{S}_{(l+1)\cdot})$, which is a multivariate normal distribution. The acceptance probabilities for a birth step and a death step are (see equations 75 and 76 at bottom of page), respectively.

Some Extensions

Founders from different source populations

If the founders are sampled from more than one reference population with heterogeneity in QTL effects, the mapping population or pedigree is a structured population. The linear mixed model described earlier can be called the homogeneity model and is insufficient to describe the genetic architecture of a quantitative trait in a structured population. This leads to the heterogeneity model approach for genetic mapping (Peterz-Enciso and Varona, 2000; Xu and Yi, 2000).

Assume that there are K source populations ($K > 1$) and an allele in the mapping population comes from the hth source population with probability $p_h(\sum_{h=1}^{K} p_h = 1)$. The expectation and the variance of the allelic effect of the jth QTL in the hth source population are denoted by b_{jh} and σ_{jh}^2, respectively. The overall allelic variance for the jth QTL can be partitioned into between- and within-population variances, i.e.:

$$\sigma_{a_j}^2 = \underbrace{\sum_{h=1}^{K} p_h (b_{jh} - \sum_{h=1}^{K} p_h b_{jh})^2}_{\text{between-population}} + \underbrace{\sum_{h=1}^{K} p_h \sigma_{jh}^2}_{\text{within-population}}$$

(77)

The constraint $\sum_{h=1}^{K} p_h b_{jh} = 0$ is needed to assure that $E(\mathbf{y}) = \mathbf{X}\boldsymbol{\beta}$. It can be seen that a

$$\min\left\{1, \frac{p(\mathbf{y}|\boldsymbol{\theta}^*)}{p(\mathbf{y}|\boldsymbol{\theta})} \cdot \frac{p(l-1)}{p(l) \cdot p(\mathbf{S}_{j\cdot}|\lambda_{j\cdot}, \mathbf{S}_{j\cdot}^L, \mathbf{S}_{j\cdot}^R)} \cdot \frac{q(l; l-1) \cdot q(\mathbf{S}_{j\cdot})}{\dfrac{q(l-1; l)}{l}}\right\}$$

(74)

$$\min\left\{1, \frac{p(\mathbf{y}|\boldsymbol{\theta}^*)}{p(\mathbf{y}|\boldsymbol{\theta})} \cdot \frac{p(l+1) \cdot p(\mathbf{a}_{l+1}|\sigma_{a_{l+1}}^2) \cdot p(\mathbf{d}_{l+1}|\sigma_{d_{l+1}}^2)}{p(l)} \cdot \frac{\dfrac{q(l; l+1)}{l+1}}{q(l+1; l) \cdot q(\mathbf{a}_{l+1}, \mathbf{d}_{l+1})}\right\}$$

(75)

$$\min\left\{1, \frac{p(\mathbf{y}|\boldsymbol{\theta}^*)}{p(\mathbf{y}|\boldsymbol{\theta})} \cdot \frac{p(l-1)}{p(l) \cdot p(\mathbf{a}_j|\sigma_{a_j}^2) p(\mathbf{d}_j|\sigma_{d_j}^2)} \cdot \frac{q(l; l+1) \cdot q(\mathbf{a}_j, \mathbf{d}_j)}{\dfrac{q(l-1; l)}{l}}\right\}$$

(76)

QTL can be detected when either the within- or between-population variance is significant. Therefore, the heterogeneity model will increase the power of gene detection for structured populations.

In a structured population, the trait phenotypes can still be expressed by equation (3). For simplicity, the dominance effects are ignored. The model now becomes:

$$\mathbf{y} = \mathbf{X\beta} + \sum_{j=1}^{l} (\mathbf{Z}_j^p + \mathbf{Z}_j^m)\mathbf{a}_j + \mathbf{e} \qquad (78)$$

Here the vector of allelic effects $\mathbf{a}_j$ is completely different from that in equation (3). Suppose that m_h founders come from the hth source population ($\sum_{h=1}^{K} m_h = m$)). Let $\mathbf{a}_j$ be decomposed into $\mathbf{a}_j = (\mathbf{a}_{j1}^T, \mathbf{a}_{j2}^T, \ldots, \mathbf{a}_{jK}^T)^T$, where $\mathbf{a}_{jh}$ is a vector containing all the founder allelic values of the jth QTL from the hth source population (see equations 79 and 80 at bottom of page),

where $\mathbf{1}_n$ is a unity vector of order n and $\mathbf{I}_n$ is an $n \times n$ identity matrix. This model assumes that the distribution of allelic effects consists of the mean and the variance of each source population, reflecting the heterogeneity for the source populations. The model is therefore called the heterogeneity model. The linear mixed model discussed in section (3) does not allow this type of heterogeneity and is therefore called the homogeneity model.

Under the heterogeneity model, QTL mapping can be carried out by either ML approach or Bayesian statistics. Peterz-Enciso and Varona (2000) developed an ML method for an F_2 population derived from two outbred populations, while Xu and Yi (2000) took a Bayesian framework implemented via the reversible jump MCMC algorithm. The latter can deal with complicated pedigrees with multiple generations.

Correlated founders

Founders are defined as individuals whose parents are unspecified in the pedigree. So far we have discussed QTL mapping methods that are suitable for pedigrees with non-inbred and unrelated founders. However, these methods can easily be modified to cases where the founders are inbred and/or genetically related. To take into consideration the genetic relationship of the founder alleles, the identity matrices in the covariances of the founder allelic and dominance effects, $\mathbf{a}_j$ and $\mathbf{d}_j$, are replaced to the expectations of IBD matrices, i.e. $\mathrm{Cov}(\mathbf{a}_j) = \mathbf{H}_a\sigma_{a_j}^2$ and $\mathrm{Cov}(\mathbf{d}_j) = \mathbf{H}_d\sigma_{d_j}^2$. Note that the matrices $\mathbf{H}_a$ and $\mathbf{H}_d$ are non-identity matrices and are determined by the genetic relationship among the founder alleles.

QTL mapping in pedigrees with inbred and/or related founders can be implemented via either ML or Bayesian methods. Note that the covariance matrices of the allelic and dominance effects for all members of the pedigree are derived as:

$$\mathrm{Cov}[(\mathbf{Z}_j^p + \mathbf{Z}_j^m)\mathbf{a}_j]$$
$$= \mathrm{E}[\mathrm{Cov}(\mathbf{Z}_j^p + \mathbf{Z}_j^m)\mathbf{a}_j] + \mathrm{Cov}[\mathrm{E}(\mathbf{Z}_j^p + \mathbf{Z}_j^m)\mathbf{a}_j]$$
$$= \mathrm{E}[(\mathbf{Z}_j^p + \mathbf{Z}_j^m)^T \mathrm{Cov}(\mathbf{a}_j)(\mathbf{Z}_j^p + \mathbf{Z}_j^m)]$$
$$+ \mathrm{Cov}[(\mathbf{Z}_j^p + \mathbf{Z}_j^m)\mathrm{E}(\mathbf{a}_j)]$$
$$= \mathrm{E}[\mathrm{Cov}(\mathbf{Z}_j^p + \mathbf{Z}_j^m)\mathbf{a}_j]$$
$$= \mathrm{E}[(\mathbf{Z}_j^p + \mathbf{Z}_j^m)\mathbf{H}_a(\mathbf{Z}_j^p + \mathbf{Z}_j^m)^T]\sigma_{a_j}^2 \qquad (81)$$

and

$$\mathrm{Cov}(\mathbf{W}_j\mathbf{d}_j) = \mathrm{E}[\mathrm{Cov}(\mathbf{W}_j\mathbf{d}_j)] + \mathrm{Cov}[\mathrm{E}(\mathbf{W}_j\mathbf{d}_j)]$$
$$= \mathrm{E}[\mathbf{W}_j^T\mathrm{Cov}(\mathbf{d}_j)\mathbf{W}_j] + \mathrm{Cov}[\mathbf{W}_j\mathrm{E}(\mathbf{d}_j)]$$
$$= \mathrm{E}[\mathbf{W}_j^T\mathrm{Cov}(\mathbf{d}_j)\mathbf{W}_j] = \mathrm{E}[\mathbf{W}_j^T\mathbf{H}_d\mathbf{W}_j]\sigma_{d_j}^2 \quad (82)$$

respectively. Therefore, the expectations of IBD matrices become

$$\mathrm{E}(\Pi_j) = \mathrm{E}[(\mathbf{Z}_j^p + \mathbf{Z}_j^m)\mathbf{H}_a(\mathbf{Z}_j^p + \mathbf{Z}_j^m)^T] \qquad (83)$$

and

$$\mathrm{E}(\mathbf{a}_j) = [\mathrm{E}(\mathbf{a}_{j1}^T)\,\mathrm{E}(\mathbf{a}_{j2}^T)\ldots\mathrm{E}(\mathbf{a}_{jK}^T)]^T = [b_1\mathbf{1}_{2_{m1}}^T\; b_1\mathbf{1}_{2_{m2}}^T\;\ldots\; b_k\mathbf{1}_{2_{mk}}^T]^T \qquad (79)$$

$$\mathrm{Cov}(\mathbf{a}_j) = \mathrm{diag}[\mathrm{Cov}(\mathbf{a}_{j1})\,\mathrm{Cov}(\mathbf{a}_{j2})\ldots\mathrm{Cov}(\mathbf{a}_{jK})] = \mathrm{diag}[\sigma_{a_{j1}}^2\mathbf{I}_{2_{m1}}\,\sigma_{a_{j2}}^2\mathbf{I}_{2_{m2}}\ldots\sigma_{a_{jk}}^2\mathbf{I}_{2_{mk}}] \qquad (80)$$

$$E(\boldsymbol{\Delta}_j) = E[\mathbf{W}_j^T \mathbf{H}_d \mathbf{W}_j] \qquad (84)$$

To implement an ML method or a Bayesian method as described in earlier sections, the first requirement is to derive $E[(\mathbf{Z}_j^p + \mathbf{Z}_j^m) \mathbf{H}_a (\mathbf{Z}_j^p + \mathbf{Z}_j^m)^T]$ and $E[\mathbf{W}_j^T \mathbf{H}_d \mathbf{W}_j]$. This can easily be done for simple pedigrees, e.g. multiple F_2 families derived from inbred lines and sib mating designs (Xie *et al.*, 1998a,b). For complex pedigrees, algorithms described previously may need to be modified. Finally, the full Bayesian analysis of QTL mapping can easily be extended to inbred and/or related founders. The prior distributions for the allelic and dominance effects become;

$$\mathbf{a}_j \sim N_{k_a}(0, \mathbf{H}_a \sigma_{a_j}^2) \qquad (85)$$

and

$$\mathbf{d}_j \sim N_{k_d}(0, \mathbf{H}_d \sigma_{d_j}^2) \qquad (86)$$

The posterior distributions for the allelic and dominance effects and variances are then modified accordingly. Yi and Xu (2001) developed a Bayesian mapping for general pedigrees. Their method can deal with inbred founders.

Combined linkage and association analysis

The theoretical basis for QTL mapping is non-random genetic association between QTL and markers or linkage disequilibrium. The most commonly employed approaches for QTL mapping are pedigree-based linkage analysis and population-based association or linkage disequilibrium analysis. With pedigree analysis, the non-random association is measured by the dependent segregation between a putative QTL and markers. This is because it is possible to trace each allele or haplotype of a pedigree member to its origin in the founders. The chromosomal regions that explain a significant proportion of the phenotypic variance are candidate positions of QTL. However, linkage analysis uses only the recombinations that occurred within the pedigree. The number of recombination events within a pedigree is usually not sufficiently large to allow fine mapping, unless the number of individuals per generation in the pedigree is very large. Therefore, pedigree linkage analysis cannot usually map genes into their location in a fine scale, although it is a powerful tool for a genome-wide scan for QTL.

Linkage disequilibrium mapping is another widely used mapping tool, especially in humans. In linkage disequilibrium mapping, the degree of non-random association, produced by various evolutionary forces such as mutation, drift, selection and admixture, is estimated and used to infer the strength of the linkage between two loci. In contrast to pedigree analysis, linkage disequilibrium mapping takes advantage of the historical recombination events between loci. The disequilibrium of two loci that occurred a long time ago decays progressively by recombination to reach an asymptotic equilibrium. The speed of the decay depends on the distance between the gene and the marker. Because of the cumulative nature of historical recombinations, linkage disequilibrium between a gene and a marker will be destroyed quickly unless the linkage is very tight. The significant linkage disequilibrium detected may suggest close physical linkage between the two loci. Therefore, linkage disequilibrium mapping seems to be more useful for precise estimation of QTL positions.

Linkage and linkage disequilibrium alone utilize only partial information available for mapping QTL. The former exploits linkage information contained in the pedigree, but not linkage disequilibrium accumulated in the base population from which the founders of the pedigree come. Most existing methods for linkage analysis are based on the assumption of linkage equilibrium between QTL and markers. In fact, QTL may be in linkage disequilibrium with their flanking polymorphic markers under certain conditions. Therefore, the assumption of linkage equilibrium can dramatically reduce the statistical power for detecting QTL and the precision for the estimation of QTL locations as well. In contrast to linkage analysis, linkage disequilibrium mapping only incorporates linkage disequilibrium information in the population, but not

linkage information in the pedigree. Therefore, much genetic information embedded in the pedigree is left out. Furthermore, information that has been collected from pedigrees in the past becomes irrelevant.

When the reference population is in linkage disequilibrium, a more desirable procedure for QTL mapping is to combine the pedigree linkage analysis and the linkage disequilibrium mapping. Such a procedure can be implemented via a two-step approach (e.g. Zheng and Elston, 1999). The first step is linkage analysis using the pedigree data only. This is a coarse-scale mapping step in which chromosome regions are identified. The second step is fine-scale mapping using the combined analysis in which the focus is only on the regions of interest. The combined analysis can increase both the power of gene detection and the precision of gene localization. The combined analysis will benefit the most when there are many founders. Several combined linkage and linkage disequilibrium mapping methods have been proposed. The transmission/disequilibrium test (TDT) is actually designed to test the presence of both linkage and linkage disequilibrium between a marker and a binary disease locus. Allison (1997) extended the TDT methods to QTL mapping. However, the TDT is only suitable for nuclear families commonly seen in human populations and is difficult to apply to arbitrary pedigrees. Fulker *et al.* (1999) proposed a combined linkage and association sib-pair analysis for quantitative traits using ML variance component approaches. The method involves modelling of the allelic means for a test of association, with simultaneous modelling of sib-pair covariance structure for a test of linkage. The method of Fulker *et al.* (1999) was extended to larger sibships by Sham *et al.* (2000) and Abecasis *et al.* (2000).

For general pedigrees, Almasy *et al.* (1999) developed an extension to the ML variance component linkage approach that exploits disequilibrium to fine-map QTL. The method uses identity-by-state (IBS) information to supplement IBD information. Only IBD information is used for modelling the covariance between non-founders, but IBS information is used for covariances between founders. Because disequilibrium information is included in IBS measurements, the method makes efficient use of the linkage disequilibrium information in the base population. Xiong and Li (2000) developed an ML-based combined analysis that can be applied to general pedigrees. The method retains the framework of the linkage analysis and only changes the haplotype frequencies at the marker and QTL of founders in the likelihood function for linkage analysis. Because the haplotype frequencies include disequilibrium coefficients, the method can exploit linkage disequilibrium to increase the power of QTL analysis.

Conclusions

Random genetic models, based on the simple premise that individuals of like phenotype are more likely to share IBD, offer a conventional statistical environment in which to separate genetic variances from environmental variance. With the availability of marker genotype information, random genetic models have been utilized by human geneticists and animal geneticists interested in partitioning the genetic variance of quantitative traits into effects due to specific chromosomal regions. A variety of statistical methods have been developed to analyse random genetic models. The IBD-based variance component approach has been recognized as a very useful QTL mapping method not only for animal populations but also for human pedigrees, in particular with the development of the software packages such as SOLAR. This approach captures all the essential features of genetic architecture without relying on the specification of unknowable parameters, such as the number of alleles, allelic frequencies and the genotype-specific means and variances. Much progress has been achieved in QTL mapping with the application of the Bayesian methodology and MCMC algorithms. However, numerous problems and improvements remain to be explored, including the incorporation of epistasis and genotype × environment interaction into random genetic model

approaches, and developing optimal sampling strategies for reversible jump MCMC. Fine-mapping methods for animal populations are much less developed than linkage methods. Statistical methods combining linkage and linkage disequilibrium mapping need to be fully developed.

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26 Structural Genomics: Integrating Linkage, Physical and Sequence Maps

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Introduction

Maps are images of the relative positions of objects in space. In that respect, mapping genes along a chromosome essentially is no different than drawing a roadmap of a particular city. Furthermore, as roadmaps exist at different levels of resolution, so do maps of chromosomes. Roadmaps can be drawn where only the highways are indicated. On the other hand, a map could be drawn where every individual house is drawn to scale.

Mapping of genes, chromosomes and whole genomes can also take place at different levels of resolution. Maps that assign genes to locations on particular chromosomes can be constructed using a number of different methods, each with its own particular features and level of resolution. The order and position of genes along the different chromosomes can be deduced based on the co-segregation of these genes in genetic pedigrees (linkage mapping) or more directly by physical localization on the chromosomes themselves (physical mapping by *in situ* hybridization on metaphase chromosomes). Both of these methods produce gene maps of medium resolution in the order of several million base pairs (bp). The use of radiation hybrid cell panels and particularly the construction of contigs of cloned DNA such as bacterial artificial chromosomes (BACs) enables a much higher level of (physical) mapping resolution, in the order of 100,000 bp. The ultimate map will eventually be the complete nucleotide sequence of each of the 39 different chromosomes of the chicken genome.

The chicken has a long-standing history as an experimental model in research areas ranging from embryology and virology to genetics and molecular biology. Easy access to the chicken embryo explains why the chicken is one of the best-characterized vertebrate models. In virology, the chicken was the first species in which a tumour-causing virus was detected (Rous, 1911). In genetics, even at the beginning of the 20th century, Bateson used the chicken for pioneering studies on Mendelian inheritance and sex determination (Bateson, 1902; Bateson and Punnett, 1906, 1908). The first linkage map that was published for a domestic animal was that of the chicken, described by Hutt (1936). In the early days of the analysis of the structure and functioning of eukaryotic genes, yet again the chicken was among the first species to be used as a model organism. Chicken ovalbumin (Breatnach *et al.*, 1977; Woo *et al.*, 1978) and globin (Dodgson *et al.*, 1979) genes were among the first eukaryotic genes for which it was shown that genes in eukaryotes were split into introns and exons.

There are a number of different reasons for the interest in the chicken also as a model organism in genomics.

1. The genome size is only one-third of that of mammals (Tiersch and Wachtel, 1991), mainly due to its low number of repetitive sequences and reduced intron sizes (Hughes and Hughes, 1995).
2. It has an interesting complex genomic structure ($2n = 78$) with two chromosomal subtypes, the macrochromosomes and microchromosomes (Bloom *et al.*, 1993). The microchromosomes appear to be somewhat more gene dense than the macrochromosomes, reaching densities comparable to that of the *Fugu* genome (McQueen *et al.*, 1998; Clark *et al.*, 1999).
3. Being a bird, it is ideally placed in the evolutionary phylogenetic tree with regard to human, mouse and zebrafish, the first higher vertebrates whose complete genome sequence will soon become available.

Linkage Mapping

Basic characteristics of linkage mapping

A genetic linkage map shows the relative locations of specific loci along the chromosomes. There are two requirements for linkage mapping: (i) pedigrees in which the relationships are known; and (ii) polymorphic loci (genetic markers). Any inherited physical or molecular characteristic that differs among individuals and is easily detectable in the laboratory can be used as a potential genetic marker. Markers must be polymorphic to be useful in linkage mapping; that is, alternative forms must exist among individuals so that they are detectable among different members in family studies. Nowadays, most markers used in linkage mapping are based on variations at the DNA level. Such variations can occur within exon sequences, in which case they can lead to observable changes, such as differences in eye colour, blood type and disease susceptibility. Most variations, however, occur within introns and have little or no effect on an organism's appearance

or function, yet they are detectable at the DNA level and can be used as markers.

Two markers located near each other on the same chromosome will tend to be passed on together from the parent to its offspring. During the normal production of sperm and egg cells, DNA strands occasionally break and rejoin in different places on the same chromosome or on the other copy of the same chromosome (i.e. the homologous chromosome). This process, called meiotic recombination, can result in the separation of two markers originally on the same chromosome. The closer the markers are to each other, the less likely it is that a recombination event will separate them. Recombination frequency thus provides an estimate of the distance between two markers. On the genetic map, distances between markers are measured in terms of centimorgans (cM), named after the American geneticist Thomas Hunt Morgan. Two markers are said to be 1 cM apart if they are separated by recombination 1% of the time.

Normally, the existence or absence of linkage is indicated by the size of the LOD score. The LOD score, Z, introduced by Morton (1955), is the logarithm of the odds that the loci are linked (with recombination fraction θ) rather than unlinked (recombination fraction 0.5). LOD scores are a function of the recombination fraction and therefore are calculated for a range of θ values. The θ for which the maximum value of Z is calculated gives the most likely recombination fraction between the two loci tested. In a two-point analysis, the LOD score is calculated for any given pair of loci. The criteria used for linkage and the rejection of linkage (exclusion) are +3 and −2, respectively. When a LOD score at a certain θ is 3, the odds of linkage against no linkage between two randomly chosen loci is 1000 (10^3) to 1. The overall odds in favour of linkage with a LOD score of 3 and a genome of 2000–4000 cM in size will then be 20 to 1.

Genetic maps are classified into a number of types, largely by the statistical criteria used to construct them. A *framework map* is a map where the placement of individual loci has a statistical support of at least 1000:1. This means that the difference in

log-likelihoods between the framework map order and any other made by changing the position of any one marker must be at least 3 (since log1000 = 3.0). An *inclusive map*, sometimes referred to as a *comprehensive map*, is a map where markers are included in their most likely positions irrespective of the statistical support. The utility of such a map is to make statements about the positions of markers that cannot be placed with framework support. An *approximate map* is one where the position of markers is shown as the range of intervals that a particular marker could occupy at framework support. These are probably more informative than inclusive maps, since the markers do not upset the stability of the framework map.

Linkage mapping in the chicken

In the chicken, the loci used to construct the first generation of linkage maps consisted primarily of morphological, immunological and physiological genetic markers and loci affecting feather colour and form. The latest update of this 'classical' linkage map (Bitgood and Somes, 1993) contained 119 loci. Only 82 loci could be grouped into eight linkage groups. Furthermore, 38 of these 82 could not be mapped precisely. These early linkage maps were strongly hampered by the limited availability of useful polymorphic loci that were segregating in chicken populations and most of these loci were segregating only in specific chicken flocks, necessitating the production of many different test crosses for linkage mapping. It was therefore not until the development of large numbers of molecular DNA markers and specific mapping populations in the last decade of the 20th century that the generation of linkage mapping in the chicken was really boosted. Integration of the classical linkage groups has been attempted by linking some of the molecular markers to classical mutant phenotypes such as extension (Takeuchi *et al.*, 1996; Sazanov *et al.*, 1998), dominant white (Ruyter-Spira *et al.*, 1997), naked neck (Pitel *et al.*, 2000), polydactyly (Pitel *et al.*,

2000), pea-comb (Bartlett *et al.*, 1996), blue eggshell (Bartlett *et al.*, 1996), dermal melanin inhibitor (Levin *et al.*, 1993), sex-linked dwarfism (Burnside *et al.*, 1991; Crittenden *et al.*, 1993) and late feathering K (Bacon *et al.*, 1988; Levin and Smith, 1990; Hamoen *et al.*, 2001). These results indicate that the classical linkage groups I, IV and Z correspond to chromosomes 1, 2 and Z, and linkage group II to linkage group E22C19W28. These results also clearly show that several of the linkages reported in the past are not correct. For example, naked neck and blood group P are located not on chromosome 1 (linkage group I) but on chromosome 3 and extension of eumelanin (*MC1R*) is not located on chromosome 1 but located on microchromosome 11 (Takeuchi *et al.*, 1996; Sazanov *et al.*, 1998; Kerje *et al.*, 2003, unpublished).

Mapping populations

In the chicken, three different populations have been used for the construction of a genetic map.

1. The East Lansing population (Crittenden *et al.*, 1993) consists of 52 BC1 animals derived from a backcross (BC) between a partially inbred jungle fowl line and a highly inbred White Leghorn line.

2. The Compton population (Bumstead and Palyga, 1992) consists of 56 BC1 animals derived from a backcross between two inbred White Leghorn lines that differed in their resistance to salmonella. Reference panel DNAs from these two mapping populations are available and have been distributed widely to the poultry community.

3. The Wageningen population (Groenen *et al.*, 1998) consists of 456 F_2 animals from a cross between two broiler dam lines originating from the White Plymouth Rock breed. The number of informative meioses for the two backcross populations varies from 20 to 56 and the average mapping resolution therefore is only 5–7 cM. In the Wageningen population, the number of informative meioses varies from 15 to

886 with an average mapping resolution of 1 cM.

Chicken linkage maps based on DNA markers

The first three linkage maps based completely on DNA markers were all comprehensive maps. The first of these was published by Bumstead and Palyga (1992); it was based on the Compton (C) reference population and consisted solely of restriction fragment length polymorphism (RFLP) markers. The second genetic map to be published (Levin *et al.*, 1993, 1994) was based on the East Lansing (EL) reference population and consisted primarily of RFLPs, random amplified polymorphic DNA (RAPD) markers and chicken repeat element 1 (CR1) markers. Since then, both populations have been used to map a considerable number of microsatellite markers (Cheng *et al.*, 1995; Crooijmans *et al.*, 1996, 1997; Gibbs *et al.*, 1997) and amplified fragment length polymorphism (AFLP) markers (Knorr *et al.*, 1999) as well. The third map (Groenen *et al.*, 1998; Herbergs *et al.*, 1999) was based on a large F_2 population and consisted solely of microsatellite and AFLP markers, although recently several single-nucleotide polymorphism (SNP) markers have been added to this map as well. Because these three maps have many markers in common, it was possible to

integrate them into one consensus linkage map comprising 1889 loci and spanning 3800 cM (Groenen *et al.*, 2000). Since that time more than 100 loci have been added to the map, bringing the current total number of loci on the genetic map for the chicken to 2012 (Fig. 26.1) and the total genetic length close to 4000 cM. The consensus linkage map still consists of 51 linkage groups, several of them probably representing the same microchromosome. So far, 26 of the linkage groups have been assigned to a particular chromosome. The remaining 25 linkage groups have an ExxCxxWxx number with reference to the linkage groups of the original three linkage maps. The consensus map and its updates (Schmid *et al.*, 2000; Fig. 26.1) are all approximate. The framework of this map is dominated by markers that have been mapped on the Wageningen mapping population. The reason for this is that the number of informative meioses for markers typed on the Wageningen map is five- to tenfold higher than those only mapped on the Compton or East Lansing populations.

Variations in recombination frequencies

Based on the genetic length of 4000 cM for the chicken genome and on the physical size of the genome of 1.2×10^9 bp, it can be calculated that on average 1 cM equals 300 kbp. However, this number is

Fig. 26.1. (pages 501–518). Cytogenetic and linkage map of the chicken genome and location of identified BACs and BAC contigs. The cytogenetic maps of the macrochromosomes are shown as the standard GTG-banded ideograms (Ladjali-Mohammedi *et al.*, 1999) with the location of the ISH mapped locus indicated by a vertical bar to the right. The linkage map was adapted from Groenen *et al.* (2000). The framework markers on the linkage map have been ordered and their positions are indicated by the number to the left. Framework loci are those loci whose order relative to one another is supported by odds larger than 3. The possible location of the loci whose order is not supported by odds larger than 3 is indicated by an error bar. Loci representing identified genes are shown in italics. Loci on the linkage map for which a BAC clone has been identified are shown in bold. The position of individual BAC clones (relative to the linkage map) are indicated by a dot whereas BAC contigs are indicated by a solid vertical bar. Numbers to the right of these dots or bars refer to the number of BAC clones that are represented. Loci on the linkage map for which a BAC clone is available are indicated in bold. Genes identified on the BACs either by sequencing or STS mapping are shown to the left of the dot or bar representing the BAC. Discrepancies between the cytogenetic and linkage map are marked by a star, i.e: *GH1* on GGA1 and GGA27; *CYP19* on GGA1 and GGA10; *HOXB@* on GGA3 and GGA27; *ACTB* and *RARB* both on GGA2 and a microchromosome and PPAT on GGA4 and GGA6. In all these cases, the linkage map location is the most likely correct location of the gene.

Chromosome 1
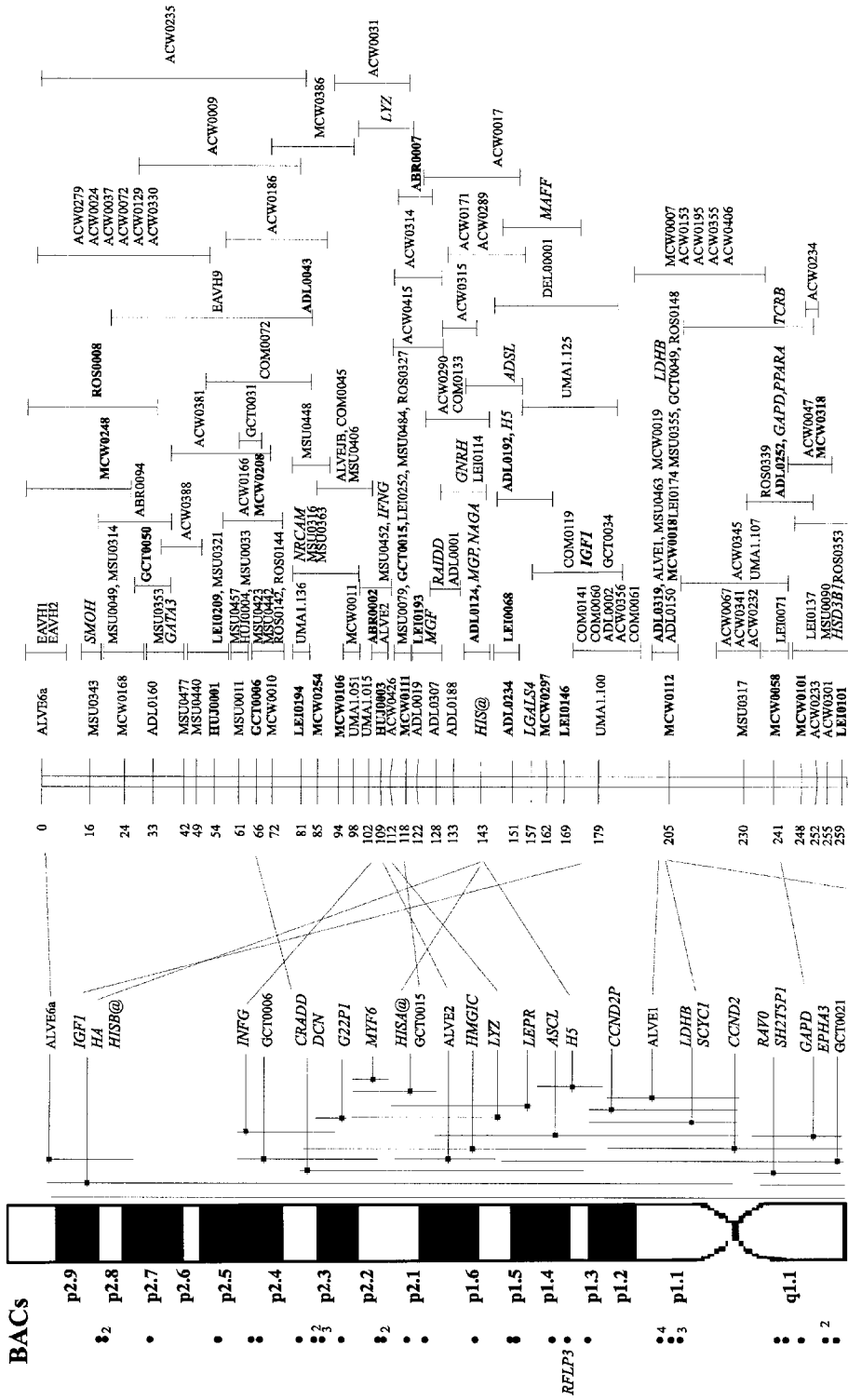
Fig. 26.1. *Continued.*
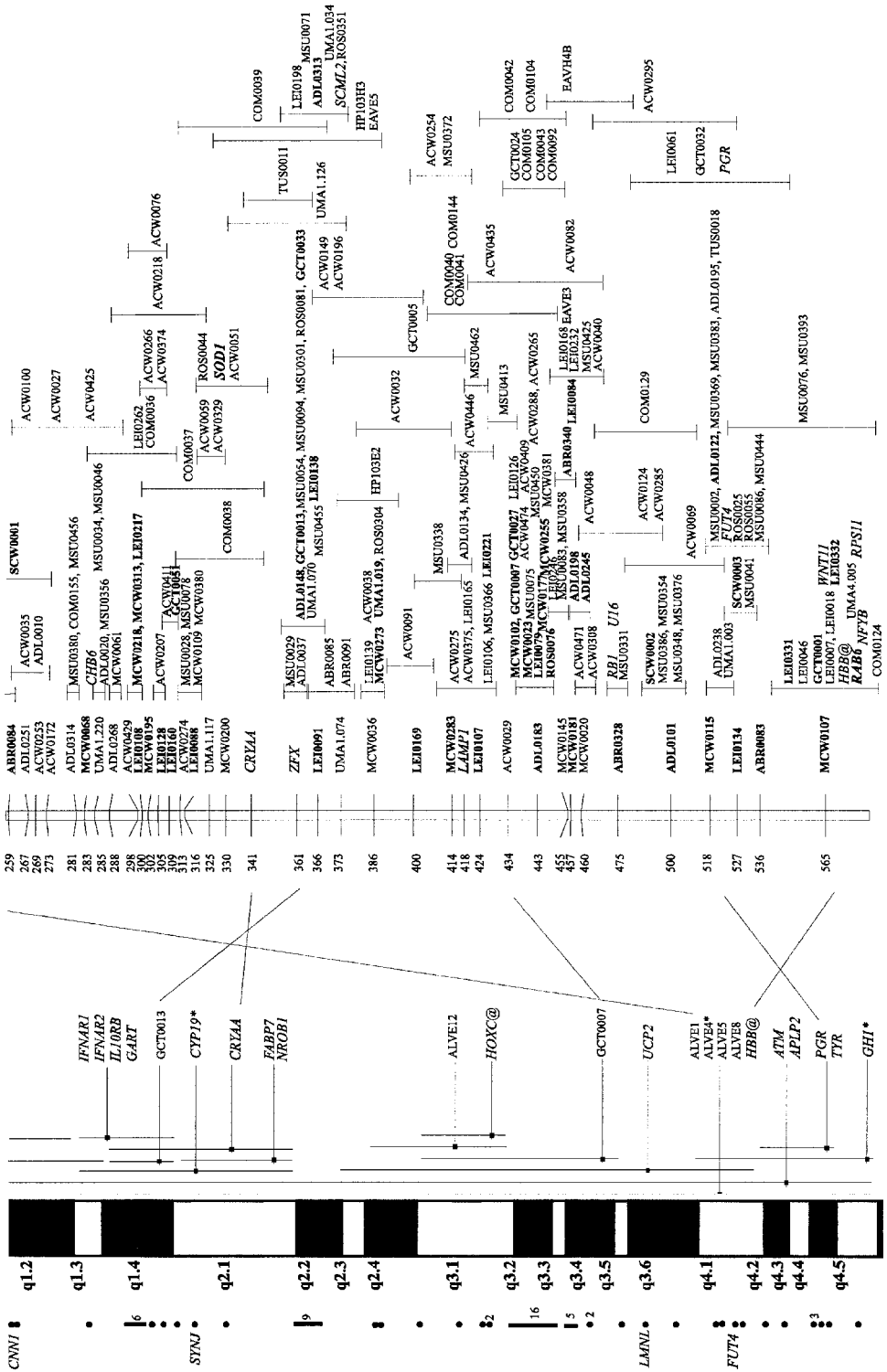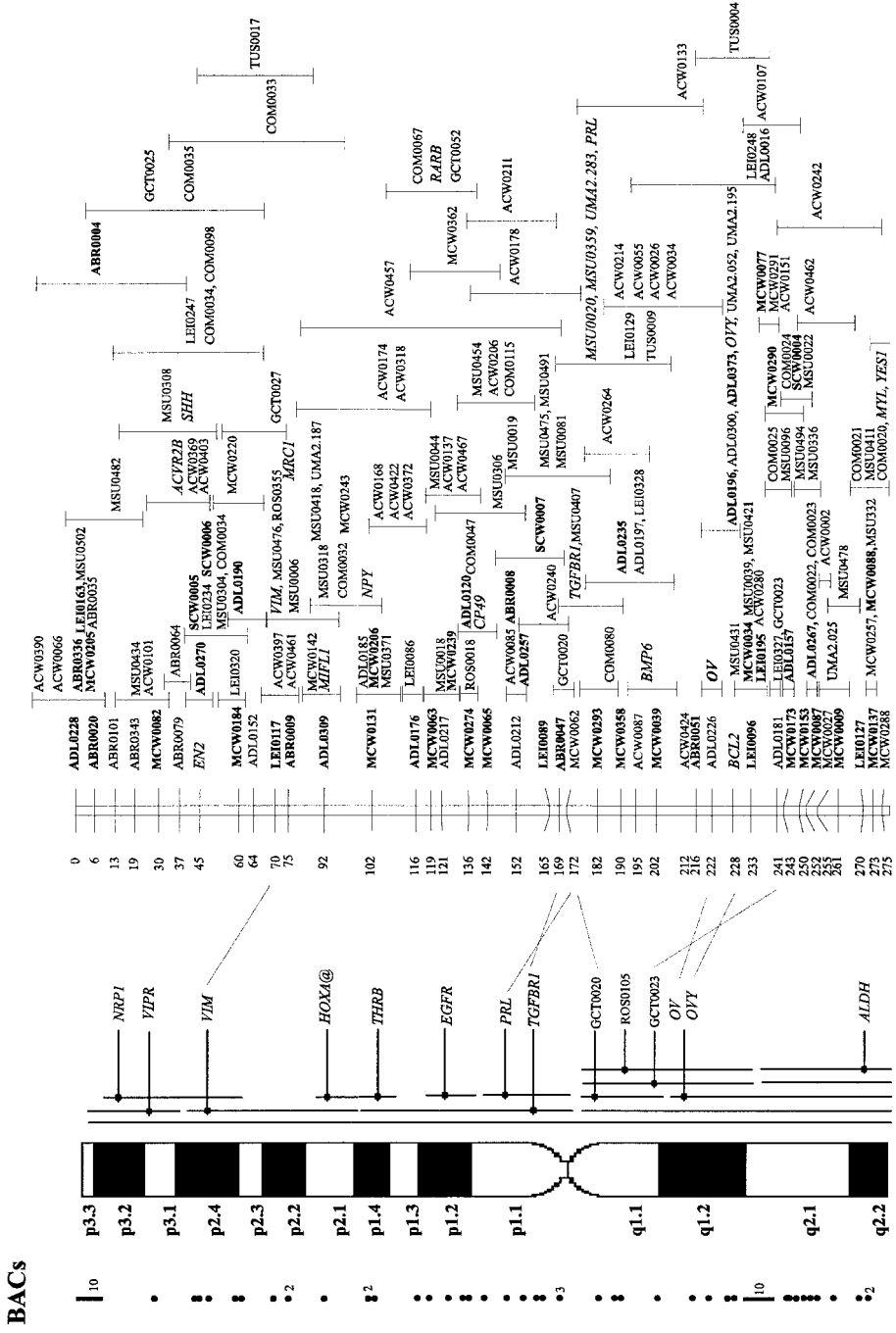
Chromosome 2

Fig. 26.1. *Continued.*
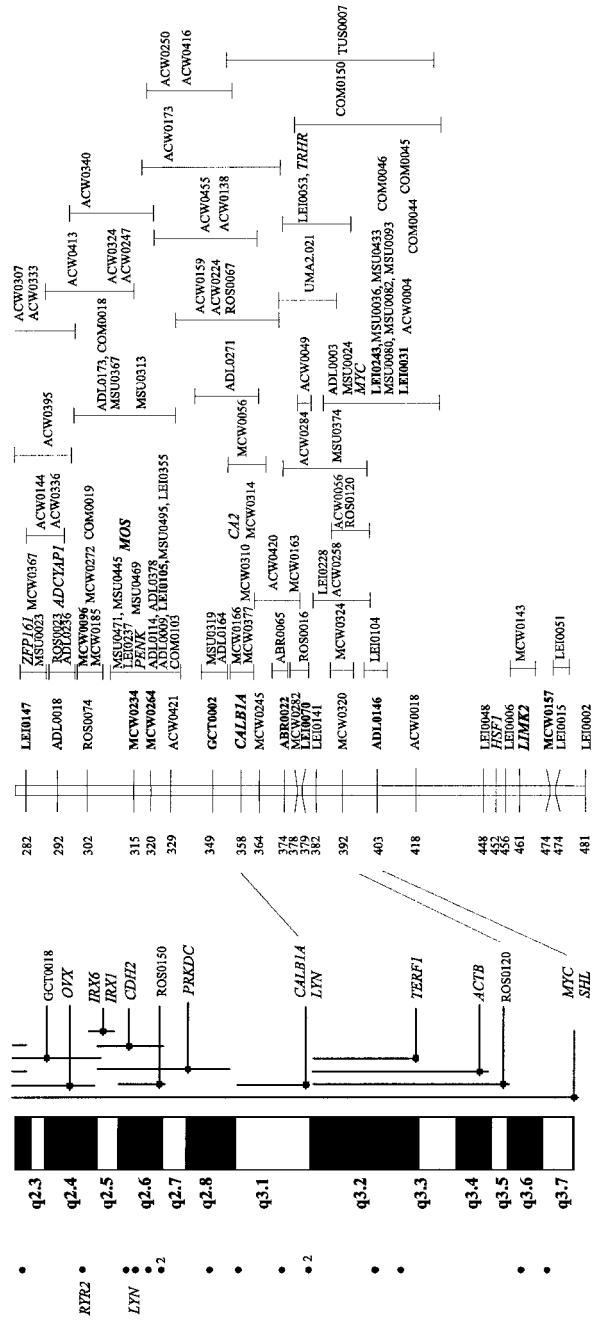
Chromosome 3
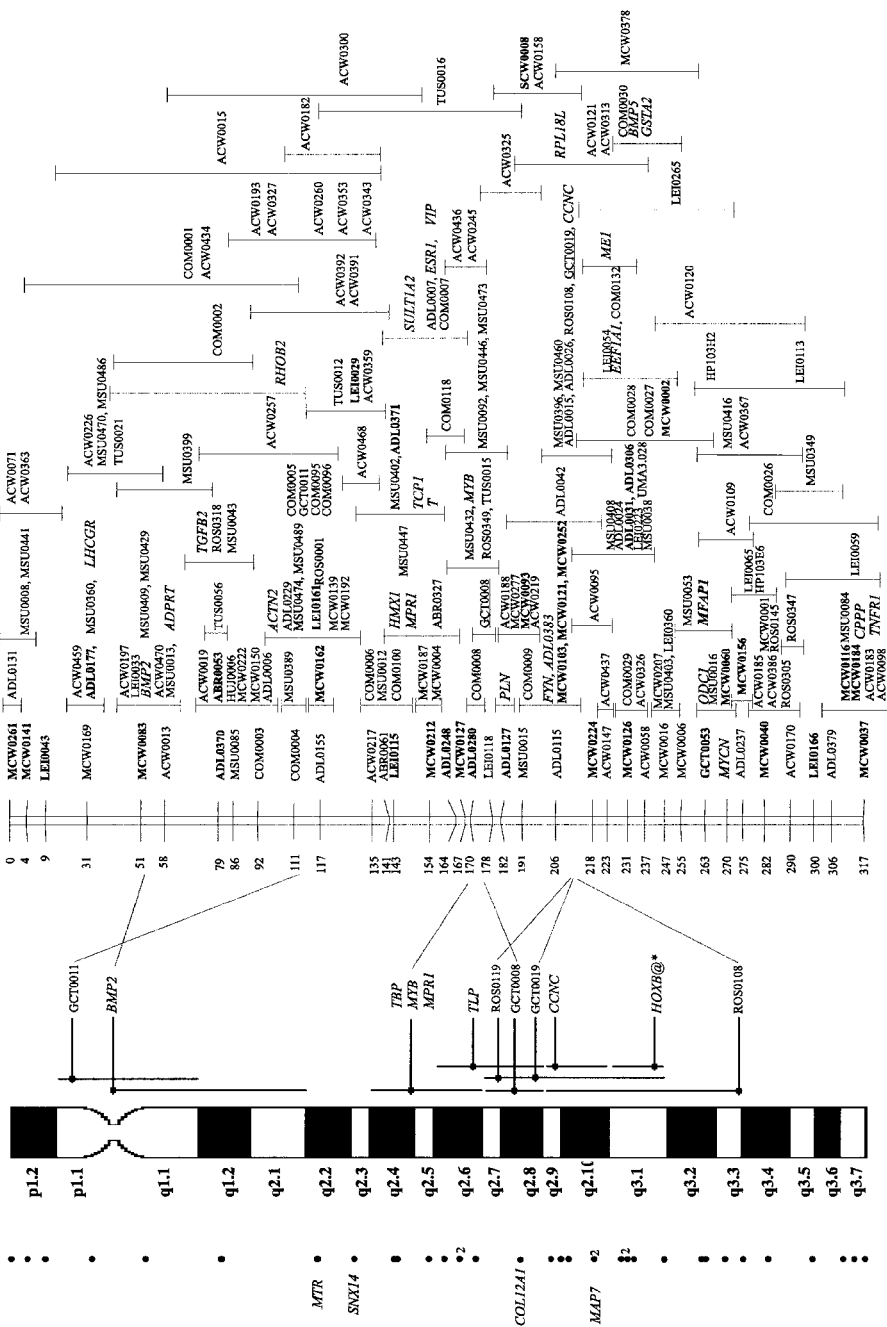
Fig. 26.1. *Continued.*
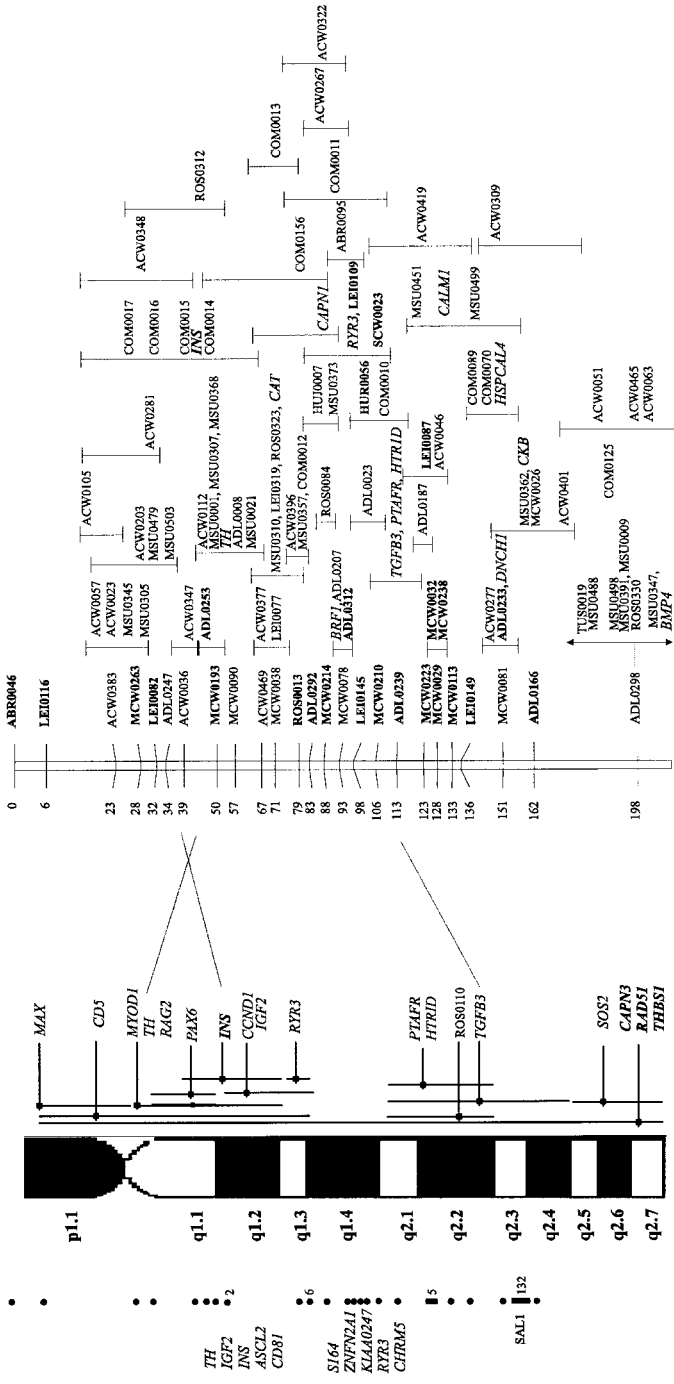
Chromosome 4

Fig. 26.1. *Continued.*

Chromosome 6

Chromosome 7

Fig. 26.1. *Continued.*

Chromosome 8

Chromosome 9

Fig. 26.1. *Continued.*

Chromosome 12

Chromosome 13
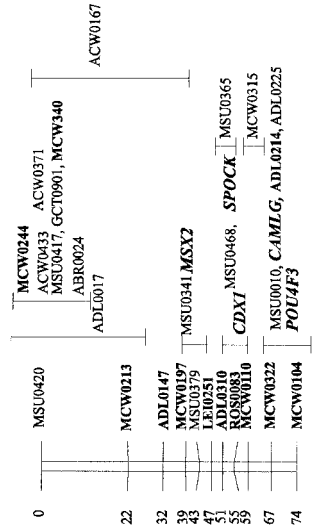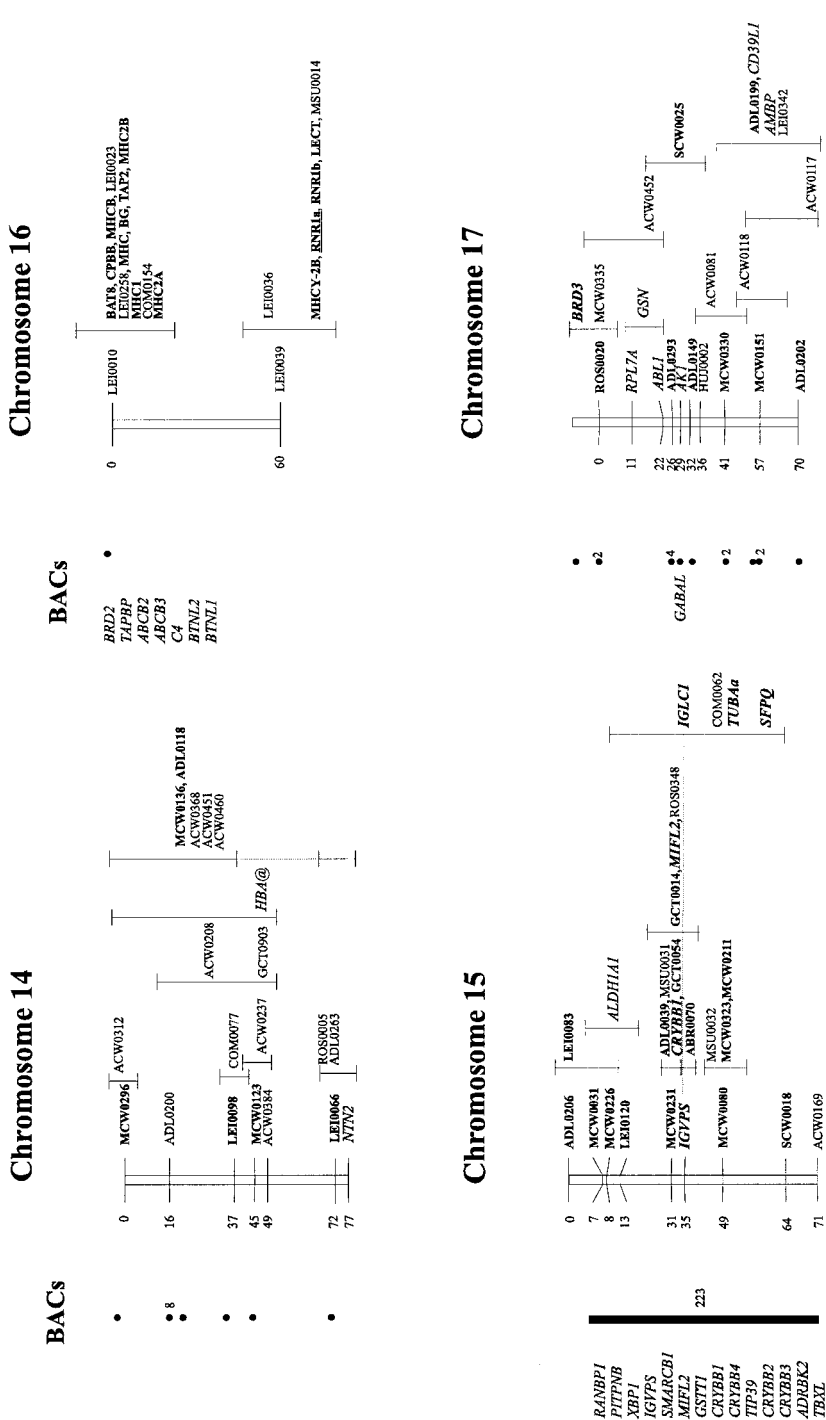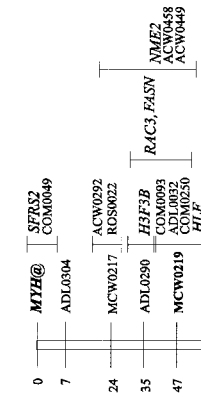
Fig. 26.1. *Continued.*

Chromosome 24
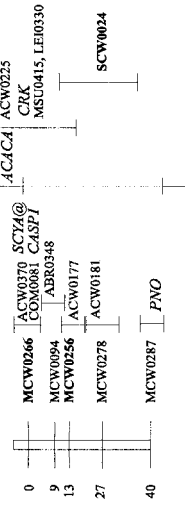
Chromosome 23
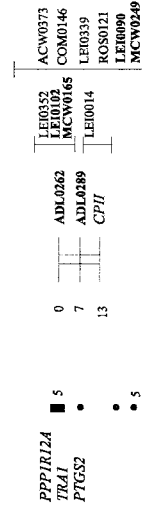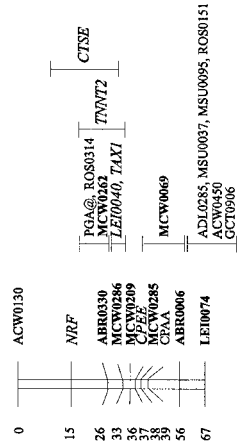
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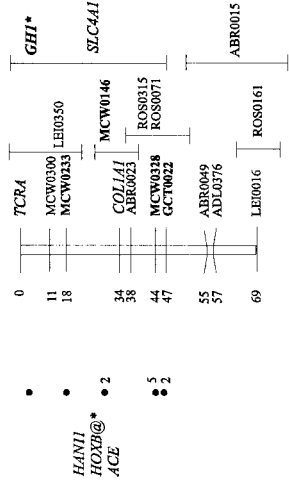
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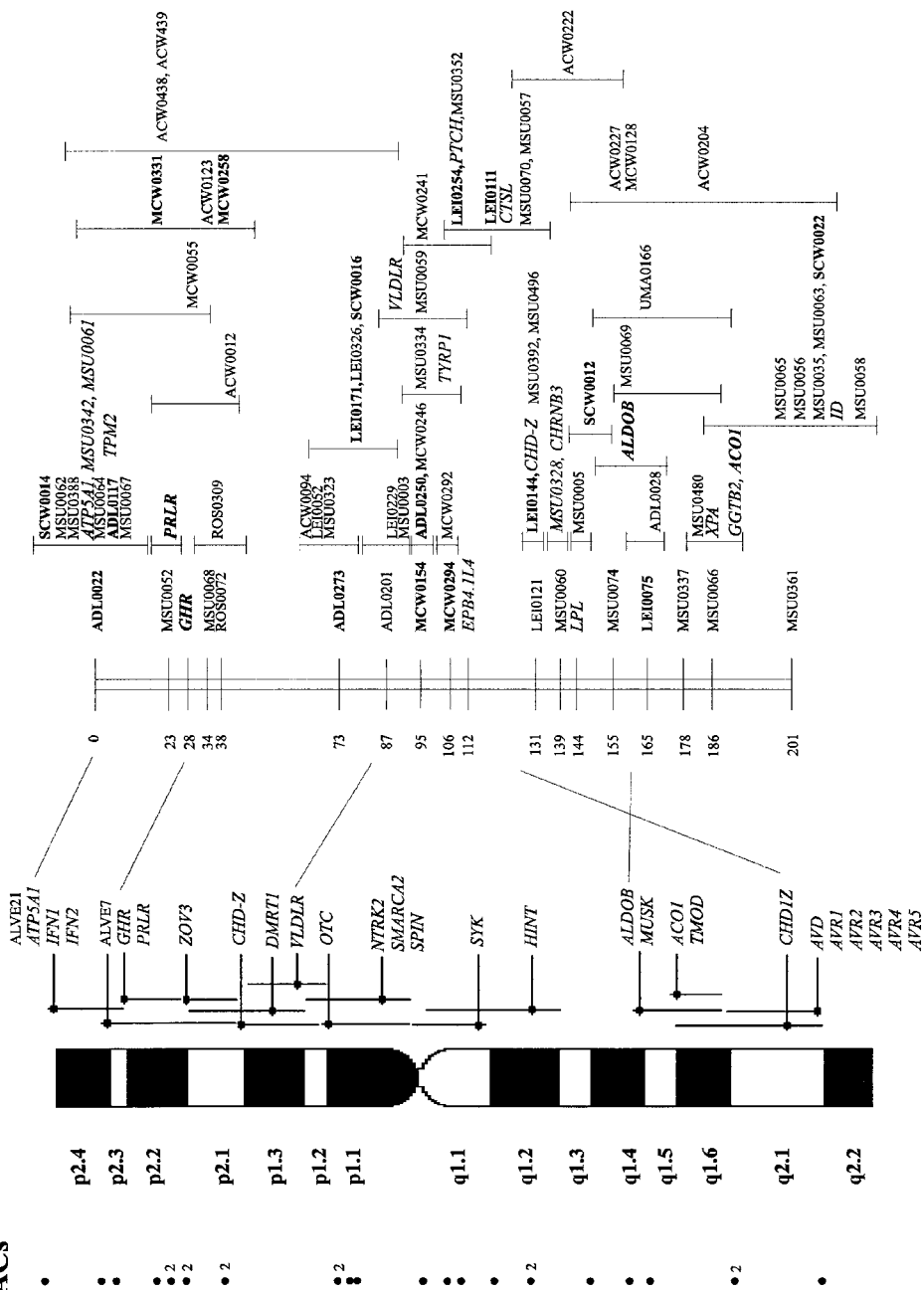
Chromosome 19
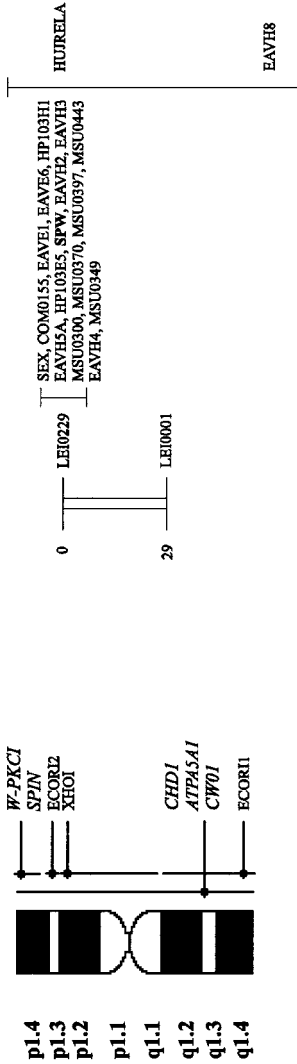
Chromosome 26
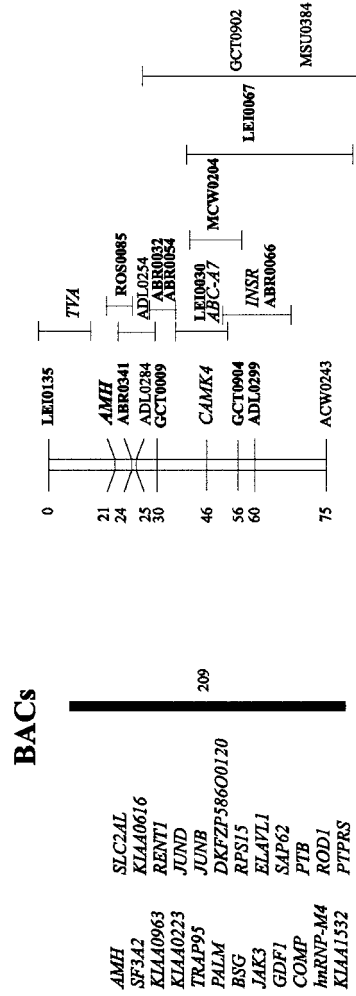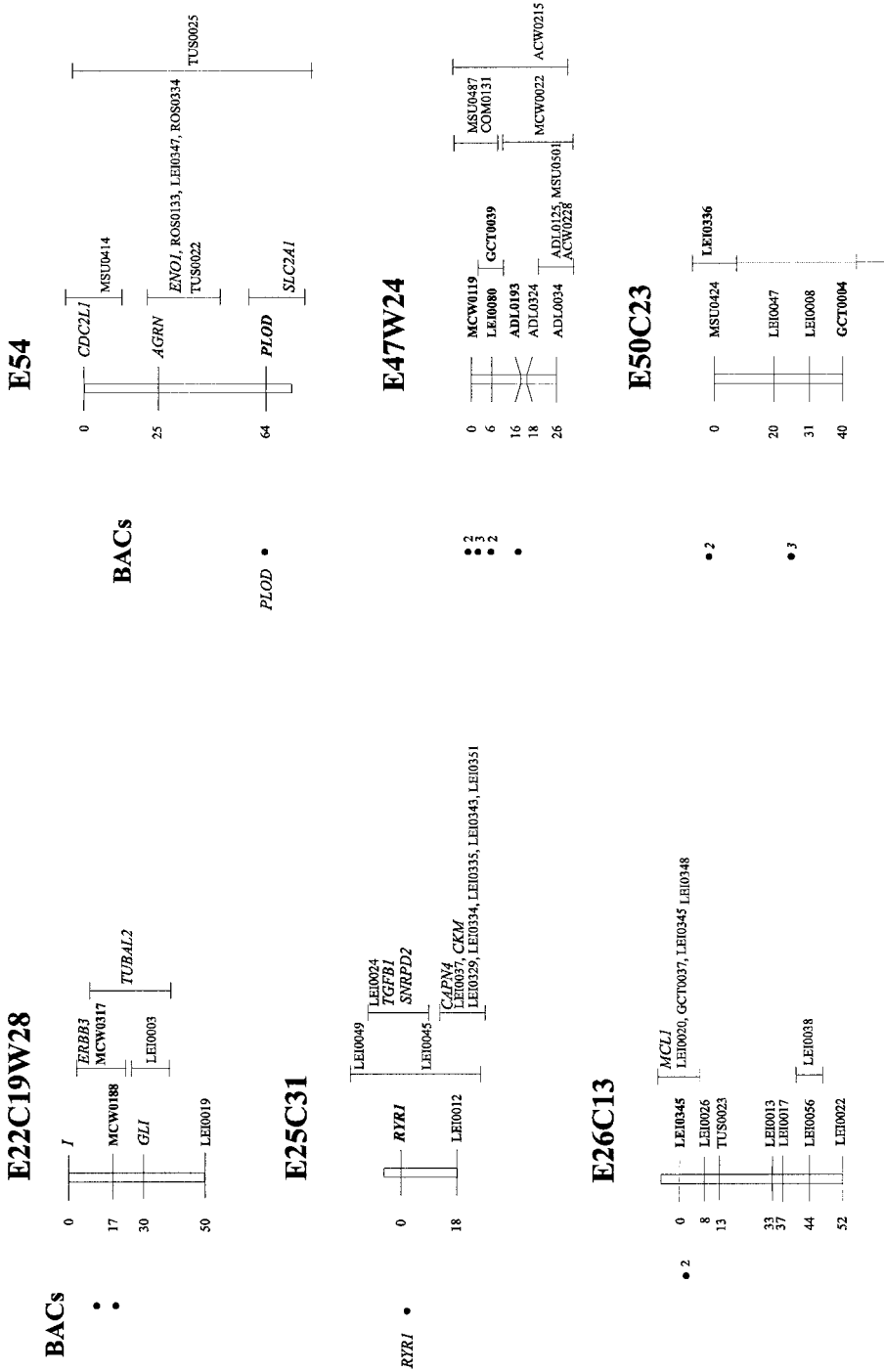
Fig. 26.1. *Continued.*
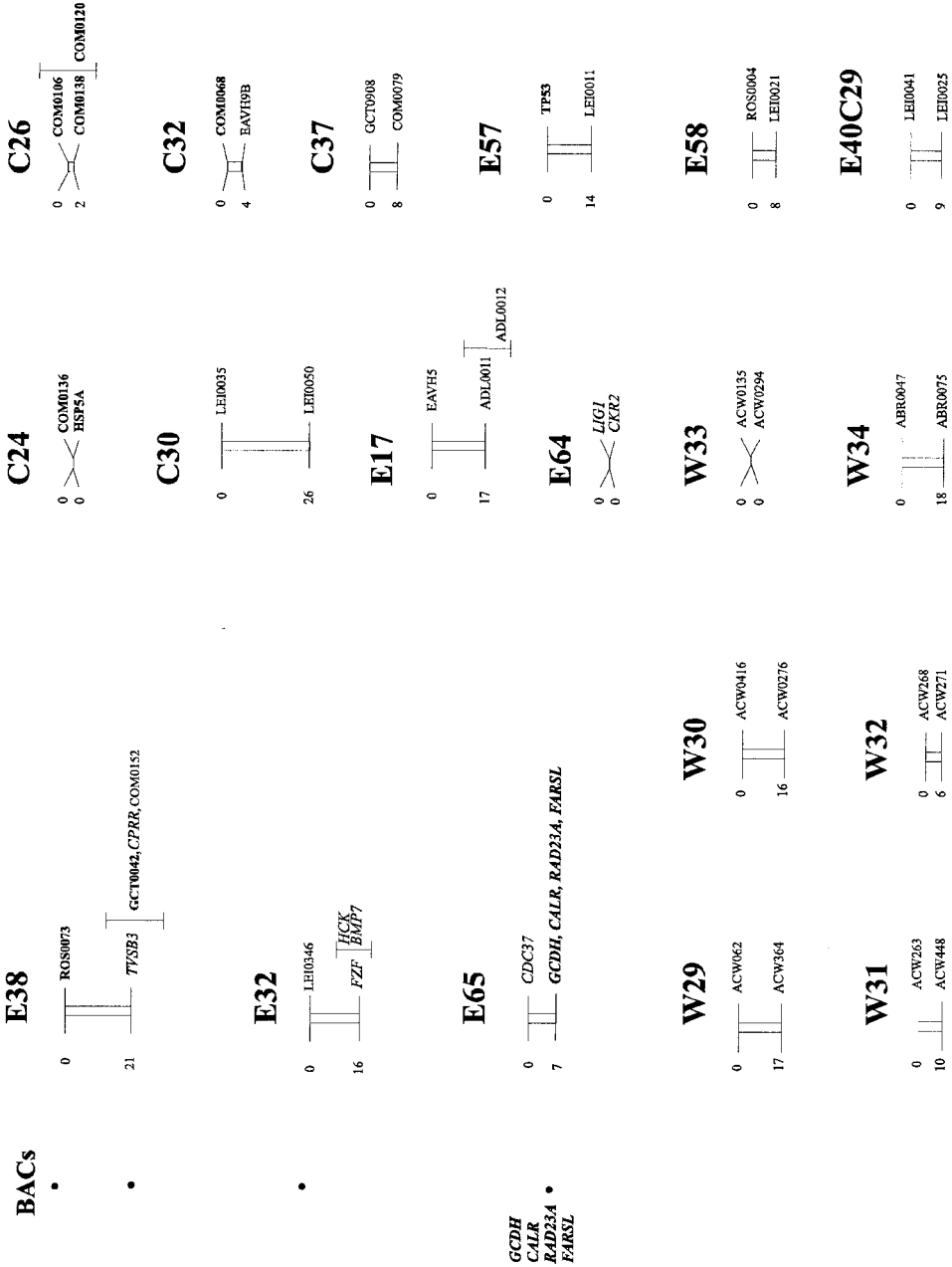
Fig. 26.1. *Continued.*

Fig. 26.1. *Continued.*

only a useful rule of thumb, as it is known that for most species recombination varies widely for different chromosomal regions. In general, recombination occurs more frequently towards the telomeres of chromosomes, whereas it is less frequent around the centromeres. This characteristic of recombination has to be kept in mind when aligning linkage with physical maps. It is also of particular relevance for studies aimed at the fine mapping and molecular characterization of quantitative trait loci (QTL).

Although clear differences are observed between many of the linkage groups of the East Lansing, Compton and Wageningen maps (see www.arkdb.org), many of these differences can probably be attributed to typing errors in the individual data sets. In addition to differences in recombination along the chromosome, differences in recombination frequencies also exist between individuals. Particularly clear is the difference in recombination between males and females in many species. In the chicken, information with regard to differences between male and female recombination is only available from the Wageningen mapping population (Groenen *et al.*, 1998). Differences in the recombination rates between the sexes were observed, but the differences were smaller than those observed in other species such as humans (Donis-Keller *et al.*, 1987) and pigs (Archibald *et al.*, 1995). Overall the length of the male map appears to be somewhat larger than the female map. This would be in agreement with the prediction (Haldane, 1922) that the linkage map of the homogametic sex (male in chicken) will be larger. However, the observed overall differences between the male and female map is only 1.15%, with several of the female linkage groups actually being larger than their male counterparts. Therefore, although the observed differences in some regions are clearly significant, the 1.15% overall difference observed might be caused by discrepancies in the number of informative meioses between males and females or by typing errors.

DNA markers mapped on the chicken linkage maps

Genetic DNA markers can be divided into two groups according to O'Brien and Graves (1991): type I loci (within or adjacent to known genes) and type II loci (random DNA markers). Different kinds of markers have been developed over the years for the two types. Several of these markers have been used in genetic mapping of the chicken genome. The most important types of markers that have been used to construct linkage maps in the chicken are described in Chapter 23 (Aggrey and Okimoto).

The most recent type of marker, the SNP, has seen an increase in its popularity mainly because of its high abundance and possibility of being used on DNA chips or other high-throughput systems. SNPs are in fact the underlying variation in many of the markers described above, including AFLPs, allele-specific oligonucleotides (ASOs), single-strand conformation polymorphisms (SSCPs) and RFLPs. The classic RFLPs, for example, are a subclass of SNP markers in which the mutation results in the creation or destruction of a restriction recognition site. Although this type of marker is primarily biallelic, its high abundance makes it very powerful. The frequency of SNPs is rather high – about 1 kb^{-1} in humans (Wang *et al.*, 1998). In the chicken a frequency as high as one per 100 bp has been observed (Vignal *et al.*, 2000). Because of their abundance these markers have a high potential for detailed haplotype analysis, e.g. association studies (Collins *et al.*, 1996). In the chicken, SNP markers located within 47 genes have already been mapped in the East Lansing (Smith *et al.*, 2001) or Wageningen (Crooijmans and Groenen, unpublished results) mapping populations. These SNP markers are indicated on the consensus linkage map shown in Fig. 26.1 with the identification symbols TUS or SCW, respectively. Most of the SNPs identified to date have been identified by a direct sequencing approach of PCR-amplified DNA. Smith *et al.* (2001) used this approach to identify 65 SNPs in 17,500 bp derived

from 37 expressed sequence tags (ESTs). A similar approach was used to detect 139 SNPs in 31,000 bp derived from 33 different genes and cosmids (Schmid *et al.*, 2000). The frequency of the SNPs detected in this study (one SNP per 225 bp) is twofold higher than in the study by Smith *et al.* (2001) (one SNP every 470 bp) probably because in the latter study, only the two East Lansing parental DNAs were used in the analysis. This is further illustrated by the findings of Crooijmans and Groenen (unpublished results) who observed an SNP frequency of one SNP per 175 bp by sequencing a larger sample of DNAs (derived from the East Lansing and the Wageningen mapping populations). In that study 170 SNPs were identified from 30,000 bp derived from sequence tagged site (STS) markers located on chicken chromosome 10.

Physical Mapping

Different types of physical maps vary in their degree of resolution. The lowest-resolution physical map is the chromosomal (sometimes called cytogenetic) map, which is based on the distinctive banding patterns observed by light microscopy of stained chromosomes. Higher-resolution maps are obtained by means of a panel of radiation hybrid cells, containing different fragments of the genome under investigation. The more detailed BAC, yeast artificial chromosome (YAC) or cosmid contig maps depict the order of overlapping DNA fragments spanning the genome. The highest-resolution physical map is the complete elucidation of the DNA base-pair sequence of each chromosome in the genome.

Chromosomal or cytogenetic maps

In a chromosomal map, genes or other identifiable DNA fragments are assigned directly to their respective chromosomes, generally using metaphase spreads of these chromosomes. These markers can be physically associated with particular bands (identified by cytogenetic staining) primarily by fluorescent *in situ* hybridization (FISH), a technique that involves tagging the DNA marker with an observable fluorescent label. The location of the labelled probe can be detected after it binds to its complementary DNA strand in an intact chromosome.

As with genetic linkage mapping, chromosomal mapping can be used to locate genetic markers defined by traits observable only in whole organisms. Because chromosomal maps are based on estimates of physical distance, they are considered to be physical maps. The number of base pairs within a band, however, can only be estimated.

In many species, and in particular in mammals (including human and mouse), the individual chromosomes are easily identified by means of simple banding techniques in combination with their size and appearance (acrocentric, sizes of the short and long arm). In the chicken, however, the number ($2n = 78$) and in particular the small size of a large proportion of these chromosomes (referred to as microchromosomes) have formed a significant obstacle for cytogenetic mapping. A standardized banded karyotype has been prepared only for the eight macrochromosomes and the two sex chromosomes, Z and W (Ladjali-Mohammedi *et al.*, 1999; Fig. 26.1).

The possibility of identifying all of the chicken chromosomes has been greatly enhanced by recent developments in chromosome painting (Griffin *et al.*, 1999; Guillier-Genick *et al.*, 1999) and the isolation of large insert clones (BACs) for a large proportion of the microchromosomes (Fillon *et al.*, 1996; Crooijmans, Fillon, Vignal and Groenen, unpublished results). By using flow sorting of chromosomes, Griffin *et al.* (1999) were able to develop chromosomal painting probes for chicken chromosomes 1–9 and Z and one microchromosome. Furthermore, amplification of single microchromosomes resulted in painting probes for a large number of the microchromosomes (Schmid *et al.*, 2000; Masabanda and Griffin, unpublished results). Probably of more general practical application for the identification of the

microchromosomes is the identification of individual large insert clones (BACs) that are known to be located on different microchromosomes. Different BACs are subsequently used as landmarks for the individual microchromosomes. Other clones that in a two-colour FISH experiment co-hybridize with such a landmark clone can then be assigned directly to a particular microchromosome. This approach was pioneered by Fillon *et al.* (1998), who generated a first set of 17 large insert clones that distinguished 16 different microchromosomes. By developing polymorphic markers for some of these, they were also able to assign some of the linkage groups to specific microchromosomes.

This work has been extended in further experiments, resulting in 22 landmark clones for chromosomes 9–31 (Fillon and Vignal, described in Schmid *et al.*, 2000). In these experiments, mainly BAC clones were used that were derived from markers with a known location on the genetic linkage map (Crooijmans and Groenen, unpublished results), thereby further integrating the chicken linkage and chromosomal maps.

Because the landmark probes have become available only recently, cytogenetic mapping information is still mostly confined to genes located on the macrochromosomes (Fig. 26.1). The cytogenetic mapping of new genes without prior mapping information is still laborious in the chicken, because different combinations of two-colour FISH experiments have to be performed to be able to map unequivocally that gene to a microchromosome. To date around 200 loci, most of them representing known genes, have been assigned cytogenetically to a particular chromosome (Fig. 26.1). An additional 44 genes have been mapped cytogentically to a microchromosome without the exact identification of that particular microchromosome (Table 26.1).

Radiation hybrid mapping

Genetic mapping resolution has been increased through the application of *in vitro* radiation-induced chromosome fragmentation and cell fusions to create panels of cells with specific and varied human chromosomal components (Walter and Goodfellow, 1993). Assessing the frequency of marker sites remaining together after radiation-induced DNA fragmentation can establish the order and distance between the markers. Because only a single copy of a chromosome is required for analysis, even non-polymorphic markers are useful in radiation hybrid mapping. (In meiotic linkage mapping, described above, two copies of a chromosome must be distinguished from each other by polymorphic markers.) Donor cells are subjected to a lethal dose of radiation that fragments their chromosomes. The average size of a fragment is a function of the dose of radiation. After irradiation the donor cells are fused with recipient cells of a different species. A selection system is used to pick out recipient cells that have taken up some of the donor chromosome fragments. When a set of DNA markers is assayed in a panel of such radiation hybrids, the patterns of cross-reactivity can be used to construct a map. The principle is very similar to meiotic linkage analysis: the nearer together two DNA sequences are on a chromosome, the lower is the probability that they will be separated by the chance occurrence of a breakpoint between them. The frequency of breakage between two markers can be defined by a value θ, analogous to the recombination frequency in meiotic mapping. The value of θ varies from 0 (the two markers are never separated) to 1.0 (the two markers are always broken apart). In radiation hybrid mapping the distance is expressed in centirays (cR). For example, a distance of 1 cR between two markers represents a 1% frequency of breakage between them.

Radiation hybrid cell lines have proved to be a powerful resource for gene mapping, particularly in mammals, and they have been used to develop detailed physical gene-dense maps in humans (Gyapay *et al.*, 1996; Stewart *et al.*, 1997), mouse (McCarthy *et al.*, 1997), rat (Watanabe *et al.*, 1999), dog (Vignaux *et al.*, 1999), cat (Murphy *et al.*, 1999), cow (Womack *et al.*, 1997) and

Table 26.1. Genes mapped cytogenetically to an unidentified microchromosome.

Symbol	Name	Publication
ACTB	Beta actin	Suzuki *et al.* (1999b)
ADORA1	Adenosine A1 receptor	Sazanov *et al.* (2000)
ADORA2B	Adenosine A2B receptor	Sazanov *et al.* (2000)
ADORA3	Adenosine A3 receptor	Sazanov *et al.* (2000)
ARVCF	Armadillo repeat gene	Suzuki *et al.* (1999b)
CDH4	Cadherin 4	Suzuki *et al.* (1999b)
CREBL1	c-AMP-responsive element binding protein-like 1	Suzuki *et al.* (1999b)
DMD	Dystrophin	Dominguez-Steglich *et al.* (1990)
EPHB1	Ephrin receptor EphB1	Suzuki *et al.* (1999b)
EPHB2	Ephrin receptor EphB2	Suzuki *et al.* (1999b)
F10	Coagulation factor X	Suzuki *et al.* (1999b)
FLNC	Filamin C, gamma (actin-binding protein-280)	Smith *et al.* (2000)
FMOB	Fibromodulin	Smith *et al.* (2000)
FUS	Fusion	Suzuki *et al.* (1999b)
GRB2	Growth factor receptor bound protein 2	Suzuki *et al.* (1999b)
ICSBP	Interferon Consensus Sequence Binding Protein	Dosch *et al.* (1998)
INPP5D	Inositol polyphosphate-5-phosphatase, 145 kD	Suzuki *et al.* (1999b)
IRX3	Iroquois homeobox protein 3	Ogura *et al.* (2001)
IRX4	Iroquois homeobox protein, clone IRX4	Ogura *et al.* (2001)
IRX5	Iroquois homeobox protein 5	Ogura *et al.* (2001)
MAPK1	Mitogen activated protein kinase	Suzuki *et al.* (1999b)
MATR3	Matrin 3	Suzuki *et al.* (1999b)
MC1R	Melancortin 1-receptor	Sazanov *et al.* (1998)
MITF	Microphthalmia-associated transcription factor	Suzuki *et al.* (1999b)
MYHL	Myosin, heavy polypeptide, adult fast-white	Dominguez-Steglich *et al.* (1993)
MYOG	Myogenin (myogenic factor 4)	Suzuki *et al.* (1999b)
NGFB	Nerve growth factor, beta polypeptide	Dominguez-Steglich *et al.* (1992a)
NOTCH1	Notch homologue 1 (translocation associated)	Suzuki *et al.* (1999b)
NR5A1	Fushi tarazu factor homologue 1	Suzuki *et al.* (1999b)
PAX7	Paired box homeotic gene 7	Suzuki *et al.* (1999b)
PER2	Period (Drosophila) homologue 2	Yoshimura *et al.* (2000)
PER3	Period (Drosophila) homologue 3	Yoshimura *et al.* (2000)
PFGFRB	Platelet-derived growth factor receptor, beta polypepetide	Suzuki *et al.* (1999b)
PLCG2	Phospholipase C, gamma 2	Suzuki *et al.* (1999b)
PPY	Pancreatic polypeptide	Smith *et al.* (2000)
PRNP	Prion protein (p27-30)	Smith *et al.* (2000)
PTPN11	Protein tyrosine phosphatase non-receptor type 11	Suzuki *et al.* (1999b)
RARB	Retinoic acid receptor, beta	Smith *et al.* (2000)
RPL13	Ribosomal protein L13	Sazanov *et al.* (1998)
SHC1	SHC (Src homology 2 domain containing) transforming protein	Suzuki *et al.* (1999b)
SLC6A4	Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	Smith *et al.* (2000)
TF	Transferrin, ovotransferrin, conalbumin	Dominguez-Steglich *et al.* (1992b)
TRAF1	TNF receptor-associated factor 1	Smith *et al.* (2000)
ZW10	ZW10 (Drosophila) homologue	Okamura *et al.* (2001)

pig (Yerle *et al.*, 1998). Although several attempts have been made in the past to construct radiation hybrid cell mapping panels in the chicken (Kwok *et al.*, 1998; Bumstead, unpublished results), only recently has such a panel become available (Morrison and Vignal, unpublished results). Preliminary mapping experiments with a subset of the

mapping panel have resulted in a radiation hybrid (RH) map of chromosome 10 consisting of 45 loci, which is in good agreement with the linkage and BAC contig map for this chromosome (Morrison, Crooijmans, Groenen and Vignal, unpublished results). The current availability of an RH mapping panel for the chicken will most certainly generate a new boost in the physical mapping of a large number of chicken genes and in particular for the mapping of the large number of ESTs that have recently become available (see www.chick.umist.ac.uk). Furthermore, the mapping of microsatellites on this RH panel, whose location on the linkage map is known and for which BAC clones are also already available, will guarantee the integration of all available maps in the chicken.

Contig maps

This bottom-up mapping approach involves cutting the chromosome into small pieces, each of which is cloned and ordered. The ordered fragments form contiguous DNA blocks (contigs). Contig building of large regions or even complete chromosomes and genomes is only feasible with clones with large inserts, such as YACs and BACs. An advantage of this approach is the accessibility of these stable clones to other researchers. Contig construction can be verified by FISH, which localizes the clones to specific regions within chromosomal bands. Contig maps thus consist of a linked library of small overlapping clones representing a complete chromosomal segment.

Two types of large insert libraries have been developed in the chicken. A chicken YAC library has been developed by Toye *et al.* (1997) and provides an 8.5-fold redundant coverage; it consists of 16,000 clones with an average insert size of 634 kb. YAC clones are efficient in covering large physical areas and are easier to develop. This system has several drawbacks, particularly, insert instability. Therefore, the establishment of another type of large insert library, the BAC library, was constructed.

Although a number of different BAC libraries have been constructed for the chicken (Zoorob *et al.*, 1996; Zimmer *et al.*, 1997; Crooijmans *et al.*, 2000; Dodgson, unpublished results) only two of these are predominantly used for the building of chicken BAC contig maps. The first of these is the Wageningen library (Crooijmans *et al.*, 2000), based on the DNA from a White Leghorn chicken. The chicken DNA fragments have been cloned in the *Hin*dIII cloning site and the library consists of almost 50,000 clones with an average insert size of 134 kb, which is equivalent to a 5.5-fold genome coverage. Over 3000 BACs with a known map location have been identified from this library (see Fig. 26.1) and a complete BAC map based on this library is currently under construction. Screening of this library is possible by two-dimensional PCR (see www/zod.wau.nl/vf) and by filter hybridization (www.hgmp.mrc.ac.uk). The other library consists of three parts, which are constructed using the *Bam*HI, *Eco*RI and *Hin*dIII cloning sites, respectively. Each of these parts comprises 39,400 clones, resulting in a tenfold genome coverage (J. Dodgson, personal communication). Screening of this library is possible by filter hybridization (see hbz.tamu.edu and poultry.mph.msu.edu). The *Bam*HI part of this library is also accessible for PCR screening (www.resgen.com) and a BAC map from this library is also under construction (J. McPherson and J. Dodgson, personal communication). Furthermore, fingerprinting data from the Wageningen and US libraries will be merged to generate a combined BAC map of 15-fold genome coverage. This physical BAC contig map of the chicken genome should be available in the first half of 2003. Copies of both libraries are available from the BAC centre in Texas (hbz.tamu.edu).

Using the Wageningen BAC library, at least one BAC clone has been isolated for markers that have been mapped at 5–10 cM intervals on the chicken linkage map. In order to be able to integrate the linkage and cytogenetic maps, BAC clones have been isolated with markers from almost every linkage group of the consensus linkage map.

BAC contigs are currently being developed for several linkage groups (Table 26.2). Much effort is being put into building a complete BAC contig map for chicken chromosome 10 by chromosome walking. This has already resulted in the identification of over 900 BAC clones, assembled into 28 contigs and covering around 75% of this chromosome. A detailed comparison of the genes located within these BAC contigs on *GGA10* with their orthologues on human chromosome 15 has identified the location of many inter- and intra-chromosomal rearrangements during evolution after the separation of these species some 300 million years ago (Crooijmans *et al.*, 2001; Fig. 26.2). The mapping results based on such BAC contigs (Crooijmans *et al.*, 2001; Buitenhuis *et al.*, 2002; Jennen *et al.*, 2002) indicate that the average size of the conserved blocks between humans and chickens is in the order of 1–4 cM. More than 10% of the chicken genome is covered by identified BAC clones.

Genomic sequencing in the chicken

Genomic sequencing in the chicken has seen a tremendous increase in recent years and it is clear that a further exponential growth can be expected in the next few years, culminating eventually in the (near) complete sequence of the entire chicken genome. Currently over 800 entries can be found in Genbank with information about genomic intron/exon sequences in chicken. An additional large number of small genomic fragments is available around genetic markers (> 1000 fragments, mostly microsatellites and SSCPs) and as a result of end sequencing and sample sequencing of BACs (> 3000 fragments) (Crooijmans *et al.*, 2001; Buitenhuis *et al.*, 2002; Jennen *et al.*, 2002; Crooijmans and Groenen, unpublished) and cosmid clones (Clarke *et al.*, 1999; Smith *et al.*, 2000). In spite of this increase in genomic sequencing, information on large contiguous stretches of genomic DNA and complete genes is still limited. By May 2002, the number of chicken genomic sequences in Genbank that were larger than 20,000 bps was 42. Two-thirds of these sequences consisted of the complete sequence of BAC clones (Table 26.3). The other one-third consisted of individual genes or gene clusters (Table 26.4). A detailed comparison of the structure and organization between chicken genes and their mammalian orthologues has shown that the number of introns and the position of these introns have been strongly conserved between these species (Table 26.5 gives some examples). Furthermore, though this is not an absolute rule, introns in the chicken are generally smaller than in their mammalian counterparts (Hughes and Hughes, 1995), resulting on average in a two- to threefold smaller size of the chicken genes with regard to their mammalian orthologues (Table 26.5). However, occasionally chicken genes can be larger than their mammalian orthologue, as is seen, for example, for the *COL6A2* gene, which is 26 kb in chickens and 21.5 kb in humans. This smaller difference in the size of the

Table 26.2. Overview of identified chicken BAC clones.

Chromosome	Size (cM)	BACs	STS	SNPs	Genes	Coverage	Human
GGA1 (NK)	–	20	–	–	–	–	HSA12
GGA5 (Sal1)	11	132	–	–	5	–	HSA14
GGA8 (qtel)	30	137	72	30	10	20%	HSA1
GGA10	120	938	453	170	126	75%	HSA15
GGA13	74	188	65	–	21	18%	HSA5
GGA15	71	223	122	–	13	25%	HSA22
GGA24	58	166	69	–	22	20%	HSA11
GGA28	75	187	101	–	23	20%	HSA19
Total genome	4000	2692				8%	
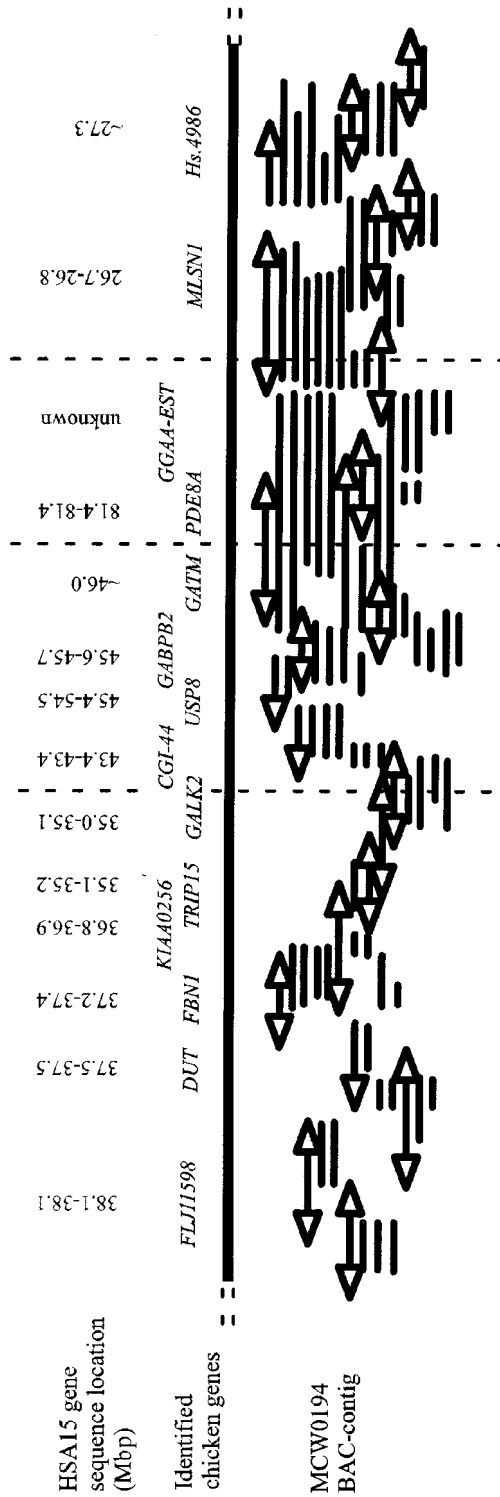
Fig. 26.2. 2.5 Mbp BAC contig around microsatellite MCW0194 on chromosome 10. The genes identified within this contig and their position on the human sequence map according to genome viewer build 25 (www.ncbi.nlm.gov) are shown at the top. BAC clones are not drawn to scale; arrows at the end of a BAC clone indicate the development of an STS marker that was used for chromosome walking. Vertical dotted lines indicate the position of an intrachromosomal rearrangement between the chicken and human chromosomes. (Adapted from Crooijmans *et al.*, 2001.)

Table 26.1. BAC clones for which the complete sequence is available in Genbank.

BAC	Size (bp)	Accession number	Genes	Human chromosomal region	Overlapping BAC clones
bW068C05	124,309	AC091708	*TES, CAV1*	7q31.2	bW065N20
bW055C14	55,589	AC087179	*MET, CAPZA2*	7q31.2–q31.3	bW105M15, bW069H02
bW105M15	98,793	AC091725	*MET*	7q31.2	bW065N20, bW055C14, bW069H02
bW069H02	135,273	AC084761	*CAPZA2, MET, ST7*	7q31.1–q31.3	bW055C14
bW100N11	145,230	AC092403	*GARS*	7p14–p15	bW077D19, bW101K06
BW101K06	140,753	AC121065		7p14–p15	bW046K16, bW100N11
bW046K11	99,032	AC120510	*TAX1, HIBADH*	7p14–p15	bW101K06
bW077D19	164,355	AC092081	*CRHR2, GARS*	7p14–p15	bW100N11
bW028H24	86,274	AC092869	*AKAP9*	7q21–q22	
bW071G10	121,851	AC091751	*CGI-97, LIMK1, FLJ10900, WBSCR1, ELN, PNUTL2*	7q11.23, 17q22–q23	bW093J15, bW126P17
bW126P17	129,285	AC091726	*WBSCR1, LIMK1*	7q11.23	bW071G10, bW039A19
bW039A19	202,128	AC120196	*CUTL1, LOC136120*	7q22 7p14.1	bW126P17
bW093J15	137,023	AC091091	*BAZ1B, FZD9, BCL7B, TBL2, WBSCR1*	7q11.23	bW071G10
bW065N20	109,569	AC084760	*CAV1*	7q31.2	bW068C05, bW105M15
bW075D11	86,551	AC120511	*CDK6, LOC135818*	7q21–q22	bW038H09, bW068G02
bW068G02	164,715	AC096683	*CCM1, LOC57798*	7q21–q22	bW050C06, bW075D11
bW092E21	170,968	AC118982	*STEAP*	7q21–q22	bW097F19
bW097F19	113,455	AC094012	*AKAP9*	7q21–q22	bW050C06, bW092E21
bW050C06	119,064	AC094011	*CCM1, CYP51*	7q21–q22	bW068G02, bW097F19
bW038H09	89,724	AC093704	*CDK6*	7q21–q22	
BAC192C9	187,259	AP003795	*IGF2*	11p15.5	BAC26D12
BAC26D12	156,014	AP003796	*IGF2, TH, ASCL2, CD81, INS*	11p15.5	BAC192C9
bW039H1	171,122	AC119050	*DKFZP564F1123, FLJ10648, ITPK1, FLJ10483*	14q31–q32.13	
bW095P15	128,031	AC119051	*TMP21, NEK8*	14q24	
TAM31–14F7	167,693	AC120501			
TAM31–9F3	171,122	AC120500			

chicken genes (and more generally, the 2.5 times smaller chicken genome) is in part the result of the lower number of repetitive elements that is found in this species. The most frequent repetitive element found in the chicken genome is the CR1 family, with an estimated 30,000 to 100,000 copies in the genome (Burch *et al.*, 1993; Vandergon and Reitman, 1994). This number is in sharp contrast to the number of repeated

Table 26.2.   Sequences of chicken genes or gene cluster whose size is larger than 20,000 bp.

Gene (cluster)	Accession number	Size (bp)	Reference
Centromeric protein CENP-C	AB042324	27,630	Okamura *et al.* (2001)
Class II cytokine receptor cluster	AF082667	46,304	Reboul *et al.* (1999)
Col6A2 gene for type VI collagen subunit alpha2	X56659	27,443	Hayman *et al.* (1991)
Lipoprotein lipase gene	X60547	22,257	Cooper *et al.* (1992)
SCL gene locus	AJ131018	35,980	Göttgens *et al.* (2000)
Gene for angiopoetin-2A	AJ289777	46,022	Mezquita *et al.* (2000)
Gene for angiopoetin-2B	AJ289778	46,022	Mezquita *et al.* (2000)
Gene for angiopoetin-2C	AJ289779	46,022	Mezquita *et al.* (2000)
MHC locus	AL023516	92,863	Kaufman *et al.* (1999)
Delta-1 and delta-2 crystallin genes	M10806	25,342	Nickerson *et al.* (1985)
Gene for transcriptional repressor deltaEF1	D76434	22,268	Sekido *et al.* (1996)
Alpha-globin gene cluster	AY016020	103,190	Flint *et al.* (2001)
Beta-globin gene cluster	L17432	30,539	Reitman *et al.* (1993)
Embryonic myosin heavy chain gene	J02714	31,111	Gulick *et al.* (1985)
T cell receptor alpha gene (TCRA) and defender against death protein I gene (DAD1)	U83833	31,793	Wang *et al.* (1997)
Vitellogenin II gene	X13607	20,343	Van het Schip *et al.* (1987)

elements in the human genome, which contains around 850,000 LINES, 1.5 million SINES and 450,000 copies of retrovirus-like elements (International Human Genome Sequencing Consortium, 2001).

Extensive sequencing of large contiguous segments of the chicken genome is still limited to a handful of regions homologous to regions syntenic to human chromosomes 7q21–q22, 7q31.2, 7q11.23, 7p14–p15, 11p15.5, 14q24, 14q31–q32, the chicken MHC, beta-globin and the class II cytokine receptor clusters and a small number of large genes including the T-cell receptor alpha, myosin heavy chain, *GART-B* and vitogenellin 2 genes. The general rule that seems to emerge from the sequence of these larger regions in the chicken genome is that they are generally 2.5–3 times smaller than the corresponding regions in the human genome and that the gene order has been conserved over regions of at least 1 cM. This is in agreement with the physical mapping results on chicken chromosome 10, where the size of the conserved segments appears to be somewhere in the range of 0.5–5 cM (Crooijmans *et al.*, 2001).

The largest contiguous chicken sequence available to date is the region syntenic to human chromosome 7q31.2 harbouring the *TES*, *CAV1*, *MET*, *CAPZA2* and *ST7* genes (Fig. 26.3, see also www.nisc. nih.gov/open_page.html?projects/zooseq. html). This sequence is derived from the complete sequence of five partially overlapping BAC clones (see Table 26.3) together spanning a region of around 414,000 bp. The order of the genes is identical to that found in the human genome. Interestingly, the size of this region in humans is 1 Mbp, which is 2.5 times the size of the chicken region. A similar situation is seen for the region on chicken chromosome 5 around the *IGF2* gene (305,000 bp). Here the gene order is *IGF2*, *INS*, *TH ASCL2* and *CD81*, again identical to the human gene order, and the size of the region is threefold smaller than in the human genome. Finally, the same situation is observed in the 310,000 bp sequence from the chicken genome containing the *CCM1*, *CYP51* and *AKAP9* genes.

Although gene order seems to be conserved over distances at the scale of centimorgans, for particular regions the situation might be much more complex. A good example is the 100 kb core region of the chicken MHC complex (Kaufman *et al.*, 1999). This region contains a number of genes that probably represent the chicken orthologues of the genes present in the mammalian MHC

Table 26.3. Comparison of a selection of sequenced chicken and human orthologous genes.

Gene symbol	Gene description	Accession number	Gene size (kbp)		Reference
			In chicken	In human	
MYHE	Myosin heavy chain homologous to human MYH1 and MYH4	J02714	22.7	24.3 and 26.3	Gulick *et al.* (1985)
ALDOB	Aldolase B	M10946	8.8	17.4	Burgess *et al.* (1985)
GAPD	Glyceraldehyde-3-phosphate dehydrogenase	M11213	4.0	6.8	Stone *et al.* (1985)
ACTA2	Alpha actin, aortic, smooth muscle	M13756	9.5	20.7	Carroll *et al.* (1986)
BAZ1B	Bromodomain adjacent to zinc finger domain, 1B	AC091091	47.0	81.9	Ayele *et al.* (unpublished)
SCL	Stem cell leukemia	AJ131018	36	197	Göttgens *et al.* (2000)
IL8	Interleukin 8	AJ009800	3.0		Kaiser *et al.* (1999)
IFNG	Interferon gamma	Y07922	4.5	5.0	Kaiser *et al.* (1998)
IL2	Interleukin 2	AJ224516	3.2	5.3	Kaiser and Mariani (1998)
B2M	Beta2-microglobulin	Z48931	2.2	4.2	Riegert *et al.* (1996)
CAPZA2	Capping protein (actin filament) muscle Z-line, alpha 2	AC087179, AC084761	30	56	Ayele *et al.* (unpublished)
AK1	Adenylate kinase 1	D00251	4.9	11.3	Suminami *et al.* (1988)
CAV1	Caveolin 1	AC091708, AC084760	18	39	Ayele *et al.* (unpublished)
TES	Testis-derived transcript	AC091708	27	51	Ayele *et al.* (unpublished)
HBB	Haemoglobin beta	L17432	1.5	2.8	Reitman *et al.* (1993)
HBE	Haemoglobin epsilon	L17432	1.5	1.8	Reitman *et al.* (1993)
COL6A2	Collagen alpha 2, subtype VI	X56659	26.0	21.5	Hayman *et al.* (1991)
BRD2	Bromodomain-containing 2	AL0323516	3.8	11.5	Kaufman *et al.* (1999)
TAP1	Transporter 1, ATP-binding cassette, sub-family B	AL0323516	4.0	8.4	Kaufman *et al.* (1999)
TAP2	Transporter 2, ATP-binding cassette, sub-family B	AL0323516	3.1	16.2	Kaufman *et al.* (1999)
C4	Complement component 4	AL0323516	14.3	20.6	Kaufman *et al.* (1999)

region such as those coding for the class I-α chain, *ABCB2*, *ABCB3*, *BRD2* and *C4A*. However, the gene order differs and genes such as the *LMP* and those genes coding for the class II-β chain are absent from this region in the chicken, while additional B-G genes and C-type leptin-coding genes are present. In many respects, the MHC region has been shown to be rather exceptional in the genome of many species and the differences observed between the chicken and human MHC regions might be yet another result of the special character of this particular genomic region.

Comparative genome analysis: the identification of conserved regulatory sequences

The chicken has a long history as a species that is used for comparative biology. Consequently, in the genomics area, an increase is seen of comparative genome analysis between long stretches of sequences between mammals (predominantly human and mouse) and the chicken as a tool for the detailed annotation of the regulatory sequences of specific genes. In a detailed analysis of the *SCL* locus, Göttgens *et al.*
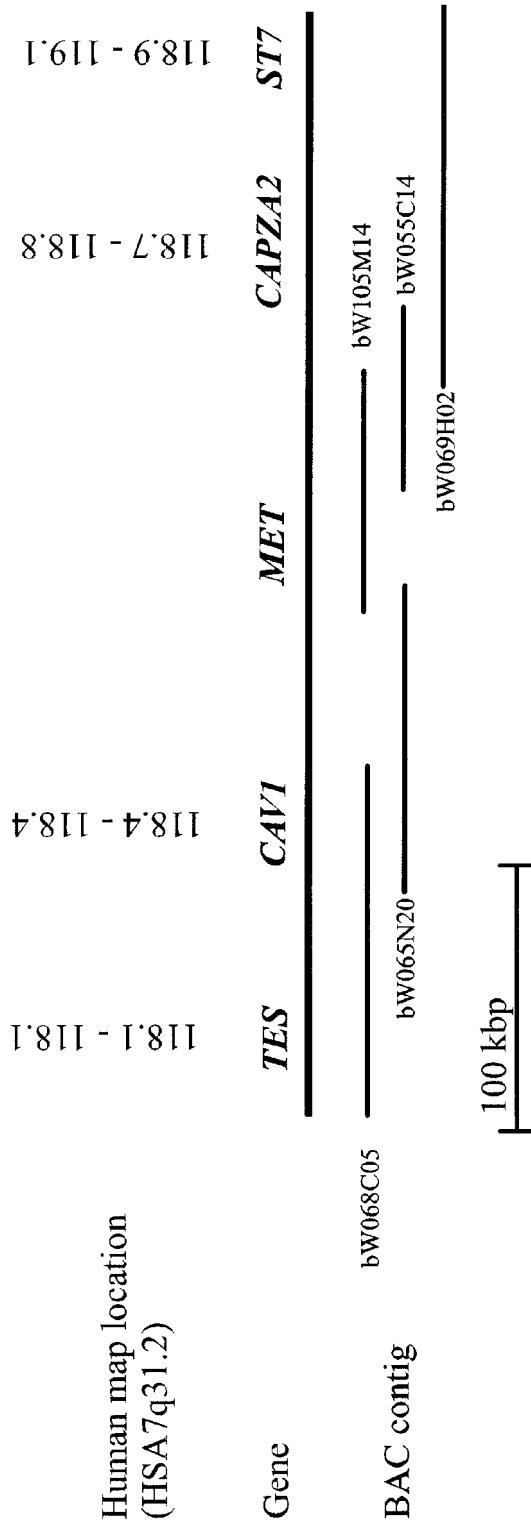
Fig. 26.3. BAC contig for which the complete sequence has been deposited in Genbank (see Table 26.3 for accession numbers). The complete sequence of this BAC contig consists of 414,000 bp. The genes identified within this contig and their position on the human sequence map according to genome viewer build 25 (www.ncbi.nlm.gov) are shown at the top. BAC clones are drawn to scale.

(2000) compared 36 kb around the chicken *SCL* locus with the corresponding regions in the mouse (86 kb) and humans (197 kb). In all three species the *SCL* gene appears to be flanked by the *SIL* and *MAP17* genes, which is in agreement with the sequencing and physical mapping results described in the previous section. That is, for relatively small stretches of the genome, gene order appears to be conserved between chickens and mammals. A detailed sequence comparison between the chicken and mammalian sequences enabled Göttgens *et al.* (2000) to identify, in addition to already known enhancers, an enhancer 23 kb downstream of the initiation start site of the *SCL* gene. Similar results have been obtained in a detailed analysis of 100 kb of the chicken α-globin gene cluster (Flint *et al.*, 2001), further illustrating the general application of this approach in the identification of regulatory elements. The results of Flint *et al.* (2001) also suggested the existence of small chromosomal segments that have been conserved in a large variety of species (human, mouse, chicken, pufferfish) and that may constitute chromosomal units that are co-regulated by common *cis*-acting regulatory elements.

In addition to these recent more extended sequence comparisons, regulatory elements have been characterized in many chicken genes and to some extent similar regulatory elements have been identified that parallel their mammalian orthologues. Examples of such investigations include the NO synthetase gene (Lin *et al.*, 1996), *TGFB3* (Jakowlew *et al.*, 1992; Burt *et al.*, 1995), *CTCF* (Klenova *et al.*, 1993), smooth muscle α-actin gene (Jung *et al.*, 1999), αA-crystallin (Ilagen *et al.*, 1999) and *CYP2H1* (Handschin and Meyer, 2000).

Besides the identification of regulatory elements, a comparative genome analysis also helps to identify orthologous genes even if they have diverged considerably. A good illustration is the identification of the *IFNAR2*, *IL10R2* and *IFNAR1* genes in the interferon/interleukin-10 receptor gene cluster on chicken chromosome 1 (Reboul *et al.*, 1999).

Future Prospects

Integration of the different chicken maps

BAC and cosmid clones for markers and genes that have been mapped on the chicken linkage map have been the key elements used for the integration of the chicken physical and linkage maps. Although these clones have allowed the assignment of 26 of the linkage groups of the consensus map to a specific chromosome (see Fig. 26.1), 25 of the linkage groups still lack a chromosomal assignment. Many of these unassigned linkage groups are very small, with a limited number of markers. Furthermore, a considerable number of markers on these linkage groups are derived from multi-locus markers, making the isolation of BACs or cosmids more difficult and time consuming.

BACs that have been isolated with markers mapped on the chicken linkage map will also be the key elements that will help to integrate the cytogenetic and linkage maps with the BAC contig maps and eventually the genomic sequences. These BACs have already resulted in aligning 3000 BACs with the linkage map, comprising approximately 10% of the chicken genome (Fig. 26.1). Fingerprinting and end sequencing of all these BACs will ensure the integration of all available maps in the chicken.

Sequencing the chicken genome, the ultimate physical map

The last decade of the 20th century saw a tremendous increase in gene mapping in the chicken, particularly with regard to the linkage mapping and its use in QTL mapping (see Chapter 24). This eventually culminated in one consensus linkage map of the chicken genome (Groenen *et al.*, 2000). The first decade of the 21st century has only just started, but now it is already clear that with regard to chicken genomics it will be dominated by the development of detailed physical maps and within a few

years will culminate in the availability of the (nearly) complete sequence of the chicken genome. On 22 May 2002, the National Human Genome Research Institute prioritized the chicken among a small group of organisms to be considered for entry into the sequencing pipeline as the current efforts with human, mouse and rat approach completion. Although filling in holes in the maps and finishing the sequence will certainly remain an important focus for the rest of this decade, the wealth of information and the tools available will contribute to continued interest in the chicken as a model and farm animal. In particular, research on the characterization of QTL at the molecular level faces exciting new times and will further increase our knowledge and understanding of such complex traits as ascites, growth, fat deposition, immune response and resistance to diseases caused by a whole range of pathogens.

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27 Incorporating Molecular Information in Breeding Programmes: Methodology

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Introduction

Selection of genetically superior individuals as parents is a key component in a breeding programme. The genes responsible for the genetic variability of quantitative traits are known as quantitative trait loci (QTL). In spite of the significant advances that have been made in molecular genetics, only a very few QTL have been identified. As a result, individuals cannot be selected by directly observing their genes, and statistical methods are used to predict the genetic potential of individuals using all available information. The process of predicting the genetic potential of individuals is known as genetic evaluation.

Until recently, the information used for genetic evaluation has been limited to pedigree relationships and trait phenotypes. Although only a few QTL have been identified, many chromosomal regions that show evidence of QTL have been identified using molecular markers. This chapter describes the methodology for genetic evaluation using these molecular markers together with pedigree relationships and trait phenotypes.

Genetic Evaluation

Let a_i be the unobservable genotypic value of candidate i, and let **D** represent all the information available to predict the unobservable genetic values of the candidates. Suppose k candidates are to be selected from a total of n candidates such that the mean of the selected candidates is maximized. It is easy to show that this can be achieved by computing the conditional mean

$$\hat{a}_i = E(a_i|\mathbf{D}) \tag{1}$$

for each candidate, and then selecting the k candidates with the largest $\hat{a}_i$ (Bulmer, 1980; Fernando and Gianola, 1986). The conditional mean also has other desirable statistical properties and is known as the best predictor (Henderson, 1984).

BLUP using trait data

When **D** consists of pedigree relationships and trait phenotypes, and when vector **y** of trait phenotypes and the vector **a** of unobservable genotypic values are assumed to have a multivariate normal distribution, the conditional mean of **a** is a linear function of **y**:

$$E(\mathbf{a}\,|\,\mathbf{D}) = \mathbf{\mu}_a + \mathbf{CV}^{-1}(\mathbf{y} - \mathbf{\mu}_y) \tag{2}$$

where, conditional on pedigree information, $\mathbf{\mu}_a$ and $\mathbf{\mu}_y$ are the expected values of **a** and **y**, **C** is the covariance matrix between **a** and **y**, and **V** is the covariance matrix of **y**. Regardless of the joint distribution of **a** and **y**, the linear function of **y** on the right side

of equation (2) gives the best linear predictor of **a** (Henderson, 1984).

Suppose **y** can be modelled as

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Za}^* + \mathbf{e} \tag{3}$$

where **X** and **Z** are known incidence matrices, **β** is an unknown vector of fixed effects,

$$\mathbf{a}^* = \mathbf{a} - E(\mathbf{a}) \tag{4}$$

and *e* is a vector of residuals with null mean and covariance matrix **R**. Then the expected value of *y* can be written as

$$\boldsymbol{\mu}_y = \mathbf{X}\boldsymbol{\beta} \tag{5}$$

and the covariance matrices **C** and **V** can be written as

$$\mathbf{C} = \mathbf{G}_P\mathbf{Z}' \tag{6}$$

and

$$\mathbf{V} = \mathbf{Z}'\mathbf{G}_P\mathbf{Z} + \mathbf{R} \tag{7}$$

where $\mathbf{G}_P$ is the conditional covariance matrix of **a** given **P** the pedigree relationships:

$$\mathrm{Var}(\mathbf{a}\,|\,\mathbf{P}) = \mathbf{G}_P \tag{8}$$

When $\boldsymbol{\mu}_a$ and $\boldsymbol{\mu}_y$ are not known, their generalized least-squares estimates can be substituted in equation (2). Doing this gives the best linear unbiased predictor (BLUP) of **a** (Henderson, 1984). In practice, Henderson's mixed model equations (HMME) are used to obtain BLUP (Henderson, 1984). Constructing HMME requires inverting $\mathbf{G}_P$, and under additive inheritance there are very efficient algorithms to compute this inverse for purebred (Henderson, 1976) as well as multibreed (Elzo, 1990; Lo *et al.*, 1993) populations.

BLUP using trait and marker data

Now, suppose **D** includes genotypes at a marker that is closely linked to one trait locus that directly contributes to the genetic variablility of **y**. Furthermore, continuing with the assumption of multivariate normality for **a** and **y**, the conditional mean of **a** is still a linear function of *y* as given in equation (2), but with $\boldsymbol{\mu}_a$, $\boldsymbol{\mu}_y$, **C** and **V**

modified to account for **M**, the marker information in **D**. This section will describe how these modified vectors and matrices are used for genetic evaluation. The next section discusses the theory for computing $\boldsymbol{\mu}_a$, $\boldsymbol{\mu}_y$, **C** and **V** conditional on marker and relationship information.

Consider a QTL that is linked to a marker. This QTL will be called the marked quantitative trait locus (MQTL). Suppose there is gametic phase equilibrium between the MQTL and the marker, and suppose that this marker is not linked to any other trait locus. In this case,

$$E(\mathbf{a}\,|\,\mathbf{P}) = E(\mathbf{a}\,|\,\mathbf{P}, \mathbf{M}) \tag{9}$$

and

$$E(\mathbf{y}\,|\,\mathbf{P}) = E(\mathbf{y}\,|\,\mathbf{P}, \mathbf{M}) \tag{10}$$

Thus, $\boldsymbol{\mu}_a$ and $\boldsymbol{\mu}_y$ are not affected by the marker information. However,

$$\mathrm{Var}(\mathbf{a}\,|\,\mathit{P}) \neq \mathrm{Var}(\mathbf{a}\,|\,\mathbf{P}, \mathbf{M}) \tag{11}$$

and thus **C** and **V** are affected by the marker information.

In a very general setting, Chevalet *et al.* (1984) described how $\mathbf{G}_{PM} = \mathrm{Var}(\mathbf{a}\,|\,\mathbf{P}, \mathbf{M})$ can be estimated by Monte Carlo simulation and how $\mathbf{G}_{PM}$ can be used for QTL mapping and marker-assisted selection. However, they did not provide an efficient algorithm to obtain the inverse of $\mathbf{G}_{PM}$. Thus, their method is not suitable for use in HMME for genetic evaluation by BLUP in large livestock populations.

Under additive inheritance, in order to use HMME for marker-assisted BLUP, Fernando and Grossman (1989) modelled a_i as

$$a_i = v_i^m + v_i^p + u_i \tag{12}$$

where v_i^m and v_i^p are the additive effects of the maternal and paternal alleles at the MQTL, and u_i is the additive effect of the remaining trait loci. Given genotypes at a co-dominant marker, the conditional covariance matrix of **v** can be computed recursively and its inverse can be obtained efficiently (Fernando and Grossman, 1989; Wang *et al.*, 1995). Using (12), **y** is modelled as

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Wv} + \mathbf{Zu} + \mathbf{e} \tag{13}$$

where $\mathbf{v}$ is the vector of gametic effects at the MQTL, and $\mathbf{W}$ is a known incidence matrix relating the gametic effects of an individual with its trait phenotypes. Suppose the vector $\mathbf{y}$ is of order n and the pedigree contains q individuals. Then, $\mathbf{W}$ will be of order $n \times 2q$. If the ith element of $\mathbf{y}$ is a trait phenotype from individual j, the ith row $\mathbf{w'i}$ of $\mathbf{W}$ will contain $2q - 2$ zeros and two ones such that the product $\mathbf{w}'_i\mathbf{v} = v_j^m + v_j^p$. Now, marker-assisted BLUP in large livestock populations can be obtained by solving HMME corresponding to mixed linear model (13):

$$\begin{bmatrix} \mathbf{X'R^{-1}X} & \mathbf{X'R^{-1}W} & \mathbf{X'R^{-1}Z} \\ \mathbf{W'R^{-1}X} & \mathbf{W'R^{-1}W} + \mathbf{\Sigma}_v^{-1} & \mathbf{W'R^{-1}Z} \\ \mathbf{Z'R^{-1}X} & \mathbf{Z'R^{-1}W} & \mathbf{Z'R^{-1}Z} + \mathbf{\Sigma}_v^{-1} \end{bmatrix}$$
$$\begin{bmatrix} \hat{\mathbf{\beta}} \\ \hat{\mathbf{v}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X'R^{-1}y} \\ \mathbf{W'R^{-1}y} \\ \mathbf{Z'R^{-1}y} \end{bmatrix} \qquad (14)$$

where $\mathbf{\Sigma}_v$ is the conditional covariance matrix of $\mathbf{v}$ given the relationship and marker information, and $\mathbf{\Sigma}_u$ is the conditional covariance matrix of $\mathbf{u}$ given the relationship information. The BLUP for $\mathbf{v}$ is given by $\hat{\mathbf{v}}$, for $\mathbf{u}$ by $\hat{\mathbf{u}}$, and for $\mathbf{a}$ by $\hat{\mathbf{v}} + \hat{\mathbf{u}}$. For a pedigree with q individuals, the order of HMME given in (14) is $3q$ + the order of $\mathbf{\beta}$.

The BLUP of $\mathbf{a}$ can also be obtained by solving HMME given below that correspond to the usual animal model, where $\mathbf{a}$ is not partitioned as in (12):

$$\begin{bmatrix} \mathbf{X'R^{-1}X} & \mathbf{X'R^{-1}Z} \\ \mathbf{Z'R^{-1}X} & \mathbf{Z'R^{-1}Z} + \mathbf{G}_{PM}^{-1} \end{bmatrix}$$
$$\begin{bmatrix} \hat{\mathbf{\beta}} \\ \hat{\mathbf{a}} \end{bmatrix} = \begin{bmatrix} \mathbf{X'R^{-1}y} \\ \mathbf{Z'R^{-1}y} \end{bmatrix} \qquad (15)$$

The covariance matrix $\mathbf{G}_{PM}$ in (15) can be written as

$$\mathbf{G}_{PM} = \mathbf{Q}\mathbf{\Sigma}_v\mathbf{Q'} + \mathbf{\Sigma}_v \qquad (16)$$

where $\mathbf{Q}$ is a $q \times 2q$ matrix with two ones and $2q - 2$ zeros in each row such that the product $\mathbf{q}'_i\mathbf{v} = v_i^m + v_i^p$. The HMME given in (15) have $2q$ fewer equations than those given in (14). However, $\mathbf{\Sigma}_v^{-1}$ and $\mathbf{\Sigma}_u^{-1}$ in (14) are very sparse, whereas $\mathbf{G}_{PM}^{-1}$ in (15) is dense. Thus, the HMME given in (14) can be solved much more efficiently than the HMME given in (15).

Now suppose that the MQTL and the marker are not in gametic phase equilibrium (linkage disequilibrium). Then, the vector $\mathbf{v}$ of gametic effects, which unconditionally has null expectation, conditional on the marker and relationship information, will have a non-null expectation:

$$\mathbf{\eta} = E(\mathbf{v} \mid \mathbf{P}, \mathbf{M}) \qquad (17)$$

To obtain BLUP under this situation, $\mathbf{y}$ is modelled as

$$\mathbf{y} = \mathbf{X}_n\mathbf{\beta}_n + \mathbf{X}_g\mathbf{\beta}_g + \mathbf{Wv^*} + \mathbf{Zu} + \mathbf{e} \qquad (18)$$

where $\mathbf{\beta}_n$ and $\mathbf{\beta}_g$ are non-genetic and genetic fixed effects; $\mathbf{X}_n$ and $\mathbf{X}_g$ are known incidence matrices that relate $\mathbf{\beta}_n$ and $\mathbf{\beta}_g$ to $\mathbf{y}$; and $\mathbf{v^*}$ are the MQTL effects deviated from their means.

Conditional means and covariances given marker and relationship information

As described in the previous section, genetic evaluation by linear methods, using marker and trait information, requires computing means and covariances of MQTL effects. The principles underlying these computations will be discussed here.

The theory for computing the MQTL covariances given by Fernando and Grossman (1989) has the implicit assumption that the maternal and paternal origin (parental origin) of the marker alleles is known. When the origin of the marker alleles is not known, theory given by Wang *et al.* (1998) can be used to accommodate unknown parental origin of marker alleles. For simplicity, in the following discussions, it will be assumed that the parental origin of the markers is known.

Conditional means of MQTL effects

If the marker and the MQTL are statistically independent, the marker does not provide any information about the mean of MQTL effects. In the genetics literature, loci that are statistically independent are said to be in gametic phase equilibrium or linkage equilibrium. So, when the marker and the

MQTL are in gametic phase equilibrium, the conditional mean of the MQTL effects is equal to its unconditional expectation. Therefore, it is only necessary to discuss the computation of the conditional means of the MQTL effects when the marker and the MQTL are in gametic phase disequilibrium.

Wang *et al.* (1998) developed a theory for modelling the conditional means of MQTL effects in a multibreed pedigree, where each individual in the pedigree is from a pure breed or from any type of breed cross originating in one of the pure breeds. They assumed that the pure breeds that make up the multibreed pedigree are in gametic phase equilibrium. However, due to differences in allele frequencies between breeds, linked loci in the breed crosses will be in gametic phase disequilibrium. The theory developed by Wang *et al.* (1998) for crosses of pure breeds that are in gametic equilibrium is presented first, followed by a brief description of how this theory can be extended to accommodate situations where the pure breeds themselves may be in disequilibrium.

CROSSES INVOLVING PURE BREEDS IN GAMETIC PHASE EQUILIBRIUM. Let i be an individual with mother d and father s. Note that any MQTL allele in a multibreed pedigree can be traced back to a founder that belongs to some pure breed. Thus, the conditional mean for v_i^m, for example, can be written as

$$\mathrm{E}(v_i^m|P, M) = \sum_l \mathrm{E}(v_i^m|Q_i^m \leftarrow B_l, \mathbf{P}, \mathbf{M})$$

$$\mathrm{Pr}(Q_i^m \leftarrow B_l|\mathbf{P}, \mathbf{M})$$

$$= \sum_l \mu_l \, \mathrm{Pr}(Q_i^m \leftarrow B_l|\mathbf{P}, \mathbf{M}) \qquad (19)$$

where $Q_i^m \leftarrow B_l$ denotes that Q_i^m originates in breed l, μ_l is the mean of v_i^x in breed l, and $\mathrm{Pr}(Q_i^m \leftarrow B_l|\mathbf{P}, \mathbf{M})$ is the conditional probability that Q_i^m originates in breed l given marker and relationship information. This probability, which can be thought of as the breed B_l composition of MQTL allele Q_i^m, can be obtained recursively as

$$\mathrm{Pr}(Q_i^m \leftarrow B_l|\mathbf{P}, \mathbf{M}) = \mathrm{Pr}(Q_i^m \leftarrow Q_d^m|\mathbf{P}, \mathbf{M})$$

$$\mathrm{Pr}(Q_d^m \leftarrow B_l|\mathbf{P}, \mathbf{M}) + \mathrm{Pr}(Q_i^m \leftarrow Q_d^p|\mathbf{P}, \mathbf{M})$$

$$\mathrm{Pr}(Q_d^p \leftarrow B_l|\mathbf{P}, \mathbf{M}) \qquad (20)$$

where $\mathrm{Pr}(Q_i^m \leftarrow Q_d^m|\mathbf{P}, \mathbf{M})$ denotes the conditional probability that Q_i^m descended from the maternal allele of d, and $\mathrm{Pr}(Q_i^m \leftarrow Q_d^p|\mathbf{P}, \mathbf{M})$ denotes the conditional probability that Q_i^m descended from the paternal allele of d. In the following, these probabilities will be referred to as the maternal and paternal probabilities of descent (PDQs) for allele Q_i^m.

Calculation of the conditional breed compositions for the MQTL alleles using (20) can be organized into a tabular algorithm. The resulting table of breed compositions will have a row for each MQTL allele and a column for each breed (Table 27.3). In the following description of the tabular algorithm, the element of the breed composition table for MQTL allele Q_i^x and breed B_l is denoted by $e(Q_i^x, B_l)$. The rows in the table are ordered such that rows for ancestors precede the rows for descendants. The algorithm is initialized by setting each element in the table to zero. Now, starting at the top of Table 27.3, the conditional breed compositions are computed as follows.

1. If individual i is from pure breed l, put 1.0 in element $e(Q_i^x, B_l)$ for $x = m, p$.
2. If individual i with father s and mother d is a cross, to compute the breed compositions for Q_i^m: (i) multiply the elements from the row for Q_d^m by the maternal PDQ for Q_i^m; (ii) multiply the elements from the row for Q_d^p by the paternal PDQ for Q_i^m; and (iii) put the sum of the rows generated in (i) and (ii) in the row for Q_i^m. The breed compositions for Q_i^p are similarly obtained from the rows for Q_s^m and Q_s^p.

Consider the pedigree given in Table 27.1. In this pedigree, because individuals 1 and 2 are founders, the parental origin of their marker alleles cannot be determined. However, the parental origin of marker alleles in the founders does not affect the results from equation (20). Thus, we will arbitrarily assign maternal origin for allele M_1 in individuals 1 and 2. Consequently, allele M_2 in individual 1 and allele M_3 in individual 2 are assigned paternal origin. The maternal and paternal PDQs for the MQTL alleles of non-founders are given in Table 27.2.

These PDQs are easily derived from the marker information. For example, given the marker genotypes for individuals 1, 2 and 3, it is clear that 3 inherited the M_1, the maternal marker allele, from individual 1. Thus, the probability that 3 also inherits Q_1^m from 1 is $1 - r$, the probability of no recombination between the marker and the MQTL; the probability that 3 receives Q_1^p is r, the probability of a recombination. It is easy to see that using the PDQs from Table 27.2 in the tabular algorithm results in Table 27.3.

For genetic evaluation by BLUP, the incidence matrix for $\boldsymbol{\beta}_g = [\mu_1, \mu_2]$ in the linear model (18) can be constructed from the breed composition table (Table 27.3). The row in $\mathbf{X}_g$ for a record from animal i is obtained as the sum of the rows for Q_i^m and Q_i^p from the breed composition table. Suppose one phenotypic record is available from each individual; then $\mathbf{X}_g$ for the pedigree in Table 27.1 is

$$\mathbf{X}_g = \begin{bmatrix} 2 & 0 \\ 0 & 2 \\ 1 & 1 \\ 2 & 0 \\ 2-r & r \end{bmatrix} \tag{21}$$

CROSSES INVOLVING PURE BREEDS IN GAMETIC PHASE DISEQUILIBRIUM. Suppose that, due to mutation, selection, drift, or population admixture, the founders of each pure breed are in gametic phase disequilibrium for the marker and the MQTL and that the degree of disequilibrium is the same for all founders of a particular breed.

In a multibreed population, any MQTL allele can be traced to some pure breed B_l and a founder haplotype with some marker allele M_k. Because of gametic disequilibrium, the distribution of MQTL alleles depends not only on the breed but also on the linked marker allele. Thus, the conditional mean for v_i^m, for example, can be written as

$$\mathrm{E}(v_i^m | \mathbf{P}, \mathbf{M}) = \sum_l \sum_k \mu_{lk} \, \mathrm{Pr}(Q_i^m \leftarrow B_{lk} | \mathbf{P}, \mathbf{M}) \tag{22}$$

where μ_{lk} is the mean of v_i^m for a founder belonging to breed B_l with maternal marker allele M_k, and $\mathrm{Pr}(Q_i^m \leftarrow B_{lk} | \mathbf{P}, \mathbf{M})$ is the probability that Q_i^m can be traced to a founder belonging to breed B_l with maternal marker allele M_k. This probability will be referred to as the breed B_{lk} composition of MQTL allele Q_i^m, and it can be computed recursively using the PDQs as

$$\begin{aligned} &\mathrm{Pr}(Q_i^m \leftarrow B_{lk} | \mathbf{P}, \mathbf{M}) \\ &= \mathrm{Pr}(Q_i^m \leftarrow Q_d^m | \mathbf{P}, \mathbf{M}) \, \mathrm{Pr}(Q_d^m \leftarrow B_{lk} | \mathbf{P}, \mathbf{M}) \\ &\quad + \mathrm{Pr}(Q_i^m \leftarrow Q_d^p | \mathbf{P}, \mathbf{M}) \, \mathrm{Pr}(Q_d^p \leftarrow B_{lk} | \mathbf{P}, \mathbf{M}) \end{aligned} \tag{23}$$

Table 27.1. A hypothetical multibreed pedigree.

Individual	Mother	Father	Breed	Marker genotype
1	0	0	B_1	$M_1 M_2$
2	0	0	B_2	$M_1 M_3$
3	1	2	–	$M_1 M_3$
4	0	0	B_1	$M_1 M_1$
5	3	4	–	$M_1 M_1$

Table 27.2. Maternal and paternal PDQs for the MQTL alleles of non-founders in the pedigree given in Table 27.1.

Allele	Maternal PDQ	Paternal PDQ
Q_3^m	$1 - r$	r
Q_3^p	r	$1 - r$
Q_5^m	$1 - r$	r
Q_5^p	0.5	0.5

Table 27.3. Conditional breed compositions of MQTL alleles for the multibreed pedigree given in Table 27.1. The pure breeds are assumed to be in equilibrium.

MQTL allele	Breed	
	B_1	B_2
Q_1^m	1	0
Q_1^p	1	0
Q_2^m	0	1
Q_2^p	0	1
Q_3^m	1	0
Q_3^p	0	1
Q_4^m	1	0
Q_4^p	1	0
Q_5^m	$1 - r$	r
Q_5^p	1	0

As in the previous section, calculation of the breed compositions can be organized into a tabular algorithm. The rules for computing the elements of the table are:

1. If individual i is a founder from breed B_l with maternal marker allele M_k and paternal marker allele $M_{k'}$, put 1.0 for elements $e(Q_i^m, B_{lk})$ and $e(Q_i^p, B_{lk'})$.

2. If i is a non-founder with father s and mother d, to compute the breed compositions for Q_i^m: (i) multiply the elements from the row for Q_d^m by the maternal PDQ for Q_i^m; (ii) multiply the elements from the row for Q_d^p by the paternal PDQ for Q_i^m; and (iii) put the sum of the rows generated in (i) and (ii) in the row for Q_i^m. The breed compositions for Q_i^p are similarly obtained from the rows for Q_s^m and Q_s^p.

Using the PDQs from Table 27.2 in this algorithm gives Table 27.4. As in the previous section, the incidence matrix for $\boldsymbol{\beta}_g = [\mu_{11}, \mu_{12}, \mu_{21}, \mu_{23}]'$ is obtained from the table of breed compositions:

$$\mathbf{x}_g = \begin{bmatrix} 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 1 \\ 1-r & r & r & 1-r \\ 2 & 0 & 0 & 0 \\ 1+(1-r)^2 & r(1-r) & r^2 & r(1-r) \end{bmatrix} \quad (24)$$

Table 27.4. Conditional breed composition of MQTL alleles for multibreed pedigree given in Table 27.1. The pure breeds are assumed to be in disequilibrium.

MQTL allele	Breed			
	B_{11}	B_{12}	B_{21}	B_{23}
Q_1^m	1	0	0	0
Q_1^p	0	1	0	0
Q_2^m	0	0	1	0
Q_2^p	0	0	0	1
Q_3^m	$1-r$	r	0	0
Q_3^p	0	0	r	$1-r$
Q_4^m	1	0	0	0
Q_4^p	1	0	0	0
Q_5^m	$(1-r)^2$	$r(1-r)$	r^2	$r(1-r)$
Q_5^p	1	0	0	0

Conditional variances and covariances of MQTL effects

Computation of the variances and covariances will be considered separately. Both will be considered for multibreed pedigrees where the pure breeds may be in gametic phase disequilibrium for the marker locus and the MQTL.

CONDITIONAL VARIANCES. As discussed in computing the conditional mean, any MQTL allele in a multibreed pedigree can be traced to a founder that belongs to some 'breed' B_{lk}. Therefore, the conditional variance for v_i^m, for example, can be written as

$$\text{Var}(v_i^m | \mathbf{P}, \mathbf{M}) = E[\text{Var}(v_i^m | Q_i^m \leftarrow B_{lk}, \mathbf{P}, \mathbf{M})] +$$
$$\text{Var}[E(v_i^m | Q_i^m \leftarrow B_{lk}, \mathbf{P}, \mathbf{M})] \quad (25)$$

The first term of (25) can be written in terms of the conditional breed compositions as

$$E[\text{Var}(v_i^m | Q_i^m \leftarrow B_{lk}, \mathbf{P}, \mathbf{M})] =$$
$$\sum_l \sum_k \sigma_{lk}^2 \Pr(Q_i^m \leftarrow B_{lk} | \mathbf{P}, \mathbf{M}) \quad (26)$$

where $\sigma_{lk}^2 = \text{Var}(v_i^m | Q_i^m \leftarrow B_{lk}, \mathbf{P}, \mathbf{M})$. The second term of (25) can also be written in terms of the conditional breed compositions as

$$\text{Var}[E(v_i^m | Q_i^m \leftarrow B_{lk}, \mathbf{P}, \mathbf{M})] =$$
$$\sum_l \sum_k (\mu_{lk} - \bar{\mu}_i^m)^2 \Pr(Q_i^m \leftarrow B_{lk} | \mathbf{P}, \mathbf{M}) \quad (27)$$

where $\bar{\mu}_i^m$ is the conditional mean of v_i^m given $\mathbf{P}$ and $\mathbf{M}$:

$$\bar{\mu}_i^m = \sum_l \sum_k \mu_{lk} \Pr(Q_i^m \leftarrow B_{lk} | \mathbf{P}, \mathbf{M}) \quad (28)$$

Substituting (26) and (27) in (25) gives

$$\text{Var}(v_i^m | \mathbf{P}, \mathbf{M}) = \sum_l \sum_k [\sigma_{lk}^2 + (\mu_{lk} - \bar{\mu}_i^m)^2]$$
$$\Pr(Q_i^m \leftarrow B_{lk} | \mathbf{P}, \mathbf{M}) \quad (29)$$

CONDITIONAL COVARIANCES. Consider the covariance between v_i^m and v_j^x, where x is m or p, and j is not a direct descendant of individual i. Note that Q_i^m is either Q_d^m, the maternal allele, or Q_d^p, the paternal allele, of d the mother of i. Thus, the conditional covariance between v_i^m and v_j^x can be computed recursively as

$$\text{Cov}(v_i^m, v_j^x | \mathbf{P}, \mathbf{M}) = \text{Cov}(v_d^m, v_j^x | \mathbf{P}, \mathbf{M})$$

$$\text{Pr}(Q_i^m \leftarrow Q_d^m | \mathbf{P}, \mathbf{M}) + \text{Cov}(v_d^p, v_j^x | \mathbf{P}, \mathbf{M})$$

$$\text{Pr}(Q_i^m \leftarrow Q_d^p | \mathbf{P}, \mathbf{M}) \tag{30}$$

This is identical to the formula used when the marker locus and the MQTL are in equilibrium. However, because the conditional variances under disequilibrium are different from the variances under equilibrium and because $\text{Cov}(v_d^m, v_j^x | \mathbf{P}, \mathbf{M})$ or $\text{Cov}(v_d^p, v_j^x | \mathbf{P}, \mathbf{M})$ may reduce to a variance, using (30) gives different results under disequilibrium than under equilibrium.

As described below, use of (30) to construct $\mathbf{\Sigma}_v$ can be expressed in matrix notation. To do so, the rows and columns of $\mathbf{\Sigma}_v$ are ordered such that those for ancestors precede those for descendants. Suppose $\mathbf{\Sigma}_s$ is the gametic covariance matrix for individuals 1, 2, . . ., $i - 1$. This matrix can be expanded to include the covariances with v_i^m, for example, as

$$\mathbf{\Sigma}_{s+1} = \begin{bmatrix} \mathbf{\Sigma}_s & \mathbf{\Sigma}_s \mathbf{q} \\ \mathbf{q}' \mathbf{\Sigma}_s & \text{Var}(v_i^m) \end{bmatrix} \tag{31}$$

where $\mathbf{q}$ is a $2(i-1) \times 1$ vector with the maternal and paternal PDQs for Q_i^m at the positions corresponding to v_d^m and v_d^p and zero at all the other positions, and $\text{Var}(v_i^m)$ is computed using (30). The genetic parameters needed for computing $\mathbf{\Sigma}_v$ are the breed by marker means, μ_{lk}, and variances, σ_{lk}^2, and the recombination rate between the marker and the MQTL, r. The theory presented here can be used to estimate these means, variances and r by maximum likelihood or Bayesian methods.

To illustrate the principles for computing $\mathbf{\Sigma}_v$ under gametic phase disequilibrium, consider the joint distributions for marker and MQTL alleles in Table 27.5. Here, the frequency for MQTL allele Q_1 is 0.85 for breed B_1 and 0.61 for breed B_2. Thus, crosses between these two breeds will result in generating gametic phase disequilibrium. Furthermore in Table 27.5, within each breed, the frequencies of Q_j depend on the allele at the marker locus. Thus, gametic phase disequilibrium is present also within each breed. Note that each of the MQTL means and variances is a function of the joint distribution of the marker and MQTL alleles. For the frequencies given in Table 27.5, and effects of $v_1 = 1$ and $v_2 = 0$ for the MQTL alleles Q_1 and Q_2, these parameters are: $\mu_{11} = 0.889$, $\mu_{12} = 0.5$, $\mu_{21} = 0.1$, $\mu_{23} = 0.667$, $\sigma_{11}^2 = 0.099$, $\sigma_{12}^2 = 0.25$, $\sigma_{21}^2 = 0.09$, $\sigma_{23}^2 = 0.222$. Given these parameters and assuming a recombination rate of $r = 0.1$, $\mathbf{\Sigma}_v$ for the hypothetical five member pedigree (Table 27.1) is given in Table 27.6.

For comparison, $\mathbf{\Sigma}_v$ was also computed assuming the marker and the MQTL are in equilibrium (Table 27.7). Here, it was assumed that the frequency of Q_1 was 0.85 in both breeds.

Finally, to construct HMME, $\mathbf{\Sigma}_v^{-1}$ needs to be computed. Using partioned matrix theory, this inverse can be computed recursively (van Arendonk *et al.*, 1994; Wang *et al.*, 1995). Suppose that $\mathbf{\Sigma}_s^{-1}$ is the inverse of the sub matrix $\mathbf{\Sigma}_s$ defined previously, then the inverse of $\mathbf{\Sigma}_{s+1}$ is

$$\mathbf{\Sigma}_{s+1}^{-1} = \begin{bmatrix} \mathbf{\Sigma}_s^{-1} & 0 \\ 0 & 0 \end{bmatrix} + \begin{bmatrix} -\mathbf{q} \\ 1 \end{bmatrix} v^{ii} \begin{bmatrix} -\mathbf{q}' & 1 \end{bmatrix} \tag{32}$$

where

$$v^{ii} = [\text{Var}(v_i^m) - \mathbf{q}' \mathbf{\Sigma}_s \mathbf{q}]^{-1} \tag{33}$$

Note that $\mathbf{q}$ has only two non-zero elements. Thus, (32) leads to an efficient algorithm, and the resulting inverse is very sparse. Also, because $\mathbf{q}$ has only two non-zero elements, only four elements from $\mathbf{\Sigma}_v$ are needed to compute v^{ii}, and these elements can be obtained efficiently without constructing the entire $\mathbf{\Sigma}_v$ matrix (Abdel-Azim and Freeman, 2001).

Table 27.5. Joint distribution of marker alleles (M_i) and MQTL alleles (Q_j) by breed.

	Breed				
	B_1			B_2	
	Q_1	Q_2		Q_1	Q_2
M_1	0.8	0.1	M_1	0.01	0.09
M_2	0.05	0.05	M_3	0.6	0.3

Table 27.6. Conditional gametic covariance matrix for MQTL for the pedigree in Table 27.1 under disequilibrium between marker and MQTL.

	v_1^m	v_1^p	v_2^m	v_2^p	v_3^m	v_3^p	v_4^m	v_4^p	v_5^m	v_5^p
v_1^m	0.099	0.0	0.0	0.0	0.089	0.0	0.0	0.0	0.08	0.0
v_1^p	0.0	0.25	0.0	0.0	0.025	0.0	0.0	0.0	0.022	0.0
v_2^m	0.0	0.0	0.09	0.0	0.0	0.009	0.0	0.0	0.001	0.0
v_2^p	0.0	0.0	0.0	0.222	0.0	0.2	0.0	0.0	0.02	0.0
v_3^m	0.089	0.025	0.0	0.0	0.127	0.0	0.0	0.0	0.115	0.0
v_3^p	0.0	0.0	0.009	0.2	0.0	0.238	0.0	0.0	0.24	0.0
v_4^m	0.0	0.0	0.0	0.0	0.0	0.0	0.099	0.0	0.0	0.049
v_4^p	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.099	0.0	0.049
v_5^m	0.08	0.023	0.001	0.02	0.115	0.024	0.0	0.0	0.144	0.0
v_5^p	0.0	0.0	0.0	0.0	0.0	0.0	0.049	0.049	0.0	0.099

Table 27.7. Conditional gametic covariance matrix for MQTL for the pedigree in Table 27.1 under equilibrium between marker and MQTL.

	v_1^m	v_1^p	v_2^m	v_2^p	v_3^m	v_3^p	v_4^m	v_4^p	v_5^m	v_5^p
v_1^m	0.127	0.0	0.0	0.0	0.115	0.0	0.0	0.0	0.103	0.0
v_1^p	0.0	0.127	0.0	0.0	0.013	0.0	0.0	0.0	0.011	0.0
v_2^m	0.0	0.0	0.127	0.0	0.0	0.013	0.0	0.0	0.001	0.0
v_2^p	0.0	0.0	0.0	0.127	0.0	0.115	0.0	0.0	0.115	0.0
v_3^m	0.115	0.013	0.0	0.0	0.127	0.0	0.0	0.0	0.115	0.0
v_3^p	0.0	0.0	0.013	0.115	0.0	0.127	0.0	0.0	0.013	0.0
v_4^m	0.0	0.0	0.0	0.0	0.0	0.0	0.127	0.0	0.0	0.064
v_4^p	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.127	0.0	0.064
v_5^m	0.103	0.011	0.001	0.115	0.115	0.013	0.0	0.0	0.127	0.0
v_5^p	0.0	0.0	0.0	0.0	0.0	0.0	0.064	0.064	0.0	0.127

Discussion

This chapter has discussed how BLUP can be used for genetic evaluation using pedigree relationships, trait phenotypes and marker genotypes. When the marker and the MQTL are in equilibrium, the means and variances of MQTL effects are not affected by the marker data. However, the markers do provide information for modelling covariances of MQTL effects. Thus, as discussed below, even markers that are in equilibrium can be used for marker-assisted selection.

When BLUP is based only on pedigree relationships and trait phenotypes, genetic covariances between relatives are computed conditional on the pedigree relationships. These genetic covariances are used in BLUP to combine optimally information from all relatives to obtain the genetic evaluation of each candidate. When, in addition to pedigree and trait information, marker genotypes are available for BLUP, genetic covariances are computed conditional on the pedigree and marker information. These covariances, which are more refined than those based only on pedigree information, lead to more accurate evaluations. In general, closer relatives contribute more than distant relatives toward the BLUP of a candidate. However, if the recombination rate between a marker and an MQTL is low,

even distant relatives that share the same marker information can make significant contributions compared with when only pedigree relationships are used.

To illustrate this, consider the pedigree given in Fig. 27.1. Assuming that the additive genetic variance is 1.0 at a QTL, Table 27.8 gives the genetic covariance matrix for
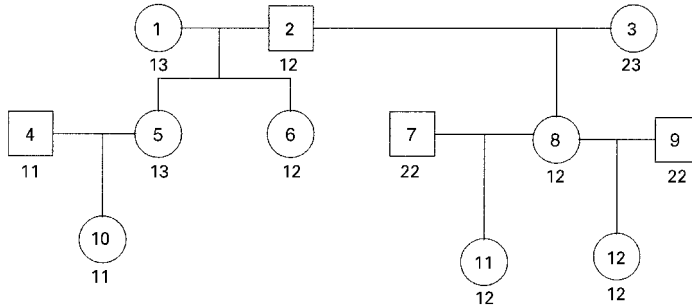
Fig. 27.1. Hypothetical pedigree to illustrate use of marker information for genetic evaluation. The marker genotype for each individual is given below the square or circle representing that individual.

Table 27.8. Genetic covariance matrix for the pedigree in Fig. 27.1 at a QTL conditional only on relationship information. The additive genetic variance at the QTL is assumed to be 1.0.

	g_1	g_2	g_3	g_4	g_5	g_6	g_7	g_8	g_9	g_{10}	g_{11}	g_{12}
g_1	1	0	0	0	0.5	0.5	0	0	0	0.25	0	0
g_2	0	1	0	0	0.5	0.5	0	0.5	0	0.25	0.25	0.25
g_3	0	0	1	0	0	0	0	0.5	0	0	0.25	0.25
g_4	0	0	0	1	0	0	0	0	0	0.5	0	0
g_5	0.5	0.5	0	0	1	0.5	0	0.25	0	0.5	0.125	0.125
g_6	0.5	0.5	0	0	0.5	1	0	0.25	0	0.25	0.125	0.125
g_7	0	0	0	0	0	0	1	0	0	0	0.5	0
g_8	0	0.5	0.5	0	0.25	0.25	0	1	0	0.125	0.5	0.5
g_9	0	0	0	0	0	0	0	0	1	0	0	0.5
g_{10}	0.25	0.25	0	0.5	0.5	0.25	0	0.125	0	1	0.0625	0.0625
g_{11}	0	0.25	0.25	0	0.125	0.125	0.5	0.5	0	0.0625	1	0.25
g_{12}	0	0.25	0.25	0	0.125	0.125	0	0.5	0.5	0.0625	0.25	1

Table 27.9. Genetic covariance matrix for the pedigree in Fig. 27.1 at an MQTL conditional on relationship and marker information. The additive genetic variance at the QTL is assumed to be 1.0 and the recombination rate between the marker and the MQTL is assumed to be 0.05.

	g_1	g_2	g_3	g_4	g_5	g_6	g_7	g_8	g_9	g_{10}	g_{11}	g_{12}
g_1	1	0	0	0	0.5	0.5	0	0	0	0.025	0	0
g_2	0	1	0	0	0.5	0.5	0	0.5	0	0.475	0.475	0.475
g_3	0	0	1	0	0	0	0	0.5	0	0	0.025	0.025
g_4	0	0	0	1	0	0	0	0	0	0.5	0	0
g_5	0.5	0.5	0	0	1	0.095	0	0.453	0	0.5	0.430	0.430
g_6	0.5	0.5	0	0	0.095	1	0	0.048	0	0.048	0.045	0.045
g_7	0	0	0	0	0	0	1	0	0	0	0.5	0
g_8	0	0.5	0.5	0	0.453	0.048	0	1	0	0.430	0.5	0.5
g_9	0	0	0	0	0	0	0	0	1	0	0	0.5
g_{10}	0.025	0.475	0	0.5	0.5	0.048	0	0.430	0	1	0.408	0.408
g_{11}	0	0.475	0.025	0	0.430	0.045	0.5	0.5	0	0.408	1	0.453
g_{12}	0	0.475	0.025	0	0.430	0.045	0	0.5	0.5	0.408	0.453	1

this pedigree at this QTL conditional only on relationship information. Table 27.9 gives the genetic covariance matrix for the same QTL conditional on relationship and marker information, assuming that the recombination rate between the marker and the MQTL is 0.05. Note that individual 8 is a half-sib to individuals 5 and 6. Thus, conditional only on relationship information, the covariance between individuals 8 and 5 is 0.25, and between individuals 8 and 6 is also 0.25 (Table 27.8). Note that individuals 8 and 5 received the same marker allele from their common sire, individual 2, while individuals 8 and 6 received different alleles from individual 2. Thus, the covariance between individuals 8 and 5 is 0.453, while that between individuals 8 and 6 is 0.048 (Table 27.9). So, when only pedigree information is used, both 5 and 6 will contribute equally to the evaluation of 8, but when relationship and marker information are combined, 5 and 6 do not contribute equally to the evaluation of 8. Also, consider individuals 10 and 11. These two individuals have a common grandparent. Thus, conditional only on relationship information, the covariance between these two individuals is 0.0625 (Table 27.8). However, these two individuals received the same marker allele from their common grandparent. Thus, conditional on relationship and pedigree information, their covariance is 0.408 (Table 27.9).

When the marker and the MQTL are in disequilibrium, the marker data can be used to model the means and variances at the MQTL as well. We have extended the theory given by Wang *et al.* (1998) for modelling the means, variances and covariances of MQTL effects in multibreed pedigrees to accommodate the situation where the pure breeds that make up the multibreed pedigree may themselves be in disequilibrium. In this situation, the effect on marker allele within breed is included in the model. If the number of levels for this effect is large, it may be beneficial to consider the effect of marker within breed as random.

A key concept used in computing the conditional means, variances and covariances is that an MQTL allele, Q_i^m for example, can be traced to the maternal

or paternal allele of its mother d. The probabilities $\Pr(Q_i^m \leftarrow Q_d^m | \mathbf{P}, \mathbf{M})$ and $\Pr(Q_i^m \leftarrow Q_d^p | \mathbf{P}, \mathbf{M})$ that Q_i^m descended from Q_d^m or Q_d^p are called the PDQs for Q_i^m (Wang *et al.*, 1995). When marker genotype for a parent is missing, the PDQs can still be computed using the observed markers in the pedigree. But using these in (30) does not give exact results (Wang *et al.*, 1995). Goddard (1998) described the use of markers flanking an MQTL for BLUP. In this case, the PDQs are computed given the genotypes at the flanking markers. If double recombinants can be ignored, when the parent of an individual is doubly heterozygous at the flanking markers, the PDQs for offspring receiving non-recombinant haplotypes are 1 and 0 or 0 and 1. In this case, the MQTL effects of the offspring are eliminated from the model. This can result in a substantial reduction in the number of equations that need to be solved. When the linkage phase of a parent is not known, the PDQs can be computed given all the marker information. Again, this leads to (30) not giving exact results.

Marker data are most useful for genetic evaluation in traits with low heritability (Smith, 1967; Meuwissen and Goddard, 1996). Low heritability suggests non-additive gene action. Under non-additive gene action, however, the inverse of the genetic variance covariance matrix is dense, and as a result HMME cannot be solved efficiently. Thus, BLUP is difficult to implement for non-additive traits, especially when inbreeding is present (De Boer and Hoeschele, 1993). BLUP under non-additive gene action is even more difficult with multibreed data (Lo *et al.*, 1997). However, non-additive gene action and multibreed data do not add to the complexity of genetic evaluation under a finite locus model. Under the assumption of a finite locus model, the conditional mean can be calculated and used as an alternative to BLUP (Fernando and Grossman, 1996; Goddard, 1998; Stricker and Fernando, 1998). This conditional mean of the genotypic values can be calculated exactly using Elston–Stewart type algorithms (Elston and Stewart, 1971), approximated using iterative peeling (van Arendonk *et al.*, 1989), or it can be estimated using Markov chain Monte Carlo

(MCMC) methods (Fernando and Grossman, 1996; Goddard, 1998; Stricker and Fernando, 1998). The computational efficiency of each of these methods is directly related to the number of loci considered in the finite locus model. For peeling-type algorithms, this relationship is exponential in the number of loci. For MCMC methods, this relationship is linear in the number of loci. When marker data are used, a finite locus model needs to accommodate MQTL effects as well as the polygenic effect. Comparison of conditional means under finite locus models with BLUP evaluations indicate that a small number of loci is sufficient to model the polygenic component (Totir *et al.*, 2001). More research is needed to study this approach further.

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28 Incorporating Molecular Information in Breeding Programmes: Applications and Limitations

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Introduction

Poultry breeding prior to this decade was based mainly on what could be observed or measured at the phenotypic level, such as egg number, body weight and feed efficiency. Unfortunately such quantitative traits are also influenced by random environmental factors such as feed quality, peck order, temperature and disease. These environmental factors can greatly decrease genetic progress because birds with good genetic merit but poor environmental effect would be culled, while birds with poor genetic merit but favourable environmental conditions would be kept for breeding. Clearly random environmental factors are a hindrance to breeding superior genetic stock. These errors in the selection process reduce the heritability of trait. However, even greater selection problems occur with sex-limited traits, which can only be measured in one sex, such as egg production, and traits that cannot be measured in either sex, such as disease resistance or meat quality. In those cases the breeder must rely entirely on the pedigree to obtain information from relatives to make selection decisions. This process can be inaccurate, costly and time consuming.

Molecular genetics can in theory address all these issues. Recent advances in biochemical techniques have allowed scientists to probe directly into the genetic code of life. These advances would seem to provide the answer to selecting superior animals without complications of environmental effects. However, this seeming Utopia for poultry breeding is not all that simple. This chapter will examine potential applications of molecular genetics to issues facing the poultry industry and limitations. Parts of this chapter have been presented elsewhere (Muir, 2002).

Issues

The traits of selection and limitations of the species are the primary determinants of where, when and on what traits molecular genetics will be used. The first issue is what traits to select, i.e. what are the problems that poultry breeders need to address? The broiler and layer segments of the poultry industry have very different problems and needs, though there are some similarities. What problems are similar and what problems are unique to the poultry industry? The answers to these issues will help to define what tools can and should be used.

Traits of selection

Preisinger (1998) noted that, in view of the high reproductive rate of hens, in the layer industry a single primary breeder could supply all the grandparents needed in the world and currently about 70% of the total world market is supplied by two groups. This structure is more pronounced in the layer business than it is in the broiler business. Preisinger (1998) concluded that all breeding plans for commercial layer breeding companies have one major objective in common: to increase the genetic potential of the stock to produce a maximum number of saleable, high-quality products at minimum cost in a given production system. Breeders of egg-type chickens concentrate on four major objectives: low mortality, maximum number of saleable eggs per hen housed, low feed cost per unit of egg production (per egg or per kilogram of egg mass) and optimal egg quality. Animal welfare issues are becoming a major concern that breeders will need to address (see Preisinger, 1998; Arthur and Albers, Chapter 1; Kjaer and Mench, Chapter 5; Muir, Chapter 14) and management practices to control these problems, such as beak trimming, may be banned in the future. As such, Preisinger (1998) concluded that egg producers are asking for a different type of hen, specifically adapted to 'animal friendly' production systems.

For broilers, growth is the most critical trait of selection, though increased white (breast) meat yield is becoming important and feed efficiency has the greatest impact on financial bottom lines for integrated production operation (see Arthur and Albers, Chapter 1). However, animal welfare concerns are also impacting management practices such as bird density of growing birds and feed restriction of breeder birds. Table 28.1 summarizes traits of selection, along with issues related to selection for those traits.

Arthur and Albers (Chapter 1) conclude that research is urgently needed for a better understanding of the biological basis of the consequences of lack of balance in the modern broiler compared with its wild ancestor. Understanding this biological basis should direct researchers and breeders to design selection approaches aimed at preventing this lack of balance from progressing further. Genomics could well play a key role in this, both in unravelling the biological mechanisms and in supporting the breeders in selection programmes.

Putative quantitiative trait loci (QTL) have been identified in chickens for feed consumption and body weight at 48 days (Van Kaam *et al.*, 1999), egg quality (Tuiskula-Haavisto *et al.*, 1998) and egg production (Lamont *et al.*, 1996). Szydlowski and Szwaczkowski (1998) used advanced

Table 28.1. Primary traits of selection and characteristics.

Trait of selection	Sex measured	Measurement cost	Heritability
Broilers			
Feed efficiency	Both	High	Moderate
Rate of gain	Both	Low	High
Dressing percentage	Both	Low	Moderate
Disease resistance	Neither	High	Low
Egg production (dam line)	Female	Moderate	Low
Layers			
Egg production	Female	High	Low
Feed efficiency (eggs/feed)	Female	High	Moderate
Well-being	Female	High	Low[a]
Disease resistance	Neither	High	Low
Egg quality	Female	Low	Moderate
Liveability	Both	Low	Low

[a]Depends on how the trait is measured.

statistical procedures to identify QTL associated with body weight, initial egg production, average egg weight and age at sexual maturity. Generally, the results showed mixed model inheritance of the traits studied, indicating high-probability QTL with large effects for all traits studied.

Maximizing genetic gain for traits of selection

The 'quest' of all animal breeding is to maximize long- and short-term response for all traits of economic importance. These responses to selection depend on four factors: (i) selection intensity; (ii) accuracy of selection; (iii) initial genetic variation for the trait of interest; and (iv) effective population size to maintain that variation. The following examines how molecular genetics can aid in each of these factors.

Selection intensity

Intensity is mainly a function of the reproductive capacity of the species and it would seem that molecular genetics would offer no advantage. For some traits, such as egg production in the dam line of broilers, costs limit the number of animals that can be tested. However, multi-stage selection can increase the selection differential in such cases (Muir and Xu, 1992; Xu and Muir, 1992) in combination with molecular genetics. If genetic markers linked to important QTL have been identified, it is possible to do a first-stage selection at a young age on a large population, then only test those in the later stage that pass the first culling.

Another example where multi-stage selection will be advantageous is lost selection intensity on males in poultry layer programmes. Due to cost constraints, usually only one or two roosters are kept from each full-sib family and those are chosen at random. By use of molecular genetics it would be possible to choose among those full-sib brothers in the first stage, then wait for the full record of sisters in the second stage for final selection. In both cases this added selection differential would be lost without molecular genetics and represents lost opportunity (Muir, 1997b, 1999).

Accuracy of selection

Preisinger (1998) concluded that there is an apparent reduction in rate of genetic progress of egg-type chickens. To reduce this problem he advocated methods to improve accuracy of estimation of breeding value. Accuracy is determined by two factors: (i) the heritability of the trait; and (ii) the amount of information available from relatives, including the individual itself.

The use of molecular genetics for sex-limited traits, or traits that cannot be measured in either sex, is perhaps one of the most compelling reasons to use molecular genetics (Lande and Thompson, 1990). For traits that cannot be measured directly in either sex, such as disease resistance, quantitative genetic techniques would require a sib or progeny test, which would be costly and/or increase the generation interval. Gavora (1998) concluded that disease resistance is particularly well suited for the application of marker-assisted selection (MAS), because most of the QTL identified are dominant for Marek's disease (MD) resistance and should facilitate their use in practical breeding programmes. Great strides toward establishing QTL for MD resistance have been made (see Cheng, Chapter 21).

Improvement of animal well-being could greatly benefit from molecular genetics. Selection to improve animal well-being is difficult and requires either direct measurement for traits related to well-being (see Faure *et al.*, Chapter 13) or indirect measurement using group selection (see Muir, Chapter 14). Markers linked to QTL that improve well-being, while at least not compromising productivity, would allow genotypic selection of hard-to-measure phenotypes.

Group selection has been very effective in eliminating cannibalism in birds with untrimmed beaks (Muir and Craig, 1998) but group selection requires that families be housed together and selected as a group. As a result, the rate of inbreeding would increase rapidly, which would be unacceptable for

commercial breeding. Furthermore, because birds in breeding programmes are housed in single-bird cages to obtain individual egg production records, aggression cannot be measured in that environment. Thus measurement of animal well-being in group cages would conflict with current selection programmes, such as best linear unbiased prediction (BLUP), which requires individual records. Thus, alternatives such as molecular genetics might offer a better solution if markers associated with well-being could be found. Because only females are housed and some traits are not measurable in males (e.g. vent picking), group selection is primarily limited to females. Males are chosen based on their sisters' performance. Again, within-family selection for behaviour in males represents a lost opportunity that molecular genetics can address.

Genetic variation for the trait of interest

Long-term response to selection for any trait is dependent on polymorphic loci that influence the trait. Quantitative and molecular genetics are limited to changing frequencies of existing alleles. Many alleles can be lost in the selection process due to random genetic drift, which is a consequence of finite populations and is proportional to the rate of inbreeding.

Beneficial alleles lost during the selection process can be found in wild ancestral populations. A beneficial use of molecular genetics is to search for alleles in wild ancestors of domesticated species that have become lost (Muir, 1994). In every instance where this technique was used, new alleles that outperformed the elite parents by as much as 20% were found (Tanksley, 1997). While it would be possible to cross such populations with domesticated lines and start selecting from the new synthetic line, the frequency of undesirable alleles would also be dramatically increased and would require long-term selection to restore the population to high productivity.

A second advantage of molecular genetics is introgression. Introgression has traditionally been used when genes must be introduced quickly and economically into poultry populations. However, undesirable genes in the donor genome must be excluded as far as possible. Theoretically, DNA-based markers can enhance the efficiency of introgression (Groen and Timmermans, 1992; Hospital et al., 1992). Ideally, introgression would employ equally spaced markers in the host genome and tightly linked flanking markers for the donor gene. The gene of interest could then be introgressed with the highest recovery of the host genome (Fairfull et al., 1998). An example of successful application of this technique is the naked neck gene desirable for production in hot environments (Yancovich et al., 1996).

Transgenic techniques present the one tool that is not available among quantitative genetics techniques (see Petitte, Chapter 33). New genes for novel functions or products can be created. For example, it is theoretically possible to produce directly, in eggs of transgenic chickens, pharmaceuticals such as growth hormone, insulin or any number of other drugs. While pharmaceutical production would be a major boon for the pharmaceutical industry, it may not have much impact on the poultry industry because of the relative few birds needed to produce the product. Genes can also be introduced directly into poultry to improve disease resistance (Gavora, 1996, 1998) or to improve feed efficiency. However, the use of transgenics to improve growth has not been beneficial.

Effective population size

Optimal long-term response to selection is achieved by minimizing loss of favourable alleles, which occurs as a result of random genetic drift and associated inbreeding depression, while maximizing the frequency of desirable alleles (Robertson, 1960, 1961). On the one hand, selection increases frequency of favourable alleles and opposes loss of alleles through drift. On the other hand, increasing selection intensity also reduces the effective population size, which increases the rate of loss of favourable alleles. Similarly, for a given selection intensity, selection programmes that increase the accuracy of selection, such

as BLUP, reduce the effective population size because relatives tend to be selected. Thus, selection programmes that optimize short- vs. long-term response are usually not the same (Muir, 1997a, 2000; see also Quinton, Chapter 12).

Molecular tools can help in this regard by limiting the rate of inbreeding. Van der Beek (1996) and Van Arendonk *et al.* (1998) suggested using mixed semen and determining the sire from parentage testing based on information from genetic markers. As a result, a factorial mating design can be implemented that leads to a higher selection response without increasing the rate of inbreeding.

Preserving genetic variation is essential for finding genes that may have value in the future to combat a new disease or to address a new selection objective, or simply to recover alleles lost during the selection process. Molecular genetic tools can help to maintain this diversity (see Delany, Chapter 15).

Summary

Marker-assisted selection is most beneficial for traits that have low heritability, are measured late in the life cycle, are costly, difficult or expensive to measure, or cannot be measured directly in one or both sexes.

Molecular genetics can also recover lost genetic variation in ancestors, and aid in introgressing such genes into elite populations. Table 28.2 presents a summary of potential benefits of molecular genetics for various traits in poultry breeding.

Limitations

Several problems have been observed with the implementation of MAS. Some of them are most likely due to genotype–environment interactions, negative epistasis between QTL or epistasis between QTL and the genetic background. Also, QTL that were detected by crossing divergent lines identify QTL that differ between breeds and have limited direct application for within-breed improvement (Dekkers and Hospital, 2002). Four overriding factors limit the application of this technology: biological, theoretical, statistical and economic.

Biological factors

One of the main limitations of MAS in within-family selection is the large number of offspring needed from each mating. Van Arendonk *et al.* (1994) concluded that the fraction of the within-family variance that can be explained by markers is of critical

Table 28.2. Potential benefits of molecular genetic techniques.

Benefit	Traits receiving most benefit
Increase selection intensity	Traits measured late in the life cycle or are costly (egg production and feed efficiency for broiler breeders)
	Selection within full-sib families for sex-limited traits (male chicks for egg production, well-being)
Increase accuracy of selection	Selection between full-sib families for sex-limited traits (roosters for egg production, well-being)
	Traits that cannot be measured directly on one or both sexes (egg production, disease resistance, carcass quality, well-being)
	Traits with low heritability (disease resistance, egg production)
	Accurate pedigrees (all traits)
Maintain, find or integrate new genetic variation	Recover lost genes from ancestral populations (all traits)
	Introgress QTL into elite populations (all traits)
	Reduce rate of inbreeding (all traits)
Transgenics	Disease resistance, digestive efficiency, pharmaceuticals
Mating systems	Predict heterosis (heterotic traits, egg production, liveability)

importance. To predict 10% of the within-family variance in grand-offspring, informative genotypes on 500 daughters of both grandsires are needed. In this case, a 20% increase in annual genetic gain is expected (Meuwissen and Van Arendonk, 1992). In contrast to dairy, an increase of only 2–4% is expected in poultry (Van der Beek and Van Arendonk, 1994), due to smaller family size. Van der Beck and Van Arendonk (1996) listed other factors that influence effectiveness of MAS, including the amount of variation explained by the QTL, map distance between the marker and QTL, marker polymorphism, structure of the breeding programme and trait of selection (sex-limited or not).

Compared with pigs and cattle, the relative per-animal replacement cost of poultry is low, reproductive capacity is high, but half-sib family sizes are relatively small.

Theoretical factors

Diversion of selection pressure

Use of MAS, for either introgression or recurrent selection programmes, diverts some selection pressure away from traits of economic importance. Thus, in introgression, the benefit of the target gene must be greater than that which could be achieved by regular selection over the same time period (Dekkers and Hospital, 2002). In recurrent selection programmes, monetary resources devoted to MAS could also be allocated to phenotypic selection programmes. Alternatively, conventional selection programmes can be enhanced by increasing the number of individuals tested and/or the effective population size, which would allow either the selection intensity to increase or the rate of inbreeding to decrease, thus increasing both short- and long-term responses (Muir 1997a, 2000; see also Quinton, Chapter 12).

Linkage disequilibrium

Use of MAS in any form requires linkage disequilibrium (LD), at either the family or population level. In the case of a randomly mating population, different individuals will tend to be in equilibrium, with QTL alleles segregating in proportion to the relative frequencies of the alleles. Alternative marker genotypes will include both positive and negative alleles at any linked QTL, and the mean quantitative value of the alternate marker genotypes will not differ even when a linked segregating QTL is present in the population. If the population under consideration has been selected for many generations without crossing to divergent lines or breeds, it is unreasonable to assume LD between markers and QTL unless they are very close together (Visscher and Haley, 1998).

However, LD always exists within families, even between loosely linked loci. Hence, most animal breeding applications will have to utilize LD within families. Therefore, specific linkage arrangements must be determined for each individual by progeny testing numerous offspring (Beckmann and Soller, 1986). A mixed model approach that utilizes within-family disequilibrium was developed by Fernando and Grossman (1989). Goddard (1992) and Meuwissen and Goddard (1996) generalized the procedure to take advantage of flanking markers. As a result, information on the value of chromosome segments does not erode so quickly from one generation to the next. Although all LD will eventually degrade or break down, with this method two things are happening: the LD established within a given family in a given generation, e.g. among half-sib progeny of a heterozygous sire, will erode over generations but, each generation, new LD will be established within the newly generated families. Because the Fernando and Grossman (1989) model utilizes the within-family LD jointly from all families, as it arises, although LD established within a family in a given generation will erode over generations the joint effect will not. Thus, the efficiency of this method will not erode over generations (J. Dekkers, Iowa State University, 2002, personal communication). In fact Fernando and Totir (see Chapter 27) conclude that, as data accumulate over the generations, the

marked QTL effects will be evaluated more accurately.

However, results presented by Ollivier and Colleau (1998) indicated difficulty in achieving efficient marker-assisted combined selection in situations of linkage equilibrium using linear models and methods of co-ancestry similar to those proposed by Fernando and Grossman (1989). They concluded that very close linkages are necessary and inducing linkage disequilibria would be the most efficient method of increasing efficiency of MAS and would be simpler to implement in practice.

Candidate genes and QTL with major effects

A method of incorporating molecular genetics in selection programmes that does not rely on linkage disequilibrium is to select directly on the candidate gene, i.e. the QTL themselves when they can be identified. The advantage of candidate loci is that the effect of the marker does not erode with recombination as happens with MAS. Templeton (1998) examined linkage mapping vs. the candidate gene approach. There are two major types of measured genotype approach in quantitative genetics: the linkage mapping approach, in which the genotypes being measured are used as markers of a chromosomal segment; and the candidate gene approach, in which prior knowledge indicates that the surveyed loci may be involved in the phenotype of interest. These two approaches are not mutually exclusive; a mixture of anonymous markers and candidate loci can be used in a linkage study. Moreover, the ultimate goal of many linkage studies is to identify the loci responsible for any detected phenotypic associations. This goal requires candidate loci (Templeton, 1998).

Hill (1998) concluded that identifying candidate genes by use of the synteny of the genetic maps of widely divergent species will provide a method for identification of genes of commercial importance in livestock. Fine mapping of QTL more precisely than to l cM is extremely difficult – and insufficient if the gene is to be identified and cloned. The complete sequences of the human and mouse genomes may enable candidate genes to be identified from them using map distances for QTL found in livestock or laboratory animals.

However, one should not expect to find loci with large effects on a trait in a population that has undergone long-term selection for that trait (Lin *et al.*, 1992). Simulations show that the probability of finding genes with major effects for traits that have undergone phenotypic selection for several generations is very low (Sehested and Mao, 1992). With phenotypic selection, genes with large effects reach fixation sooner than those with small effects, and after several generations of selection the likelihood of finding genes with major effects is very low. Traits that show substantial genetic variation after several generations of selection are likely to be determined by a large number of loci, each with trivial effects.

Linkage, mutation, genetic correlations and non-additive genetic effects such as overdominance can keep genes segregating in populations. Unfortunately maintenance of polymorphism through a genetic correlation implies a negative pleiotropic effect on fitness or other traits of selection (Lin *et al.*, 1992; Muir, 1994). Also, deleterious alleles may be maintained in a population by heterozygous advantage (Lin *et al.*, 1992). In either case, superiority cannot be fixed, because of low fitness in the former case and by definition in the latter (Muir, 1994). Thus, if genes with large effects are found to be segregating in a highly selected population with a moderate heritability, the chances are that selecting on the gene would have a negative effect on overall productivity.

From a trait analysis in a poultry layer strain segregating for two GH alleles, Kuhnlein and Zadworny (1994) found that Marek's disease resistance was co-selected with production traits. However, the resistance allele was associated with late sexual maturity and high rate of egg production, whereas the susceptibility was associated with earlier age at first egg and a low rate of egg production. Similarly, Webb and Jordan (1978) found that the halothane gene, a major gene in pigs, had antagonistic effects on percentage lean vs. meat quality and

mortality. These results demonstrate that QTL of large effect can be maintained segregating in a population due to pleiotropic effects with opposite desirability.

Relative efficiency in the short- vs. long-term response

Muir and Stick's (1998) study indicates that, when candidate genes are identified, the optimal programme is not to fix the gene as rapidly as possible. In doing so, animals with many favourable alleles will also be discarded and long-term response will suffer. Smith and Webb (1981) compared the efficiency of direct selection on major genes, on the trait, and an index combining the two. They concluded that the efficiency depends largely on the ratio of additive genetic variance due to the major locus to the total additive genetic variance. When phenotypic selection is effective (high heritability), further information on a major gene will add little to the rate of improvement. If phenotypic selection is not very effective, as for traits with low heritability or if indirect selection must be used, then selection on the candidate gene can add significantly to the rate of genetic improvement (Smith and Webb, 1981).

Several computer-based simulations have been completed comparing MAS with phenotypic selection for populations in linkage equilibrium utilizing within-family LD. Ruane and Colleua (1995) observed in their simulations that MAS resulted in substantially greater increase in the candidate gene than BLUP but lower polygenic response. Meuwissen and Goddard (1996) showed that if the marker QTL explained 33% of the genetic variance, but selection was before recording the trait, MAS increased genetic gain by 9%, if selection occurred after recording the trait, by 38%, if on a sex limited trait, by 38%, and if on traits which could only be measured destructively (carcass traits), by 64%. Meuwissen and Van Arendonk (1992) concluded that conventional selection would be superior to MAS in the long term because conventional selection allocates less selection differential to the fixation of major genes and more to

selection differential of polygenes with small effects. Our results (Muir, 1997b; Muir and Stick, 1998) support the conclusion of Meuwissen and Van Arendonk (1992) and Ruane and Colleau (1995).

Van der Werf (1999) considered the relative advantage of combined selection for a sex-limited and non-sex-limited traits as compared with conventional BLUP. When phenotypic information was available on both sexes, combined selection was initially about 9% better than BLUP selection, but this advantage is lost over the longer term. When phenotypic information was available on females only, initial benefit from combined selection was up to 95%. In general, van der Werf (1999) concluded that combined selection not only increases selection accuracy, but to some extent also accommodates better variances changes. Postponing fixation of the QTL by using a lower weight for its genotypic value is generally beneficial for medium- and long-term profit.

Henshall and Goddard (1998) reported that where the desirable allele was dominant, there was less QTL variance available for selection, and using markers did not result in a large improvement over selection without markers. Under these conditions, by the 7th year the response from BLUP without markers was better than the response from BLUP with markers.

Optimizing long-term selection response is much more difficult than optimizing short-term response. Existing quantitative genetic theory is only adequate for strategies that maximize short-term response to selection and is much less developed for long-term response. Selection on an index of molecular score and phenotype will only optimize short-term selection; for the long term, phenotypic selection alone is superior because it better distributes pressure over all loci (Dekkers and Hospital, 2002).

Additivity and background genotype

There is an abundance of evidence indicating that perhaps epistasis is the norm and additivity is a statistical artefact. From studies published for agronomic traits in plants,

Mayo and Franklin (1998) concluded that it is becoming increasingly clear that epistasis among loci with large effects on a quantitative trait are common and, biologically, additivity is rare. The implications of epistasis for both detecting and using QTL in breeding programmes are obvious. For example, if interactions exist, the effects of individual QTL cannot be meaningfully isolated and such QTL will not contribute as predicted to selection response.

The results of Mackay (1998) support this conclusion. In long-term populations of *Drosophila* she estimated epistatic effects for mapped factors affecting selection response by pairwise effects of non-adjacent intervals containing bristle number QTL. She found that epistatic effects were as large or larger than the main effects, and were also sex-specific.

Non-additivity would also affect expression of a gene in different backgrounds. For example, Rothschild *et al.* (1994) found a positive effect on an oestrogen receptor allele on litter size but the size of the effect was dependent on the genetic background of the pigs (Nielsen and Sørensen, 1998).

In wide crosses of tomatoes, De Vicente and Tanksley (1993) found that at least one QTL had an effect opposite of that expected due to overdominance. In inbred line crosses, Edwards *et al.* (1987) found that overdominance occurred frequently for yield traits. Evaluating expression of QTL in different dams, Dunnington *et al.* (1993) concluded that associations between band patterns and quantitative traits may not be consistent in different genetic backgrounds. Fairfull *et al.* (1987) found that epistatic effects (additive × additive, additive × dominance, and dominance × dominance) were significant for egg production traits.

Statistical factors

Estimation

The effect of the QTL must be established empirically on the basis of statistical associations between markers and phenotype, and hence suffers from the same limitations as quantitative genetic selection. Thus, although combined selection is most effective with traits of low heritability or traits that are difficult to measure (Lande and Thompson, 1990), the ability to detect QTL also requires phenotypic data and is similarly limited in such cases. Thus, the 'greatest opportunities for MAS might exist for traits with moderate rather than low heritability' (Dekkers and Hospital, 2002).

Implementation

Another statistical issue that remains is determining which of the many QTL-marker associates to include in the marker score. Both false positives and false negatives in QTL selection are an issue, but false negatives have a greater impact on efficiency of MAS (Moreau *et al.*, 1998; Spelman and Garrick, 1998). Alternatively, by use of BLUP technology, Meuwissen *et al.* (2001) were able to obtain a molecular score with high predictive ability based on high-density marker genotyping data by using all estimated marker effects, regardless of their statistical significance.

Power

In experiments with low power, QTL effects are overestimated (Georges *et al.*, 1995). Incorrect estimation of QTL effect can lead to the wrong weights being used in selection programmes (Spelman and Van Arendonk, 1997), with corresponding reduced genetic gain.

Economics

Integration of present and new genetic approaches to problems requires consideration of only one factor: economics. Breeders will use any approach that increases their overall profitability. Profitability is determined by the difference in income generated by sales less cost of development. Sales are mainly determined by the genetic superiority of stocks. Geneticists in academia are usually only concerned with the first aspect of profitability (genetic superiority) and often

neglect the equally important consideration of costs to implement the programme. (Muir, 1994).

The major detriments to molecular genetics at this time are the expense and speed of analysis. From economic considerations, at present markers can only be established for those animals that are in an elite breeding nucleus and each selected individual must be mated to enough individuals to pay for the extra cost of genotyping. Dairy cattle fall into this category because of five factors, three of which were listed by Dentine (1992): (i) milk production is a sex-limited trait; (ii) genetic progress in cattle occurs mainly on the sire side because of reproductive limits of the dam; and (iii) selection is multi-stage – the first stage is intense selection among young bulls, where accuracy is low, followed by extensive and costly progeny testing in the second stage. To these factors should be added: (iv) dairy bulls are used extensively due to artificial insemination; and (v) the worth of each bull is many times that of the cost of genotyping each bull.

Colleau (1998) assessed the superiority of MAS over conventional schemes in dairy cattle selection nuclei by deterministic prediction of genetic gains for a given overall investment, in a situation where a single QTL was involved in addition to polygenes. This superiority was moderate (< 5%) and depended on the heritability and the typing costs. An inferiority of MAS was observed for a moderate heritability (0.25), when the cost of typing 20 calves exceeded that of obtaining a calf from embryo transfer. When no typing costs occurred, the percentage of MAS superiority was generally small. It became relatively substantial (around 5%) for the lowest heritability and for the highest proportion of genetic variance explained by the QTL. Thus, Visscher and Haley (1998) concluded that it may only be feasible to use microsatellite markers to select on limited areas of the genome (e.g. to introgress a QTL from one breed to another) but using many such markers at the same time in a breeding programme may be impossible, until costs are reduced.

Dekkers and Hospital (2002) stated that MAS will be most cost effective when molecular costs are less than those of phenotypic observations, such as genotype building and population-wide LD, or the ability to select early. But costs are a greater issue with combined selection because molecular information is in addition to, rather than in place of, phenotypic information, in which case the cost:benefit of MAS may not be more effective than simple phenotypic selection. Arthur and Albers (see Chapter 1) agree with this conclusion: 'As the economic value of individual chickens is relatively low, DNA-based genotyping of individual breeding candidates must be done at low cost per bird. Therefore commercial application of genotyping at the DNA level will largely be through direct genotyping for critical genes and not through marker-assisted selection approaches per se that are being designed for larger species'.

Prospects and Future Directions

With recurrent animal breeding programmes, based primarily on within-line or breed selection, the primary limitations are: (i) the inability to utilize population-wide LD; and (ii) the ability to utilize the power of molecular genetics at the cellular level.

Population-wide LD

Utilization of population-wide LD would allow less expensive MAS programmes with potentially greater relative efficiency than is possible with within-family LD. Increasing flanking marker density to 1–2 cM would uncover substantial population-wide LD (Dekkers and Hospital, 2002).

Utilizing the power of molecular genetics

The real power of molecular genetics is the ability to sort genotypes by some criteria at any age after fertilization. More specifically, the process of animal breeding has been to

increase the frequency of favourable alleles into one genotype. The problem is that these alleles are scattered in the population and possibly linked to undesirable loci. Even assuming that the locations and functions of all the genes in the genome of an animal were known, and assuming these all acted additively, there would still be the formidable task of trying to get all the best alleles into one genotype and it would require several generations of selection on the genotype to bring about the desired combinations.

To address this problem, Visscher and Haley (1998) and Meuwissen *et al.* (2001) suggested breeding strategies to select on molecular score at an early age. For example, with selection only on molecular score, the only limitation is the reproductive cycle. Some technologies may be able to break this limitation by recovery of oocytes before puberty or even the unborn fetus. This is then combined with *in vitro* fertilization and embryo transfer. Further reductions in generation interval would be possible if meiosis could be conducted *in vitro*. Such advances would allow several generations of selection to occur in the laboratory in what has been termed velogenetics (Georges and Massey, 1991). However, as with most new technologies, there is a downside. With each meiotic event and union of gametes (or whatever process is used to recombine the haploid cells), random loss of alleles will occur and inbreeding will increase. This loss of alleles will primarily affect traits not under selection but will also impact to some degree those under selection, because not all QTL for a given trait will be marked. Thus four or five quick cycles of velogenetics could increase short-term response but limit long-term response and result in other fitness problems associated with inbreeding depression. This problem can only be addressed by using a large effective population size of selected cells or embryos. A large effective population size of selected cells would require an even larger number of cells to be genotyped so as to allow selection among them.

There are clear advantages for such technologies for species that have longer reproductive cycles, such as cattle, but for chickens, which reach sexual maturity at 18 weeks, the advantages may not offset the added costs. Arthur and Albers (see Chapter 1) conclude that the ability to reproduce at an earlier age would support the increase of genetic progress by a reduction of the generation interval. The technical problems associated with this, the acceptability of the technologies to be used, and the relatively small impact make such a new development unlikely in poultry. Nevertheless, five or ten generations of genotypic selection within possibly a few weeks would give a company a definite competitive advantage.

Conclusions

Webb (1998) concluded that, for the present, the cost of MAS is still high in relation to the benefit; he indicated a need for caution since the performance benefits may be small, there may be undesirable correlated effects and there is a risk that marker selection may be unsuccessful while slowing down progress from conventional selection. He further concluded that it is very evident that current quantitative methods, with or without assistance from markers, can continue to provide competitive rates of improvement for at least the next decade and so the pig industries of the world will not be under great pressure to venture hastily into the molecular technologies. However, all of us will have to share the responsibility of entering the debate about their role. Similar reasons for concern exist in the poultry industry.

If there were to be experimental validation of these methods, industry and scientists would be less hesitant. Eisen (1998) provided examples of developments in selection theory that have not been adequately tested with artificial selection experiments, including MAS. Hard evidence of the advantages of MAS in the way of selection response from model species or economic species is needed before large commitments of time and money are made by animal breeders. Dekkers and Hospital (2002) concluded that

as the theoretical and experimental results of QTL detection have accumulated, the initial enthusiasm for the potential genetic gains allowed by molecular genetics has been tempered by evidence for limits to the precision of the estimates of QTL effects. The present mood is one of cautious optimism.

However, the recent purchase of Dekalb (a pig-breeding company) by Monsanto (a company known for molecular genetic innovation) indicates that some major companies are taking the opportunities of molecular genetics seriously.

Dodgson (see Chapter 34) offers an alternative view:

The real question is whether continued progress in quantitative genetic theory will be made. Certainly computer technology will make it possible to collect and process more data and to employ more sophisticated models, but how far can one go with a technology based on *not knowing* what the genes/alleles are actually doing? Meanwhile, molecular genetics (genomics) technology continues to move forward at breathtaking speed.

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29   Comparative Genomics

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Introduction

Comparative genomics provides a link between the 'gene-rich' maps of human and other model organisms, with the 'gene-poor' maps of farm animals, including poultry (Andersson *et al.*, 1996; Andersson, 2001). In its simplest definition, comparative genomics applies to comparisons made between any organisms based on the maps of their genomes. These maps can be at a variety of levels of resolution, including chromosome or cytogenetic maps, maps based on genetic linkage, radiation hybrid or physical mapping and, at the highest level, the DNA sequence itself (Nadeau and Sankoff, 1998b; Burt, 2001).

Nomenclature Used in Comparative Genomics

Homologues, orthologues and paralogues

The creation of comparative maps is based on establishing similarities or *homologies* between genomes. These can be based on DNA or protein sequences, cross-hybridization between DNA probes, etc. (Andersson *et al.*, 1996). In practice, homologies are usually based on DNA sequence alignments. Sequence similarities between any two species may be a reflection of a common origin through a speciation event and would represent *orthologous* sequences. Sequence homologies may also result from gene duplication events within a species, e.g. gene families, and represent *paralogous* sequences. For most purposes, comparative maps between species are based on *gene orthologues* and care must be taken to avoid misclassification due to *gene paralogues.*

Conserved synteny, conserved segments and conserved gene orders

Once orthologous genes or orthologues have been defined, conserved chromosome segments can be identified. This is an area of much confusion and the resolution of comparative maps is very dependent on the resolution of the gene maps being compared (Nadeau and Sankoff, 1998a; Burt, 2001). The nomenclature of conserved regions used by Murphy *et al.* (2000) will be used throughout this chapter. If two genes are on the same chromosome they are said to be *syntenic*, and if they are also syntenic in another species they show *conserved synteny* (CS). With more orthologues mapped, it is possible to define *conserved segments unordered* (CSU), where an uninterrupted group of orthologues shows conserved synteny, without regard to gene order. When more orthologues are mapped it is then possible to examine *conserved segments with conserved gene order* (CSO), where an uninterrupted group of three or more orthologues has the same gene order in the two species being compared.

Tools and Resources for Comparative Mapping

The key resources for comparative mapping are *expressed sequence tags* (ESTs, a source of gene markers and sequence homologies), genetic and physical markers, genome maps (genetic and physical) and databases providing summaries of genome data for each species under comparison (Gellin *et al.*, 2000; see also Groenen and Crooijmans, Chapter 26).

Cytogenetics

The karyotypes of most birds are remarkably similar, having $2n = 76–84$, consisting of a few large macrochromosomes (14–16) and a variable number of microchromosomes (60–64), though the diploid number can vary from $2n = 40$ in the stone curlew to $2n = 126$ in the hoopoe. The number and shape of chromosomes have been used to compare the genomes of avian species. This is usually limited to the size and shape of the macrochromosomes and the number of microchromosomes. More details on chromosome structure can be gained using a variety of banding techniques (GTG, RBG, etc.), but again this approach is limited in detail, mostly for the macrochromosomes and the larger microchromosomes (Auer *et al.*, 1987). A standardized banded karyotype is available for the chicken as an aid for comparison (Ladjali-Mohammedi *et al.*, 1999).

Genetic linkage mapping

So far, gene maps based on genetic linkage have produced the most detailed comparative maps between the chicken and other species (Burt and Cheng, 1998; Groenen *et al.*, 2000; Schmid *et al.*, 2000). This approach suffers from the need for genetic mapping populations and polymorphic genetic markers. A number of approaches have been taken to map genes, mostly within the reference mapping populations,

including the use of restriction fragment length polymorphism (RFLP), single strand conformation polymorphism (SSCP), heteroduplex polymorphisms, PCR-RFLP, mismatch primer PCR, microsatellite markers within genes and single-nucleotide polymorphism (SNP)-based assays (Bumstead and Palyga, 1992; Bumstead *et al.*, 1993a,b, 1994a,b, 1995; Khatib *et al.*, 1993; Burt, 1994a,b,c; Levin *et al.*, 1994; Thorp *et al.*, 1994; Burt *et al.*, 1995a,b; Crooijmans *et al.*, 1995; Morrice *et al.*, 1995; Ruyter-Spira *et al.*, 1996, 1998; Smith *et al.*, 1996, 1997, 1998; Girard-Santosuosso *et al.*, 1997; Groenen *et al.*, 1999, 2000; Daval *et al.*, 2000; Noakes *et al.*, 2000; Shi *et al.*, 2001). 'Comparative anchor tagged site' primers, or CATS, were also used, in a limited way, to increase the sequences and genes available for mapping in the chicken (Smith *et al.*, 1997). These primers are based on conserved exon sequences of mammalian species (Lyons *et al.*, 1997).

Fluorescence *in situ* hybridization (FISH)

In the past relatively few genes were mapped by *in situ* hybridization (Shaw *et al.*, 1989, 1991; Tereba *et al.*, 1991; Dominguez-Steglich *et al.*, 1992a,b,c, 1993; Nanda *et al.*, 1996; Riegert *et al.*, 1996) and the earliest reliable result was for the *MYB* proto-oncogene (Soret *et al.*, 1990). This was partly due to technical difficulties with *in situ* hybridization and the lack of rapid methods for isolation of physical probes. Since the mid–late 1990s, physical mapping resources have become readily available and allow the rapid isolation of gene-specific cosmid (Buitkamp *et al.*, 1998) or bacterial artificial chromosome (BAC) clones (Crooijmans *et al.*, 2000). This has resulted in an uptake of FISH as a rapid and reliable gene-mapping tool (Klein *et al.*, 1996; Fillon *et al.*, 1997, 1998, 2001; Mager *et al.*, 1998; Masabanda *et al.*, 1998; Fukagawa *et al.*, 1999, 2001; Suzuki *et al.*, 1999a,b; Matsishima *et al.*, 2000; Carre *et al.*, 2001; Habermann *et al.*, 2001a,b; Kansaku *et al.*, 2001; Koskinen *et al.*, 2001; Ladjali-

Mohammedi *et al.*, 2001; Ogura *et al.*, 2001; Okamura *et al.*, 2001; Shibusawa *et al.*, 2001; Yokomine *et al.*, 2001). In chicken, only the eight largest chromosomes and the Z/W sex chromosomes can be unambiguously identified by size and shape alone. The specific position of genes on these macrochromosomes can be defined at the level of a cytogenetic band when combined with G- or R-banding techniques (Suzuki *et al.*, 1999a). Many authors often define the position as the distance from the telomere on the short arm relative to the length of the whole chromosome (*FLpter*). Recently, it has been possible to differentiate 23 of the 30 microchromosomes in the chicken by two-colour FISH using a set of chromosome-specific probes (Fillon *et al.*, 1998; Schmid *et al.*, 2000).

Chromosome paints for hybridization to chicken and other avian genomes have been produced, either by chromosome sorting or microdissection of single chromosomes and amplification by degenerate oligonucleotide primer (DOP)-PCR (Ambady *et al.*, 1997; Griffin *et al.*, 1999; Guillier-Genecik *et al.*, 1999). Zoo-FISH (chromosome painting between species) is a rapid method for creating comparative maps between species but has a limited resolution: consequently it underestimates the number of conserved segments. Also, Zoo-FISH cannot provide any information about conservation of gene order within conserved segments.

High-resolution genetic and physical mapping

The availability of arrayed yeast artificial chromosome (YAC) (Toye *et al.*, 1997c) and BAC (Crooijmans *et al.*, 2000) large-insert genomic libraries, combined with the public availability of the human genome sequence (International Human Genome Sequencing Consortium, 2001) and the increasing amount of chicken EST sequence data (Abdrakhmanov *et al.*, 2000; Schmid *et al.*, 2000; Tirunagaru *et al.*, 2000), soon to reach over 350,000 partially sequenced cDNA clones (Boardman *et al.*, 2002; Burt

et al., 2002), has facilitated a bi-directional approach to target high-resolution physical mapping to specific chromosomal regions in the chicken. For example, this approach was used to create a high-resolution comparative map between human chromosome 15 and chicken chromosome 10 (Crooijmans *et al.*, 2001). This and other examples of this approach are discussed in more detail by Groenen and Crooijmans in Chapter 26 and so will not be discussed in any detail here. Programmes are underway to create a complete chicken BAC map, which will be the next-generation chicken genome map. With progress in the construction of a genome-wide BAC map and the extensive conservation of genome organization between chicken and human, prospects for a chicken genome sequence in the next few years are also good.

Bioinformatics

One of the goals of comparative genomics is to integrate information from diverse species and sources (Law and Archibald, 2000). In this way, information on orthologues can be shared between species, including map locations but also gene functions, expression patterns, disease models, etc. To facilitate this integration, species-specific databases have been created for all the major farm animal species, including the chicken (Law and Archibald, 2000; Hu *et al.*, 2001). The URL addresses of the key chicken, human and mouse databases are listed in Table 29.1.

To search for gene homologies, a number of tools are available, e.g. NCBI BLAST (www.ncbi.nlm.nih.gov), and when combined with mapping data these can establish gene orthologies. With the publication of the draft sequence of the human genome (International Human Genome Sequencing Consortium, 2001) it is usually worth searching for homology with anonymous (genomic or EST) chicken sequences. Homologies will indicate a potential or known gene and a conserved gene location. An early example of this approach was the identification of

Table 29.1. URL addresses for species-specific and comparative mapping databases.

Species	URL	Database name
Chicken	www.arkdb.org	*Arkdb*, Roslin Institute, UK
Chicken	poultry.mph.msu.edu/resources/Resources.htm	East Lansing, USA
Chicken	www.zod.wau.nl/vf/research/chicken/frame_chicken	*Chickace*, The Netherlands
Mouse	www.informatics.jax.org	*MGD*, Jackson Lab, USA
Human/mouse	www.ncbi.nlm.nih.gov/homology	*NCBI*, USA

an *EV3*-retroviral insertion in the *HCK* gene (Benkel *et al.*, 1995). Shi *et al.* (2001) published two anonymous EST sequences AI979770 (*TUS0019*) and AI979776 (*YUS0022*) mapped on to the East Lansing reference map. A BLAST search of the human genome showed that AI979776 was orthologous to human *CAB45* (stromal cell-derived factor) on chromosome 1p36.3–p36.2 (Burt, 2002, unpublished data). Using these sequence-based approaches, 39 genes have been mapped in the chicken with orthologues in human and mouse (Schmid *et al.*, 2000; Burt, 2002, unpublished data).

Statistical analyses

Until full genome sequences are available, comparative maps can only provide an estimate of the true number and location of conserved segments. Various authors have developed statistical models to estimate the true number of conserved segments from the observed data (Nadeau and Taylor, 1984; Ehrlich *et al.*, 1997; Nadeau and Sankoff, 1998a; Schoen, 2000; Waddington, 2000; Waddington *et al.*, 2000; Kumar *et al.*, 2001). With gene maps based on only 100–200 genes, only the largest conserved segments will be detected (Ehrlich *et al.*, 1997). So 'large conserved segments' may actually be interrupted by smaller conserved segments. These and other conserved segments will only be revealed when larger numbers of genes are mapped, possibly 500 or more. Consequently, low-resolution maps will underestimate the true number of conserved segments. Once the number and location of conserved segments has been defined, it is possible to define evolutionary breakpoints in gene arrangements.

Once chromosomal breakpoints have been established, phylogenetic tools can then be used to calculate lineage-specific rates of chromosome rearrangement that generate these breaks in gene order (Ehrlich *et al.*, 1997; Burt *et al.*, 1999).

Comparative maps and the detection of errors

Comparative mapping is a powerful tool for identifying conserved segments but two major sources of error in defining these segments are misclassification of orthologues with paralogues and mapping errors (Nadeau and Sankoff, 1998a). It is important that everyone should recognize that comparative maps are an estimate of the underlying comparative map between any two species and errors will be present, but should be minimized. The comparative map itself can be used as a tool to detect potential errors. After mapping a new gene, a new conserved segment may be identified, not predicted from the available data. Is it real or an artefact? The mapping data may be incorrect or the gene orthology may be misclassified with a paralogue. The comparative map can therefore highlight a potential problem and the investigator can then recheck the data.

Mapping errors can arise due to either errors in genetic linkage, cross-hybridization between probes in genetic linkage or FISH experiments or incorrect assignment of chromosomes in FISH or somatic cell hybrids. The first example of comparative mapping between the chicken and mammals was demonstrated using chicken–Chinese hamster hybrid cells and identified the *ALB-GC-PPAT-PAICS* conserved synteny (Juneja

et al., 1982; Palmer and Jones, 1986). This syntenic group was thought to lie on chicken chromosome 6 (Kao, 1973) until recently, when genetic and FISH mapping re-assigned this group to chromosome 4 (Fillon *et al.*, 1997; Pitel *et al.*, 1998b).

Genetic linkage using PCR-based assays (e.g. microsatellite markers) can give rise to errors when the primers amplify another locus. The mapping of *LEP* (Pitel *et al.*, 1999, 2000) and *MAX* (Crooijmans *et al.*, 1995; Nanda *et al.*, 1997) loci are two recent examples, highlighted at first by a break in the conservation of synteny between the chicken and human maps. The *LEP* experiment was resolved by showing that the PCR products were not of the expected leptin sequence (Pitel *et al.*, 2000). The *LEP* locus has now been withdrawn from the chicken map. A microsatellite marker mapped a *MAX*-like locus (now renamed *MAXL*) to chromosome 3 (Crooijmans *et al.*, 1995). FISH mapping (Nanda *et al.*, 1997), however, mapped a locus *MAX* to the expected position on chromosome 5p11 (Schmid *et al.*, 2000).

Ten or more years ago *in situ* hybridization tended to use radioactively labelled DNA probes. These were not as sensitive or reliable as modern FISH techniques and most mapping results from this period have tended to be incorrect. Shaw *et al.* (1989, 1991, 1996) described the mapping of the loci for *GH* and *ACTB* to chromosomes 1 and 2, respectively. Both results were later shown to be incorrect (Suzuki *et al.*, 1999a; Schmid *et al.*, 2000). The error in mapping *GH* was also perpetuated by Toye *et al.* (1997b). Similarly, Tereba *et al.* (1991) mapped *CYP19* (P450arom) to the long arm of chromosome 1, later to be shown by physical and genetic linkage to map to microchromosome 10 (Dunn *et al.*, 2000; Crooijmans *et al.*, 2001). Dominguez-Steglich and Schmid (1993), using similar methods, mapped *OTC* to the Z chromosome, later to be correctly mapped to chromosome 1 by genetic linkage and FISH mapping (Shimogiri *et al.* 1998). At the time *OTC* was thought to be the only sex-linked gene in the chicken that was also sex-linked in mammals. This was used as evidence of a common origin of avian and mammalian sex chromosomes. The correct data now shows that sex chromosomes in birds and mammals have had independent origins (see later section on the evolution of sex chromosomes). Ladjali-Mohammedi *et al.* (2001) mapped by FISH the four *HOX* gene clusters in the chicken, to chromosomes 2 (*HOXA*), 3 (*HOXB*), 1 (*HOXC*) and 7 (*HOXD*). The *HOXA*, *C* and *D* results confirmed conservation of synteny between chicken chromosomes 2, 1 and 7 and human chromosomes 7, 12 and 2, respectively. The *HOXB* result was unexpected and appeared to define a new conserved segment. Recent genetic linkage studies have mapped *HOXB6* (*MCW0328*) to chicken chromosome 27 (Schmid *et al.*, 2000; Groenen, Wageningen, 2001, personal communication). This region is orthologous to human chromosome 17q21–q22, the expected homology with the human *HOXB* gene cluster.

Misclassification can also occur based on poor sequence homologies – a risk that may arise in any EST project. For example, Spike *et al.* (1996) mapped a putative locus for *G6PD* on to chicken chromosome 1 with weak homology with human *G6PD*. This locus has now been withdrawn from the chicken map. These gene-mapping errors are summarized in Table 29.2 and are unlikely to be the last.

Vertebrate phylogeny

To interpret comparative mapping data requires a phylogeny of birds and other vertebrates with approximate divergence dates between living species. These phylogenies are increasingly being refined using sequence data on mitochondrial and nuclear DNA sequences (Cooper and Penny, 1997). Most of what is known about avian phylogeny is based on the DNA–DNA hybridization studies of Sibley and Ahlquist (1990). However, the pattern of avian evolution is not established and much controversy remains between classical and molecular approaches (Feduccia, 1995; Cooper and Penny, 1997; Hedges and Poling, 1999).

The phylogenetic relationship of species within the order Galliformes (chickens,

Table 29.2. Gene mapping errors.

Locus symbol(s)	Status	Chromosome[a]	Comment[b]	Reference
LEP	Withdrawn	19	Non-specific PCR	Pitel *et al.* (1999, 2000)
MAXL	New symbol	4	Non-specific PCR	Crooijmans *et al.* (1995)
MAX	Approved	5	FISH	Nanda *et al.* (1997)
ACTB	Withdrawn	2	Non-specific ISH	Shaw *et al.* (1989, 1996)
ACTB	Approved	Micr.	FISH	Suzuki *et al.* (1999b)
GH	Withdrawn	1	Non-specific ISH	Shaw *et al.* (1991)
GH	Approved	27	Linkage	Schmid *et al.* (2000)
CYP19	Withdrawn	1	Non-specific ISH	Tereba *et al.* (1991)
CYP19	Approved	10	Linkage	Crooijmans *et al.* (2000)
OTC	Withdrawn	Z	Non-specific ISH	Dominguez-Steglich *et al.* (1993)
OTC	Approved	1	Linkage/FISH	Shimogiri *et al.* (1998)
HOXB@	Withdrawn	3	Non-specific FISH	Ladjali-Mohammedi *et al.* (2001)
HOXB@	Approved	17	Linkage	Groenen, Wageningen (2000, personal communication)
G6PD	Withdrawn	1	Weak BLAST hit	Spike *et al.* (1996)
ALB . . . PGM2	Corrected	4→6	Chromosome no.	Kao (1973)

[a]Micr., undefined microchromosome; [b]ISH, *in situ* hybridization with ^{3}H-labelled probes and position based on silver grain counts.

quail, turkeys, etc.) is well established, mostly from molecular and cytogenetic data (Stock and Bunch, 1982). In particular, chromosome-banding patterns define two major groups, with chicken and turkey, respectively (Fig. 29.1). Until recently, it was generally thought that the basal divergence among modern birds was between Palaognathae (kiwis, ostrich, emus, cassowaries and rheas) and Neognathae (all other birds), based on morphological and molecular studies (Sibley and Ahlquist, 1990; Caspers *et al.*, 1997; Cooper and Penny, 1997). Analysis of complete mitochondrial DNA sequences of Passeriformes, Galliformes (chicken) and Struthioniformes (ostrich and rhea), however, provide strong support for a split between Passeriformes and a branch including Galliformes and Struthioniformes (Härlid *et al.*, 1998). These results suggest that ratites are descendants of flying, neognathous ancestors and their specific characteristics are not primitive but have been acquired since divergence from a common ancestor 50–90 million years ago.

It is generally accepted that the split between mammals (Synapsida) and birds–crocodiles (Diapsida) was 310 million years ago (Mya) (Benton, 1990). Using this split, Janke and Arnason (1997) estimated the divergence of birds and crocodiles to have occurred 254 Mya (Fig. 29.2). This and more recent fossil evidence from China reinforces the view that birds evolved from the dinosaurs, 150–250 Mya (Stokstad, 2001). More controversial is the relationship of turtles to birds, crocodilians and squamates (lizards and snakes). Traditionally, modern turtles have been considered to be the only the living survivors of the Anapsida reptiles, but phylogenetic analysis of the complete mitochondrial DNA sequences of turtles, birds and crocodiles strongly supports the placement of turtles as the sister group of birds and crocodilians, to the exclusion of squamates (Zardoya and Meyer, 1998). More detailed comparisons made by Hedges and Poling (1999) clarify the relationship of turtles to these groups (Fig. 29.2). Their analysis shows that squamates branched off from all other groups 245 Mya and that turtles–crocodilians branched off from birds about 228 Mya.

Gene Families and the Definition of Orthologues and Paralogues

The problem of misclassification of orthologues and paralogues is well illustrated with avian cytokines. Few have been cloned
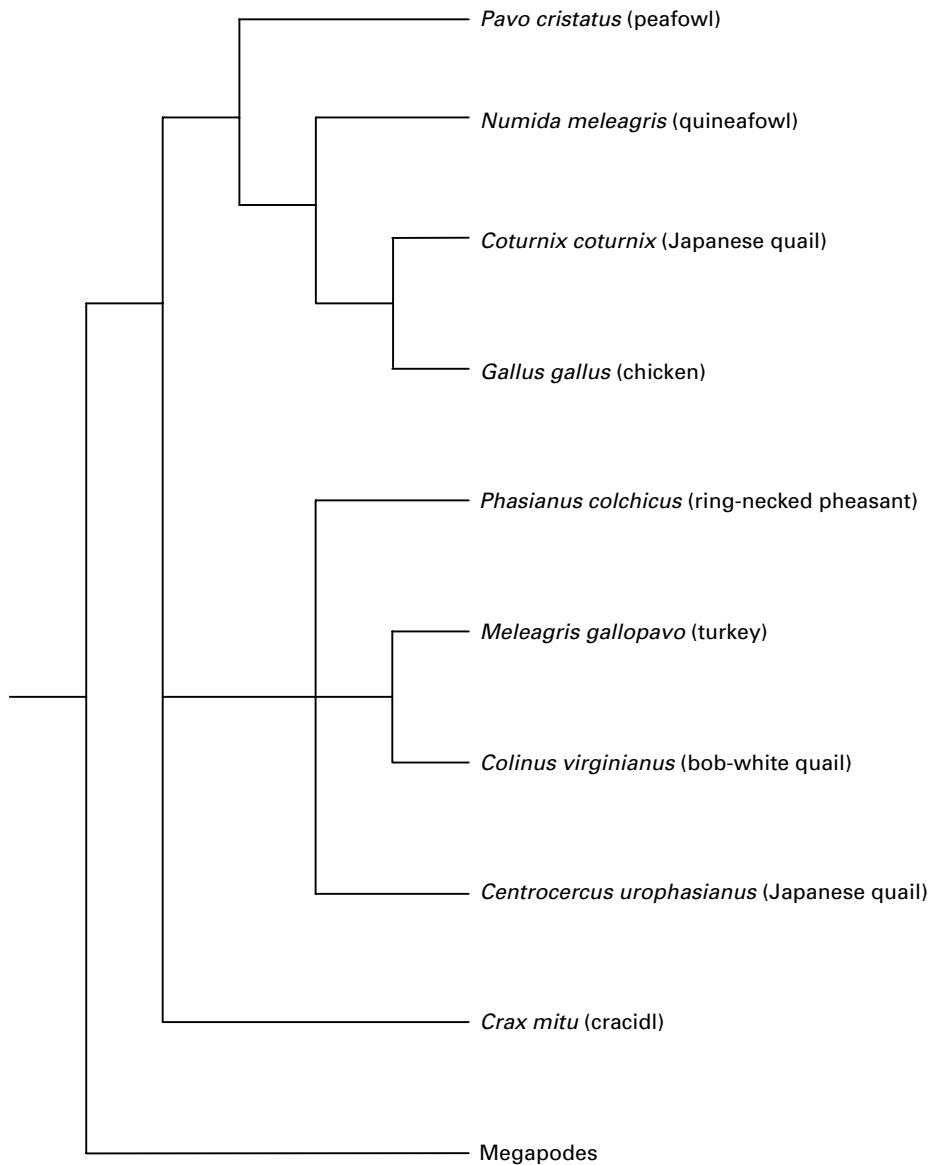
Fig. 29.1. Phylogeny of major avian groups (Stock and Bunch, 1982)

in the chicken, due in part to the low level of sequence homology between avian and mammalian cytokines, which may reflect pathogen-driven selection (Hughes *et al.*, 2001). Consequently, even when a putative chicken cytokine has been cloned it can be difficult to establish orthology with its mammalian homologue. Kaiser and colleagues (Kaiser and Mariani, 1999; Kaiser *et al.*, 1999) were able to prove that they had cloned chicken *IL2* and *IL8* (*SCYB8*) orthologues by showing conservation of coding sequences, promoters, exon–intron structure and conservation of synteny between chicken and human chromosome 4. In much the same way, orthologues to the mammalian CC chemokines *SCYA20* (*LARC*) on human chromosome 2 and the *SCYA-chemokine cluster* on human chromosome 17 were mapped to chicken
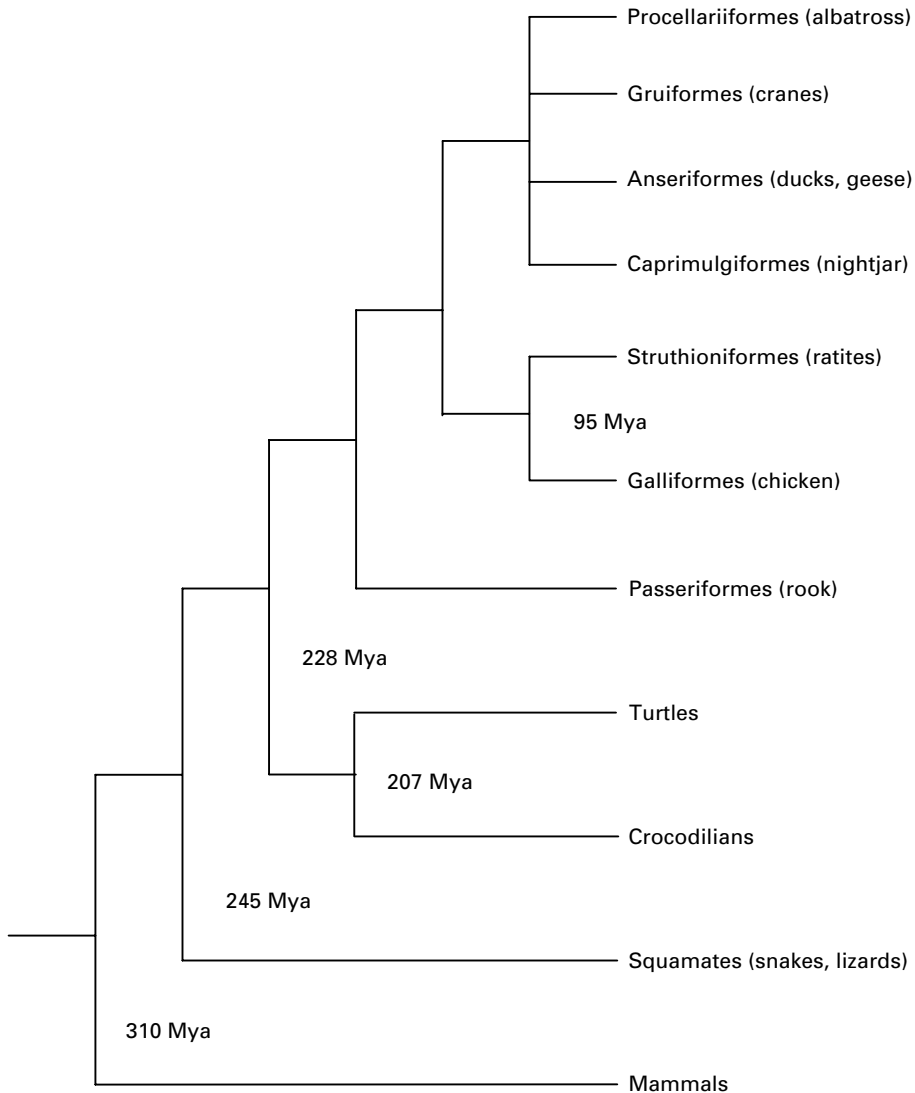
Fig. 29.2. Phylogeny of birds, reptiles and mammals (Benton, 1990; Janke and Arnason, 1997; Härlid *et al.*, 1998; Zardoya and Meyer, 1998; Stokstad, 2001).

chromosomes 9 and 19, respectively (Hughes *et al.*, 2001).

The problem of orthology and paralogy is frequently encountered during the process of gene identification from sequence sampling of BAC clones. For example, shotgun sequencing of a BAC clone identified with the marker *ABR0012* (Crooijmans *et al.*, 2001) produced sequences homologous to the transducin-like enhancer protein family (*TLE1–4*). Sequence identities were very

similar (77–88%) but *TLE3* maps to human chromosome 15q22. This region is orthologous with chicken chromosome 10 and provided the necessary information to predict that *TLE3* is the orthologue on this chromosome.

Paralogues within receptor gene families can be difficult to classify by sequence alone. This is illustrated in the cloning of the chicken vasoactive intestinal polypeptide receptor (*VIPR*; Kansaku *et al.*, 2001). At the

amino sequence level this chicken gene shows 65 and 60% conservation with the human *VIPR1* and *VIPR2* genes, respectively. Phylogenetic analysis suggested a closer similarity to *VIPR1*. FISH mapped a single *VIPR1*-like gene to chicken chromosome 2p32; this is known to be orthologous to human chromosome 3p22–p21, the location of human *VIPR1* and thus confirming orthology. It is of interest that human *VIPR2* maps to chromosome 7q36.3, which is also orthologous to chicken chromosome 2p32 (Lewis *et al.*, 1999; Schmid *et al.*, 2000). Thus these regions are paralogous and derived from a local chromosomal duplication, and the chicken arrangement may be the ancestral one (Burt, 2002, unpublished data).

Evolution of Sex Chromosomes

Of all vertebrates, the sex chromosomes in mammals, reptiles and birds are the most differentiated. In mammals, females have two X chromosomes and males a single X and a small, heterochromatic Y. In birds, the picture is reversed: males have two Z chromosomes and females a single Z and usually a small, heterochromatic W. Early cytogenetic comparisons of karyotypes suggested extensive conservation of sex chromosomes in Galliformes (Stock and Bunch, 1982). Saitoh *et al.* (1993) showed that the chicken Z-linked genes, *IREBP* and *ZOV3*, were also Z-linked in a range of other avian genera. A cluster of at least ten genes encodes chicken *IFN1* and a single gene encodes *IFN2*, and all are located on the short arm of the chicken Z chromosome (Nanda *et al.*, 1998). Sex linkage of *IFN* genes was also observed on the short arm of the Z chromosome of ducks. Using gene-specific probes for comparative FISH mapping of the Z chromosomes of chicken and Japanese quail, Suzuki *et al.* (1999c) were able to confirm the evolutionary conservation of avian sex chromosomes. In contrast to most other avian species, the ratite W chromosome is usually indistinguishable from the Z and completely lacks constitutive heterochromatin (Ansari *et al.*, 1988).

A chicken Z chromosome paint hybridized to both the emu Z and most of the W sex chromosomes (Shetty *et al.*, 1999). This confirmed directly that the ratite sex chromosomes are largely homologous and have not undergone the drastic W reduction as seen in other birds. These conclusions were confirmed and extended by Nishida-Umehara *et al.* (1999) using gene-specific probes hybridized to the sex chromosomes of the double-wattled cassowary. Besides showing homology of the Z and W chromosomes in the ratite, using a probe for *IREBP* showed, at least, that this gene was deleted from the W chromosome. Thus structural rearrangements such as deletions/inversions may have been involved in the evolution of the ancestral pair of sex chromosomes in ratites.

Comparative maps show the independent evolution of the avian and mammalian sex chromosomes from a different pair of autosomes in a primitive reptile (Fridolfsson *et al.*, 1998; Nanda *et al.*, 1999, 2000; Schmid *et al.*, 2000; McQueen *et al.*, 2001). In the chicken, homologues of the mammalian sex chromosomes map to chromosomes 1 and 4. Comparative mapping between human and the chicken sex chromosomes identified extensive homology with human chromosome 9 and some smaller conserved segments with human chromosomes 5, 8 and 18. Previous work had identified male fertility defects on human chromosome 9 that co-localized with the sex differentiation factor, *DMRT1*. Further work mapped the *DMRT1* orthologue to the chicken Z sex chromosome and demonstrated a potential regulatory role for this gene in sex determination in birds (Nanda *et al.*, 1999, 2000). This was supported by expression patterns in the genital ridge and Wolffian duct prior to sexual differentiation and expression at higher levels in ZZ than in ZW embryos (Raymond *et al.*, 1999). *DMRT1* expression becomes testis-specific after onset of sexual differentiation. These observations have led to speculation that two gene dosages of *DMRT1* are required for testis formation in ZZ males, and a single copy in ZW females leads to female sexual differentiation (Shan *et al.*, 2000).

Comparative Maps between Birds

Comparative mapping between avian species is in its infancy. Early work was based on crude measures of chromosome number, shape and size (Tegelström *et al.*, 1983). This early work suggested that the avian karyotype was stable, with few chromosome rearrangements during its evolution. This contrasted with the wide range of karyotypes found in mammals. A number of chromosome rearrangements including chromosome fissions/fusions and inversion were detected from the G-banding patterns of Galliformes (Stock and Bunch, 1982).

Low-resolution genetic linkage maps of the turkey (Reed *et al.*, 2000; E. Smith *et al.*, 2000; Burt, 2001, unpublished data) and the Japanese quail (Kayang *et al.*, 2000; Inoue-Murayama *et al.*, 2001) are underway. Physical mapping of these and other avian genomes is a more rapid method for comparing their genomes. Chicken genomic clones, previously mapped on to chicken macrochromosomes, have been used for comparative mapping of the Japanese quail (Panasenko and Rodionov, 1999; Shibusawa *et al.*, 2001). Most genes were mapped to similar locations in both species but some chromosomal rearrangements were defined for Japanese quail chromosomes 1, 2, 4 and 8. These results confirmed and extended the conclusions made from G-banding (Stock and Bunch, 1982). The location of pericentric inversions in chromosomes 1, 2 and 8 were defined relative to specific genes and genomic clones. At least two rearrangements were mapped to the centromeric region of chromosome 4, including a translocation between chicken chromosome 1 and Japanese quail chromosome 4. Also, all the genomic clones that mapped to chicken microchromosomes also mapped to Japanese microchromosomes, suggesting conservation of these chromosomes. Together these results suggest that the avian genome has changed very little during the past 250 million years.

Chicken chromosome paints for the nine largest autosomes and the Z chromosome (Griffin *et al.*, 1999) were used for Zoo-FISH analysis of 11 avian species belonging to six different orders (Schmid *et al.*, 2000). In most cases, the chicken paints labelled single chromosomes of similar size or chromosome arms in all species tested (Table 29.3). In different pheasant species, chicken chromosome 2 was orthologous to two chromosomes (3 and 6), thus confirming the origin of these chromosomes by fission of the ancestral chromosome 2 of Galliformes. Chicken chromosome 4 orthologues were detected on a chromosome 4 and a microchromosome, not only in pheasants and owl, but also in emus (Shetty *et al.*, 1999). Thus the chicken chromosome 4 arose by fusion of an ancestral chromosome 4 and a microchromosome (Schmid *et al.*, 2000).

Comparative Maps between Birds and Mammals

One of the early goals in chicken genomics was to examine the possibility of building a chicken–human comparative gene map. The first clue that this would be possible was the detection of close linkage between *GC*, *ALB*, *PPAT* and *PGM2* loci in the chicken, a conserved syntenic group found in all mammals (Juneja *et al.*, 1982; Palmer and Jones, 1986). Our initial results and those of others (Burt *et al.*, 1995a; Klein *et al.*, 1996; Smith *et al.*, 1997; Fridolfsson *et al.*, 1998; Smith and Cheng, 1998) showed that indeed there were extensive regions of conserved synteny between the human and chicken genomes. This was highlighted recently by successful Zoo-FISH with a human chromosome 4 'paint' on to chicken chromosome 4 (Chowdhary and Raudsepp, 2000).

The most detailed comparative maps are between those of chicken, mouse and human. These maps are mostly based on data from genetic linkage and FISH mapping experiments. The recent integration of genetic and physical maps of the chicken (J. Smith *et al.*, 2000a; Schmid *et al.*, 2000) facilitated the creation of a consensus comparative map between chicken, human and

Table 29.3. Comparative mapping between chicken macrochromosomes and other birds (Shetty *et al.*, 1999; Schmid *et al.*, 2000; Yoshimura *et al.*, 2000; Shibusawa *et al.*, 2001).

Species	2n	1	2	3	4	5	6[a]	7[a]	8[a]	9[a]	Z
Chicken (*Gallus gallus*)	78	x[b]	x	x	x	x	x	x	x	x	x
Japanese quail (*Corturnix corturnix*)	78	x[c]	x[c]	x	x[c]	x	x	x	x[c]	x	x
Turkey (*Meleagris gallopavo*)	80	x	3 + 6	2	4 + μ	5 + p	8	x	9		(inv)
Pheasant (*Phasianus colchicus*)	82	x	3 + 6	2	4 + μ	x	x		x		x
Golden pheasant (*Chrysolophus pictus*)	82	x	3 + 6	2	4 + μ	x	x	x	x	x	
Silver pheasant (*Lophura nycthemera*)	80	x	3 + 6	2	4 + μ	x	x	x	x	x	x
African collared dove (*Streptopelia roseogrisea*)	78	x		x	4–7	4–7	4–7	4–7	4–7	4–7	
Blackbird (*Turdus merula*)	80			x			x		x	x	
Great grey owl (*Strix nebulosa*)	82	x		x	4 + μ	x	x	x	x		
Eagle owl (*Bubo bubo*)	80								x		
Duck (*Cairina moschata*)	78						x				
Greylag goose (*Anser anser*)	80						x		x		
Rhea (*Rhea americana*)	80						x	x	x	x	
Emus (*Dromaius novaehollandiae*)	80	x	x	x	4 + μ	x	x	x	x	x	x
Double-wattled cassowary (*Casuarius casuarius*)											x

[a]The exact target chromosomes are to be determined.
[b]Indicated probe detects corresponding chromosomes.
[c]Pericentric inversion on chromosomes 1, 2 and 8, inversions on chromosome 4 and small translocation to chromosome 1.

mouse. Recent high-resolution mapping experiments are being based on BAC contig building (Crooijmans *et al.*, 2001; Buitenhuis *et al.*, 2002; Jennen *et al.*, 2002), a trend likely to increase in parallel with more detailed QTL mapping studies. Table 29.4 provides a summary of all comparative mapping data between the chicken and the mouse and human genomes. The comparative maps were constructed based on the principles described by Sankoff *et al.* (1997).

Conservation of Gene Order between Birds and Mammals

Early low-resolution comparative maps (Rettenberger *et al.*, 1995; Frönicke *et al.*, 1997; Chowdhary *et al.*, 1998; Schibler *et al.*, 1998; Burt *et al.*, 1999) suggested a low rate of chromosomal evolution in birds and carnivores and an intermediate level in ruminants, far less than observed between mouse and human. Recent high-resolution maps based on RH panels suggest that these estimates were underestimated by at least a factor of two (Schibler *et al.*, 1998; Band *et al*., 2000; Murphy *et al*., 2000). For

example, comparative mapping between human chromosome 17 and cattle chromosome 19 showed complete conservation of synteny using Zoo-FISH approaches (Chowdhary *et al.*, 1998), but parallel RH mapping revealed three evolutionary breakpoints (Yang and Womack, 1998). This is not surprising, since unless large numbers of genes (500 or more) are mapped to high resolution, the order of genes cannot be compared. Without this gene order information, it is difficult to define internal chromosomal rearrangements.

Large conserved segments are found on chicken chromosomes 4, 5, 6, 7 and 8. Closer inspection reveals one or more intrachromosomal rearrangements, with the possible exception of chicken chromosome 6 (Table 29.4). With more genes mapped at a higher resolution by BAC contig building, it is now possible to examine the question of conservation of gene order and identify intrachromosomal rearrangements within these microchromosomes.

As part of a project to create a detailed human–chicken comparative gene map targeted to the proximal distal end of chicken chromosome 5, Boyd *et al.* (2001b)

Table 29.4. Comparative map of chicken, mouse and human genomes based on loci mapped by genetic linkage and physical mapping.

Chick locus	Chick chr.	Chick consensus	Human chr.	Human pos.	Mouse chr.	Mouse pos.	References
SMOH	1	16.0	7	7q32.3-q32.3	6	7.00	Schmid et al., 2000
GATA3	1	38.0	10	10p15-p15	2	7.00	Schmid et al., 2000
NRCAM	1	87.0	7	7q31.1-q31.2	12	22.00	Schmid et al., 2000
G22P1	1	94.5	22	22q13-q13	15	47.50	Suzuki et al., 1999a
LYZ	1	105.0	12	12q13.3-q14	10	66.00	Schmid et al., 2000
HMGIC	1	105.1	12	12q15-q15	10	67.50	Schmid et al., 2000
IFNG	1	105.2	12	12q24.1-q24.1	10	67.00	Habermann et al., 2001a
MYF6	1	106.0	12	12q21-q21	10	59.00	Suzuki et al., 1999a
DCN	1	115.5	12	12q21.3-q23	10	55.00	Schmid et al., 2000
MGF	1	126.0	12	12q22-q22	10	57.00	Suchyta et al., 2001
BTG1	1	127.0	12	12q22-q22	UN	N/A	Habermann et al., 2001a
CRADD	1	132.0	12	12q21.33-q23.1	10	52.00	Schmid et al., 2000; Smith et al., 2000c
GNRH1	1	137.0	8	8p21-p11.2	14	39.50	Schmid et al., 2000
HIS@	1	143.0	6	6p21.3-p21.3	13	12.00	Schmid et al., 2000
A2M	1	143.0	12	12p13.3-p12.3	6	62.00	Bumstead, Compton, 2001, personal communication
MGP	1	143.2	12	12p13.1-p12.3	6	syntenic	Suchyta et al., 2001
EMP1	1	143.2	12	12p12-p12	6	65.00	Bumstead, Compton, 2001, personal communication
NAGA	1	143.2	22	22q11-q11	15	syntenic	Schmid et al., 2000
ADSL	1	151.0	22	22q13.1-q13.1	15	46.00	Schmid et al., 2000
H5	1	155.0	22	22q13.1-q13.1	15	46.75	Schmid et al., 2000
LGALS4	1	157.0	22	22q12-q13	7	4.00	Schmid et al., 2000
MAFF	1	163.0	22	22q12.2-q12.2	7	syntenic	Schmid et al., 2000
ASCL1	1	170.0	12	12q22-q23	10	syntenic	Schmid et al., 2000
IGF1	1	172.0	12	12q22-q23	10	48.00	Schmid et al., 2000
TRA1	1	174.0	12	12q22-q23	10	49.00	Van Hest et al., 1994; Schmid et al., 2000
ITPR2	1	189.0	12	12p11-p11	6	syntenic	Schmid et al., 2000
LDHB	1	204.0	12	12p12.2-p12.1	6	62.00	Schmid et al., 2000
CCND2	1	230.0	12	12p13-p13	6	61.10	Suzuki et al., 1999a

SCNN1A	1	233.0	12	12p13–p13	6	60.60	Habermann et al., 2001a
GAPD	1	241.0	12	12p13–p13	6	56.00	Schmid et al., 2000
CD4	1	241.0	12	12pter–p12	6	60.18	Bumstead, Compton, 2001, personal communication
USP5	1	241.0	12	12p13–p13	6	60.20	Bumstead, Compton, 2001, personal communication
PPARA	1	241.0	22	22q13.31–q13.31	15	48.80	Schmid et al., 2000
TCRB@	1	241.0	7	7q35–q35	6	20.50	Schmid et al., 2000
HSD3B1	1	254.0	1	1p13.1–p13.1	3	49.10	Schmid et al., 2000
SCYC1	1	266.0	1	1q23–q25	1	87.00	Hughes and Bumstead, 2000
EPHA3	1	283.1	3	3p11.2–p11.2	16	syntenic	Suzuki et al., 1999a
IFNAR2	1	317.2	21	21q22.1–q22.1	16	61.00	Smith et al., 2000c
IL10RB	1	317.2	21	21q22.1–q22.2	16	61.00	Smith et al., 2000c
IFNAR1	1	317.3	21	21q22.1–q22.1	16	64.00	Smith et al., 2000c
GART	1	317.4	21	21q22.1–q22.1	16	63.00	Smith et al., 2000c
SOD1	1	330.0	21	21q22.1–q22.1	16	61.00	Liu et al., 2001
CRYAA	1	341.0	21	21q22.3–q22.3	17	17.40	Schmid et al., 2000
FABP7	1	356.5	6	6q22–q23	10	syntenic	Guttenbach et al., 2000; Schmid et al., 2000
MAOA	1	357.0	X	Xp11.4–p11.3	X	5.20	Habermann et al., 2001a
OTC	1	358.0	X	Xp21.1–p21.1	X	3.00	Schmid et al., 2000
DAX1	1	358.1	X	Xp21.3–p21.2	X	33.00	C.A. Smith et al., 2000
ZFX	1	361.0	X	Xp22.1–p22.1	X	34.80	Schmid et al., 2000
SCML2	1	362.7	X	Xp22–p22	X	syntenic	Schmid et al., 2000
LAMP1	1	418.0	13	13q34–q34	8	1.00	Schmid et al., 2000
RB1	1	474.0	13	13q14.3–q14.3	14	41.00	Schmid et al., 2000
U16	1	474.1	13	13q14.2–q14.2	14	41.00	Schmid et al., 2000
HMGB1	1	475.0	13	13q12–q12	5	83.00	Schmid et al., 2000
HOXC@	1	480.0	12	12q13–q13	15	57.40	Ladjali-Mohammedi et al., 2001
FLI1	1	490.0	11	11q24.1–q24.3	9	16.00	Mager et al., 1998
APLP2	1	500.0	11	11q24–q24	9	13.00	Jennen et al., 2002
ATM	1	510.0	11	11q22–q23	9	30.00	Jennen et al., 2002
PGR	1	520.0	11	11q22.1–q22.3	9	syntenic	Schmid et al., 2000
FUT4	1	527.0	11	11q21–q21	9	3.00	Schmid et al., 2000

Table 29.4. *Continued.*

Chick locus	Chick chr.	Chick consensus	Human chr.	Human pos.	Mouse chr.	Mouse pos.	References
TYR	1	556.5	11	11q21–q21	7	44.00	Suzuki *et al.*, 1999a
THRSP	1	556.6	11	11q13.5–q14.1	UN	N/A	Carre *et al.*, 2001
UCP2	1	556.7	11	11q13–q13	7	50.00	Schmid *et al.*, 2000
WNT11	1	564.0	11	11q13.5–q13.5	7	48.00	Schmid *et al.*, 2000
HBB@	1	565.0	11	11p15.4–p15.4	7	50.00	Schmid *et al.*, 2000
RPS11	1	565.0	19	19q13.3–q13.3	7	syntenic	Smith *et al.*, 2002
GABRB3	1	565.1	15	15q11.2–q12	7	30.00	Crooijmans *et al.*, 2001
UBE3A	1	565.1	15	15q11–q13	7	28.65	Crooijmans *et al.*, 2001
GABRA5	1	565.1	15	15q11.2–q13	7	28.50	Crooijmans *et al.*, 2001
OCA2	1	565.1	15	15q11.2–q13	7	28.50	Crooijmans *et al.*, 2001
ATBA2	1	565.2	15	15q11.2–q13	7	27.00	Crooijmans *et al.*, 2001
TJP1	1	565.2	15	15q11.2–q13	7	28.50	Crooijmans *et al.*, 2001
HERC2	1	565.4	15	15q11–q13	7	27.00	Crooijmans *et al.*, 2001
RAB6	1	569.0	2	2q14–q21	2	syntenic	Schmid *et al.*, 2000
NFYB	1	570.0	12	12q22–q24.1	10	43.70	Schmid *et al.*, 2000
WNT3A	2	–1.0	17	17pter–qter	11	32.00	Hughes *et al.*, 1999; Schmid *et al.*, 2000
VIPR1	2	9.0	3	3p22–p22	9	71.00	Kansaku *et al.*, 2001
ACVR2B	2	36.0	3	3p22–p21.3	9	syntenic	Schmid *et al.*, 2000
CCR5	2	36.1	3	3p21–p21	9	72.00	Boyd, Compton, 2001, personal communication
SHH	2	38.0	7	7q36–q36	5	16.00	Schmid *et al.*, 2000
EN2	2	45.0	7	7q36–q36	5	15.00	Schmid *et al.*, 2000
Hs.70333	2	62.0	10	10p11.2–p11.2	18	syntenic	Schmid *et al.*, 2000
NRP1	2	72.0	10	10p12–p12	8	73.00	Suzuki *et al.*, 1999a
VIM	2	76.0	10	10p13–p13	2	7.00	Schmid *et al.*, 2000
MRC1	2	77.0	10	10p13–p13	2	5.00	Schmid *et al.*, 2000
NPY	2	98.0	7	7p15.1–p15.1	6	26.00	Schmid *et al.*, 2000
HOXA@	2	115.5	7	7p15–p14	6	26.30	Ladjali-Mohammedi *et al.*, 2001

Gene		Position	Chrom	Band		Value	Reference
CP49	2	138.0	3	3q21.2–q22.3	UN	N/A	Wallace et al., 1998; Schmid et al., 2000
THRB	2	140.0	3	3p24.1–p22	14	syntenic	Suzuki et al., 1999a
EGFR	2	175.5	7	7p12–p12	11	9.00	Suzuki et al., 1999a
TGFBR1	2	179.0	9	9q33–q34.1	4	19.30	Suchyta et al., 2001
PRL	2	186.0	6	6p22.2–p22.1	13	14.00	Miao et al., 1999; Schmid et al., 2000
BMP6	2	200.0	6	6p24–p23	13	20.00	Schmid et al., 2000
ALDH1A5	2	227.0	9	9q21–q21	19	12.00	Schmid et al., 2000
BCL2	2	228.0	18	18q21.3–q21.3	1	59.80	Schmid et al., 2000
YES1	2	274.0	18	18p11.3–p11.2	5	18.20	Schmid et al., 2000
MYL	2	274.1	18	18p11.3–p11.3	5	syntenic	Schmid et al., 2000
ZFP161	2	282.0	18	18pter–p11.21	17	41.00	Schmid et al., 2000
ADCYAP1	2	282.1	18	18p11–p11	18	syntenic	Schmid et al., 2000
CDH2	2	282.2	18	18q12.1–q12.1	18	6.00	Suzuki et al., 1999a
RYR2	2	302.0	1	1q42.1–q43	13	7.00	Schmid et al., 2000
PRKDC	2	319.9	8	8q11–q11	16	9.20	Suzuki et al., 1999a
MOS	2	320.0	8	8q11–q11	4	0.00	Schmid et al., 2000
PENK	2	322.0	8	8q11.23–q12	4	0.80	Schmid et al., 2000
IRX1	2	332.0	5	5p15.3–p15.3	13	syntenic	Ogura et al., 2001
IRX6	2	332.0	UN	UN	UN	N/A	Ogura et al., 2001
TERF1	2	357.0	8	8q13–q13	1	11.80	Fillon et al., 2001
LYN	2	357.1	8	8q13–q13	4	0.00	Suzuki et al., 1999a
CALB1	2	358.1	8	8q21.3–q22.1	4	10.50	Schmid et al., 2000
CA2	2	358.2	8	8q22–q22	3	10.50	Schmid et al., 2000
TRHR	2	383.0	8	8q23–q23	15	24.70	Schmid et al., 2000
MYC	2	401.0	8	8q24.12–q24.13	15	32.00	Schmid et al., 2000
HSF1	2	452.0	8	8q24.3–q24.3	15	43.00	Schmid et al., 2000
LIMK2	2	461.0	22	22q12–q12	1	syntenic	Schmid et al., 2000
LHCGR	3	32.0	2	2p21–p21	17	46.50	Ge et al., 2001
JAG1	3	43.1	20	20p12.2–p12.2	2	77.00	Guillier-Gencik et al., 2001
SNAP25	3	43.2	20	20p12–p11.2	2	78.20	Guillier-Gencik et al., 2001
BMP2	3	52.0	20	20p12–p12	2	76.00	Schmid et al., 2000

Table 29.4. *Continued.*

Chick locus	Chick chr.	Chick consensus	Human chr.	Human pos.	Mouse chr.	Mouse pos.	References
ADPRT	3	54.0	1	1q41–q42	1	98.60	Suchyta *et al.*, 2001
TGFB2	3	77.0	1	1q41–q41	1	101.50	Schmid *et al.*, 2000
ACTN2	3	110.0	1	1q42–q43	13	7.00	Suchyta *et al.*, 2001
HMX1	3	151.0	4	4p16.1–p16.1	5	18.00	Schmid *et al.*, 2000
SULT1A2	3	151.1	16	16p12–p11.2	17	34.00	Liu *et al.*, 2001
T	3	151.2	6	6q26–q27	17	4.02	Schmid *et al.*, 2000
TCP1	3	151.3	6	6q25.3–q26	17	7.50	Schmid *et al.*, 2000
MPR1	3	153.0	6	6q25.3–q25.3	17	7.35	Yokomine *et al.*, 2001
ESR1	3	153.1	6	6q25.1–q25.1	10	12.00	Schmid *et al.*, 2000
VIP	3	153.2	6	6q24–q27	10	syntenic	Schmid *et al.*, 2000
MYB	3	170.0	6	6q23.3–q24	10	16.00	Schmid *et al.*, 2000
PLN	3	182.0	6	6q22.1–q22.1	10	syntenic	Schmid *et al.*, 2000
FYN	3	200.0	6	6q21–q21	10	25.00	Schmid *et al.*, 2000
CCNC	3	205.9	6	6q21–q21	10	syntenic	Schmid *et al.*, 2000
EEF1A1	3	218.0	6	6q14–q14	4	syntenic	Schmid *et al.*, 2000
ME1	3	222.0	6	6q12–q12	9	48.00	Pitel *et al.*, 1998c; Schmid *et al.*, 2000
BMP5	3	238.0	6	6q12–q13	9	42.00	Schmid *et al.*, 2000
GSTA2	3	239.0	6	6p12–p12	9	44.00	Schmid *et al.*, 2000
MFAP1	3	263.0	15	15q15–q12	14	28.50	Schmid *et al.*, 2000
ODC1	3	265.0	2	2p25–p25	12	6.00	Schmid *et al.*, 2000
MYCN	3	270.0	2	2p24.3–p24.3	12	4.00	Schmid *et al.*, 2000
POMC	3	300.0	2	2p23.3–p23.3	12	4.00	Cheng, East Lansing, 2001, personal communication
TNFRSF1A	3	320.0	12	12p13–p13	6	60.55	Liu *et al.*, 2001
BTK	4	–9.1	X	Xq21.33–q22	X	51.00	Suzuki *et al.*, 1999a
HPRT1	4	–9.0	X	Xq26.1–q26.1	X	17.00	Schmid *et al.*, 2000
PGK1	4	48.0	X	Xq13.3–q13.3	X	45.00	Rauen *et al.*, 1994; Schmid *et al.*, 2000

UBE2A	4	66.0	Xq24–q25	X	syntenic	Suchyta et al., 2001
CUL4B	4	75.0	Xq23–q23	X	syntenic	Schmid et al., 2000
FMR1	4	82.0	Xq27.3–q27.3	X	24.50	Schmid et al., 2000
TLR2	4	89.5	4q32–q32	3	40.50	Boyd et al., 2001
FGB	4	89.6	4q28–q28	3	48.20	Suzuki et al., 1999a
EDNRA	4	105.0	4q27–q28	UN	N/A	Suchyta et al., 2001
MADH1	4	109.0	4q28–q28	13	35.00	Schmid et al., 2000
CLOCK	4	131.5	4q11–q12	5	43.00	Noakes et al., 2000; Yoshimura et al., 2000
FGF2	4	146.1	4q25–q27	3	19.30	Schmid et al., 2000
Hs.264330	4	148.0	4pter–qter	UN	N/A	Schmid et al., 2000
IL2	4	150.0	4q26–q27	3	19.20	Schmid et al., 2000
ANXA5	4	155.9	4q26–q28	3	19.20	Bumstead et al., 1994a; Schmid et al., 2000
IRF2	4	156.0	4q35.1–q35.1	8	syntenic	Marienfeld et al., 1997; Schmid et al., 2000
SPP1	4	156.1	4q11–q21	5	56.00	Schmid et al., 2000
SCYB8	4	158.0	4q13–q21	5	syntenic	Schmid et al., 2000
ALB	4	158.3	4q11–q13	5	50.00	Suchyta et al., 2001
GC	4	158.4	4q12–q13	5	50.00	Suchyta et al., 2001
CENPC1	4	159.0	4q12–q13.3	5	syntenic	Okamura et al., 2001
PPAT	4	173.0	4q12–q12	5	syntenic	Suchyta et al., 2001
PAICS	4	173.1	4q12–q12	5	syntenic	Gavalas et al., 1993; Schmid et al., 2000
PGM2	4	173.2	4p14–q12	5	38.00	Gavalas et al., 1993; Schmid et al., 2000
KDR	4	173.3	4q12–q12	5	42.00	Schmid et al., 2000
KIT	4	180.0	4q12–q12	5	42.00	Schmid et al., 2000
NFKB1	4	189.0	4q24–q24	3	68.90	Suchyta et al., 2001
FGFR3	4	200.0	4q24–q28	5	20.00	Suchyta et al., 2001
MSX1	4	217.0	4p16.1–p16.1	5	21.00	Schmid et al., 2000
BNC1	4	217.1	4p16–p16	5	syntenic	Schmid et al., 2000
TGFBR2	3	217.2	3p22–p22	9	69.00	Schmid et al., 2000
CD8A	2	244.0	2p12–p12	6	30.50	Schmid et al., 2000
CTNNA2	2	246.0	2p12–p11.1	6	34.20	Schmid et al., 2000
NP220	2	250.0	2p24.3–p24.1	6	syntenic	Matsushima et al., 2000
CTNNA1	5	256.0	q31–q31	18	11.0	Suzuki et al., 1999a

Table 29.4. *Continued.*

Chick locus	Chick chr.	Chick consensus	Human chr.	Human pos.	Mouse chr.	Mouse pos.	References
MAX	5	4.0	14	14q23–q23	12	33.00	Schmid *et al.*, 2000
CD5	5	19.1	11	11q13.1–q13.1	19	5.00	Koskinen *et al.*, 2001
PAX6	5	26.0	11	11p13–p13	2	58.00	Suzuki *et al.* 1999a
RAG2	5	46.0	11	11p13–p13	2	56.00	Suzuki *et al.*, 1999a
MYOD1	5	47.0	11	11p15.4–p15.4	7	23.50	Suzuki *et al.*, 1999a
TPH	5	51.0	11	11p15.1–p14.3	7	23.50	Habermann *et al.*, 2001b
IGF2	5	57.0	11	11p15.5–p15.5	7	69.09	Yokomine *et al.*, 2001
INS	5	57.1	11	11p15.5–p15.5	7	69.10	Habermann *et al.*, 2001b
TH	5	57.2	11	11p15.5–p15.5	7	69.20	Schmid *et al.*, 2000
CCND1	5	60.5	11	11q13.3–q13.3	7	72.30	Schmid *et al.*, 2000
CAT	5	71.0	11	11p13–p13	2	57.00	Liu *et al.*, 2001
Hs.151050	5	73.0	11	11p11.12–p11.12	UN	N/A	Schmid *et al.*, 2000
CAPN1	5	76.0	11	11q13–q13	19	3.00	Suchyta *et al.*, 2001
KIAA0247	5	88.0	14	14q22–q22	UN	N/A	Schmid *et al.*, 2000
BRF1	5	92.0	14	14q22–q24	12	syntenic	Schmid *et al.*, 2000
RYR3	5	106.1	15	15q14–q15	2	syntenic	Schmid *et al.*, 2000
ACTC	5	106.2	15	15q11–q14	2	64.00	Crooijmans *et al.*, 2001
TYRO3	5	106.3	15	15q15.1–q21.1	2	67.10	Crooijmans *et al.*, 2001
CAPN3	5	106.4	15	15q15.1–q21.1	2	67.20	Crooijmans *et al.*, 2001
RAD51	5	106.5	15	15q15.1–q15.1	2	66.80	Schmid *et al.*, 2000
THBS1	5	106.6	15	15q15–q15	2	65.00	Schmid *et al.*, 2000
HTR1D	5	110.1	1	1p36.3–p34.3	4	66.00	Smith *et al.*, 2000b; Schmid *et al.*, 2000
PTAFR	5	110.1	1	1p35–p34.3	4	62.40	Smith *et al.*, 2000b; Schmid *et al.*, 2000
TGFB3	5	110.2	14	14q24–q24	12	41.00	Schmid *et al.*, 2000
CALM1	5	130.1	14	14q32–q32	7	4.50	Schmid *et al.*, 2000
HSPCAL4	5	151.1	14	14q32.3–q32.3	12	syntenic	Schmid *et al.*, 2000
IGH@	5	151.2	14	14q32.3–q32.3	12	58.00	Schmid *et al.*, 2000
NDUFB1	5	152.0	14	14q32–q32	12	syntenic	Boyd *et al.*, 2002

Gene		cM		Cytoband			Reference
DNCH1	5	160.0	14	14q32.3–qter	12	55.00	Schmid et al., 2000
DIO3	5	160.0	14	14q32–q32	12	F1	Boyd et al., 2002
CKB	5	160.0	14	14q32.3–q32.3	12	55.00	Schmid et al., 2000
EIF5	5	160.0	14	14q32–q32	12	syntenic	Boyd et al., 2002
CDC42BPB	5	162.0	14	14q32–q32	12	syntenic	Boyd et al., 2002
KLC	5	162.0	14	14q32–q32	12	57.00	Boyd et al., 2002
AKT1	5	164.0	14	14q32–q32	12	57.00	Boyd et al., 2002
JAG2	5	166.0	14	14q32–q32	12	57.90	Boyd et al., 2002
HSPA2	5	170.0	14	14q22–q22	12	34.00	Schmid et al., 2000
SOS2	5	192.0	14	14q21–q22	12	30.00	Suzuki et al., 1999a
BMP4	5	202.0	14	14q22–q23	14	14.00	Schmid et al., 2000
TFAM	6	42.5	10	10q21–q21	10	38.00	Suzuki et al., 1999a
PSAP	6	59.0	10	10q22.1–q22.1	10	35.00	Azuma et al., 1998; Schmid et al., 2000
SUPV3L1	6	59.1	10	10q22.1–q22.1	10	syntenic	Schmid et al., 2000
PLAU	6	59.2	10	10q24–q24	14	2.50	Schmid et al., 2000
SCD	6	63.1	10	10q24–q24	19	43.00	Schmid et al., 2000
CYP2C	6	64.0	10	10q24.1–q24.3	19	27.00	Carre et al., 2001
ACTA2	6	65.0	10	10q22–q24	7	syntenic	Schmid et al., 2000
PDE6C	6	65.1	10	10q24–q24	7	68.00	Schmid et al., 2000
CYP17	6	75.0	10	10q24.3–q24.3	19	46.00	Suzuki et al., 1999a
DNTT	6	90.0	10	10q23–q24	19	39.50	Lee et al., 2002
Hs.11859	6	111.0	1	1q25–q31	1	syntenic	Schmid et al., 2000
COL3A1	7	−12.0	2	2q31–q32.3	1	21.10	Schmid et al., 2000
NAB1	7	0.0	2	2q32.3–q33	1	27.00	Schmid et al., 2000
FN1	7	5.0	2	2q34–q34	1	36.10	Schmid et al., 2000
RXRG	7	13.0	1	1q22–q23	1	88.10	Guttenbach et al., 2000
GBX2	7	34.0	2	2q37–q37	1	65.00	Schmid et al., 2000
GDF8	7	34.1	2	2q32.1–q32.1	1	27.80	Sazanov et al., 1999; Schmid et al., 2000
EEF1B2	7	51.0	2	2q33–q34	1	syntenic	Hu et al., 1995; Schmid et al., 2000
NDUFS1	7	51.1	2	2q33–q34	1	syntenic	Hu et al., 1995; Schmid et al., 2000

Table 29.4. *Continued.*

Chick locus	Chick chr.	Chick consensus	Human chr.	Human pos.	Mouse chr.	Mouse pos.	References
CD28	7	59.0	2	2q33–q33	1	30.10	Hu et al., 1995; Schmid et al., 2000
SLC11A1	7	78.0	2	2q35–q35	1	39.20	Hu et al., 1995; Girard-Santosuosso et al., 1997; Schmid et al., 2000
VIL1	7	79.1	2	2q35–q35	1	40.80	Girard-Santosuosso et al., 1996b; Suchyta et al., 2001
INHBB	7	92.0	2	2cen–q13	1	64.10	Schmid et al., 2000
Hs.11360	7	101.0	3	3q13.3–q13.3	16	syntenic	Schmid et al., 2000
HOXD@	7	109.1	2	2q31–q32	2	45.00	Ladjali-Mohammedi et al., 2001
MCM6	7	122.0	2	2q21–q21	2	syntenic	Schmid et al., 2000
SELE	8	–15.3	1	1q23–q25	1	85.80	Morroll et al., 2001
GLUL	8	1.0	1	1q25–q25	1	syntenic	Suchyta et al., 2001
PTPRC	8	4.0	1	1q31–q32.3	1	74.00	Suzuki et al., 1999a
LOC51164	8	14.0	5	5pter–qter	UN	N/A	Schmid et al., 2000
AT3	8	31.0	1	1q23–q25.1	1	84.60	Suzuki et al., 1999a
PTGS2	8	31.1	1	1q25.2–q25.3	1	76.20	Suchyta et al., 2001
PLA2G4A	8	31.2	1	1q25–q25	1	syntenic	Suchyta et al., 2001
PLA2G2A	8	32.0	1	1p36.1–p35	4	68.00	Schmid et al., 2000
Hs.155983	8	66.0	1	1p33–p31	4	syntenic	Schmid et al., 2000
B4GALT2	8	67.0	1	1p33–p34	4	18.60	Shaper et al., 1997; Schmid et al., 2000
Hs.12413	8	90.0	1	1p33–p31.1	UN	N/A	Schmid et al., 2000
LEPR	8	90.1	1	1p31.2–p31.1	4	46.70	Schmid et al., 2000
JAK1	8	92.0	1	1p32.3–p31.3	4	46.30	Suchyta et al., 2001
GADD45A	8	94.0	1	1p31.2–p31.1	3	70.50	Schmid et al., 2000
ZNF265	8	94.1	1	1p22.1–p21.3	3	syntenic	Schmid et al., 2000
RPL5	8	94.2	1	1p13.3–p11	3	syntenic	Schmid et al., 2000
TFRC	9	90.0	3	3q26.2–qter	16	22.50	Schmid et al., 2000
EIF4A2	9	90.5	3	3q24–q27	16	14.20	Schmid et al., 2000

SCYA20	9	91.0	2	2q33–q37	1	52.00	Hughes et al., 2001
NCL	9	93.1	2	2q12–qter	1	48.40	Schmid et al., 2000
PAX3	9	99.0	2	2q35–q35	1	44.00	Girard-Santosuosso et al., 1996a; Schmid et al., 2000
SKIL	9	105.0	3	3q24–q27	3	13.00	Schmid et al., 2000
Hs.9414	9	132.0	3	3q25.2–q25.2	3	syntenic	Schmid et al., 2000
MYO5A	10	0.0	15	15q21–q21	9	42.00	Crooijmans et al., 2001
MAPK6	10	0.0	15	15pter–qter	9	38.00	Crooijmans et al., 2001
MYO1E	10	0.0	15	15q21–q22	9	41.00	Crooijmans et al., 2001
KIAA0377	10	0.0	15	15q11.2–q11.2	UN	N/A	Crooijmans et al., 2001
CKMT1	10	0.0	15	15q15–q15	2	67.60	Schmid et al., 2000
GRP58	10	0.0	15	15pter–qter	2	69.00	Crooijmans et al., 2001
B2M	10	0.0	15	15q21–q22.2	2	69.00	Schmid et al., 2000
PPIB	10	0.1	15	15q21–q22	UN	N/A	Crooijmans et al., 2001
RPS17	10	0.2	15	15cen–qter	UN	N/A	Crooijmans et al., 2001
CPEB1	10	0.3	15	15cen–qter	7	syntenic	Crooijmans et al., 2001
SNX1	10	0.3	15	15pter–qter	UN	N/A	Crooijmans et al., 2001
CSK	10	0.5	15	15q23–q25	9	32.00	Crooijmans et al., 2001
CYP11A	10	0.5	15	15pter–qter	9	31.00	Crooijmans et al., 2001
TRIP4	10	0.5	15	15q11.2–q11.2	UN	N/A	Crooijmans et al., 2001
CYP1A2	10	0.5	15	15pter–qter	9	31.00	Crooijmans et al., 2001
DRIL2	10	0.5	15	15pter–qter	UN	N/A	Crooijmans et al., 2001
NEO1	10	36.0	15	15q22.3–q23	9	syntenic	Schmid et al., 2000
CHRNA5	10	44.0	15	15q24–q24	9	32.00	Crooijmans et al., 2001
CHRNB4	10	44.1	15	15pter–qter	9	syntenic	Crooijmans et al., 2001
CRABP1	10	44.2	15	15q24–q24	9	31.00	Schmid et al., 2000
CHRNA3	10	44.3	15	15q24–q24	9	51.00	Crooijmans et al., 2001
RCN2	10	45.0	15	15q22.33–q24.1	UN	N/A	Crooijmans et al., 2001
PSTPIP1	10	45.1	15	15q24–q25.1	UN	N/A	Crooijmans et al., 2001
NR2F2	10	47.0	15	15q26–q26	7	33.00	Schmid et al., 2000
MLSN1	10	48.0	15	15q13–q14	7	27.00	Crooijmans et al., 2001

Table 29.4.   *Continued.*

Chick locus	Chick chr.	Chick consensus	Human chr.	Human pos.	Mouse chr.	Mouse pos.	References
PDE8A	10	48.1	15	15q25.3-q26.1	UN	N/A	Crooijmans *et al.*, 2001
GATM	10	48.2	15	15q11.2-q22.33	2	65.00	Crooijmans *et al.*, 2001
GABPB2	10	48.3	15	15cen-qter	3	42.70	Crooijmans *et al.*, 2001
USP8	10	48.4	15	15q11.2-q21.1	UN	N/A	Crooijmans *et al.*, 2001
GALK2	10	48.5	15	15pter-qter	UN	N/A	Crooijmans *et al.*, 2001
TRIP15	10	48.6	15	15q21.2-q21.2	2	70.00	Crooijmans *et al.*, 2001
DUT	10	48.7	15	15q15-q21.1	2	70.00	Crooijmans *et al.*, 2001
KIAA0256	10	49.0	15	15pter-qter	UN	N/A	Crooijmans *et al.*, 2001
TPM1	10	51.0	15	15q22-q22	9	40.00	Schmid *et al.*, 2000
ANXA2	10	51.5	15	15q21-q22	9	37.00	Schmid *et al.*, 2000
RORA	10	52.0	15	15q22-q22	9	36.00	Crooijmans *et al.*, 2001
TLN2	10	52.5	15	15q15-q21	UN	N/A	Crooijmans *et al.*, 2001
FLJ11896	10	53.0	15	15pter-qter	UN	N/A	Crooijmans *et al.*, 2001
FLJ20086	10	54.0	15	15q15-q15	UN	N/A	Crooijmans *et al.*, 2001
TCF12	10	54.5	15	15q21-q21	9	42.00	Crooijmans *et al.*, 2001
ALDH1A2	10	55.0	15	15q11.2-q11.2	UN	N/A	Crooijmans *et al.*, 2001
CYP19	10	60.0	15	15q21-q21	9	31.00	Schmid *et al.*, 2000
RP11-105D1	10	60.1	15	15q21.2-q21.2	UN	N/A	Crooijmans *et al.*, 2001
FBN1	10	66.0	15	15q21.1-q21.1	2	71.00	Schmid *et al.*, 2000
RANBP7	10	70.0	13	13pter-qter	UN	N/A	Crooijmans *et al.*, 2001
SWAP70	10	70.5	11	11p15-p15	7	50.00	Crooijmans *et al.*, 2001
POLG	10	71.0	15	15q26.1-q26.1	7	syntenic	Schmid *et al.*, 2000
HMX3	10	71.5	10	10q25-q25	7	61.00	Schmid *et al.*, 2000
NTRK3	10	71.6	15	15q24-q25	7	39.00	Crooijmans *et al.*, 2001
AGC1	10	72.0	15	15q26-q26	7	39.00	Schmid *et al.*, 2000
BTBD1	10	80.0	15	15q21-q21	7	syntenic	Crooijmans *et al.*, 2001
HOMER-2B	10	81.0	15	15pter-qter	7	syntenic	Crooijmans *et al.*, 2001
LBC	10	82.0	15	15pter-qter	UN	N/A	Crooijmans *et al.*, 2001

Gene		Position	Chr	Band		Value	Reference
MPLRB11	10	82.5	9	9pter–qter	UN	N/A	Crooijmans et al., 2001
ENC1	10	83.0	5	5q13–q13	13	syntenic	Crooijmans et al., 2001
IGF1R	10	88.0	15	15q25–q26	7	33.00	Schmid et al., 2000
MEF2A	10	100.0	15	15q26–q26	7	33.00	Crooijmans et al., 2001
ALDH1A3	10	101.0	15	15pter–qter	UN	N/A	Crooijmans et al., 2001
PACE4	10	101.0	15	15q26–q26	7	28.50	Suchyta et al., 2001
FLJ22551	10	102.0	15	15pter–qter	UN	N/A	Crooijmans et al., 2001
MADH6	10	105.0	15	15q21–q22	UN	N/A	Crooijmans et al., 2001
GNRHR	10	106.0	4	4q21.2–q21.2	5	44.00	Schmid et al., 2000
PUNC	10	106.1	15	15q22.3–q23	9	syntenic	Crooijmans et al., 2001
RPL4	10	107.0	15	15cen–qter	9	syntenic	Schmid et al., 2000
KNSL5	10	107.1	15	15pter–qter	UN	N/A	Crooijmans et al., 2001
CORO2B	10	107.2	15	15pter–qter	UN	N/A	Crooijmans et al., 2001
RAB11A	10	108.0	15	15pter–qter	9	syntenic	Crooijmans et al., 2001
HK1	10	108.1	10	10q22–q22	10	30.00	Crooijmans et al., 2001
PRKCD	10	108.2	3	3pter–qter	14	11.00	Crooijmans et al., 2001
TLE3	10	109.0	15	15q14–q15.1	UN	N/A	Crooijmans et al., 2001
DDXBP1	10	109.1	15	15cen–qter	UN	N/A	Crooijmans et al., 2001
MAP2K5	10	109.2	15	15q11.2–q22.33	UN	N/A	Crooijmans et al., 2001
SLC24A1	10	109.3	15	15q22–q22	UN	N/A	Crooijmans et al., 2001
FEM1B	10	109.4	15	15pter–qter	9	28.00	Crooijmans et al., 2001
FLJ20509	10	111.0	15	15pter–qter	UN	N/A	Crooijmans et al., 2001
ADAM10	10	117.0	15	15q22–q22	9	41.00	Crooijmans et al., 2001
CCNB2	10	117.1	15	15pter–qter	UN	N/A	Crooijmans et al., 2001
MYO1C	10	117.2	15	15pter–qter	11	44.13	Crooijmans et al., 2001
76P	10	118.0	15	15q14–q15.1	UN	N/A	Crooijmans et al., 2001
TP53BP1	10	118.5	15	15q15–q21	UN	N/A	Crooijmans et al., 2001
IQGAP1	10	119.0	15	15q26.1–q26.1	UN	N/A	Crooijmans et al., 2001
FLJ21140	10	120.0	15	15q26.1–q26.1	UN	N/A	Crooijmans et al., 2001

Table 29.4. *Continued.*

Chick locus	Chick chr.	Chick consensus	Human chr.	Human pos.	Mouse chr.	Mouse pos.	References
CSNK2A2	11	0.0	16	16p13.3–p13.2	8	50.00	Smith *et al.*, 2002
UQCRFS1	11	32.0	19	19q12–q13.1	UN	N/A	Smith *et al.*, 2002
CCNE1	11	38.0	19	19q13.1–q13.1	7	16.00	Smith *et al.*, 2002
KIAA0355	11	44.0	19	19q13.1–q13.1	UN	N/A	Smith *et al.*, 2002
GPI	11	44.1	19	19q13.1–q13.1	7	11.00	Smith *et al.*, 2002
MAF	11	55.0	16	16q22–q23	8	61.00	Schmid *et al.*, 2000
TERF2	11	55.1	16	16q22.1–q22.1	8	52.50	Fillon *et al.*, 2001
ARHA	12	10.1	3	3p21.3–p21.3	2	37.00	Carre *et al.*, 2001
Z75743	12	10.2	3	3p21.3–p21.3	9	syntenic	Schmid *et al.*, 2000
ARF4	12	10.3	3	3p21.2–p21.1	9	syntenic	Schmid *et al.*, 2000
ALAS1	12	10.4	3	3p21.1–p12	9	syntenic	Carre *et al.*, 2001
GABRA6	13	32.0	5	5q34–q34	11	23.00	Buitenhuis *et al.*, 2002
GABRA1	13	39.0	5	5q34–q35	11	19.00	Buitenhuis *et al.*, 2002
GABRG2	13	39.1	5	5q31.1–q33.1	11	19.00	Buitenhuis *et al.*, 2002
KIAA1673	13	39.2	5	5q21–q21	UN	N/A	Buitenhuis *et al.*, 2002
MSX2	13	41.0	5	5q34–q35	13	32.00	Schmid *et al.*, 2000
FLJ12686	13	47.0	5	5pter–qter	UN	N/A	Buitenhuis *et al.*, 2002
KIAA0731	13	48.0	5	5pter–qter	UN	N/A	Buitenhuis *et al.*, 2002
C5ORF4	13	50.0	5	5q31–q32	UN	N/A	Buitenhuis *et al.*, 2002
CNOT8	13	51.0	5	5q31–q33	UN	N/A	Buitenhuis *et al.*, 2002
SPARC	13	53.0	5	5q31–q33	11	29.90	Schmid *et al.*, 2000
FLJ10290	13	55.0	5	5p14.2–q31.3	UN	N/A	Buitenhuis *et al.*, 2002
CDX1	13	56.0	5	5q31–q33	18	30.00	Khatib and Soller, 1995; Schmid *et al.*, 2000
MADH5	13	59.0	5	5q31–q31	13	35.00	Buitenhuis *et al.*, 2002
CAMLG	13	66.0	5	5q23–q23	13	34.00	Schmid *et al.*, 2000
UBE2B	13	67.0	5	5q23–q31	11	syntenic	Buitenhuis *et al.*, 2002
IRF1	13	72.0	5	5q23–q31	11	29.00	Mariani *et al.*, 1999; Schmid *et al.*, 2000

Gene	Chr	cM	Chr	Band	Chr	Pos	Reference
DTR	13	70.0	5	5q23–q23	18	15.00	Buitenhuis et al., 2002
NR3C1	13	74.0	5	5q31–q32	18	20.00	Buitenhuis et al., 2002
POU4F3	13	71.0	5	5q31–q31	18	24.00	Heltemes et al., 1997; Schmid et al., 2000
KIAA0837	13	71.5	5	5q31–q31	UN	N/A	Schmid et al., 2000
HBA@	14	26.0	16	16p13.3–p13.3	11	16.00	Schmid et al., 2000
NTN2	14	77.0	16	16p13.3–p13.3	17	syntenic	Schmid et al., 2000
CRYBB1	15	31.0	22	22q11.2–q11.2	5	59.00	Schmid et al., 2000
CRYBA4	15	31.1	22	22q11.2–q13.1	5	59.00	Schmid et al., 2000
MIF	15	32.0	22	22q11.2–q11.2	10	40.90	Schmid et al., 2000
IGVPS	15	35.0	22	22q11.2–q11.2	16	13.00	Schmid et al., 2000
B@	16	0.0	6	6p21.30p21.3	17	23.00	Schmid et al., 2000
ABCB2	16	0.1	6	6p21.3–p21.3	17	18.60	Schmid et al., 2000
ABCB3	16	0.2	6	6p21.3–p21.3	17	18.62	Schmid et al., 2000
B-DMA	16	0.3	6	6p21.3–p21.3	17	18.56	Schmid et al., 2000
B-DMB	16	0.4	6	6p21.3–p21.3	17	18.58	Schmid et al., 2000
BRD2	16	0.5	6	6p21.3–p21.3	17	18.53	Schmid et al., 2000
C4A	16	0.6	6	6p21.3–p21.3	17	18.80	Schmid et al., 2000
G9A	16	0.7	6	6p21.3–p21.3	17	18.87	Spike and Lamont, 1995; Schmid et al., 2000
TAPBP	16	0.8	6	6p21.3–p21.3	17	18.41	Schmid et al., 2000
BTN1A1	16	0.9	6	6p22–p21.3	17	20.30	Schmid et al., 2000
GSN	17	7.0	9	9q33–q33	2	24.50	Suchyta et al., 2001
BRD3	17	7.1	9	9q34–q34	2	syntenic	Suchyta et al., 2001
SURF5	17	11.0	9	9q33–q34	2	18.00	Colombo et al., 1992
RPL7A	17	11.0	9	9q33–q34	2	18.00	Schmid et al., 2000
SURF1	17	11.0	9	9q33–q34	2	18.00	Colombo et al., 1992
SURF2	17	11.0	9	9q33–q34	2	18.00	Colombo et al., 1992
SURF4	17	11.0	9	9q33–q34	2	18.00	Colombo et al., 1992
ABL1	17	22.0	9	9q34.1–q34.1	2	21.00	Suchyta et al., 2001
AK1	17	29.0	9	9q34.1–q34.1	2	21.60	Schmid et al., 2000
ENTPD2	17	56.0	9	9q34–q34	2	syntenic	Schmid et al., 2000

Table 29.4. *Continued.*

Chick locus	Chick chr.	Chick consensus	Human chr.	Human pos.	Mouse chr.	Mouse pos.	References
HXB	17	56.1	9	9q32–q34	4	32.20	Schmid *et al.*, 2000
AMBP	17	58.0	9	9q32–q33	4	30.60	Schmid *et al.*, 2000
MYH@	18	0.0	17	17p13.1–p13.1	11	35.00	Schmid *et al.*, 2000
SFRS2	18	0.1	17	17cen–qter	11	73.00	Suchyta *et al.*, 2001
NME1	18	31.0	17	17q21–q22	11	72.00	Schmid *et al.*, 2000
HLF	18	34.0	17	17q22–q22	11	52.00	Schmid *et al.*, 2000
H3F3B	18	35.0	17	17q25–q25	11	syntenic	Schmid *et al.*, 2000
RAC3	18	40.0	17	17q24–qter	11	syntenic	Schmid *et al.*, 2000
FASN	18	40.1	17	17q25–q25	11	72.00	Pitel *et al.*, 1998a; Schmid *et al.*, 2000
CASP1	19	–2.0	11	11q22.2–q22.3	9	1.00	Suchyta *et al.*, 2001
ACACA	19	–1.0	17	17q21–q21	11	syntenic	Pitel *et al.*, 1998a; Schmid *et al.*, 2000
CRK	19	0.0	17	17p13–p13	11	44.15	Schmid *et al.*, 2000
SCYA4	19	1.0	17	17q11–q21	11	47.60	Hughes and Bumstead, 1999
SCYA5	19	1.0	17	17q11.2–q12	11	47.40	Hughes *et al.*, 2001
SCYA3	19	17.0	17	17q11–q21	11	47.59	Hughes *et al.*, 2001
ETS1	24	0.0	11	11q23.3–q23.3	9	15.00	Jennen *et al.*, 2002
SEMA3C	24	20.0	7	7q21–q31	5	syntenic	Jennen *et al.*, 2002
HNT	24	20.1	11	11q22–q24	9	syntenic	Jennen *et al.*, 2002
OPCML	24	20.2	11	11q23–qter	9	10.00	Jennen *et al.*, 2002
HSC70	24	28.0	11	11q23.3–q25	9	24.00	Jennen *et al.*, 2002
AP001924	24	29.5	11	11cen–qter	UN	N/A	Jennen *et al.*, 2002
SORL1	24	30.0	11	11q23.2–q24.2	UN	N/A	Jennen *et al.*, 2002
TECTA	24	31.0	11	11q22–q24	9	25.00	Jennen *et al.*, 2002
AP002348	24	31.1	11	11cen–qter	UN	N/A	Jennen *et al.*, 2002
GRIK4	24	39.0	11	11q22.3–q22.3	9	23.00	Jennen *et al.*, 2002
POU2F3	24	40.0	11	11pter–qter	9	23.00	Heltemes *et al.*, 1997; Jennen *et al.*, 2002

KIAA0196	24	42.0	8	8p22–p22	UN	N/A	Jennen et al., 2002
CGI-07	24	45.0	7	7pter–qter	UN	N/A	Jennen et al., 2002
KPNA4	24	45.1	11	11q22–q22	UN	N/A	Jennen et al., 2002
DKFZP434F162	24	47.0	11	11q22–q24	UN	N/A	Jennen et al., 2002
MLL	24	47.1	11	11q23–q23	9	26.00	Jennen et al., 2002
PLZF	24	48.0	11	11q23.1–q23.1	UN	N/A	Jennen et al., 2002
DKFZP566E144	24	49.0	11	11q23.1–q23.2	UN	N/A	Jennen et al., 2002
AP000997	24	52.0	11	11q23.2	UN	N/A	Jennen et al., 2002
AP000462	24	52.1	11	11cen–qter	UN	N/A	Jennen et al., 2002
AW239635	24	58.0	11	11cen–qter	UN	N/A	Jennen et al., 2002
AP001481	24	58.1	11	11cen–qter	UN	N/A	Jennen et al., 2002
ZPR1	24	58.2	11	11q22–q24	UN	N/A	Jennen et al., 2002
APOA4	24	58.3	11	11q23–q23	9	27.00	Jennen et al., 2002
APOA1	24	58.4	11	11q23.3–q23.3	9	27.00	Jennen et al., 2002
KIAA0999	24	58.5	11	11pter–qter	UN	N/A	Jennen et al., 2002
PAFAH1B2	24	58.6	11	11q23–q23	UN	N/A	Jennen et al., 2002
TAGLN	24	58.7	11	11q23.2–q23.2	9	27.00	Jennen et al., 2002
AJ394231	24	58.8	11	11pter–qter	UN	N/A	Jennen et al., 2002
EVA1	24	59.0	11	11q24–q24	9	26.00	Jennen et al., 2002
CD3E	24	59.1	11	11q23.3–q23.3	9	26.00	Jennen et al., 2002
CD3G/D	24	59.2	11	11q23.3–q23.3	9	26.00	Jennen et al., 2002
ZW10	24	59.3	11	11q22–q24	UN	N/A	Jennen et al., 2002
RPS25	24	60.0	11	11q23.3–q23.3	9	syntenic	Jennen et al., 2002
ORP150	24	60.1	11	11q22–q24	UN	N/A	Jennen et al., 2002
NCAM1	24	60.2	11	11q22.2–q22.3	9	28.00	Jennen et al., 2002
KIAA1735	24	60.3	11	11pter–qter	UN	N/A	Jennen et al., 2002
CRYAB	24	60.4	11	11q22.3–q23.1	9	29.00	Jennen et al., 2002

Table 29.4. *Continued.*

Chick locus	Chick chr.	Chick consensus	Human chr.	Human pos.	Mouse chr.	Mouse pos.	References
NFASC	26	–7.0	1	1q31–q32.3	1	70.00	Schmid *et al.*, 2000
NRF1	26	15.0	7	7q31–q32	6	syntenic	Schmid *et al.*, 2000
PGA@	26	25.0	11	11q13–q13	UN	N/A	Schmid *et al.*, 2000
Hs.80464	26	25.1	1	1p13.3–q12	3	syntenic	Schmid *et al.*, 2000
TNNT2	26	27.0	1	1q32–q32	1	60.00	Suchyta *et al.*, 2001
CNTN2	26	33.0	1	1q32.1–q32.1	1	syntenic	Suchyta *et al.*, 2001
CTSE	26	67.0	1	1q31–q31	1	69.10	Suchyta *et al.*, 2001
TCRA@	27	0.0	14	14q11.2–q11.2	14	19.50	Wang *et al.*, 1997; Schmid *et al.*, 2000
DAD1	27	0.0	14	14q11–q12	14	24.00	Wang *et al.*, 1997; Schmid *et al.*, 2000
GH	27	10.0	17	17q22–q24	11	65.00	Liu *et al.*, 2001
ACE	27	34.0	17	17q23–q23	11	65.00	Groenen, Wageningen, 2001, personal communication
SLC4A1	27	35.0	17	17q12–q21	11	62.00	Toye *et al.*, 1997a; Schmid *et al.*, 2000
COL1A1	27	36.0	17	17q21.3–q22	11	56.00	Schmid *et al.*, 2000
HOXB@	27	44.1	17	17q21–q22	11	56.00	Groenen, Wageningen, 2001, personal communication
HOXB1	27	44.1	17	17q21–q22	11	56.00	Groenen, Wageningen, 2001, personal communication
HOXB6	27	44.1	17	17q21–q22	11	56.00	Groenen, Wageningen, 2001, personal communication
ACLY	27	73.0	17	17q21.1–q21.1	UN	N/A	Daval *et al.*, 2000
KIAA1532	28	2.0	19	19p13.3–p13.2	UN	N/A	Smith *et al.*, 2002
HNRPM	28	2.0	19	19p13.3–p13.2	UN	N/A	Smith *et al.*, 2002
SF3A2	28	21.1	19	19p13.3–p13.3	10	43.00	Smith *et al.*, 2002
AMH	28	21.2	19	19p13.3–p13.3	10	43.00	Smith *et al.*, 2002
PTBL1	28	30.1	19	19p13.3–p13.3	10	43.00	Smith *et al.*, 2002
ROD1	28	30.2	19	19p13.3–p13.3	10	syntenic	Smith *et al.*, 2002
TRAP95	28	30.3	19	19p13.3–p13.3	10	syntenic	Smith *et al.*, 2002
ABCA7	28	46.0	19	19p13.3–p13.3	10	44.00	Smith *et al.*, 2002

Gene	Clone	cM	Chr	Band	Chr	Distance	Reference
GDF1	28	48.0	19	19p12–p12	8	syntenic	Smith et al., 2002
JUND	28	48.1	19	19p13.1–p13.1	8	33.00	Smith et al., 2002
COMP	28	48.2	19	19p13.1–p13.1	8	33.00	Smith et al., 2002
RENT1	28	48.3	19	19p13.2–p13.11	8	33.50	Smith et al., 2002
INSR	28	58.0	19	19p13.3–p13.3	8	1.00	Smith et al., 2002
PTPRS	28	60.0	19	19p13.3–p13.3	17	33.80	Smith et al., 2002
IGLC1	C15	0.0	22	22q11.2–q11.2	16	13.00	Schmid et al., 2000
TUBAL2	C15	10.0	22	22p11.2–q11.2	10	syntenic	Schmid et al., 2000
SFPQ	C15	25.0	1	1p33–p31.1	4	syntenic	Schmid et al., 2000
HSPA5	C24	0.0	9	9q33–q34.1	2	22.50	Schmid et al., 2000
ALDH1	E18C15W15	0.0	9	9q21–q21	19	12.00	Suchyta et al., 2001
ERBB3	E22C19W28	14.0	12	12q13–q13	10	70.00	Schmid et al., 2000
TUBAL1	E22C19W28	25.0	12	12pter–qter	10	syntenic	Suchyta et al., 2001
GLI	E22C19W28	30.0	12	12q13.2–q13.3	10	69.00	Schmid et al., 2000
SNRPD2	E25C31	–2.0	19	19q13.3–q13.3	7	syntenic	Smith et al., 2002
RYR1	E25C31	0.0	19	19q13.1–q13.1	7	10.00	Smith et al., 2002
TGFB1	E25C31	1.0	19	19q13.2–q13.2	7	6.50	Smith et al., 2002
CAPN4	E25C31	16.0	19	19q13.1–q13.1	UN	N/A	Smith et al., 2002
CKM	E25C31	20.0	19	19q13.2–q13.2	7	4.00	Smith et al., 2002
MCL1	E26C13	–1.0	1	1q21–q21	3	43.60	Suchyta et al., 2001
HCK	E32W24	15.0	20	20q11–q12	2	86.00	Benkel et al., 1995; Schmid et al., 2000
BMP7	E32W24	16.0	20	20q13.3–q13.3	2	102.00	Schmid et al., 2000
CRMP1	E38	0.0	4	4p16.1–p15	5	21.00	Suchyta et al., 2001

Table 29.4. *Continued.*

Chick locus	Chick chr.	Chick consensus	Human chr.	Human pos.	Mouse chr.	Mouse pos.	References
CDC2L1	E54	0.0	1	1p36.3–p36.3	4	79.40	Schmid et al., 2000
AGRN	E54	25.0	1	1p36.3–p32	4	syntenic	Schmid et al., 2000
ENO1	E54	30.0	1	1p36–p36	4	79.00	Schmid et al., 2000
CAB45	E54	30.1	1	1p36–p36	4	syntenic	Shi et al., 2001
PLOD	E54	64.0	1	1p36.3–p36.2	4	76.50	Suchyta et al., 2001
SLC2A1	E54	70.0	1	1p35–p31.3	4	52.00	Schmid et al., 2000
TP53	E57	14.0	17	17p13.1–p13.1	11	39.00	Schmid et al., 2000
LIG1	E64	0.0	19	19q13.3–q13.3	7	4.00	Smith et al., 2002
EZFIT	E64	0.0	19	19q13.4–q13.4	UN	N/A	Smith et al., 2002
CALR	E65	0.0	19	19p13.2–p13.2	8	37.00	Smith et al., 2002
GCDH	E65	0.1	19	19p13.2–p13.2	8	38.60	Smith et al., 2002
RAD23A	E65	0.2	19	19p13.2–p13.2	8	syntenic	Smith et al., 2002
FARSL	E65	0.3	19	19p13.2–p13.2	8	syntenic	Smith et al., 2002
CDC37	E65	5.0	19	19p13.2–p13.2	UN	N/A	Smith et al., 2002
ATP5A1	Z	–1.0	18	18q12–q21	18	51.00	Schmid et al., 2000
IFN1@	Z	2.5	9	9p22–p22	4	42.60	Schmid et al., 2000
IFNB1	Z	2.5	9	9p22–p22	4	42.60	Schmid et al., 2000
RPS6	Z	3.0	9	9p21–p21	4	syntenic	Schmid et al., 2000
TPM2	Z	10.0	9	9p13.2–p13.1	UN	N/A	Suchyta et al., 2001
FST	Z	13.0	5	5p15–p15	UN	N/A	McQueen et al., 2001
PRLR	Z	24.0	5	5p14–p13	15	4.60	Schmid et al., 2000
GHR	Z	28.0	5	5p14–p12	15	4.60	Schmid et al., 2000
DMRT1	Z	29.0	9	9p24.3–p24.3	19	syntenic	Schmid et al., 2000
SMARCA2	Z	37.5	9	9p24–p23	19	17.00	Schmid et al., 2000

CENPH	Z	56.0	5	5q21	13	51	Fukagawa et al., 2001
HINT	Z	58.5	5	5q31.2–q31.2	UN	N/A	Schmid et al., 2000
SPIN	Z	58.6	9	9q22.1–q22.3	13	syntenic	Itoh et al., 2001; Schmid et al., 2000
SYK	Z	59.0	9	9q22–q22	13	37.00	Schmid et al., 2000
VLDLR	Z	73.0	9	9p24–p24	19	20.00	Suchyta et al., 2001
TYRP1	Z	87.0	9	9p23–p23	4	38.00	Suchyta et al., 2001
NTRK2	Z	125.0	9	9q22.1–q22.1	13	36.00	Suchyta et al., 2001
PTCH	Z	126.0	9	9q22.3–q22.3	13	36.00	Schmid et al., 2000
CTSL	Z	127.0	9	9q22.1–q22.2	13	30.00	Schmid et al., 2000
CHD1	Z	131.0	5	5q15–q21	17	7.45	Griffiths and Korn, 1997; Suchyta et al., 2001
CHRNB3	Z	139.0	8	8p11.2–p11.2	8	syntenic	Schmid et al., 2000
LPL	Z	144.0	8	8p22–p22	8	33.00	Schmid et al., 2000
ALDOB	Z	160.0	9	9q22.3–q22.3	4	22.30	Schmid et al., 2000
TMOD	Z	175.0	9	9q22.2–q22.3	4	21.50	Schmid et al., 2000
XPA	Z	186.0	9	9q22.3–q22.3	4	21.50	Suchyta et al., 2001
SCII	Z	187.0	9	9q31.1–q31.1	4	23.10	McQueen et al., 2001
MUSK	Z	187.1	9	9q31.3–q32	4	26.30	Schmid et al., 2000
B4GALT1	Z	187.2	9	9p21–p13	4	18.60	Shaper et al., 1997; Schmid et al. 2000
ACO1	Z	187.3	9	9p22–p13	4	20.90	Schmid et al., 2000

Conserved segments in the mouse and human maps are highlighted with double outlines. Evolutionary breakpoints within conserved segments are indicated with a single line. The locations of chicken loci are shown relative to the consensus genetic linkage map and microchromosome numbering system (Schmid et al., 2000). Chicken genes mapped by FISH were integrated into the consensus genetic linkage map using integrated genetic and physical mapping data of macrochromosomes (Schmid et al., 2000). Mouse and human data from WWW sites listed in Table 29.1.

identified seven genes in a 4.3 Mb region orthologous with human chromosome 14q24–q32 and the distal 20 cM of mouse chromosome 12. This region was of direct interest because it contains a gene (*SAL1*) implicated in resistance to *Salmonella* infection (Mariani *et al.*, 2001). Initially human BAC clones were identified from this region and analysed using gene annotation software. In total, 24 known genes and 50 ESTs were identified in this human sequence. Seven chicken orthologues for the known genes were found in the available chicken EST databases. The locations of these chicken genes were mapped using a limited chicken RH panel (Haynes and Bumstead, Compton, 2001, personal communication) to the distal region of chromosome 5, as expected. Within the limits of experimental resolution, the order of the genes appears to be conserved between the distal end of chicken chromosome 5 and orthologous regions in human and mouse.

Although the numbers of loci that are available for comparative mapping are still limited, most of the small linkage groups in chicken (10, 12–19, 24, 27–28, E22, E25, E32, E54, E64 and E65), which most likely represent different microchromosomes, seem to represent whole chromosome or large conserved syntenies with the human genome (Table 29.4). How well the gene order is conserved on the microchromosomes is difficult to determine from genetic or FISH mapping studies alone.

A comparison of human chromosome 15 (Crooijmans *et al.*, 2001) identified seven conserved segments on chicken chromosome 1 (two CSU), chromosome 5 (two CSU) and most on chromosome 10 (three CSU), based on 91 orthologues. A high-resolution comparative gene map of chicken chromosome 10 and human chromosome 15 identified 19 CSO. This result indicates that there have been at least 16 intrachromosomal rearrangements since the divergence of humans and chickens, 300 Mya. This result does not indicate any lineage-specific rates of intrachromosomal rearrangement – that requires an outgroup species, such as the zebrafish, as suggested by Crooijmans *et al.* (2001). However, specific rearrangements in the mouse or human lineage were identified, using the chicken as the outgroup species.

The same approach has been used by the Wageningen group to construct high-resolution comparative maps of chicken chromosomes 13 (Buitenhuis *et al.*, 2002) and 24 (Jennen *et al.*, 2002). Genes mapped to chicken chromosome 13 have orthologues on mouse chromosomes 11, 13 and 18, and only human chromosome 5. At least one intra-chromosomal rearrangement was detected between chicken and human. At first sight the comparison with chicken chromosome 24 looks simpler, with orthologues on mouse chromosome 9 and human chromosome 11q22–q24. Examination of the high-resolution comparative maps, however, reveals at least four intra-chromosomal rearrangements.

A physical map of human chromosome 19 was one of the first to be completed and is virtually sequenced (Dehal *et al.*, 2001). This physical and sequence resource has been exploited in combination with chicken genome resources to create a comparative map, based on 32 chicken genes, of this chromosome and its chicken orthologues (Smith *et al.*, 2002). Six conserved segments were defined in the chicken–human comparison, which is fewer than the 12 found between mouse and human, confirming earlier conclusions about the higher rate of chromosomal rearrangement in the mouse lineage (Burt *et al.*, 1999). However, the gene order is not conserved and several intra-chromosomal rearrangements were defined in the chicken comparisons. Comparisons between the three species allowed lineage-specific evolutionary breaks to be defined. Comparisons between mouse and human genomic sequences revealed that, in many cases, the evolutionary breakpoints were located within clusters of zinc-finger genes, which would facilitate illegitimate recombination between non-homologous chromosomes (Dehal *et al.*, 2001).

Similar approaches will be needed to determine the nature of the rearrangements on chicken chromosomes 4, 7 and 8, and whether the gene order on chromosome 6 and other microchromosomes is conserved between chicken and human.

What these examples illustrate is the extensive conservation of gene arrangements between the chromosomes of birds and mammals. This also extends to most microchromosomes and to some large conserved segments on the larger chromosomes (Table 29.4). However, it is clear that the total number of chromosomal rearrangements based on the number of CSU alone will be an underestimate when compared with the number of CSO. This is likely to be more significant for the chicken–human than for the chicken–mouse comparison, due to the longer conserved segments found between chicken and human comparative maps.

Genomes Evolve at Different Rates

Comparative maps between chicken, mouse and human genomes have been used to estimate the total number of autosomal CSU between these species using observed data and a chromosome-based model of chromosomal rearrangement (Burt *et al.*, 1999; Waddington *et al.*, 2000). The number of expected autosomal CSU between chicken and human is 154, of which 126 (82%) have been defined. In contrast, 312 CSU are expected between chicken and mouse, of which 172 (55%) have been defined. Given that the total number of CSO between mouse and human is 190–230 (Hudson *et al.*, 2001), the number of chromosomal rearrangements can be estimated for each lineage (Burt *et al.*, 1999). This analysis assumes that 310 Mya, when birds and mammals diverged, the ancestral karyotype had 24 pairs of chromosomes (Morizot, 1983; Rodionov, 1997). It also assumes that the estimate of the number of chromosomal rearrangements based on the number of CSU is not a serious underestimate of the total number if based on the number of CSO. High-resolution comparative mapping, as discussed in earlier, shows that this is not true for a few conserved segments and so the total number of chromosomal rearrangements will be an underestimate. Detailed examination of the data in Table 29.4 shows an underestimate of 30% (126 CSU vs. 168 CSO) in the total number of chromosomal

rearrangements in the chicken–human comparison and 10% (172 CSU vs. 190 CSO) in the chicken–mouse comparison based on the data so far. Since the conserved segments in the chicken–mouse comparison are smaller (i.e. less conserved overall) this difference is to be expected – smaller segments are less likely to be fragmented. So the estimates of the total number of rearrangements are likely to be approximately 200 for the chicken–human comparison and approximately 350 for the chicken–mouse comparison. By exactly how much is only likely to be known when a complete genome comparison is made between chicken, mouse and human DNA sequences.

With these caveats in mind, the main conclusion to be drawn from these comparisons is that the rate of chromosome rearrangement has been higher in the mouse than in the human lineage (Burt *et al.*, 1999). The possible causes for this are many, but the main reason is likely to be a higher rate of interchromosomal rearrangement in the rodent lineage (Ehrlich *et al.*, 1997). In addition, the chicken (and avian/reptilian?) and human lineages appear to have the lowest rate of change when compared with other mammals (Burt *et al.*, 1999). Further support for this view is the apparent stability of the avian genome (Tegelström *et al.*, 1983). Comparison of avian karyotypes has revealed a striking similarity between the number and size distribution of macro- and microchromosomes, and chromosomal banding patterns of many avian species (Rodionov, 1996). The avian genome has been more stable than that of mammals. Why? There are many models to explain these differences in rates. The simplest would be that the potential for chromosome rearrangement in mammals has been greater than in birds, such as more homologous sites for illegitimate recombination (Burt *et al.*, 1999). Dispersed repeats are very abundant in mammals, making up to 50% of the average mammalian genome. These sequences have been implicated as a major cause of chromosomal rearrangements in mammals (International Human Genome Sequencing Consortium, 2001). In the chicken, and in all birds, dispersed repeats make up less than

15% of the genome and so the potential for illegitimate recombination between homologous sites will be less (Burt *et al.*, 1999).

Examination of the estimates for other species (Burt *et al.*, 1999; Murphy *et al.*, 2000) suggests that vertebrates have experienced two rates of chromosomal change, a slow rate of 0.1 changes per million years and a high rate of over 1 per million years. Consequently, the number of rearrangements cannot be used as a molecular clock to date the divergence of species, but the pattern of chromosome change is very specific and can be used to unravel the evolution of the vertebrate genome.

Deducing the Ancestral Vertebrate Genome

One of the aims of comparative genomics is to deduce the gene arrangement of ancestral species (Andersson *et al.*, 1996). Given the slow rate of chromosome rearrangement in many species, it should in principle be possible to 'reconstruct' ancestral genomes using these 'stable genomes' as anchor species and the principle of parsimony. Deducing ancestral chromosomes depends on distinguishing ancestral and derived arrangements. This can only be done with reference to an *outgroup species* – a more distantly related group than the species under comparison. Given the extensive conservation of synteny, the chicken is an ideal outgroup for all mammals. The following are examples in which a chicken outgroup has been used to deduce ancestral gene arrangements.

Genes on chicken chromosome 1 are orthologous to regions on human chromosomes 12 and 22 (J. Smith *et al.*, 2000c). Synteny of chromosome segments that are homologous to these human chromosomes has been found in many mammals (Chowdhary *et al.*, 1998) and can be considered as ancestral for mammals and birds, at least 310 Mya. The evolutionary breakpoint defined by the fusion of human chromosomes 12 and 22 therefore represents an event unique to the lineage leading to the human genome (J. Smith *et al.*, 2000c). Chromosomal segments on chicken chromosome 1, orthologous to human chromosomes 3 and 21, define another evolutionary breakpoint at least 20 million years old (J. Smith *et al.*, 2000d). Comparative mapping between mammals (Chowdhary *et al.*, 1998) showed ancestral linkage between chromosomal segments orthologous to human chromosome segments 19q and 16q. This ancestral arrangement is also found in the chicken on chromosome 11 (Smith *et al.*, 2002).

Microchromosomes may represent whole chromosome or large conserved syntenies with the human genome (see above). This observation has formed the basis of speculations about the origin of micro- and macrochromosomes. If they have not undergone chromosomal rearrangement during avian evolution, then they may represent ancestral structures (Jones *et al.*, 1997; Fillon, 1998; Masabanda *et al.*, 1998; Sazanov *et al.*, 1998). Did microchromosomes originate by fission of larger ancestral chromosomes (Takagi and Sasaki, 1974) or were the macrochromosomes derived by fusion of microchromosomes? Comparisons between avian species (see above) have identified examples of both fission and fusion in the evolution of avian chromosomes. The macrochromosomes are likely to be the product of multiple fusions and internal rearrangements between macro- and microchromosomes (Rodionov, 1996, 1997). Comparisons between chicken and human maps and an outgroup species such as the zebrafish are needed to examine the fission/fusion theories on the origin of avian microchromosomes. For example, will an ancestral arrangement be found in the zebrafish orthologous to two or more chicken chromosomes, including a microchromosome?

The Future

The creation of comparative maps between the chicken and human genomes progressed rapidly in the late 1990s. These maps were constructed to create a tool to predict

candidate genes at QTL for specific traits in the chicken. QTL mapping in plants and a few animal experiments suggest that QTL for the same trait map to similar locations in different species (Chagnon *et al.*, 1998; Mackay, 2001). Future comparative maps are therefore likely to include both orthologous genes and 'orthologous QTL'. Plans are in progress to create physical maps of most of the major farm animal species, including the chicken, as a prelude to the determination of the genome sequence of these organisms. It can therefore be predicted that by 2005–2010, comparative maps based on the genome sequences of human, mouse, cow, pig and chicken will be available. This will mark the end of comparative maps as a simple tool to predict gene location. Instead, comparative genomics will be the tool for predicting gene function in farm animals based on a shared evolutionary history with humans and other model organisms.

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30 Functional Genomics: Development and Gene Regulation

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Introduction

This chapter is divided into two main sections. The first section provides an overview of the basic events and processes necessary for the expression of genes in general and the mechanisms used to provide tissue- and stage-specific gene expression. The controlled transfer of information from the DNA level to the protein, and the many points of regulation are presented. The second section provides a more detailed look at avian development and the role that specialized genes play in coordinating the complex growth and development of an early embryo into a fully formed chick. Section two introduces the general growth-promoting, general growth-inhibiting and local growth-promoting or growth-inhibiting genes. A more detailed description of gene expression and its role in myogenesis is used to illustrate the specificity and intricacy of regulation that is required for proper muscle development. The rapid developments in the fields of molecular biology and molecular genetics have not only allowed dissection of the normal processes of growth and development, but also have provided the means to manipulate these processes to achieve modified phenotypes and even new genotypes.

General Development and Regulatory Mechanisms

Research into development in the chick, or for that matter any species, has been primarily limited to descriptive studies focusing on major structural and metabolic alterations. When viewed under the microscope, it can be observed that the two gametes involved in the conception of the chick resemble neither the parents nor any other animal. Yet, through a series of very predictable developmental processes, a single-celled zygote becomes a multicellular mass of cells and ultimately hatches as a chick (Hamburger and Hamilton, 1951; Hill, 1996). This chick can further develop, again through very predictable stages, into an adult animal of relatively defined phenotypic characteristics. This predictability in the processes of development suggests an underlying, rather rigid programme of cellular and metabolic changes that confer the phenotypic changes observed by the classic developmental biologists.

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Only recently has it become possible to observe and manipulate predictably (to the limits of current science) the processes of development in higher organisms. It is known that the 'instructions' for how the zygote develops into an adult are contained in the linear string of DNA bases in the genetic material of the germ cells. In order for the genetic material to be of value, this set of instructions must be accurate, its expression controllable, and responsive to both internal and external signals. For proper development, expression of the myriad of genes must be controlled both temporally and spatially. It would not be sufficient for the genes encoding the chick eye to be expressed at the tip of the wing and during the late stages of post-hatch development. Higher animals are complex organisms comprising many different cell types. This level of complexity requires not only that genetic information can be regulated at the cellular level but also that the expression of genes can be controlled in an intercellular fashion. Cells must be able to communicate with each other properly in order to form complicated multicellular organs, limbs and skeletal structures.

Proliferation and differentiation

Blastula formation

The unfertilized oocyte is an extremely large cell relative to the other somatic cells of a multicellular organism. This cell contains a $1n$ complement of genetic material (one-half of the normal amount found in most cells), a large number of cellular organelles and vast quantities of maternal messages and proteins. Spermatocytes, on the other hand, are very small flagellated cells that contain little more than a corresponding $1n$ complement of genetic material. The oocyte must provide all the materials and nutrients necessary for early embryonic development. This is particularly true for avian systems, which isolate the developing embryo within a semi-porous shell, making exogenous sources of nutrients and information almost non-existent.

Upon fertilization, the one-celled zygote begins very rapid cell division that results in a tremendous increase in cell number without any increase in egg size. After fertilization, the cells become progressively smaller and smaller – the result of a process referred to as cleavage division (Heasman *et al.*, 1984). This is followed by an extremely rapid increase in total cell number and proportional cell mass that, for pre-hatch birds, is restricted to the confines and nutrient content of the egg. It is only upon hatch, when the chick has access to external sources of nutrients, that real growth in mass occurs.

During cleavage division there is very little expression (transcription) of the genes contained in the DNA of the cells. The process of cell division is totally dependent upon the messages and protein machinery provided by maternal sources when the yolk and albumin were laid down. The process of cleavage division is tightly controlled in a temporal and quantitative fashion, suggesting that a 'clock' or counting mechanism is present. Research into the cellular mechanisms controlling such mechanisms will most likely provide a more complete understanding of the events of very early development.

Gastrulation

Once the fertilized egg has reached the blastula stage containing approximately 4000 cells, the rate of division slows to that more typical of common somatic cells. It is at this mid-blastula transition stage of development that expression, or transcription, of the cellular genes becomes active. The trigger for the mid-blastula transition appears to reflect the ratio of DNA to a pre-formed DNA binding factor that may serve as a counter of the amount of DNA synthesized, and thus cell number (Newport and Kirschner, 1982; Kirschner *et al.*, 1985).

The next stage of development is gastrulation, where the cells become motile and the blastula begins to invaginate, forming the cell layers that ultimately become the endoderm, mesoderm and ectoderm. It is at this point that the competing phases of proliferation and differentiation that give

rise to further specialized precursor cells and finally to the fully differentiated cells of the entire organism begin to be seen. The extremely complex processes of cellular proliferation and differentiation are orchestrated by the controlled expression of the nearly 45,000 genes contained in the genomes of every cell. It is the specific temporal expression of these genes and the interaction of the gene products that result in the characteristics that are typical of any specialized cell type.

The process of cellular differentiation often results in genetic expression patterns that are irreversible. That is, once pluripotent cells have been committed to a specific cell lineage, the cellular destiny of the cell and its progeny is determined. This dogma, in vertebrates, was challenged by Gurdon (1968) when nuclei from multipotent (not fully differentiated) cells from the intestine of a tadpole were transferred to an enucleated egg and resulted in the generation of a complete frog. For nearly three decades this phenomenon was thought to be restricted to the plants and lower vertebrates. Only recently, with the cloning of 'Dolly' the sheep from its mother's fully differentiated mammary cell, has it been demonstrated that the process of genetic commitment in the process of differentiation is reversible (Wilmut *et al.*, 1997). The interaction of the many different types of differentiated cells in a multicellular organism requires not only that the cells act in an autonomous fashion, but also that they are able to send signals to, and be responsive to signals received from, other cells. The autocrine, paracrine and endocrine signalling processes of multicellular organisms require elegant and precise interaction of signals with the genome of each cell. Although the processes regulating the expression of specific genes must necessarily be quite different, the general mechanisms are quite similar.

Regulation of gene expression

Aside from genotype–environment interactions, the phenotypes of the many different cell types in a multicellular organism are primarily due to differences in the gene expression patterns. Each cell type has its own characteristic gene expression (messenger RNA) profile and resultant protein products. These messages are the products of the selective transcription of genes by RNA polymerase II (Mooney and Landick, 1999). The regulation of gene expression, i.e. the production of functional protein products, can occur at a number of levels within the process of expression. Because gene expression is the result of a sequential series of events, there is the possibility for numerous points of control. In general, gene expression can be subdivided into a minimum of five distinct events: (i) activation of chromatin structure; (ii) initiation of gene transcription; (iii) processing of the primary transcript; (iv) translation into protein; and (v) post-translational modification of the protein.

The gene loci of higher eukaryotes can have very complex organization and structure. The split gene organization of eukaryotic genes may occupy hundreds of kilobases of DNA. Additionally, the regions of the genes that confer proper spatial and developmental expression may lie in regions that extend severalfold beyond the termini of the actual coding domains.

Activation of chromatin structure

A large number of *cis*-regulatory elements, distributed across vast regions of DNA, allow for the precise and elegant expression of eukaryotic genes. The regions of the chromosome that contain genes that are to be expressed must first be made accessible to the transcriptional machinery (Felsenfeld, 1992). Genes that are in the active state are found only in the cells in which they are expressed. The fact that the pattern of active genes found in a specific cell type is consistent (e.g. a liver cell and its progeny all have a common pattern of active genes and message expression profiles) suggests that the mechanisms are cell-type specific and that maintenance of an active transcriptional unit must be stable through multiple rounds of DNA replication. The interaction of

specific DNA-binding proteins and DNA modifications appear to regulate the transition from the inactive to active states. The acquisition of the active state must therefore be the first step in gene expression (Tsukiyama and Wu, 1997; Mizzen and Allis, 1998). The analysis of DNA–protein interactions at a single-nucleotide level of resolution has demonstrated that, depending upon the stage of development, different combinations of transcription factors can interact with the same *cis*-regulatory elements.

Initiation of gene transcription

Once a region of chromatin is in the active state, the DNA is accessible to the transcriptional machinery. Initiation of the transcription process serves as the point of control for this stage of expression. Initiation requires the binding of RNA polymerase II to the DNA and identification of the promoter sequence for the gene that is to be expressed. For most genes, this is the most common form of regulation. From a cellular perspective, regulation at the level of initiation of transcription will allow for the greatest conservation of energy and cellular resources. By controlling gene expression at this early stage, the large commitment of cellular resources to gene expression can be regulated. The chromatin is active, meaning that the genes present in this region are transcribable, but whether any particular gene is expressed (and to what degree) is further controlled at the transcriptional level.

The formation of the intitiation complex at the promoter of a gene is a very complicated and gene-specific process. In addition to RNA polymerase II there are a number of other proteins (transcription factors) that can either enhance or repress the formation of an active promoter complex (Roeder, 1996). The transcription factors, themselves products of other regulated genes, recognize specific sequences within the DNA and typically enhance the rate of recognition of the promoter site by the RNA polymerase. Although most eukaryotic genes contain a TATA box and Inr (initiation) elements that

are very close to the site of initiation, there is great variation in the number and location of other sites necessary for regulation of gene expression. These additional regulatory sequences are called enhancers and may be located hundreds or thousands of bases either upstream or downstream of the transcription start site. The binding of appropriate transcription factors to these enhancer elements typically increases the level of transcription of nearby promoters. A typical gene will involve the binding of six transcription factors, with many genes involving significantly more transcription activators (Fig. 30.1). Transcriptional activation in higher vertebrates includes three classes of proteins: (i) the basal transcriptional activators that act at the level of the promoter itself; (ii) the DNA-binding transactivators that bind to the enhancer elements; and (iii) a class of proteins called transactivators that do not bind directly to the DNA but interact with the class I and II transcriptional activators (Roeder, 1996; Shilatifard, 1998).

Although activation of gene transcription is the most efficient and logical way to control gene expression, there are also examples of repressors that act to inhibit the formation of active promoter complexes. Activation is the more efficient means of regulation, because this mechanism of control does not require the cell to commit valuable resources to the continuous production of a myriad of repressor proteins for each gene that is to remain inactive or expressed at a low level. Keeping all genes turned off unless specific activators are present allows the cell to conserve energy and metabolic substrates, resulting in the expression of only tissue- and stage-specific genes.

Processing of primary transcript

In eukaryotes, the events of transcription of the information contained in the DNA into RNA are separate from the process of translation into protein. This separation is achieved spatially by restricting transcription to the cell nucleus and translation to the cytoplasmic compartment. The product of transcription found in the nucleus is a primary messenger RNA termed hnRNA

(heterogeneous nuclear RNA). This hnRNA must be post-transcriptionally processed into the mature mRNA form before it can be exported to the cytoplasm (Fig. 30.2) (Sharp, 1994). Two steps in the processing involve addition of a 7-methyl guanosine cap to the 5′ end, and cleavage and addition of a poly-adenosine tail to the 3′ end of the transcript. The third step, called splicing, involves the specific excision of introns and the ligation of exons (the coding portion of eukaryotic genes). It is during the splicing process that the cell has the ability to regulate which exons will be retained in the mature mRNA and thus control the sequence of amino acids in the resulting protein (Breibart, *et al.*, 1987). Similarly to the process of transcription, post-transcriptional processing is dependent upon a large number of proteins and is thus highly regulable and modifiable. Although post-transcriptional processing may have
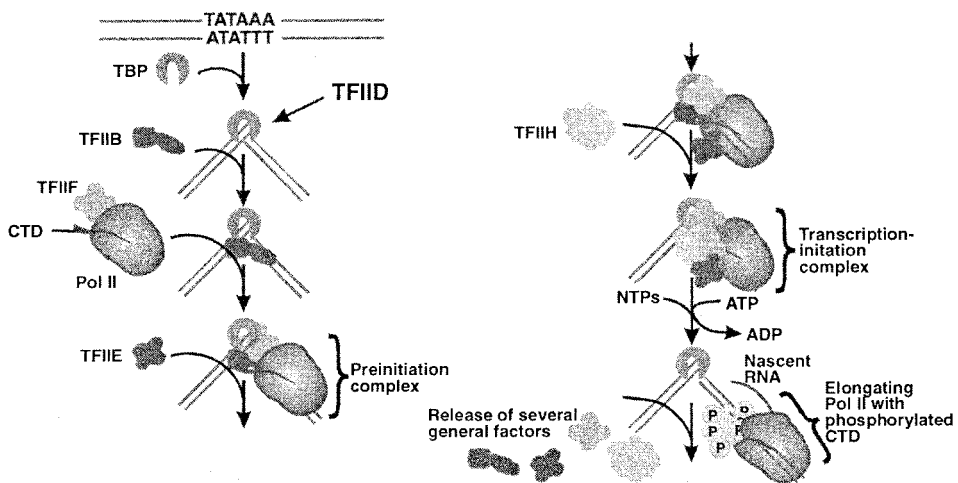
Fig. 1.1. Stepwise assembly of RNA Pol II transcription-initation complex. Formation of an active initiation complex requires the stepwise assembly of numerous initiation site recognition proteins, transcription factors and RNA polymerase II. The complement of proteins utilized imparts sequence specificity and represents a key site of regulation.
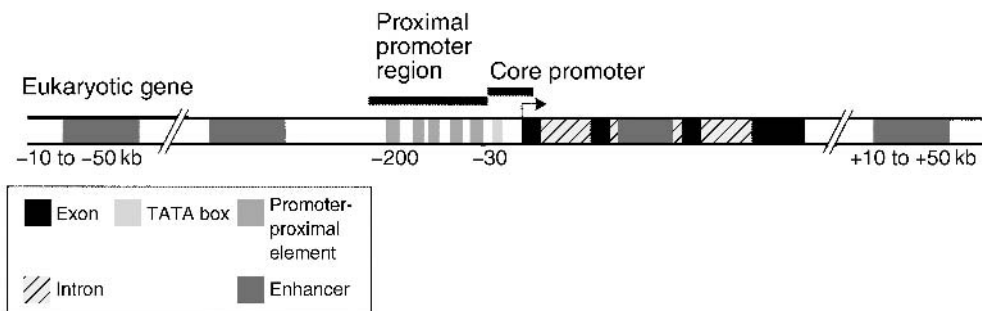
Fig. 1.2. Most eukaryotic genes are regulated by multiple transcription control mechanisms. The majority of eukaryotic genes exist in a split gene organization at the DNA level. Illustrated are the general components of the gene that allow for regulation of expression and sequence content of the mRNA. Removal of the non-coding introns and splicing of selective exons results in the ability of the cell to modify the sequence of translation products through splice variation. The enhancer elements represent distant consensus sequences within the DNA that bind additional protein factors, resulting in an increased rate of gene transcription.

tremendous influence on the sequence of the protein produced, this stage in gene expression has a lesser influence on rate of expression. Following processing of the hnRNA, the mature mRNA is transport into the cytoplasm and ultimate translation into protein at the level of the ribosome.

Translation

The process of translation requires that the proper host of structural components, nucleic acids, amino acids, and enzymatic and regulatory proteins be present. A single mRNA can be translated multiple times to yield numerous identical protein products before it is degraded by cellular ribonucleases. Interaction of cellular components with characteristics intrinsic to the mRNA determine the half-life and efficiency of translation of the message (Kozak, 1992). It is widely accepted that sequences at the 5′ end of the mRNA determine efficiency of binding to the ribosome and associated translation factors, thus regulating initiation of this stage of gene expression. It is during the formation of the initiation complex that there is the greatest potential for regulation. Several of the eukaryotic initiation factors are acted upon by kinases to alter their state of phosphorylation and thus their activities. Through this mechanism, the cell has the potential to regulate, to a limited degree, the rate of translational initiation (Kozak, 1992). Sequences present at the 3′ end of the mRNA are thought to influence stability of the message and thus its half-life and ability to be translated. Once initiated, translation proceeds at a relatively constant rate.

Post-translational modification

Typically, once an mRNA is translated into its product, the resultant protein must be further processed to achieve a conformation and structure consistent with its intended function. This post-translational processing may include, but is not limited to, cellular targeting signals, proteolytic cleavage, covalent modification, association with other proteins or cofactors, chaperone-guided folding and ultimately degradation. Regulation of each of these steps is characteristic of the nearly 100,000 different proteins that are found in higher eukaryotes and varies with the multitude of developmental stages. Typically it is only when a protein attains its functional confirmation that there is the potential to see the outcome of the information that was contained in the cellular genome of the organism. It is through a series of complex and coordinated biochemical processes that the cell is able to convert information contained in a linear string of DNA bases into a biologically functional protein. The following section examines the process of development and gene expression in greater detail with respect to avian development and, in particular, myogenesis. Special attention will be paid to the limited number of genes and their products that have been demonstrated to play pivotal roles in the processes of growth and development. The section will cover early embryonic development, somite formation, tissue modelling and multicellular and endocrine regulation.

Early Avian Embryo Development and Genetic Regulation

Embryonic and fetal growth is a multifactorial process in which the basic morphology of the embryo is derived from a pleuropotential inner cell mass as a result of selective cell proliferation, induction, migration, differentiation and programmed cell death (Hill, 1996). These processes are a coordinated complex pattern of signalling at the intracellular, cell–cell and cell–environment levels. Several hormones, growth factors and transcription factors are involved in this well-orchestrated communication network.

Pattern formation, differentiation and morphogenesis

The fate of the cells during early embryonic development is primarily regulated by

signals generated by neighbouring cells. The production of the signals or ligands and their range of action can control the extent of induction. The neighbouring cells respond to these signals by modifying the expression or function of the appropriate receptors, the intracellular signal transduction pathway, or the transcripts of target genes.

Muscle of the body and bones of the axial skeleton are derived from specialized regions of somites. The somites are first seen as morphologically distinct segmental units that are characteristic of the chordates. They are formed as pairs of epithelial spheres that bud off from the paraxial mesoderm on either side of the axial organs (Pourquie *et al.*, 1995). This process begins at the head level and continues in a craniocaudal direction (Fig. 30.3) (reviewed in Christ and Wilting, 1992; Christ and Orahl, 1995). Unlike other compartments of the mesoderm, paraxial mesoderm undergoes

segmentation and formation of somites originates from the primitive streak during gastrulation (Christ and Orahl, 1995; Stockdale *et al.*, 2000). Mice containing a mutant basic helix–loop–helix (bHLH) gene *paraxis* do not form epithelial somites, suggesting that its expression is required for epithelization and somite formation (Burgess *et al.*, 1996). It is suggested that signals such as *Wnt* genes and/or BMPs (bone morphogenic proteins) from surface ectoderm (Liem *et al.*, 1995; Munsterberg *et al.*, 1995) regulate proper *paraxis* gene expression (Sosic *et al.*, 1997). On the other hand, *Notch 1* (Reaume *et al.*, 1992), which belongs to the *Notch* family of transmembrane receptors, Delta homologue *Dll 1* (Bettenhausen *et al.*, 1995) and *Her 1*, a homologue of the *Drosophila* pair rule gene (*hairy*) that encodes a bHLH protein (Muller *et al.*, 1996), have been demonstrated to be expressed in the presomitic mesoderm and somites of mouse embryos.
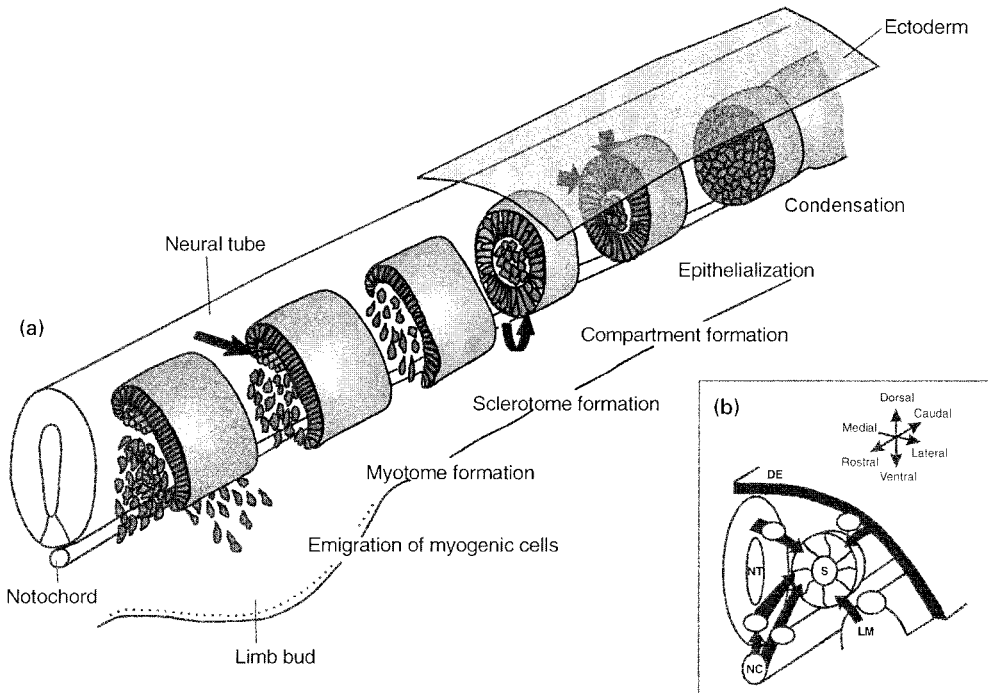
Fig. 30.3. (a) Schematic diagram of segmentation, somite formation and differentiation. The sequence of steps starts on the right, which also represents the caudal part of the chick embryo (modified from Christ *et al.*, 1998). (b) Schema of transverse section through a maturing interlimb mouse somite (modified from Tajbaksh and Cossu, 1997). Arrows indicate the origins of the signals that regulate somite formation and differentiation. DE, dorsal epidermis; NT, neural tube; S, somite; LM, lateral mesoderm; NC, notochord.

Therefore, these genes are considered as major somitogenic genes in the mice embryos. However, there is not sufficient data to speculate on their function in avian somite formation.

Each somite splits into the dermomyotome, dorsally, which gives rise to the dermis of the trunk and to skeletal muscle and the sclerotome, ventrally, which gives rise, along with the notochord, to the axial skeleton (reviewed in Christ *et al.*, 1998; Stockdale *et al.*, 2000). There are boundaries established between dorsal and ventral halves of each somite as shown by mutation in *N-cadherin* and *cadherin 11*, in which dorsal and ventral halves of a somite become separated (Horikawa *et al.*, 1999). Additionally, differential gene expression reveals the distinctions between the dorsal and the ventral part of somite. *Pax 1*, *Pax 9* and *Mfh 1* (*Mesenchymal forkhead 1*) genes are primarily expressed in the ventral somite, while *Pax 3*, *Pax 7*, *Noggin*, *Wnt 11* and *follistatin* are expressed in the dorsal portion (Stockdale *et al.*, 2000). Nevertheless, cells in newly formed somites are not definitely committed to different lineages, which implies that signals that arise from adjacent tissues, such as neural tube and notochord, mainly control their differentiation (Christ *et al.*, 1992; Ordahl and Le Duarin, 1992). For example, if somites are rotated in a way that only the dorsal and ventral positions are reversed, or if the dorsal half is replaced by a ventral half, all the elements such as muscle and bone form normally in accordance with their new environment (Christ *et al.*, 1992; Dockter and Ordahl, 2000).

Epitheliomesenchymal transition of the ventral part, forming the sclerotome, is a unique event during somite maturation (Duband *et al.*, 1987; Balling *et al.*, 1996). Signals from the notochord control this process (Fig. 30.3). Expression of TGF-β and its potential function during very early stages of avian development have been studied extensively (Jakowlew *et al.*, 1994; Sanders *et al.*, 1994). It appears that TGF-β influences epitheliomesenchymal transition (Potts *et al.*, 1991; Nakajima *et al.*, 1994) and extracellular matrix deposition that surrounds somites (Chong *et al.*, 1991). Down-

regulation of the cell adhesion molecule *N-cadherin* is also required for this transition process (Duband *et al.*, 1987; Hatta *et al.*, 1987). As previously mentioned, the sclerotome forming part of the somite is marked by the expression of *Pax 1*, *Pax 9*, and *Mfh 1* genes. Both *Pax 1* and *cFkh-1* (chicken homologue of *Mfh 1*) gene expressions are controlled by the notochord and sonic hedgehogs (*Shh*) (Muller *et al.*, 1996; Furumoto *et al.*, 1999). *Pax 1*, *Pax 9* and *Mfh 1* regulate the proliferation of sclerotome cells and are also important for the proper development of dorsal sclerotome (Furumoto *et al.*, 1999). In addition to a variety of signals and other molecules, *Noggin* along with *Shh* are probably the most important molecules in terms of initiating and maintaining the sclerotome (McMahon *et al.*, 1998). It has been suggested that there are two steps to generate cartilage from the sclerotome: (i) initiation of competence to respond to BMPs, which are the main cartilage-forming growth factors; and (ii) exposure to BMPs to trigger the chondrogenic differentiation (Murtaugh *et al.*, 1999). Because *Noggin* antagonizes the actions of BMPs, it implies that noggin is absolutely necessary for proper sclerotome and then cartilage formation (Murtaugh *et al.*, 1999). Furthermore, the sclerotome is divided into cranial and caudal halves, that differ in terms of gene expression and function. For instance, *Pax-9*, *twist* and *Dll 1* are strongly expressed in the caudal half of the sclerotome (Bettenhausen *et al.*, 1995; Neubuser *et al.*, 1995). Thus, differential expression profiles of genes and different composition of sclerotome halves give rise to segmentation of the peripheral nervous system (Rickmann *et al.*, 1985; Loring and Erickson, 1987).

The dorsal part of the somite remains epithelialized and forms dermomyotome that continues to express *Pax 3* and *Pax 7* genes, which are the principal markers of the dermomyotome (Goulding *et al.*, 1993, 1994). Dorsalization and muscle differentiation of somites are controlled by signals that originate from the surface ectoderm (Fig. 30.3) (Fan and Tessierlavigne, 1994) and the neural tube (Christ *et al.*, 1992; Buffinger and

Stockdale, 1994, 1995). In order to differentiate to muscle lineages, somatic cells must down-regulate *Pax-3* expression (Williams and Ordahl, 1994). Then, myogenic genes (*myoD*, *Myf-5*, *MRF-4* and *myogenin*) need to be activated for proper muscle lineage formation (Ott *et al.*, 1991; Pownall and Emerson, 1992). It has been demonstrated that members of the *Wnt* family of signalling molecules, expressed in the neural tube and the ectoderm, might be responsible for the induction of myogenic precursor cells in the dermomyotome of chick embryos (Wagner *et al.*, 2000). Ectopically implanted *Wnt-1-*, *-3-* and *-4-* expressing cells alter the process of somite compartmentalization towards muscle-forming precursor cells at the expense of the sclerotome (Wagner *et al.*, 2000). In combination with *Wnt* genes, *Shh* promotes both sclerotome and muscle differentiation (Munsterberg *et al.*, 1995; Stern and Hauschka, 1995). Interestingly, muscle markers *Myf5* and *MyoD* and one of the sclerotome markers *Pax 1* are activated in *Shh* knockout mice, suggesting that *Shh* and possibly *Wnt* genes primarily maintain and amplify expression rather than the activation of lineage-specific genes (Cossu *et al.*, 1996). Dermomyotome can be further divided into medial and lateral halves in terms of gene expression profile and muscle cell lineages. For instance, *flik*, *myoD*, *Wnt 11* and *Noggin* (Pownall and Emerson, 1992; Ampthor *et al.*, 1996; Marcelle *et al.*, 1997) are expressed in the cells of the medial half of the dermomyotome, whereas the expression of *follistatin*, *Sim 1*, *c-met* and *flik-1* are detected in the lateral half (Eichmann *et al.*, 1993; Ampthor *et al.*, 1996; Pourquie *et al.*, 1996). The medial and lateral halves of the somite lead to formation of different muscle groups in the body (Ordahl and Le Douarin, 1992).

The discovery of homeobox (*Hox*) genes and their role in controlling embryo development has led to a better understanding of segment-specific functions of somites (regionalization) (Kessel and Gruss, 1991; Gehring, 1993; Manak and Scott, 1994; Morgan, 1997). It is suggested that they are not required for the formation of segmentation but only for the imposition of fate on those

segments (reviewed in Manak and Scott, 1994; Morgan, 1997). *Hox* genes spread predominantly along the craniocaudal axes of embryos (Slack and Tannahill, 1992). Once the embryonic axes are determined in fertilized chick eggs, *Hox* genes, both in the anteroposterior and the dorsoventral axes, help to establish the characteristics of different regions, which are crucial for the patterning of the body (Bellairs, 1993). For example, alterations in *Hox* gene expression cause a shifting of the regional borders and axial identities (Kessel *et al.*, 1990), such as the loss of rib-forming ability of the thoracic-level somites (Selleri *et al.*, 2001). Although it is not known how *Hox* genes regulate cell proliferation in a position-dependent manner, there is increasing evidence that their patterned expression determines which cells make the growth-regulating molecules and which cells respond to them (Duboule, 1991).

Programmed cell death (apoptosis)

Regionalized cell death occurs at specific stages of development in many multicellular animal embryos (reviewed in McCabe and Noveroske, 1997). This death, commonly referred to as developmentally programmed cell death, is normal rather than pathological. However, it has to be carefully controlled in the embryo, because abnormal development can result if it fails to occur, or if it occurs excessively (Thompson, 1995). How is this programmed cell death controlled? One widely accepted theory is that animal cells need extracellular signals to survive, just as they need them to grow (Raff, 1992). When the survival factors are not provided to them, cells activate an intracellular death programme and kill themselves. For example, many of the nerve cells in the vertebrate nerve system are generated in excess and thus they compete with one another for limiting amounts of survival factors produced by target cells (Conlon and Raff, 1999). Only the nerve cells that get enough factors survive, while the rest undergo apoptosis. On the other hand, some

of the extracellular signals that can function either systemically or locally induce programmed cell death. For instance, BMPs induce apoptosis in precursor neural crest cells and undifferentiated limb mesoderms before they migrate (Graham *et al.*, 1994; Merino *et al.*, 1998). The anterior and posterior necrotic zones, along the position border of the wing, and opaque patch in the centre of the wing undergo cell death during early stages (stages 23–24; Hamburger and Hamilton, 1951) of chick limb development (Wyllie, 1995). Among the several growth factors examined in these regions of the forelimb (FGFs, BMPs, EGF, TGF-β, PDGF, IGF-II and TNF-α), there has been a spatiotemporal correlation between TNF-α and BMPs (BMP-2, -4 and −7) immunoreactivity and the incidence of cell death (Wride *et al.*, 1994; Francis-West *et al.*, 1995; McCabe and Noveroske, 1997). TNF-α has been shown to have a well-established effect on cell death and tissue reorganization in pathological situations (reviewed in Wride and Sanders, 1995). Evidence suggests that fibroblast growth factors (FGFs) are also involved in the regulation of avian embryonic cell death. For example, administration of FGFs into the areas of programmed cell death inhibits apoptosis (Macias *et al.*, 1996) and also local administration of FGF into the developing interdigital duck webs potentiates the apoptotic effect of exogenously administered BMPs (Ganan *et al.*, 1998). The role of FGFs as survival factors for avian limb mesoderm through controlling the BMP signalling pathway responsible for establishing areas of cell death has been confirmed (Montero *et al.*, 2001). It is also suggested that combined action of FGFs and BMPs is required for the expression of *MSX2* and *Snail* genes, associated with the molecular cascade responsible for apoptosis (Montero *et al.*, 2001).

In summary, given a complex network of interacting embryonic structures coordinated by a variety of signalling and regulatory genes, little is known about the precise mechanisms of action of genes associated with the developmental changes observed within the somite. For a better understanding of somite development, it is necessary to address the mechanisms that connect cell proliferation, apoptosis and the differentiation process.

Genetic control of late-term embryonic growth

Simply, growth is increase in size. It is well known that the size of any given tissue or organ depends on the number and size of the cells it contains as well as on the amount of extracellular matrix and fluid (Conlon and Raff, 1999). Cell division and cell death determine appropriate cell number in a tissue at any time during development. Also, both cell number and cell size in a tissue depend on interaction between intracellular programming and extracellular signalling. Most cells grow and duplicate their content before they divide. Thus, for conceptual clarity, growth and cell proliferation are not the same phenomenon, even though they complement each other to give rise to most of the embryonic tissue or organ development. Additionally, a distinction must be drawn between growth factors, which stimulate cell growth, and mitogens, which stimulate cell cycle progression (Conlon and Raff, 1999), though some signalling molecules do both (e.g. the insulin-growth factor IGF-I, discussed below). To increase tissue or organ growth: (i) growth factors should activate intracellular signalling pathways that stimulate biosynthetic process in the cell; and (ii) regulatory molecules (growth factors and/or mitogens) must act either by interrupting normal cell cycles through changing the activity and/or amount of regulatory enzymes and/or other components, or by preventing apoptosis.

Even in the presence of saturating amounts of growth factors and nutrients, maximum growth potential is determined by the genes that the cell expresses. Moreover, the rate of the embryonic growth is determined by the cumulative outcome of continually shifting imbalances between positive and negative factors (Efstratiadis, 1998). Therefore, to facilitate a better

understanding of how the intracellular programmes (genes) and extracellular signals work together to determine total cell mass and to coordinate growth in the different parts of a vertebrate (as elegantly described for mouse embryonic development by Efstratiadis, 1998), the genes will be categorized based upon their effects on embryo development and growth, as follows: (i) those having a general growth-promoting function (lack of function causes, directly or indirectly, an overall growth retardation); (ii) those having a general growth-inhibiting function (lack of function causes overgrowth); and (iii) those having a local growth-promoting or growth-inhibiting function (tissue-specific growth regulation).

General growth-promoting genes

Hormones, particularly growth factors, and their receptors exert marked tissue-specific and age-specific effects on embryonic and postnatal growth (Dauncey and Gilmour, 1996). Thyroid hormones, insulin and their receptors are considered as general growth-promoting hormones. For instance, chemical thyroidectomy attenuates the growth of the chick embryo (McNabb et al., 1984). Additionally, it has been shown that thyroidotyronin (T_3) enhances chick embryo cartilage growth both in vitro and in vivo, implying that normal thyroid functioning is required for optimal embryonic chicken growth (Fugo, 1940). However, thyroid hormone receptor-deficient mice do not display growth defects or other abnormalities other than auditory defects (Forrest et al., 1996), implying that thyroid hormones appear to be active during postnatal growth, a widely held belief for the majority of hormones. The somatotrophic axis (growth-hormone releasing hormone; somatostatin; growth hormone; and insulin-like growth factors) plays a major role in embryonic and postnatal growth of vertebrates (reviewed in Buyse and Decuypere, 1999; Duclos et al., 1999). Mice deficient in growth hormone (GH) and growth-hormone releasing hormone (GHRH, Andersen et al., 1995; Gage et al., 1996; Baumann and

Maheshwari, 1997) demonstrate severe growth retardation. Additionally, GH and GHRH transgenic mice exhibit overgrowth, with some reaching nearly twice the size of control animals at 9–10 weeks of age (Palmitier et al., 1983; Mayo et al., 1988). However, as explained below, the GH–GHRH–IGF-I axis is minimally developed in the avian embryo. It is widely believed that growth factors functioning through autocrine and paracrine, as well as endocrine fashions, are major determinants of embryonic growth, particularly in avian embryos.

IGFS. IGFs appear to play the central role in the main growth signalling system of vertebrate organisms, since this function of other classic growth factors such as FGF, platelet-derived growth factor (PDGF) and epidermal growth factors (EGFs) has not been clearly established. Therefore, the focus here will be on IGF involvement in embryo development and growth. IGF components include IGFs (IGF-I and IGF-II), type-I and type-II receptors and a family of six secreted IGF-binding proteins (IGFBPs) (reviewed in LeRoith and Bondy, 1996; Stewart and Rotwein, 1996). IGF-I and IGF-II are structurally very similar polypeptides of approximately 7.5 kDa. They are ubiquitously expressed during embryonic and postnatal development. In addition to their endocrine effects, locally produced IGFs exert autocrine/paracrine effects on cell proliferation and differentiation (Jones and Clemmons, 1995). Gene knockouts, alone or in combination, are used to demonstrate the role of IGF signalling in embryonic and postnatal development (Baker et al., 1993, Liu et al., 1993; Louvi et al., 1997). IGF-I knockout mice have birth weights approximately 60% of normal, with defects in ossification. IGF-II-deficient homozygotes have a growth deficiency similar to that of animals lacking IGF-I but do not exhibit developmental abnormalities. Deletion of the type-I IGF receptor yields homozygous animals that are only 45% of normal weight at delivery and die within minutes of birth (type-II receptor knockout is discussed below). They also demonstrate generalized organ hypoplasia.

Compared with avian species, much more information is available on mammalian IGFs and binding proteins (McMurtry *et al.*, 1997). Although differences in amino acid composition between chicken and mammalian IGFs exist, the physiological significance of this heterogeneity is not known (Ballard *et al.*, 1990). IGF-I gene expression is first detected soon after fertilization (late blastula stages) in the whole chick embryo (Serrano *et al.*, 1991), implying that the importance of yolk-originated IGF-I (Scavo *et al.*, 1989) to embryo development beyond this embryonic stage is probably not significant. Type-I IGF receptors have also been detected as early as the blastoderm stage (day 0, oviposition), during neurulation (day 1) and in early (days 2–3) and late (day 9) organogenesis (Scavo *et al.*, 1991). Thus, expression of the type-I receptor in early embryonic development indicates a potential for IGF signalling before neurulation or even gastrulation. Even though IGF-II transcripts have been detected in eye, heart and developing limb bud of 4-day-old chick embryos (Engstrom *et al.*, 1987), the regulation of IGF-II synthesis and secretion in the developing avian embryo remains to be clarified.

The hypothalamo–pituitary–GH–IGF-I axis does not appear to be well established during avian embryonic development. For example, pituitary secretion of GH does not begin until day 12 of chick embryonic development, due to the late formation of somatotrophs (McCann-Levorse *et al.*, 1993). As the original somatomedin hypothesis states (Salmon and Daughaday, 1987), growth hormone is one of the primary regulators of IGF-I synthesis and secretion in vertebrates. However, based on the tissue and age of the avian species, the synthesis and secretion of IGF-I are under both GH-dependent and GH-independent control mechanisms. IGF-I mRNA expression is detected in various early embryonic tissues (Serrano *et al.*, 1991; Tanaka *et al.*, 1996) before the onset of GH secretion (Porter *et al.*, 1995), implying that the GH–IGF-I axis is minimally functional during embryogenesis. Nevertheless, there is an observed increase in serum IGF-I in mid-embryogenesis, which probably has an influence on embryo growth, because the chick embryo increases about 35-fold in wet weight and completes most of its organogenesis during this period (Robcis *et al.*, 1991).

Bioactivity of IGFs is modulated by IGFBPs found in extracellular fluids and serum of the vertebrate (reviewed in Clemmons, 1997). Mammals have six distinct classes of IGFBPs, termed IGFBP-1 through IGFBP-6. IGFBP-2, -3 or -6 knockout mice do not exhibit any obvious phenotype, while mice deficient in IGFBP-4 show mild growth retardation (Wood *et al.*, 1993). Only IGFBP-2 (Schoenle *et al.*, 1995) and IGFBP-5 (Allender *et al.*, 1995) have been isolated from chicken serum. Whether avian IGFBPs are similar in structure and function to those in mammals is unresolved.

General growth-inhibiting genes

The mechanisms that halt cell growth during development are as important as those that stimulate it. Precursor cells in most vertebrate lineages, such as muscle, bone, skin, gut cells and so on, divide a limited number of times and then stop and differentiate into special post-mitotic cells. Although the proliferation stopping mechanism is not yet completely resolved, a controlled decline in a cell-cycle promoter such as a cyclin-dependent kinase (CDK) or a cyclin and/or a controlled increase in a cell-cycle inhibitor such as a CDK inhibitor are believed to be major determinants of this process. Mice that are deficient in p27[kip1], a CDK inhibitor, are about 30% larger than normal, with more cells in all organs that have been examined, such as the thymus and spleen (Fero *et al.*, 1996; Kiyokawa *et al.*, 1996; Nakayama *et al.*, 1996). When p18[ink], another CDK inhibitor gene, is knocked out, a general growth-inhibiting function of this gene is revealed (Franklin *et al.*, 1998). Moreover, mice deficient in both p27[kip1] and p18[ink] (double knockout) have some organs even larger than they are in mice deficient in either protein alone (Franklin *et al.*, 1998). Therefore, both of the CDK inhibitors are considered as general growth-inhibiting

genes. Although strong p21 expression is detected in 6-day-old embryonic chicken brain (Klinz, 1989), neither p21 nor p18 has been shown to affect avian embryo development.

Another growth-inhibitory gene is type-II IGF receptor. Type-II IGF receptor gene expression is regulated by parental imprinting in mammals (Baker et al., 1993). It is thus suggested that type-II IGF receptor protects the developing embryo from unregulated, elevated IGF-II blood concentrations. Type-II IGF receptor-deficient mice demonstrate overgrowth and organomegaly (Ludwig et al., 1996). Because avian species do not possess the classic type-II IGF receptor (Yang et al., 1991), which molecule or molecules possess this function remains to be solved.

Local growth-promoting or growth-inhibiting genes

There has to be a mechanism to coordinate the growth of each tissue so that they can produce concinnity in the whole organism. Several theories have been suggested to explain this phenomenon. The one most favoured by scientists in the field of growth biology is that each tissue produces an inhibitor that specifically suppresses the growth of itself (Bullough, 1962). As the organ or tissue grows, the inhibitory substance accumulates until it reaches a threshold, causing cessation of growth of the tissue or organ from which it is produced (Goss, 1978). This theory can relate to liver regeneration as described even in ancient Greek mythology and in grafting experiments (reviewed in McPherron and Lee, 1999). For instance, the removal of part of the liver causes rapid compensatory growth of the remainder. Also, when a small liver is transplanted to a large host, it will grow much faster than the normal rate until it has reached proportional size of the host (Kam et al., 1987). Because of the lack of direct evidence for tissue-specific inhibitory molecules, this once widely accepted theory fell out of favour. However, based on recent findings, this type of mechanism can operate in skeletal muscle growth. When

myostatin (also known as growth and differentiation factor-8), a member of the TGF-β family, is disrupted in mice, skeletal muscle mass increases up to three times normal size (McPherron et al., 1997). Additionally, myostatin mutation has been linked to double-muscled cattle breeds (Grobert et al., 1997; Kambadur et al., 1997). It is suggested, therefore, that myostatin is the negative regulator of muscle growth in normal animals.

Myostatin is mainly synthesized in skeletal muscle as a 376 amino acid propeptide, which gives rise to a 15 kDa active, processed and mature protein (McPherron et al., 1997). Structurally, it contains all the characteristic features of the TGF-β family, such as a proteolytic processing signal site and an active carboxy-terminal region that has a highly conserved pattern of cysteine knots (McPherron et al., 1997). Myostatin is detected very early in the myotome of developing mouse (McPherron et al., 1997) and cattle embryos (Kambadur et al., 1997) and expression continues in the adult muscle. Myostatin gene expression is first seen during the blastoderm stage of the chick embryo (unincubated embryo, E0 (embryo day 0)) and remains constant through E1 (Kocamis et al., 1999a) (Fig. 30.4). Myostatin mRNA dramatically declines on E2 and remains lower through E6. Levels then sharply increase on E7 and plateau through E16. Myostatin mRNA increases by E17 and remains high through E19, then decreases prior to hatching. The highest and lowest myostatin mRNA levels are detected in the 1- and 2-day-old chicken embryo, respectively. Strong expression in the early chicken embryo (E0 and E1) (Kocamis et al., 1999a) is intriguing and suggests the possibility that myostatin has an important role during early chicken embryonic development even before myogenic identity is established. However, the strong myostatin mRNA expression seen during the blastoderm stage of Cobb × Cobb chick embryos (Kocamis et al., 1999a) was not observed in Ross × Ross chicken embryos (Fig. 30.4) (Kocamis et al., 2002). Because the Ross × Ross strain is considered a fast-growing chicken line, the different myostatin expression pattern between these two strains
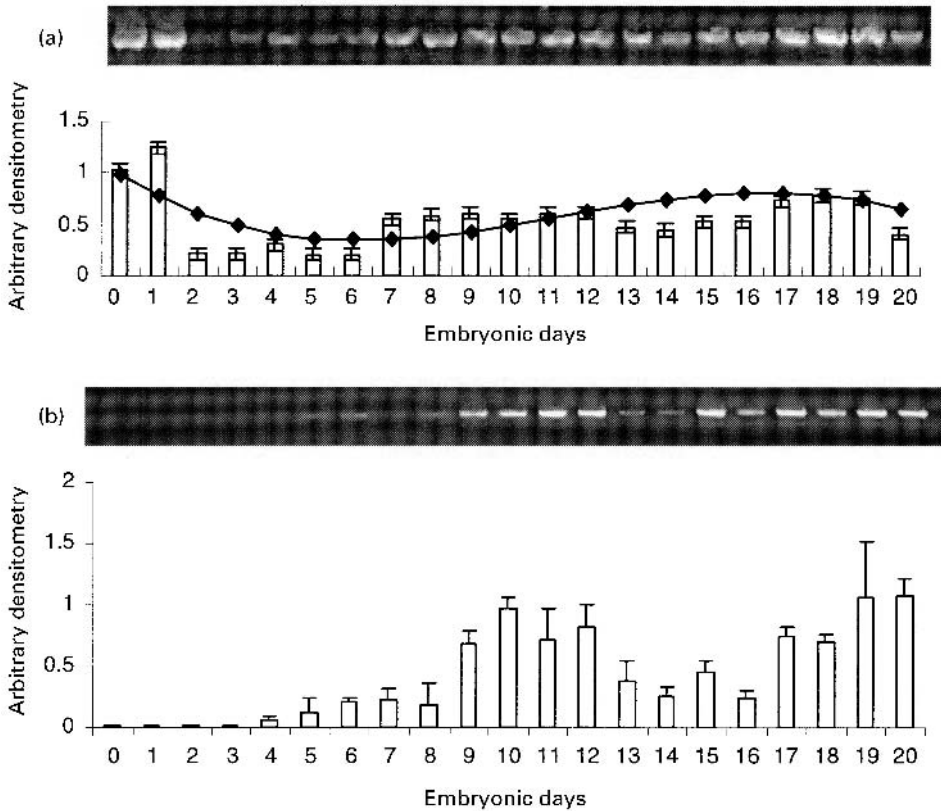
Fig. 30.4. Representative steady-state levels of myostatin mRNAs in whole embryo (E0 to E6, $n = 6$ per day), thoracic/abdominal halves (E7 and E8, $n = 6$ per day) and pectoralis muscle (E9 to E20, $n = 4$ per day) during (a) Ross × Ross and (b) Cobb × Cobb chicken embryonic development. The bands for myostatin mRNA were analysed by densitometry and the integration values (mean ± SD), after normalization to β-actin, were expressed in arbitrary densitometric units at each sampling day (Kocamis *et al*., 1999a, 2002, respectively).

could be related to the different growth/developmental rates of these birds. Therefore, early myostatin expression may not only determine muscle growth but may also play a pivotal role in early embryonic growth.

In vitro studies demonstrate that recombinant myostatin inhibits the proliferation of C2C12 myoblasts and bovine fetal myoblasts obtained at 160 days of gestation (Thomas *et al*., 2000). Extra myostatin specifically upregulates P[21] and decreases the amount of cyclin-dependent kinases (especially cdk2), a family of enzymes that catalyse events required for cell-cycle transition, in C2C12 cells (Thomas *et al*., 2000; Rios *et al*., 2001). Both research groups suggested that myostatin blocks the myoblast transition in the

G1/S and/or G2/M phases of the cell cycle. On the other hand, the two studies found conflicting results in terms of myostatin effects on apoptosis, even though they used the same myoblast cell culture lines. For instance, Thomas *et al*. (2000) demonstrated that myostatin does not affect apoptosis in C2C12 cells as shown in terminal deoxynucleotidyl transfer mediated dUTP nick end labelling (TUNEL) assays, whereas Rios *et al*. (2001) show that myostatin overexpression inhibits apoptosis in the same cells. Therefore, further studies should be conducted for a better understanding of myostatin involvement in apoptosis.

Although the phenotype of myostatin-deficient animals allows the possibility

that myostatin may be the specific growth inhibitor that was speculated to exist in the early 1960s (Bullough, 1962), several questions remain in terms of exact mechanisms of myostatin function. First, does myostatin circulate in the blood of any given animal? If so, do binding proteins in the TGF-β family such as follistatin and noggin regulate its activity in circulation? Secondly, can myostatin inhibit the growth of skeletal muscle in adult animals? If so, is it dose dependent? Thirdly, is myostatin involved in highly muscled callipyge sheep (Cockett et al., 1994), Piétrain pigs (Brenig and Brem, 1992) or selected poultry? Fourthly, how does myostatin interact with the growth factors that have been well documented to stimulate skeletal muscle growth, such as insulin-like growth factors (IGF-I and IGF-II)?

Another tissue-specific local growth-inhibitor gene is FGF receptor 3 (*FGFr3*). It is expressed in cartilage during mouse endochondral ossification. Thus, *FGFr3* knockout mice show overgrowth of long bones and vertebrae with variable growth retardation (Deng *et al.*, 1996). *FGFr3* expression is detected in the interdigital areas of 7-day-old chick and duck embryos, which is closely correlated with the areas of cell death (Montero *et al.*, 2001). Not only is its expression regulated by local treatments with BMPs and FGFs, but it also follows the same expression pattern as FGFs and BMPs. It is thus suggested that FGFs, as survival factor for the limb mesoderm, specifically use *FGFr3* for the control of BMP signalling pathways responsible for establishing the areas of cell death (Montero *et al.*, 2001).

Summary

As illustrated in this chapter, nature has provided a very elegant cellular coordination that results in an animal developing from a one-cell zygote to a fully functional multicellular organism. Advances in science have led to a greater understanding of the regulatory mechanisms controlling proliferation, differentiation and development, allowing manipulation of these processes.

As will be described in later chapters, the expression patterns of genes associated with specific phenotypes or responses to treatments can be examined. These patterns can now be altered to manipulate the growth and developmental processes of the animal via ectopic application of growth factors (Kocamis *et al.*, 1998, 1999b) or by altering endogenous gene expression levels or timing. With the development of transgenic technologies it is even possible to introduce new or altered genes to create entirely new phenotypes. The advent of new molecular genetic technologies and their application to animal agriculture have created new opportunities and exciting possibilities for this essential and vibrant industry.

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31   Expressed Sequence Tags, DNA Chip Technology and Gene Expression Profiling

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Introduction

The efforts of the human genome project have led to the identification of thousands of genes in many different organisms, though functional information for most of the newly identified genes is lacking. Quantitative analysis of gene expression is one step towards an understanding of gene function. DNA microarrays were initially developed for analysing the expression of large numbers of genes simultaneously (Schena *et al.*, 1995). The microarray technique uses a robot for precise application of micro volumes of DNA solutions to a matrix (i.e. glass slide or nylon membrane) (Fig. 31.1). The total pool of mRNA from experimentally manipulated cells, tissues or animals can be labelled using an oligo-deoxy-thymidine (dT)-primed reverse transcriptase reaction and fluorescent (or radioactive) nucleotides (Freeman *et al.*, 2000; Hegde *et al.*, 2000). The labelled cDNA targets (synthesized from mRNAs) are allowed to bind to the DNA probes immobilized on the glass slide (or nylon membrane). The intensity of the hybridization signal is related to the amount of RNA that is present for the corresponding species. Thus, one can readily evaluate the differential expression of thousands of genes in a single microarray experiment. A challenge for functional genomics will be the reverse engineering and modelling of gene regulatory networks that are revealed by global gene expression profiling.

Expressed Sequence Tags (ESTs)

Major chicken EST sequencing projects

The initial step in functional genomics is to make an extensive catalogue of genes expressed in each tissue. Microarray analysis requires careful preparation of tissue-specific cDNA libraries for high-throughput DNA sequencing: normalization of abundant clones (Soares *et al.*, 1994) and eventual subtraction of redundant clones (de Fatima Bonaldo *et al.*, 1996). High-throughput, single-pass DNA sequencing from either the 5′- or 3′-end of randomly picked cDNA clones yields ESTs, representing 500–800 bp reads, that are then used in BLAST searches for gene identity (Altschul *et al.*, 1997). Typically, the approach of sequencing from the 5′-end of EST clones favours gene identity because of the likelihood of finding coding region sequence. The overlapping EST sequences from the same gene (contigs) can be used to assemble full-length *in silico* cDNA sequences (Fig. 31.2). The number of contigs assembled by this bioinformatic process also gives a good estimate of the number of genes expressed in a given tissue.

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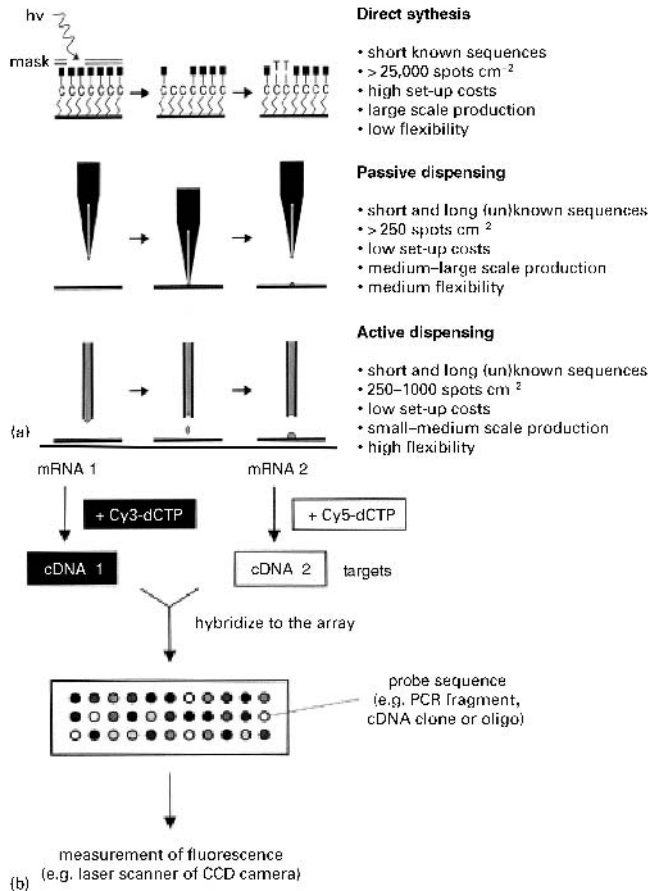
Fig. 2.1. Principles of DNA microarray technology. (a) Oligonucleotides (oligos) are directly synthesized on to glass slides. Alternatively, cDNA inserts are PCR amplified, using vector-specific primers, and printed at high density on a solid matrix by a computer-controlled high-precision XYZ robot. (b) Pools of RNA are reverse transcribed in the presence of fluorescently labelled (or radiolabelled) nucleotides (i.e. targets) and allowed to hybridize to the cDNA sequence immobilized on the array (i.e. probes). Hybridizing species are detected with a laser scanner (or a phosphor-imager for nylon membrane arrays). (Originally published in an article by van Hal *et al.*, 2000; reproduced with permission of Elsevier Science, BV.)

Genome-scale cDNA sequencing and DNA microarray technology, initiated by the Human Genome Project, have enabled the exploration and discovery of thousands of genes in model organisms (humans, mice, fruit flies, yeast, etc.). Until now, the greatest obstacle to implementing similar studies of functional genomics in chickens has been the lack of a complete catalogue of tissue-specific gene sequences.

Before 2001, there was only a modest collection of chicken ESTs (< 22,000) cataloged in public databases maintained at EMBL/GenBank, Roslin Institute, University of Delaware and University of Hamburg. Although the chicken once lagged behind other animals in genomic research, a milestone in avian genomics was reached in 2001 with the culmination of several independent chicken EST sequencing efforts. Using high-throughput DNA sequencing, the University of Delaware has identified 37,388 ESTs from several primary and normalized tissue-specific chicken cDNA libraries: lymphoid tissue, liver, abdominal fat, breast and leg muscle/bone
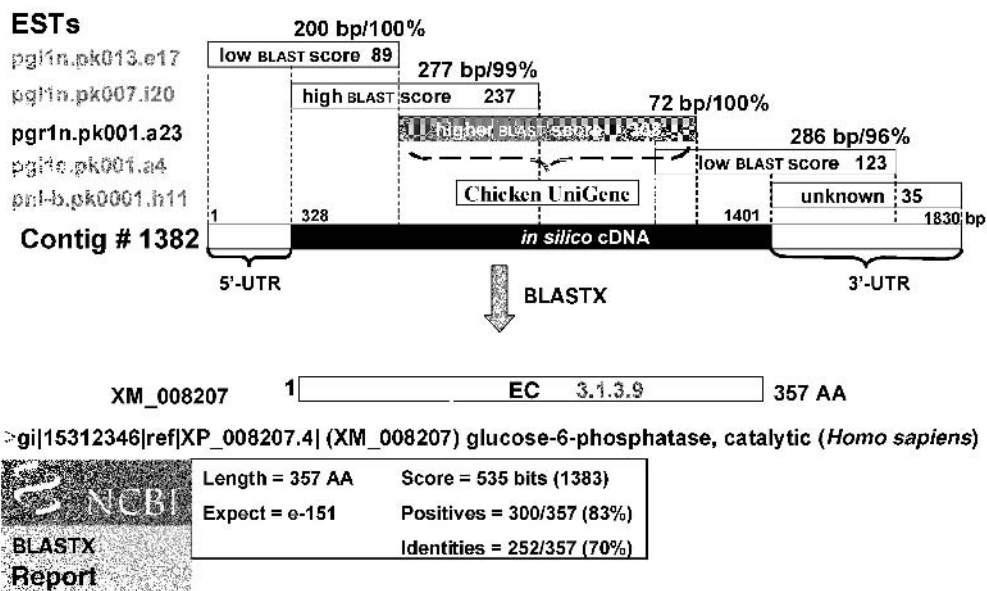
Fig. 31.2. CAP3 assembly of ESTs sequenced from different chicken cDNA libraries in the University of Delaware collection. The CAP3 sequence assembly program (Huang and Madan, 1999) was used to build contigs of EST sequences using a 40-base overlap and 95% sequence identity. The overlapping sequence of each EST is shown by the vertical dashed lines and the percentage identity is indicated above each bar. The BLASTX score for each individual EST is given within the bar. The BLASTX score for the high fidelity *in silico* cDNA was 535. UTR, untranslated region; bp, base pairs.

growth plate, pituitary/hypothalamus/pineal, and reproductive tract (testis/ovary/oviduct) prepared mainly from broiler chickens (Table 31.1). These EST sequences were entered into GenBank and the original chicken EST database (www.chickest.udel.edu). Another chicken EST database of clones that were sequenced from chicken bursa cells (swallow.gsf.de/dt40.html) presently lists 11,260 ESTs sequenced from the unnormalized dkfz426 library and 12,982 ESTs from the normalized riken1 library (Abdrakhmanov *et al.*, 2000; Buerstedde *et al.*, 2002) (Table 31.2). On 17 December 2001, a consortium of British institutions, funded by the British Biotechnology and Biological Sciences Research Council (BBSRC), unveiled a much larger chicken EST database (www.chick.umist.ac.uk) of 299,506 clones that were derived from high-throughput sequencing of 21 tissue-specific cDNA libraries (Table 31.3) prepared from Leghorn (layer type) chickens (Chambers, 2002). The addition of chicken EST sequences to the BBSRC database since

Table 31.1. University of Delaware Chicken EST Sequencing Projects (www.chickest.udel.edu).

Chicken cDNA libraries	No. of ESTs
Lymphoid tissue (activated T cells, bursa, thymus, spleen)	8870
Liver	5611
Abdominal fat	6742
Breast/leg muscle/growth plate	5511
Pituitary/hypothalamus/pineal	8742
Testis/ovary/oviduct	1912
Total	37,388

Table 31.2. University of Hamburg Chicken EST Sequencing Project (swallow.gsf.de/dt40.html).

Chicken bursa cDNA libraries	No. of ESTs
dkfz426	11,260
riken1	13,332
Total	24,592

the first release now brings the total to 330,388 chicken ESTs. The combined efforts from these high-throughput chicken EST

Table 31.3. BBSRC Consortium Chicken EST Sequencing Project (www.chick.umist.ac.uk).

Tissue-specific cDNA libraries	No. of ESTs
Adult brain – cerebellum	10,451
Adult brain – cerebrum	14,072
Adult brain – other parts	15,526
Adult heart	9,530
Adult pancreas	1,233
Adult adipose	2,811
Adult kidney + adrenal	14,863
Adult liver	13,177
Adult small intestine	17,838
Chicken chondrocytes isolated from growth plates	31,601
Chicken muscle	4,126
Chicken ovary	29,725
Embryo brain, 16 days	16,830
Stage 10, whole chick embryos	12,709
Stage 20–21, whole chick embryos	18,769
Stage 22 heads	17,955
Stage 22 limbs	15,820
Stage 36 heads	15,413
Stage 36 hearts	8,789
Stage 36 limbs	14,729
Stage 36 trunks	13,539
Embryo	**134,553**
Adult	**164,953**
Total	**299,506**

Note: This download of chicken EST sequences was made on 1 February 2002. The table was compiled from the first release of BBSRC chicken EST sequence data (17 December 2001). The database now contains a total of 330,388 chicken ESTs.

sequence projects have provided thousands of ESTs from almost all chicken tissues, with minimal overlap. The next goal will be to develop a complete gene catalogue for the chicken genome from this bonanza of ESTs.

Bioinformatics

Once the genomics bottleneck, scores of bioinformatic programs and tools have been developed to compress, analyse and visualize enormous volumes of gene sequence and related information from high-throughput DNA sequencing projects. The identification of the complete repertoire of expressed chicken genes

(some 30,000–40,000 genes) will require bioinformatic processing of this massive volume of chicken gene sequence information (representing about 353,500 ESTs) into contigs or *in silico* cDNAs. A number of bioinformatic tools have been developed to assemble the overlapping ESTs derived from the same gene into tentative consensus sequences or contigs (Burke *et al.*, 1999; Huang and Madan, 1999; Miller *et al.*, 1999; Zhuo *et al.*, 2001).

The following example illustrates the use of the CAP3 sequence assembly program (Huang and Madan, 1999) to build contigs (or high fidelity *in silico* cDNAs) of chicken ESTs from several tissue-specific cDNA libraries in the University of Delaware collection (Fig. 31.2). The CAP3 program assembled five EST clones (one unknown EST, two low-BLAST-score ESTs, and two high-BLAST-score ESTs) into a contig (or *in silico* cDNA) that has a high translated-protein sequence identity with human glucose-6-phosphatase (EC 3.1.3.9). The last EST clone was initially classified as unknown (BLAST score = 35) with BLASTX because it corresponds to the 3′-non-coding (untranslated) region (UTR). The first clone has a low BLASTX score of 89 because part of its sequence corresponds to the 5′-UTR. The key to solving this chicken contig assembly was an EST clone (BLAST score = 308) sequenced from the normalized reproductive tract library (*pgr1n.pk001.a23*); this EST sequence represents only coding regions and it overlaps two adjacent ESTs. The complete *in silico* cDNA sequence for this contig has a high BLASTX score (535) with the catalytic enzyme, human glucose-6-phosphatase. In this case, the chicken EST clone *pgr1n.pk001.a23* had the highest individual BLASTX score and was selected to represent chicken glucose-6-phosphatase for printing on the liver array.

In order to obtain a good estimate of the number of genes expressed in the chicken transcriptome, we have analysed all of the chicken EST sequences found in public databases, as of 1 February 2002, and assembled a preliminary chicken gene index (Table 31.4). We used the CAP3 sequence assembly program (Huang and Madan, 1999)

to build contigs from the world's current public collection of chicken ESTs (353,484 EST sequences) (Carre *et al.*, 2002). This CAP3 assembly reveals tentative consensus sequence for 32,374 contigs and 76,060 unclustered singletons (singlets), which together represent 108,434 non-redundant chicken cDNA sequences. Although contig assembly underestimates the total number of genes, the singlets group contains artefactual and contaminant sequences in addition to the bona fide cDNAs. For example, an analysis of human ESTs indicated that there are about 35,000 human genes, which is much lower than initial estimates (Ewing and Green, 2000). The cattle gene index, assembled with the CAP3 program from 127,279 bovine EST sequences, comprised 16,740 tentative consensus sequences or contigs and a total of 47,891 non-redundant bovine cDNA sequences (Smith *et al.*, 2001). Suddenly, the chicken seems to have surpassed other farm animals (i.e. cattle) in accruing ESTs that will be used to estimate the total number of genes expressed by its genome.

This large international collection of chicken ESTs will provide the avian genomics community with the resources and tools needed to explore the chicken's functional genome. Genome-wide gene expression profiling will enable the discovery of new genes and detailed mapping of the genetic circuits that control expression of important production traits in poultry.

Array Methodology

Types of arrays

Two types of microarrays that are currently used can be categorized according to the size of the immobilized DNA: either short oligonucleotides (15–25 bases) or cDNAs (200–2000 bp). Oligonucleotide DNA chips are produced either by combinatorial chemistry coupled with photolithographic masking technology for *in situ* synthesis (i.e. Affymetrix GeneChip probes) or by deposition of small oligonucleotides of defined

Table 31.4. Chicken gene index compiled from University of Delaware (UD), BBSRC and public collections.[a]

	UD ESTs	BBSRC ESTs	All public ESTs
Total ESTs	37,388	299,506	353,484
Contigs	5,629	29,852	32,374
Singlets	11,197	70,440	76,060
Total non-redundant sequences	16,826	100,292	108,434

[a]See 'Notes', p. 642.

composition produced by routine oligonucleotide synthetic technology. Robotic-driven micro- deposition technology is used to prepare oligonucleotide arrays or cDNA arrays. In the case of cDNA arrays, inserts from chosen EST clones are amplified using PCR and spotted on to either glass slides or nylon membranes (Fig. 31.1).

Preparation of oligonucleotide DNA chips is the most efficient method for preparing microarrays since it lends itself to almost total automation, high production and very high density (250,000 probes cm^{-2}). The technologies for producing oligonucleotide or DNA microarrays on glass slides or nylon membranes are essentially identical, but there are advantages and disadvantages with each method. Glass slides permit high-density spotting (> 4000 targets) but require unique hybridization chambers and specialized confocal laser scanners for detection of hybridization signals. Nylon membranes are used for less dense spotting (< 4000 probes) and have the advantage of requiring no special equipment for detection, since a phosphor-imager (or X-ray film) can be used to record hybridization signals. Essentially, there are four steps in the DNA microarray procedure: array construction, sample preparation/hybridization, image processing and data analysis.

Preparation of arrays

An array is essentially a reverse Northern (or dot) blot, in which the probe is immobilized to a solid phase prior to hybridization. Drawing parallels to this convention, the

editor of *Nature Genetics* has encouraged the adoption of the term 'probe' for the DNA spotted on the array and 'target' for the labelled (sample) DNA hybridized to the array (Phimister, 1999). The cDNAs used for arrays can be chosen from an uncharacterized collection (i.e. clones from a cDNA library) or a 'named' collection (i.e. a group of annotated ESTs). Probes for the arrays are generated using PCR and primers present in the plasmid vector. Because of the number of individual samples handled, a 96-well or 384-well format is normally used for PCR. The PCR products are evaluated by agarose gel electrophoresis to ensure adequate PCR amplification and then purified for spotting on the array. For membrane-based arrays, a simple alcohol precipitation is sufficient, which also allows concentration of the DNA fragments to be adjusted to the desired level of 500–1000 ng μl^{-1} for printing. Glass slide arrays require more scrupulous purification, since great care must be taken to eliminate particulate material that can interfere with spotting and fluorescence detection.

A robot is used to print spots of each DNA sample on to multiple slides or membranes in a serial operation. The robot can position the print head with a high degree of precision. Glass microscope slides coated with poly-lysine or aminosilanes are used to anchor the DNA. Charged nylon is commonly used for membrane-based arrays. After printing, the DNA is denatured to produce single-stranded polymers and then anchored by UV cross-linking or chemical bonding.

Different types of pens or tips are used for making the different arrays. For glass slide arrays, stainless steel pins or 'quills' take up the DNA solution and deposit it in spots of 75–500 μm. Apart from the instrumentation, the preparation of the cDNAs for arraying represents the largest expense in the analytical procedure. Thus, careful selection of the collection of cDNAs to be arrayed is the most cost-effective approach to studying global changes in gene expression. Commercial vendors of arrays have taken this approach and even produce tissue-specific or thematic arrays. While it would be ideal to have every gene expressed in an organism represented on an array, this is not currently feasible for vertebrate genomes.

The most challenging tasks in array manufacture are organization of the deposition of thousands of probes on the array and management of the information used for identification of each mobilized probe. Commercial vendors of arrays usually provide web-based access to this information. In addition, several software programs are available to handle image processing and data management.

Labelling of mRNA and hybridization to arrays

Labelling of the target mRNAs isolated from cells or tissue is accomplished in an oligo-dT-primed reverse transcriptase reaction that incorporates fluorescent (or radiolabelled) nucleotides into the first strand cDNA molecule. Either ^{32}P- or ^{33}P-nucleotides can be used for radioactive labelling, although ^{33}P prevents 'bleed-over and blooming' from adjacent spots and gives a crisper image with phosphor-imaging.

A number of fluorescent-tagged nucleotides are commonly used for glass slide arrays. Cy3- and Cy5-labelled nucleotides can be detected by most laser scanners and provide high sensitivity. The difference in their emission wavelength prevents an overlap in the signal from differently labelled samples. The obvious advantage of this method of labelling (as opposed to radioactive labelling) is that two differently labelled samples can be hybridized simultaneously to the same array. The major drawback to two-channel dye labelling is the difference of the two dyes in labelling efficiency, signal intensity, photo-bleaching and background fluorescence (van Hal *et al.*, 2000). However, dye-swapping techniques and rigorous normalization methods (Tseng *et al.*, 2001; Yang *et al.*, 2002) have been developed to eliminate fluorescent dye bias as a source of systematic error in glass microarray experiments (Nadon and Shoemaker, 2002). Relative signal intensity should be independent

of variations in slides or hybridization conditions that could occur when using a single fluorophore to measure changes in expression in small quantities of RNA. In the case of only a very small quantity of mRNA available for labelling, one common amplification method involves the inclusion of a T7 RNA polymerase promoter into the oligo-dT primer, which allows for near-linear amplification of the first strand in an *in vitro* transcription reaction (Lee *et al.*, 1999).

Factors that affect the hybridization reaction include the amount of the cDNA or oligonucleotide probe immobilized on the slide (or filter), the concentration of the labelled mRNA species, DNA sequence composition of the duplexes, salt concentration and temperature. For membrane filters, the amount of DNA immobilized should be at least in tenfold excess of the cognate species in the labelled target mixture in order to achieve pseudo first-order kinetics of hybridization. Under these conditions, the hybridization signal is determined by the concentration of the labelled species; a twofold increase in the amount of target should yield a twofold difference between two mRNA populations competing for the same immobilized probe.

Salt concentration and temperature for hybridization can be manipulated in much the same way as for traditional Northern blot analysis, with the caveat that the labelled mRNA is a complex mixture and hybridization conditions must be determined empirically. An additional complication arises with the presence of repetitive elements, which give rise to strong hybridization signals due to saturation of the immobilized probe. Hybridization solutions routinely contain appropriate blocking DNA (polyAT, Cot1 DNA, sheared salmon sperm DNA) to minimize this problem.

Signal detection of arrays

Confocal laser scanners are used to read glass slide arrays hybridized to fluorescent-labelled targets. The fluorophore (Cy3 or Cy5) is excited by a laser and the emission signal is collected through a confocal lens for detection by a photomutiplier tube. Although the original laser scanners used in microarray analysis could detect only two colours, four-colour laser scanners are now available from several vendors. A phosphor-imager is most commonly used to measure hybridization signals in radioactively labelled arrays. Images are then imported into an analysis program, which quantifies the signal intensity of the spots. Several commercially available programs, which also incorporate tools for data analysis, have been developed.

The thousands of data points generated in typical microarray experiments pose new challenges in sample tracking and data analysis. Standardized methods for analysing arrays do not exist, but attempts are under way to develop industry-wide standards in normalization, data analysis and reporting gene expression data (Brazma *et al.*, 2001).

Computational and statistical analysis of gene expression data

Although DNA microarray technology has been universally adopted for large-scale gene expression profiling, the development of appropriate statistical analysis programs is still in its infancy. A unique feature of microarray analysis is the need for preprocessing of raw data to remove artefacts or background noise and for normalization of signal intensities between dyes and across slides in replicated experiments. Global (non-linear) normalization is widely used for multiple slide experiments, where all intensity values on an array are divided by the mean intensity of each slide. Data filtering is used to eliminate uninformative genes (i.e. those with hybridization signals below an arbitrary threshold) or genes with expression levels that do not change across slides or test conditions.

Numerous methods have been developed to identify differentially expressed genes or genes grouped into similar expression patterns. The most simple and informative method of detecting differentially expressed

genes is to determine the fold difference of each gene between two conditions (control and experimental RNA samples) and to plot these ratios in a histogram. Although the expression of most genes will not change, genes that show a twofold difference or higher are considered as differentially expressed genes. This method has the serious disadvantage of reduced specificity (introducing false positives at low expressions levels) and reduced sensitivity (missing true positives at high expression levels) (Draghici, 2002; Liang, 2002; Nadon and Shoemaker, 2002).

Another goal of exploratory gene expression profiling is to identify basic patterns of gene expression with the underlying assumption that genes with similar expression patterns should be functionally related (Eisen *et al.*, 1998; Tamayo *et al.*, 1999). Hierarchial clustering is a descriptive tool that groups genes together into a phylogenetic tree or dendrogram according to the closeness of their expression patterns (Eisen *et al.*, 1998). Another clustering method, called self-organizing maps (SOMs), is well suited for determining gene expression patterns in time-series experiments (Tamayo *et al.*, 1999; Toronen *et al.*, 1999). SOMs analysis is a robust descriptive tool for analysing exploratory data and discovering basic patterns of gene expression. The SOMs program (GENECLUSTER) provides graphic displays of genes clustered according to their expression pattern, which often contains a group of functionally related genes (Tamayo *et al.*, 1999). More elaborate computational analyses such as support vector machines (SVM) (Chow *et al.*, 2001; Taniguchi *et al.*, 2001) and principal component analysis (PCA) (Cunningham, 2000; Crescenzi and Giuliani, 2001; Liang, 2002; Peterson, 2002) have been developed to identify sets (clusters) of marker genes that characterize distinct biological processes from large gene expression data sets. The SVM method uses prior knowledge of gene expression to classify functional clusters of co-regulated genes from new expression profiles (Taniguchi *et al.*, 2001). Both computational approaches (SVM and PCA) hold promise for identification of marker genes

in cancer profiling studies, novel drug discovery and classification of candidate genes in regulatory pathways (Cunningham, 2000; Chow *et al.*, 2001; Crescenzi and Giuliani, 2001; Taniguchi *et al.*, 2001).

There has been a great deal of interest in improving the reliability of conclusions drawn from vast volumes of data generated from gene expression profiling with DNA microarrays (Brazma and Vilo, 2000; Altman and Raychaudhuri, 2001; Dopazo *et al.*, 2001; Thomas *et al.*, 2001). A number of very robust statistical modelling approaches have been developed to provide normalization of expression data across many arrays and to improve the signal-to-noise ratio (Alter *et al.*, 2000; Dopazo *et al.*, 2001; Thomas *et al.*, 2001; Tseng *et al.*, 2001; Yue *et al.*, 2001). Global gene expression experiments generate missing data that can be estimated and replaced by imputed values prior to statistical analysis by sensitive methods such as K-nearest neighbour (Troyanskaya *et al.*, 2001). One method, called significance analysis of microarrays (SAM), uses permutations of repeated measurements to estimate the false discovery rate (FDR) (Tusher *et al.*, 2001). A Bayesian probabilistic modelling method has been developed to provide statistical inferences of gene changes and to accelerate gene discovery by avoiding false identification of large numbers of genes (Baldi and Long, 2001); a high FDR is particularly prevalent when gene expression data are presented merely as fold differences between two conditions (i.e. control vs. experimental samples). The most effective way to reduce the FDR in gene expression experiments is to increase the number of replicate microarrays (Lee *et al.*, 2000; Pan, 2002). The general recommendation is that a minimum of three replicates be used in designing microarray experiments (Lee *et al.*, 2000). Thus, a great deal of care and caution must be taken in the construction of DNA microarrays, in preprocessing of raw data and in the statistical analysis of large gene expression data sets.

Most DNA microarray studies have included the additional step of an independent measurement of differential gene expression by either Northern blot analysis

(Schena *et al.*, 1995; Iyer *et al.*, 1999; Taniguchi *et al.*, 2001) or real-time quantitative RT-PCR (Q-RT-PCR) (Wurmbach *et al.*, 2001). Validation of differential gene expression by these traditional 'gold standard' methods provides an assessment of the sensitivity and specificity of microarray gene expression (Nadon and Shoemaker, 2002). A parallel comparison of the relative expression of 70 genes by DNA microarray and Northern blot analysis showed that differences in mRNA expression levels are underestimated by the DNA microarray (Taniguchi *et al.*, 2001). Furthermore, both commercial oligonucleotide (GeneChip, Affymetrix) and custom cDNA microarray platforms consistently underestimate the relative changes in mRNA expression levels between control and experimental samples, when compared with absolute quantification by Q-RT-PCR (Yuen *et al.*, 2002). However, this study clearly showed that a custom DNA microarray can be easily calibrated with a simple algorithm from measurement of a few differentially expressed genes by the more sensitive Q-RT-PCR method. Thus, transcriptional profiling with microarrays is a powerful and rapidly developing technology for the accurate identification of functional genes and regulatory networks in many organisms, including the chicken.

Gene Expression Profiling in Chicken Tissues

Lymphoid tissue and chick embryo fibroblasts

There have been only three papers published on gene expression profiling with DNA microarrays in chickens (Liu *et al.*, 2001; Morgan *et al.*, 2001; Neiman *et al.*, 2001). An extensive catalogue of chicken lymphoid ESTs has been made from high-throughput sequencing of an activated T cell (Tirunagaru *et al.*, 2000) and bursal cDNA libraries (Abdrakhmanov *et al.*, 2000; Buerstedde *et al.*, 2002). DNA microarrays have offered the first look at global gene expression in the chicken during normal development of the bursa (Neiman *et al.*, 2001) and during Marek's disease virus (MDV) infection (Liu *et al.*, 2001; Morgan *et al.*, 2001).

Neiman *et al.* (2001) developed a chicken immune system cDNA microarray (representing 2000 genes) to analyse broad changes in gene expression that occur during normal embryonic B-cell development and during *myc*-induced neoplastic transformation in the bursa. In follicles, *myc* induction of B-cell neoplasia requires a target cell population present during early bursal development, and progresses through pre-neoplastic transformed follicles to metastatic lymphomas. The number of mRNAs showing at least a threefold change was greater during *myc*-induced lymphomagenesis than during normal development, and hierarchical cluster analysis of expression patterns revealed that levels of several hundred mRNAs varied in concert with levels of *myc*-overexpression. A group of 41 mRNAs was consistently elevated in *myc*-overexpressing pre-neoplastic and neoplastic cells. Another cluster of genes was overexpressed in neoplasia independent of *myc* expression level, including a small subset with the expression signature of embryonic bursal lymphocytes. Overexpression of *myc*, and some genes co-expressed with *myc*, could be important for generation of pre-neoplastic transformed follicles. However, expression profiles of late metastatic tumours showed a large variation in concert with *myc* expression levels and some showed minimal *myc* overexpression. Therefore, a high level of *myc* overexpression could be more important in the early induction of these lymphomas than in maintenance of late-stage metastases.

We have used microarrays containing 1126 non-redundant cDNAs selected from our chicken activated T-cell database to profile changes in gene expression that accompany infection of chicken cells with MDV (Morgan *et al.*, 2001). MDV is an oncogenic avian herpesvirus that induces T-cell lymphomas in susceptible chickens in a matter of weeks (Calnek and Witter, 1997). Field viruses isolated over the second half of the

20th century are classified as 'mild', 'virulent', 'very virulent', or 'very virulent plus', depending on the type of lesions they cause and the vaccination schemes required to protect chickens from the disease. Marek's disease is one of the most serious infectious diseases of chickens, and also serves as an important model for studies on herpesvirus oncology, viral latency and control of herpetic disorders by vaccination.

Our initial experiments focused on changes in gene expression that accompany infection of chicken embryo fibroblasts (CEFs) with MDV. We have identified a group of 13 genes whose expression was consistently up-regulated following MDV infection of CEFs (Morgan *et al.*, 2001). Several of these genes – macrophage inflammatory protein (MIP), interferon (IFN) response factor-1, and IFN-inducible protein – express products expected to be involved in inflammation and cellular stress. In particular, members of the MIP family of $C\frac{1}{m}C$ chemokines have proinflammatory activity (Cook, 1996), and Kaposi's sarcoma-associated herpesvirus encodes three MIP-related chemokines in its genome (Boschoff *et al.*, 1997; Endres *et al.*, 1999). Two other induced genes – quiescence-specific protein and thymic shared antigen-1 (TSA-1) (also known as stem cell antigen-2) – express products involved in cell growth and differentiation. In addition, a group of genes whose products are expected to participate in antigen presentation (MHC class I, MHC Class II and β_2-microglobulin) were identified with microarrays.

We have constructed a number of mutants of MDV and two of these (RB1B-*meqlac* and RB1BLAT*lac*) have lost their ability to induce tumours in chickens. In both cases, these mutant viruses also fail to establish the expected peripheral blood viraemia in the weeks that follow the initial infection. Microarrays offer a means to determine if loss-of-function mutants have altered the profiles of host gene expression following infection. In CEFs, we found that the RB1BLAT*lac* mutant induced a host gene expression profile that was essentially identical to that induced by the parent RB1B strain. In the case of RB1B*meqlac*, however,

the host gene expression profile induced by the mutant was similar to that induced by the parent virus, except for the induction of a host heat-shock binding protein by the mutant. Although we are not convinced at this time that the absence of Meq protein affects the pool of heat-shock and heat-shock binding proteins within MDV-infected CEFs, it is interesting that the *meq* promoter contains a heat-shock binding element (Jones and Kung, 1992) and preliminary studies using the yeast two-hybrid system suggest that Meq could bind to HSP70 and HSP90. Other herpesviruses are known to induce heat-shock proteins following infection (Ohgitani *et al.*, 1998, 1999).

Gene expression profiling has been applied to quantitative trait loci (QTL) mapping to identify genes conferring genetic resistance to MD. In a preliminary study, Liu *et al.* (2001) have shown that DNA microarrays, containing approximately 1200 genes or ESTs, are able reproducibly to detect differences in gene expression of uninfected and MDV-infected peripheral blood lymphocytes isolated from two inbred lines of chickens: East Lansing Avian Diseases and Oncology Laboratory (ADOL) lines 63 (MD resistant) and 72 (MD susceptible). Microarray data were validated by quantitative PCR and found to be consistent with previous literature on gene induction or immune response. Fifteen genes identified by microarray analysis were integrated into the chicken genetic map. Twelve of these mapped genes had human orthologues. Seven genes were located on the chicken linkage map as predicted by the human–chicken comparative map, while two other genes defined a new conserved syntenic group. More importantly, one of the differentially expressed genes is known to confer genetic resistance to MD, while another gene identified by cluster analysis is a prime positional candidate for a QTL.

Metabolic tissue

We have used DNA microarrays made from EST clones sequenced from an activated

T-cell cDNA library (Tirunagaru *et al.*, 2000) to conduct some preliminary gene expression studies in liver of broiler chickens divergently selected for growth rate (Beccavin *et al.*, 2001). We isolated mRNA from the liver of one bird per age per genetic line (fast-growing line, FGL; slow-growing line, SGL) at 3, 5, 7 and 9 weeks of age for preparation of ^{32}P-labelled targets. After hybridization, a data variation filter was used to eliminate genes with a spot intensity below the detection threshold and those without hybridization to both spots for a given gene across all four age groups. Patterns of gene expression in each tissue were examined by SOMs analysis (Tamayo *et al.*, 1999). The GENECLUSTER program contains a data variation filter whose limits

are set by the user, usually to reject a gene whose spot intensity does not change by more than twofold between any two time points in a time series (at least three time points are required). Thus, this variation filter rejected genes whose expression levels did not change across time (3–9 weeks). In our preliminary gene clustering study with a microarray of 1200 cDNAs printed in duplicate, only 171 genes passed the first variation filter and were used in the SOMs analysis (see udgenome.ags.udel.edu/~cogburn).

The SOMs analysis identified 59 differentially expressed genes, displaying four unique expression patterns, in liver of FGL birds (Fig. 31.3a) and 76 differentially expressed genes, displaying six unique
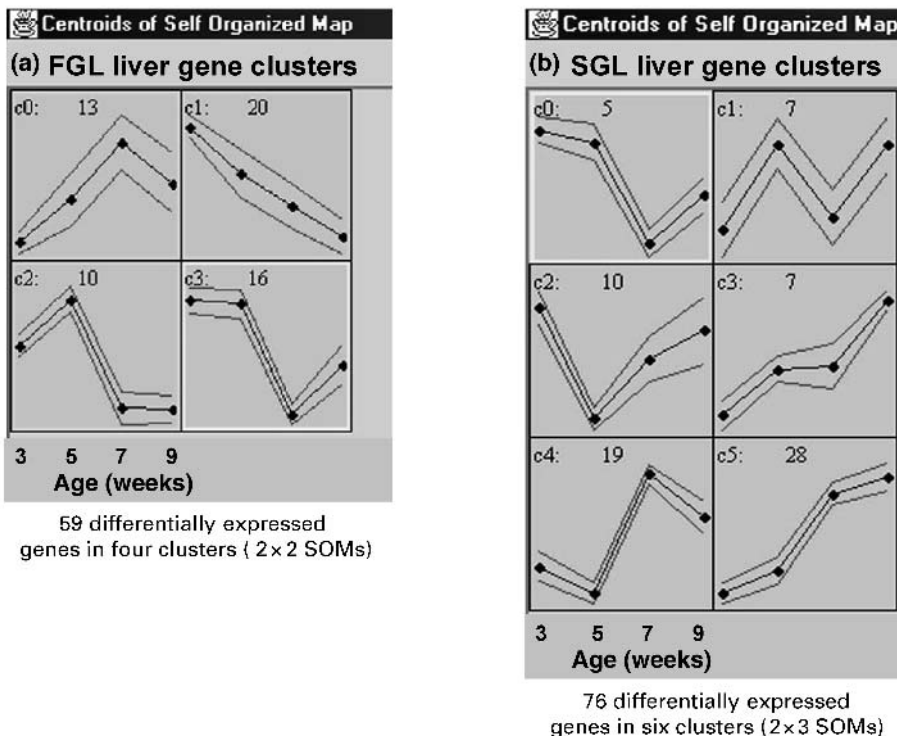
Fig. 31.3. Self-organizing maps (SOMs) analysis of gene expression in liver of broiler chickens divergently selected for growth rate. The centroids represent the average (± SD expression level over time. The number of genes in each cluster is indicated in the top centre of the graph. A total of 59 genes were assigned to four clusters (c0–c3) of gene expression in liver of the fast-growing line (FGL) (a). In the slow-growing line (SGL) (b), there were 76 genes expressed in six distinct clusters (c0–c5). These results are based on hybridization of eight membrane arrays (one bird per genetic line at four ages). For a complete list of genes in each cluster, see udgenome.ags.udel.edu/~cogburn

expression patterns, in liver of SGL birds (Fig. 31.3b). In the FGL liver, the expression of 13 genes in cluster 0 (*SURF-3*, *L3-phosphoserine phosphatase*, *endotransgly-cosylase*, *reductase*, etc.) increases in liver from 3 to 7 weeks, then decreases at 9 weeks. The largest cluster of genes (c1) (*SNARE*, two heat-shock proteins (*71 and 90a*), *Spot 14*, *apolipoproteinA1*, etc.) showed a progressive decline in expression levels in the FGL. The developmental expression profile of Spot 14 revealed by microarray analysis was confirmed by Northern blot analysis. Expression levels in cluster 2, containing ten genes (*lunatic fringe*, *superoxide dismutase*, a RasGAP protein, *WASP*, etc.), and cluster 3, containing 16 genes (*GAPDH*, *G-protein*, *tapasin*, etc.), were higher at 3 and 5 weeks than at 7 and 9 weeks. Although there were some subtle differences in the patterns of the SGL liver, two clusters (c3 and c5) containing seven and 28 genes, respectively, showed increased expression of a number of transcription factors (*Spi.1/PU.1*, *vigilin*, etc.) with age. One differentially expressed gene in cluster 5 of SGL birds (growth hormone responsive gene-2, or *GHRG2*, a novel chicken sulfotransferase) was identified by differential mRNA display in liver of the growth hormone receptor gene-deficient dwarf chicken (Cao *et al.*, 1999). A similar expression pattern was found for cluster 3 in FGL and cluster 0 in SGL livers, where expression levels of these genes (including *GAPDH* and *22S ribosomal protein*) were higher at 3 and 5 weeks than at 7 and 9 weeks.

A major disadvantage of SOMs analysis is that genes are clustered only within a given data set. SOMs analysis cannot be used to detect differences between two genetic lines (i.e. FGL vs. SGL) because this must be done visually by examining different clusters and looking for the same genes within similar cluster patterns. Additional data analysis (pair-wise correlation statistical analysis) must be used in conjunction with SOMs analysis for full interpretation of expression data (Tamayo *et al.*, 1999; White *et al.*, 1999). In this experiment, we also used Wilcoxson positive signed-rank test (Lehmann, 1975) to make pair-wise

comparisons (FGL vs. SGL) of each gene across time points. Using this method, we found a cluster of 21 genes whose expression was significantly higher ($P < 0.05$) in liver of FGL than in liver of SGL birds across the four ages (3, 5, 7 and 9 weeks). A small group of three genes was expressed at higher ($P < 0.05$) levels in the liver of the SGL when compared with FGL during this period. Thus, a number of new differentially expressed genes (i.e. different between FGL and SGL birds) were found with the pair-wise statistical method. Despite some limitations, SOMs analysis is an excellent tool for the visualization of different profiles of clustered genes (Tamayo *et al.*, 1999; Toronen *et al.*, 1999). Furthermore, most studies using SOMs analyses have shown that groups of genes clustered into a similar expression pattern contain a number of functionally related genes (Tamayo *et al.*, 1999; Toronen *et al.*, 1999).

From these initial profiling studies, we have identified a number of differentially expressed candidate genes that could contribute to expression of the extreme phenotypes found in broiler chickens divergently selected for growth rate (Beccavin *et al.*, 2001). One candidate gene, called *Spot 14*, encodes a thyroid hormone-induced hepatic protein (Brown *et al.*, 1997) that regulates expression of all six enzymes in the lipogenesis pathway. Spot 14 (Liaw and Towle, 1984) is a nuclear protein found primarily in liver and adipose tissue of the rat (Jump *et al.*, 1990; Kinlaw *et al.*, 1995), human and mouse (Grillasca *et al.*, 1997). *Spot 14* is transcriptionally up-regulated by glucose, triiodothyronine (T_3) and insulin (Jump *et al.*, 1990). Furthermore, *Spot 14* regulates expression of type 1 deiodinase (Brown *et al.*, 1997), which is responsible for peripheral conversion of inactive T_4 to metabolically active T_3 and induces expression of the thyroid hormone receptor (TR)-β. Microarray analysis has shown that *Spot 14* is the most abundantly induced gene in liver of hyperthyroid (T_3-fed) mice (Feng *et al.*, 2000). Targeted disruption of the *Spot 14* gene in the liver of knockout mice increases *de novo* lipogenesis (Zhu *et al.*, 2001). In humans, Spot 14 expression has become an

important link between two prognostic indicators of human breast cancer – enhanced lipogenesis and 11q13 amplification that result in overexpression of genes that are critical for lipogenic tumour growth (Moncur *et al.*, 1998). Thus, the thyroid hormone-induced gene (THIG) (*Spot 14*) is a very interesting candidate gene for further study of the functional genes that control energy metabolism, especially since lipogenesis is a critical metabolic pathway that is overly active in broiler chickens. Interestingly, a number of thyroid-related candidate genes are clustered near an obesity QTL (*Fob3*) that was mapped to chromosome 15 in divergently selected obese mice (Horvat *et al.*, 2000).

We have recently printed a higher-density chicken liver DNA microarray (Chicken LiverArray v1) on 8 × 12 cm nylon membranes for expression profiling of hepatic genes (Fig. 31.4). Each liver-specific microarray contains 3456 elements printed in grids of 6 × 6 spots in a 96-well microtitre plate format. Each cDNA insert was printed as a single spot on the array. To validate the liver microarray, we examined expression of hepatic genes during the peri-hatch period – or the metabolic jump from chorioallantoic to pulmonary respiration (Glass *et al.*, 2002). A comparison of day 18 embryos (*n* = 4) with 3-day-old chicks (*n* = 4) shows up-regulation (> twofold difference) of 17 genes (*liver fatty acid binding protein* (*FABP*), *adipophilin*, *Spot 14*, *fatty acid synthase*, *ATP-citrate lyase*, *glycogen phosphorylase*, *fumerase*, *hexokinase*, etc.). The expression of another group of 11 genes (*glucagon*
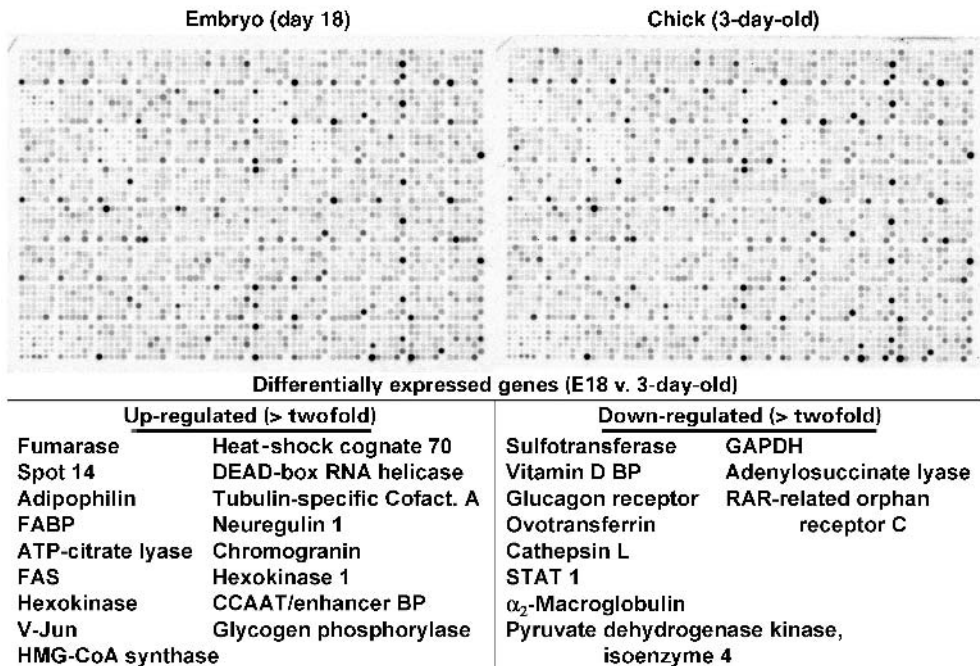
Fig. 31.4. Gene expression profiling in liver of embryos and newly hatched chicks with a chicken liver cDNA microarray containing 3456 elements (Chicken LiverArray v1) printed in 6 × 6 grids in a 96-well format. Liver samples were collected from four 18-day embryos and four 3-day-old broiler chicks. To label the liver mRNAs (cDNAs) 25 μg of total RNA and 100 μCi ^{33}P-dCTP were used in a reverse transcriptase reaction. The ^{33}P-dCTP-labelled cDNA targets from each liver sample were hybridized to separate liver DNA microarrays. After stringent washing, the membranes were exposed to a phosphor-imager screen (Storm 840, Molecular Dynamics) for 4 h. The image files were imported into Pathways 4 software (Invitrogen) for determination of spot intensities and data analysis.

receptor, *vitamin-D binding protein*, *ovo-transferrin* (*conalbumin*), *GAPDH*, *cath-epsin L*, *STAT 1*, *pyruvate dehydrogenase kinase*, etc.) was down-regulated. A number of the up-regulated genes found in the newly hatched chick are involved in lipogenesis and energy metabolism. Although the newly hatched chick is actively catabolizing fat stored in the absorbed yolk sac, our micro-array data indicate that a number of genes in the adipogenic pathway are up-regulated in the hatchling chicks.

Genome-scale cDNA sequencing and DNA microarray analysis have opened the door to the exploration and discovery of thousands of new chicken genes. Genome-wide gene expression scans will enable us to map functionally the genetic pathways that control immunity, metabolism, growth, development, reproduction and environ-mental adaptation. Many of the current problems associated with intensive genetic selection and intensive poultry production systems can be addressed with this new knowledge about the chicken's functional genome.

Notes

We used the CAP3 sequence assembly pro-gram (Huang and Madan, 1999) to build contigs of EST sequences using a 40-base overlap and 95% sequence identity. This assembly was made from chicken ESTs downloaded from all public databases on 1 February 2002. The assembly was first made on the UD and BBSRC collections separately. The final assembly was made on chicken ESTs found in all public databases (i.e. UD, BBSRC, GenBank, Roslin Institute, Stratagene and INRA-Rennes). The sing-lets represent unique non-overlapping sequences that include both known and unknown ESTs from BLASTX searches and non-coding RNA (ncRNA). Chicken EST sequences added to BBSRC (www.chick.umist.ac.uk) and bursal (swallow.gsf.de/dt40.html) databases since 1 February 2002 are not included in this assembly.

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32 DNA Polymorphisms in Functional Genes

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Introduction

One may be content to map a quantitative trait locus (QTL) and use linked markers in marker-assisted selection. However, the ultimate goal in QTL identification is to identify the mutation responsible for the trait variation itself. This requires a variety of different approaches, each providing corroborative evidence for the involvement of a gene or a mutation in trait variation. Examples are association analyses between nucleotide variations in genes with the trait variations, the size and abundance of gene transcripts and analysis of the protein encoded by the gene. Further inference may be made from the location of the mutation in regulatory sequences or in protein encoding sequences or even from the evolution of genes and strains of poultry.

A search for 'functional' DNA polymorphisms starts with the choice of genes to be analysed. Genes may be chosen on the basis of their location in chromosomal areas identified as harbouring a QTL. Alternatively, genes may be chosen based on their biological properties or even from genome-wide association studies between traits and markers in genes. It has been suggested that such genome-wide studies are more powerful in detecting QTL than mapping based on within-family segregation of traits and markers. This appears to be particularly important for traits that have low penetrance and are subject to epistasis.

As a consequence, in human genetics comprehensive catalogues of single nucleotide polymorphisms (SNPs) in genes are being established to be used in epidemiology to relate SNPs with traits of low penetrance. A recent example is the collection of SNPs in about 100 candidate genes for late onset of diabetes. As a consequence, high-throughput methodologies have been developed that permit SNP analysis in a large number of samples. It is hoped that some of these methodologies will also become accessible to the poultry geneticist.

This chapter will review the methodology of identifying mutations in or near genes, the establishment of haplotypes (i.e. groups of co-segregating alleles) and the association of mutations with traits. It will then discuss a series of DNA polymorphisms for which trait associations have been established and what insights into the genetic architecture of complex traits have been gained. Most of the data presented is unpublished, from collaborative studies between other laboratories and ours. The emphasis is on White Leghorns, which have been the focus of our research.

Identification of Markers in Functional Genes

The most convenient genetic markers within genes are restriction fragment length polymorphisms (RFLPs), i.e. changes in the nucleotide sequence that lead to the gain or loss of a restriction site. To detect such RFLPs, genomic DNA is digested with a restriction enzyme and analysed by Southern blotting using the cDNA of the gene of interest as a probe. The hybridization pattern will reveal RFLPs in exons as well as introns and flanking sequences containing promoter or terminal regions. The RFLP markers can then be mapped and confirmed by sequencing. Based on the sequence, a PCR can be designed and the RFLP detected by gel electrophoresis of the digested product. PCR-RFLP detection is amendable to automation and high-throughput analysis.

Various pooling strategies can be used to expedite the search for RFLPs segregating in strains of interest. As an example, the DNA of individuals can be pooled and run in parallel to a single individual. If an RFLP is present, the profile of the hybridization signal of the pooled DNA is expected to differ quantitatively from that of an individual. Such differences may be additional bands or differences in relative band intensities. With this approach, RFLPs that occur at a frequency above 10% can easily be detected.

This approach can be used to couple the search for markers with the identification of candidate genes. As an example, pools of DNA of divergently selected strains can be digested with a restriction enzyme (e.g. one pair of strains and five different enzymes on a 20-slot gel) and hybridized with one or several cDNA probes. Again, differences in the hybridization signal will reflect the presence of an RFLP that differs in its frequency and may indicate that the particular RFLP is associated with a QTL that responded to selection. The same strategy can be used to analyse pooled DNA from individuals of the same strain but representing extremes of a trait distribution.

The cDNA probes used for hybridization may be from an anonymous cDNA library. Promising clones can than be sequenced, identified and further analysed. Li *et al.* (1998a) used this approach and showed that in a series of White Leghorn strains more than 50% of liver-expressed nuclear genes revealed polymorphism at *Msp*I and/or *Taq*I sites. This was the first indication that SNPs in White Leghorns are very abundant. Furthermore, a cDNA clone that segregated for an RFLP that was consistently co-selected with Marek's disease resistance was identified as phosphoenol-pyruvate carboxykinase-M, a key regulatory gene of gluconeogenesis (Li *et al.*, 1998b). They speculated that this observation may reflect the importance of glucose metabolism in tumour growth and/or resilience to the disease. A second clone, which revealed an RFLP that responded to selection for egg production traits and/or disease resistance, was identified to be of mitochondrial origin (Li *et al.*, 1998c).

A more efficient approach than using anonymous cDNA clones is the usage of candidate cDNA clones chosen on the basis of expression profiling or protein analyses. In particular, expression profiling of divergently selected strains using DNA chip technology may provide a more educated selection of candidate genes. Furthermore, high-throughput sequencing may soon supplant a search for RFLP markers.

Abundance of DNA Polymorphisms and the Need for Establishing Haplotypes

RFLP analyses indicate that DNA polymorphisms in or near genes are very abundant. For example, the growth hormone (*GH*) gene is only about 2000 bp in length but, when analysed with only two restriction enzymes, revealed five RFLPs in White Leghorn chickens (Kuhnlein *et al.*, 1997; Kuhnlein, unpublished data) and additional RFLPs are present in meat-type chickens (Fotouhi *et al.*, 1993). Sequence analysis will certainly reveal additional DNA polymorphisms but has not been conducted systematically. We have sequenced a 4000 bp region of the 5′ end of the gene coding for

phosphoenolpyruvate carboxy kinase (PEPCK-C) in 32 chickens (64 genome equivalent) from commercial White Leghorn strains and found 19 DNA polymorphisms, which amounts to an average of one SNP per 200 bp. Another gene under analysis in our laboratory is ornithine decarboxylase (ODC), which appears to be even more polymorphic. One marker may be tested at a time but considerable economy in typing can be achieved by first establishing the major segregating haplotypes (i.e. combination of alleles) in a particular strain of interest. Typing can then be restricted to DNA polymorphisms diagnostic for different haplotypes or sections of the gene tree.

In all cases, markers within genes that we have analysed were at a maximal linkage disequilibrium, i.e. one of the four possible combinations of the alleles at two loci was always missing. It is compatible with the parsimonious model that each mutation has only occurred once in evolution and that recombination between the two markers has been absent. As a consequence, gene trees can be delineated and the evolutionary history of a gene can be established.

The gene tree for *PEPCK-C* is shown in Fig. 32.1. The 19 DNA polymorphisms at the 5' end of this gene potentially give rise to more than 500,000 allelic combinations. However, among the 64 genes analysed, only six combinations or haplotypes were observed. Among the 19 polymorphic sites, two coincided with RFLPs as indicated in Fig. 32.1. They are convenient for dissecting the gene tree and conducting QTL association studies within distinct groups of alleles.

Figure 32.2 explains how a gene tree can be used to identify the haplotype that segregates for a putative QTL. Assuming that a QTL represents a single mutational event
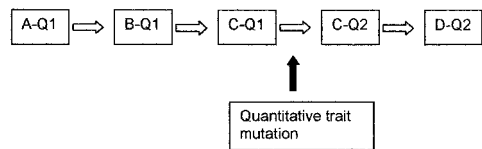
Fig. 32.2. A gene tree can be used to identify the haplotype that segregates for a putative QTL. In the above example it is assumed that the quantitative trait mutation has occurred in haplotype *C*. In a strain that segregates for these haplotypes, *A* and *B* homozygotes as well as *AB* heterozygotes will be homozygous for Q_1, while the *D* homozygote will be homozygous for Q_2. All other six genotypes will be a mixture of Q_1Q_2 heterozygotes and both Q_1 and Q_2 homozygotes.
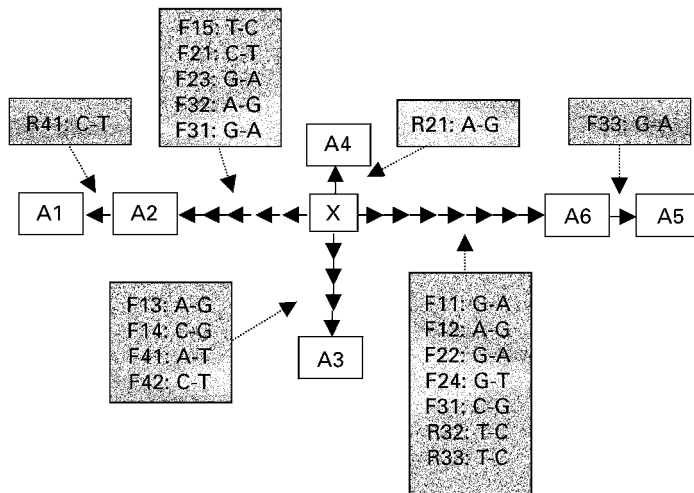
Fig. 32.1. Gene tree of the chicken *PEPCK-C* haplotypes in White Leghorns. The difference between two most distant haplotypes (*A1* and *A5*) is 14 SNPs. X is a putative branch-point haplotype and may represent the ancestral gene. All DNA polymorphisms were either transitions or transversion. The markers R41 and F22 coincide with an *Acl*I and *Bst*EII RFLP, respectively. They can be used to subdivide the tree into the haplotypes (*A1*), (*A2*, *A3*, *A4*) and (*A5*, *A6*).

during evolution, it must have occurred in one of the haplotypes of the gene tree. Hence, homozygotes and heterozygotes of haplotypes that have arisen prior to the QTL mutation will be homozygous for one of the QTL alleles, while genotypes representing more recent haplotypes will be homozygous for the other QTL allele. When association analyses are conducted, the number of genotypes in each class may not be sufficient and one may simply compare genotypes comprising the most recent with the most ancient haplotypes. Alternatively, when the evolutionary tree is branched as for PEPCK-C, one may compare genotypic classes containing the genetically most distant haplotypes.

Implicating DNA Polymorphisms as Being Responsible for Trait Variations

There are many approaches to implicate DNA polymorphisms (or haplotypes) as being responsible for trait variations, each differing in the amount of work invested and in the power of resolution. They may be grouped into: (i) selection response in closed breeding populations; (ii) trait association studies in closed populations; and (iii) segregation analysis within families. Ultimately molecular evidence should be obtained, whether a particular haplotype affects gene expression or leads to an altered protein.

In the 'response to selection approach', the haplotype frequencies in divergently selected strains or in a selected strain and its matched control strain are compared. Selection pressure will result in a change of the haplotype frequencies and hence may provide evidence of an involvement of that particular gene. The main drawback of this approach is that the strains have to be of large population sizes to exclude the possibility that differences in haplotype frequencies may be due to genetic drift. An alternative is to compare several different pairs of strains selected for the same trait. A uniform direction of change of the haplotype frequencies in all pairs of strains supports

the observation that the frequency changes are a consequence of selection of the particular trait. Examples of this approach are the uniform response of endogenous viral gene frequencies to selection for egg production traits in a pair of White Leghorn strains (Kuhnlein *et al.*, 1997) or the co-selection of a DNA polymorphism in the mitochondrial phosphoenolpyruvate carboxykinase gene with Marek's disease resistance (Li *et al.*, 1998b).

The reverse strategy is also possible. One may divergently select for the two alleles at a DNA polymorphism and record the influence of such selection on the phenotype. Again, care should be taken to conduct selection in a sufficiently large population in order to exclude the possibility that genetic drift is responsible for the change in phenotype.

Association analyses are searches for correlations between DNA polymorphisms and traits in closed random-bred populations. Ideally, the strain should have been propagated for many generations to minimize long-range linkage disequilibrium between putative QTL in different genes. Otherwise a QTL associated with a DNA polymorphism in a gene may be located in adjacent genes that are at linkage disequilibrium. Some linkage disequilibrium cannot be avoided. We have noted the latter in our analysis of a strain that had been propagated at a large population size for more than 20 generations. While most single loci in this strain were at Hardy–Weinberg equilibrium, there was significant disequilibrium between pairs of loci, presumably due to interactive effects on hatchability and fertility. A further problem stems from interactions between major genes. In this case the effect of a haplotype of one gene may be dependent on the segregation of haplotypes in a second gene. Hence the trait association of an allele or haplotype may be strain specific (Feng *et al.*, 1997; Aggrey *et al.*, 1998; Nagaraja *et al.*, 2000).

A very powerful approach is a segregation analysis within families. In its simplest version it involves setting up crosses segregating for different haplotypes and testing for haplotype/phenotype associations.

Again, problems arise from interactions with other genes. Since the putative unknown interacting gene will segregate differently in each family, the haplotype being tested will have different effects in different families. As a consequence many different families have to be tested. The effort to set up crosses and conduct phenotypic evaluations may therefore only be warranted if prior evidence for a QTL in a gene is available.

Homeostasis and Gene Interaction

Complex traits are determined by networks of interacting genes. Such networks are overlapping and may have evolved to buffer the phenotype of an organism against variations caused by the environment, by stochastic events and by genetic variations (Hartman *et al.*, 2001). As a consequence, mutations in one gene may only have phenotypic consequences in the presence of a mutation in another gene. The importance of such buffering has now been fully recognized and it is thought that many human diseases with low penetrance are the consequences of a breakdown of homeostatic processes due to the co-inheritance of mutations in several different genes.

Generally a QTL is defined as affecting the mean of a trait. However, based on homeostatic mechanisms some mutations may stabilize the buffering of a trait against variations in other genes. In an outbred population that segregates for alleles in many other genes, such mutations are expected to increase the standard deviation of a trait while the effect on the mean may be small. This is indeed the rule rather than the exception. Hence markers that affect the standard deviation rather than the mean should also be classified as QTL.

Homeostatic mechanisms are also evident at the level of trait correlations. An example is given in Fig. 32.3, which lists the partial correlation coefficient between the rate of egg laying, egg weight, body weight and feed consumption in a strain of White Leghorns at peak of laying. All four traits were significantly correlated, ensuring that a chickens lay eggs at a size and frequency commensurable with its body weight and feed intake. Again, some mutations may affect the underlaying regulatory network and hence lead to changes in trait correlations. This may result in the paradoxical situation that some QTL may have opposite effects on a trait, depending on whether chickens are below or above the median of a second trait.

In summary, in the case of a continuous trait, analysis of a DNA polymorphism for trait associations should include the effects on trait means, the variance and the effect on trait correlations. Changes in means indicate a change of the homeostatic set-points, while
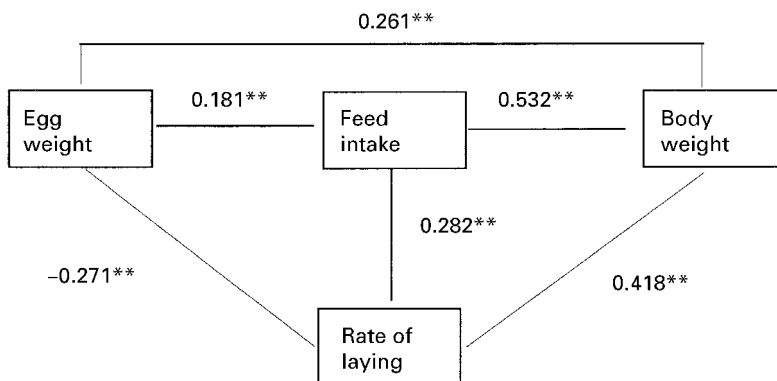
Fig. 32.3. Partial correlation coefficients between traits in a White Leghorn strain at the peak of egg laying. All correlations were significant at $P < 0.01$. The strain is the control strain 7 developed at Agriculture Canada (Gowe *et al.*, 1993). Correlations in other strains may differ.

changes in the variance and in the interaction between traits indicate changes in the mechanisms that maintain homeostasis.

Examples of DNA Polymorphisms in Functional Genes

Production traits in White Leghorns

Effects of single marker or marker haplotypes on trait means

Production traits generally recorded in layers are age at first egg, body weight, rate of egg laying, egg weights and egg specific gravity, and feed consumption. These traits represent a very diverse range of biological activities, including onset of sexual maturity, feed intake, growth, reproduction and calcium metabolism. Traits measured in meat-type birds are more restricted, concerning primarily the rate of growth, yield of breast meat, feed consumption and the size of fat pads. Most of the research relating

traits with mutations in genes have been carried out in White Leghorn strains and will therefore be the main focus of this discussion. Unless otherwise stated, the examples given are from an analysis in strain 7, a control strain that had been established at Agriculture Canada in 1956 by crossing four North American commercial lines. The strain was subsequently propagated without selection, using 100 sire families and mating each sire to two females each. From its breeding history it is expected that linkage disequilibrium in this strain is relatively small (Gowe *et al.*, 1993).

Table 32.1 summarizes the significance of the association of single marker genes with egg production traits. The genes analysed were candidate genes selected on the basis of their function. They comprise four genes of the neuroendocrine axis: growth hormone (*GH*), growth hormone receptor (*GHR*), insulin-like growth factor 1 (*IGF-1*) and pituitary regulatory factor 1 (*Pit-1*); and genes involved in energy metabolism: the mitochondrial

Table 32.1. Single marker effects (P > F) on production traits in the White Leghorn strain 7.

Production trait	IGF-1 PstI	ODC HindIII	ODC MspI	Pit-1 SSCP-Ax	Mitochondria MspI	Mitochondria AsnI	PEPCK-C AclI	PEPCK-C BstEII	PEPCK-M AccI
AFE	n.s.	0.025 ↓a	0.001 ↑a	n.s.	n.s.	n.s.	0.099 ↓	n.s.	0.056 △
HBWT	n.s.	n.s.	n.s.	n.s.	n.s.	0.007 ↓	n.s.	0.008 ▽	n.s.
MBWT	n.s.	n.s.	n.s.	n.s.	n.s.	0.005 ↓	n.s.	n.s.	n.s.
FBWT	n.s.	n.s.	n.s.	n.s.	n.s.	0.037 ↓	n.s.	n.s.	n.s.
HDR1	n.s.	n.s.	n.s.	0.022 ▽	n.s.	n.s.	n.s.	n.s.	n.s.
HDR2	n.s.	n.s.	n.s.	0.145 ▽	n.s.	n.s.	n.s.	n.s.	n.s.
HDR3	n.s.	n.s.	n.s.	0.012 ▽	n.s.	n.s.	n.s.	n.s.	n.s.
EWT1	0.037 ↓d	n.s.	n.s.	n.s.	n.s.	0.037 ↓	n.s.	n.s.	n.s.
EWT2	0.067 ↓d	n.s.	n.s.	n.s.	n.s.	0.060 ↓	n.s.	n.s.	n.s.
EWT3	0.018 ↓d	n.s.	n.s.	n.s.	n.s.	0.021 ↓	n.s.	n.s.	n.s.
SP1	n.s.	0.007 ▽	n.s.	n.s.	0.047 ↑	n.s.	0.031 ▽	n.s.	n.s.
SP2	n.s.	n.s.	n.s.	n.s.	0.069 ↑	n.s.	n.s.	n.s.	n.s.
SP3	n.s.	n.s.	n.s.	n.s.	0.053 ↑	n.s.	n.s.	n.s.	n.s.
Feed cons.	n.s.	n.s.	0.039 ↓r	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

The table lists the significance (*P*-value) of the trait association of markers in a series of genes. The genes were insulin-like growth factor (*IGF1*), ornithine decarboxylase (*ODC*), pituitary transcription factor 1 (*Pit-1*), mitochondria and the cellular and mitochondrial form of the phosphoenolpyruvate caboxykinase (*PEPCK-M* and *PEPCK-C*). Markers were restriction fragment polymorphism for the enzymes indicated with the exception of *Pit-1*, where genotypes were defined by a single-strand conformational polymorphism (SSCP) in the promoter region. ↓, association of the loss of the restriction site with a lower trait value; a, additivity; d, dominance of the +allele; r, recessiveness of the +allele; ▽, underdominance and △, overdominance of the +allele, respectively.

genome, the cytosolic and mitochondrial phosphoenolpyruvate carboxykinase (*PEPCK-C* and *PEPCK-M*) and ornithine decarboxylase (*ODC*), a major rate-limiting enzyme in the synthesis of polyamines and the target of many pharmaceutical agents.

Significant associations were surprisingly frequent. Among 12 markers in eight genes, nine were associated with traits. Only a marker in the *GHR* gene (*Hin*dIII RFLP) and two markers in *GH* gene (*Sac*I and *Msp*I RFLP) did not show any trait associations. At face value, it indicates that the number of genes affecting a trait is very large, although the possibility of being biased by the selection of genes cannot be excluded. Despite significant correlation between traits (Fig. 32.2), single marker effects were trait specific. For example, the *IGF-1 Pst*I marker was associated with egg weight in all three laying periods, but with none of the other production traits. Similarly, Pit-1 *SSCP-B* only affected the rate of egg laying (Kansaku *et al.*, 2000). Some marker effects were restricted to a particular time period, indicating that they are not only trait specific but also age specific. Thus, *PEPCK-C Bst*EII only affects the body weight prior to ovulation (HBWT), but not at a later age (MBWT, FBWT). A further surprise was the frequent occurrence of underdominance, i.e. the trait value for the heterozygotes was lower than for either of the two homozygotes. As an example, the median value of the overall hen day rate of egg laying is for the *Pit-1* SSCP marker was 73% and 71% for the two homozygotes, respectively, but only 63% for the heterozygote (*P* = 0.02, Kruskal–Wallis rank test). When two markers in the same gene were scored, they often differed in their trait associations. They either affected different traits or only one of the markers showed significant association. Association of one marker but not the other may reflect that only one of the markers was in phase with the putative QTL. Association with different traits may reflect that the two markers were in phase with different QTL.

As pointed out above, a higher resolution and more meaningful interpretation may be obtained when alleles are grouped into haplotypes. In the absence of recombinations and repeat occurrence of the same mutation, the alleles of two RFLP markers give rise to a maximum of three different haplotypes. In the case of *ODC*, the three haplotypes were haplotype *A* (*Hin*dIII+, *Msp*I+), haplotype *B* (*Hin*dIII−, *Msp*I+) and haplotype *C* (*Hin*dIII−, *Msp*I−), while the fourth potential haplotype (*Hin*dIII+, *Msp*I−) was absent. The three haplotypes gave rise to a total of six different genotypes. Figure 32.4 shows the trait distributions for onset of sexual maturity (age at first egg, AFE) for the *ODC* genotypes *AC*, *BC* and *CC*. The trait distributions for genotypes *AC* and *BC* are identical and different from genotype *CC*. It indicates that haplotype *C* is in phase with one of the putative QTL alleles and haplotypes *A* and *B* with the other. Furthermore, *A* and *B* appear to be dominant over *C*.

A marker in the *GHR* genes (*Hin*dIII RFLP) and two markers in the *GH* gene (*Sac*I RFLP and *Msp*I RFLP) were not associated with production traits. However, in another strain of White Leghorns – strain S, originally developed by Hutt and Cole and later maintained without selection at Agriculture-AgriFood Canada – markers in both genes were significantly associated with body weight (*GHR*) and the rate of egg production (*GH*) (Feng *et al.*, 1997). Lack of association in strain 7 (Feng *et al.*, 1998) may indicate differences in the genetic background of the two strains or differences in the linkage with other markers in the gene. In particular, delineation of the haplotypes of the *GH* genes on the basis of four different RFLPs revealed the presence of at least four different haplotypes in White Leghorn chickens. Strain S segregated for two of the most distant haplotypes separated by four RFLPs, while strain 7 segregated for three haplotypes, which differed by one or two RFLPs, respectively (Kuhnlein *et al.*, 1997).

Interactions between genes and effects on trait correlations

As indicated above, complex phenotypes such as production traits are determined by a network of interacting genes. Gene interactions can be demonstrated directly by

comparing effects of marker combinations in two different genes. An example is given in the discussion of DNA polymorphisms with immune traits below. Other more indirect evidence comes from linkage disequilibrium analysis and an analysis of trait correlations.

In the absence of extensive trait selection, markers in a closed breeding population are expected to be in Hardy–Weinberg
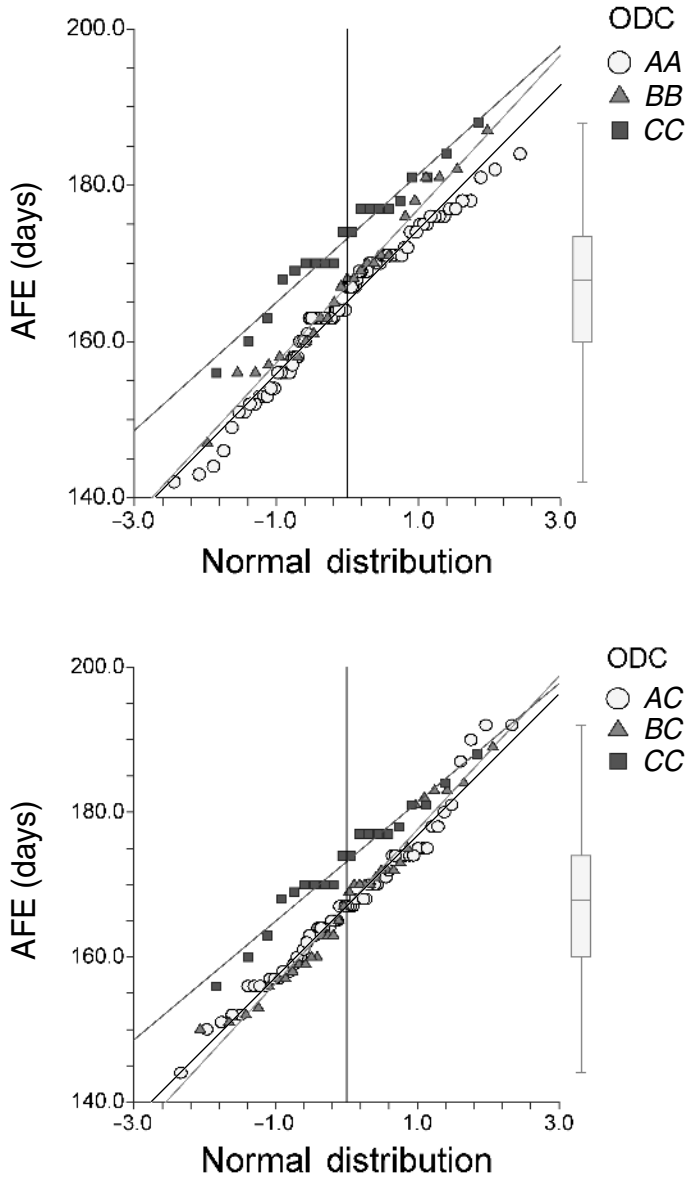
Fig. 32.4. Comparison of the distribution of the age at first egg (AFE) for different *ODC* genotypes. The intercept with the 0-value of the normality scale is equal to the mean of the trait, while the slope is equal to the standard deviation. The graph indicates that haplotype *C* is in phase with one QTL allele, and haplotypes *A* and *B* with the other. Further, *A* and *B* are dominant over *C*.

equilibrium. This is the case for nearly all the markers we have analysed in the random-bred strain 7. Exceptions are expected from markers in genes that affect fitness (i.e. rate of egg laying, fertility, embryonic development and hatchability). An example is the *Pit-1* SSCP described above, which has a significant deficiency of heterozygotes. This may reflect the fact that the late rate of egg laying of *Pit-1* SSCP heterozygotes is nearly 20% lower than that of either of the homozygotes. Hence, there are fewer heterozygous offspring than expected from Mendelian inheritance. Another example is PEPCK-C, the main regulatory enzyme for gluconeogenesis. Analysis of large families showed deviation from Mendelian inheritance of the *PEPCK-C* genotypes, indicating that the PEPCK-C may affect fertility traits (Parsandejad *et al.*, 2000).

When digenic disequilibria of the markers listed in Table 32.1 are analysed, significant deviations from equilibrium are frequent. This is even the case for markers that have been mapped to different chromosomes. Hence physical linkage can be excluded and the deviation from equilibrium may be due to an effect of haplotype combinations in different genes on fitness. Clearly, a direct analysis of fitness traits is warranted and may reveal many DNA polymorphisms that have biological consequences.

Indirect evidence for gene interactions also comes from correlations and regression analyses of traits. Feng *et al.* (1997) showed that the correlation between the rate of egg laying and body weight differed for the two *GHR Hin*dIII genotypes. As a consequence, the effect of the *GHR Hin*dIII genotypes on the rate of egg laying in chickens with low body weight was opposite to that in chickens with high body weight. Similarly, the magnitude of the effect of the *GH* haplotypes on the rate of egg laying increased with increasing AFE. Nagaraja *et al.* (2000) compared partial correlation coefficients between the production traits in chickens homozygous and heterozygous for a *Pst*I RFLP in *IGF-1* (+/+ vs. −/−), respectively. The two genotypes were associated with differences in the partial correlation coefficients between feed consumption and body weight and feed consumption and egg weight. Additionally, some of the partial correlation coefficients in the heterozygous class were age dependent. Another example is the association of the mitochondrial genotype on the interdependence of egg weight with body weight (Fourtunis *et al.*, 2000). The effect of the mitochondrial marker on egg weight described in Table 32.2 was entirely due to individuals with body weights below the median, while among heavy chickens the mitochondrial genotype did not affect egg weight. Figure 32.5 shows that the mitochondrial haplotypes affect egg weight of chickens with a low body weight to a much greater extent than chickens with a high body weight.

Table 3.2. Association of mitochondrial haplotype with egg weights in chickens with body weights below and above the median.

| | MBWT < 1.76 kg[a] | | | | MBWT > 1.76 kg[a] | | |
| | Haplotype | | | | Haplotype | | |
	P > F	A (*n* = 23)	B (*n* = 66)	C (*n* = 49)	*P* > F	A (*n* = 33)	B (*n* = 70)	C (*n* = 42)
EWT1	0.043	51.9	50.6	51.9	0.641	54.0	53.5	53.9
EWT2	0.007	57.6[b]	55.7[c]	58.0[b]	0.637	59.7	59.0	59.6
EWT3	0.005	62.0[b]	57.8[c]	59.4	0.724	61.8	61.9	62.5

[a]Median mature body weight.
[b,c]Different superscripts in a line indicate that the means are significantly different (Bonferroni multiple comparison test).
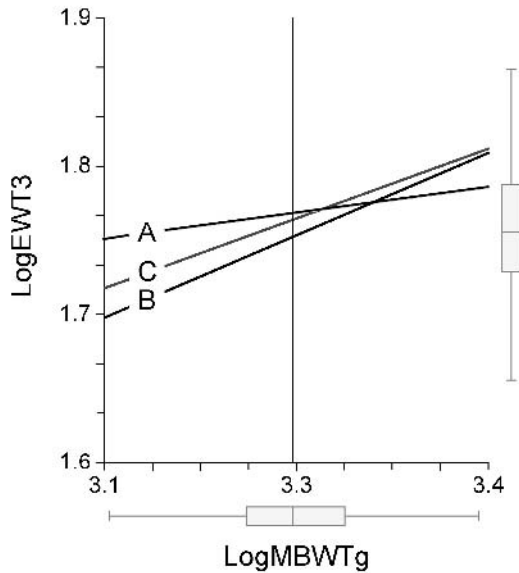
Fig. 32.5. Regression lines of egg weight against mature body weight for three mitochondrial haplotypes. Haplotypes are characterized by an *Msp*I RFLP and an *Asn*I RFLP. The regression lines indicate that the haplotypes affect egg weights in chickens with a low body weight to a much greater extent than in chickens with a high body weight.

The effects of DNA polymorphisms on trait correlations result in an interdependency of QTL in genes affecting different traits. Such interdependencies are difficult to dissect. Specifically, if two major loci are analysed, the combination of three genotypes at each locus will lead to a total of nine different genotypic combinations. Hence, the number of observations in some classes may be very small and not amenable to statistical analysis.

Strain comparisons

In some cases, comparisons of DNA polymorphisms in strains with qualitatively different phenotypes may provide convincing evidence for genotype/phenotype associations. A classical example of this approach is the identification of the *GHR* gene as being responsible for sex-linked dwarfism in chickens. Based on analogy with the Laron-type dwarfing syndrome, the *GHR* gene was targeted as a candidate gene. Subsequent analysis revealed a lack of GH binding sites in the liver and a shift of the abundance of *GHR* transcripts

to shorter sizes. It was subsequently found that sex-linked dwarf chickens differed from normal chickens by a mutation in a splice acceptor site. As a consequence, a polyadenylation site within this intron may be utilized as a processing signal and lead to the presence of a short mRNA and ultimately a trunctuated GHR receptor (Huang *et al.*, 1993).

A more recent example is the identification of a putative DNA polymorphism responsible for broodiness (Zadworny *et al.*, 2000). An analysis of the promoter sequence of prolactin (pos −2700 to +88) revealed the presence of six SSCP. Three SSCP were linked, giving rise to two haplotypes that differed by a transversion, a transition and a 24 bp insertion. Analysis of different strains of chickens (White Leghorn, Silky, Geline, Gifuijidori, Nagoya) showed that the frequency of the 'short' haplotype was correlated with the incidence of broodiness behaviour in these strains. Together with the observation that the prolactin level increases severalfold upon induction of broodiness, the genetic data provides strong evidence that the insertion in the prolactin gene may

be directly responsible for the absence of broodiness in the White Leghorn. This insertion may have occurred late in the evolution since the red jungle fowl, Silkies, turkeys, quail, pheasant and duck all carry the short form of the prolactin gene.

Selection at the DNA level

Trait associations with DNA polymorphisms may be validated by selection at the DNA level. We are not aware of any published studies of this type, presumably due to costs associated with such breeding programmes. However, we have conducted a small-scale breeding programme for two strains that were homozygous for contrasting alleles of the *GHR* gene and the *GH* gene, while maintaining the genetic diversity in other genes. The parent strain was the Ottawa strain 7. As indicated above, in this strain markers in the *GHR* and *GH* gene were not significantly associated with production traits. However, the female housing body weight of genotypes *GHR Hin*dIII+, *GH Sac*I+/+ was lower than that of the contrasting *GHR Hin*dIII−, *GH Sac*I−/− (Kuhnlein and Aggrey, unpublished data). After breeding for two generations with

selection aimed at increasing the frequency of the rare genotype *GHR Hin*dIII−, *GH Sac*I−/−, the difference in body weight was maintained and, due to an increase in the number of observations, difference in body weight between the two genotypes became highly significant (Table 32.3). Comparison of the distribution of housing body weight at generation two for the two genotypes are shown in Fig. 32.6.

Table 32.3. Effect of marker-assisted selection on housing body weight.

Gener-ation	Body weight		Difference in body weight	$P > F$
	Genotype A^a	Genotype B^b		
0	1293 (*n* = 18)	1313 (*n* = 69)	20 ± 36	0.074
1	1266 (*n* = 19)	1392 (*n* = 19)	126 ± 49	0.014
2	1372 (*n* = 35)	1488 (*n* = 64)	116 ± 30	0.0002

[a]Genotype *A*: Homozygous for *GH Sac*I− and *GHR Hin*dIII−
[b]Genotype *B*: Homozygous for *GH Sac*I+ and *GHR Hin*dIII+
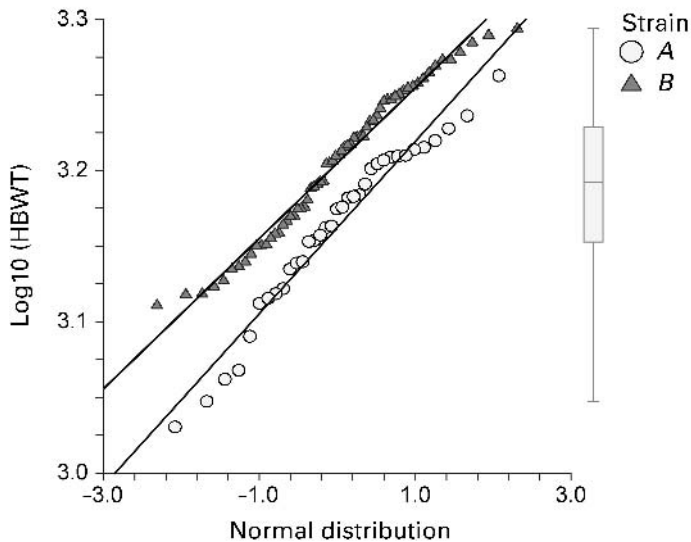
Fig. 32.6. Comparison of the distribution of housing body weight at generation two for the two genotypes. Genotype (strain) *A* is homozygous for *GH Sac*I− and *GHR Hin*dIII−. Genotype (strain) *B* is homozygous for *GH Sac*I+ and *GHR Hin*dIII+.

Effects on immune responsiveness and disease resistance

Immune responsiveness

Immune responsiveness is expected to depend on the physiological state of the chickens as well as the nature of the antigen, the dose and time course of administration, the previous history of antigen exposure and the type and time point of data gathering. It is therefore time consuming and difficult to create the type of database needed to identify DNA polymorphisms in functional genes.

We have analysed a data set created at Agriculture and AgriFood Canada in Ottawa, using the same White Leghorn strain used to assess the influence of DNA polymorphisms on production traits. Mature chickens were challenged with attenuated Newcastle disease virus (NDV) and avian encephalomyelitis virus (AEV) and the antibody response was determined. In parallel, cell-mediated immunity was assessed by measuring the inflammation response to subcutaneous injection of heat-inactivated virus. The two viruses provoked distinct responses. AEV elicited a strong cell-mediated immune response but virtually no antibody response. NDV, on the other hand, was a strong antibody producer but provoked a weak inflammation response.

Analysis of markers in the *GH* gene (*Sac*I RFLP), *GHR* and *IGF-1* revealed that the *GHR* marker was significantly associated with antibody response to NDV (Aggrey *et al.*, 1996), while the main effect of the GH and *IGF-1* markers was on the cell-mediated immune response to AEV (Joseph, 1999). This succinct difference was surprising and may indicate a prominent role of the GH receptors in B-cell maturation while GH and IGF-1 may affect T-cell activation. However, since attenuated virus was used when eliciting the antibody response, it may also reflect that these genes have different effects on the proliferation of the two viruses.

In the above analysis the less frequent homozygote was omitted. Yet even when comparing the more frequent genotypic classes, extensive gene interactions were revealed. Figure 32.7 shows the influence of the *GH* marker genotype on the distribution of the delayed type hypersensitivity (DTH) response to AEV in chickens either homozygous or heterozygous for the *IGF-1* marker genotype. The *GH* marker only has an effect among homozygotes for *IGF-1*, but not among heterozygotes. Hence, in a strain that does not segregate for the particular *IGF-1* haplotype, the *GH* marker would not affect cell-mediated immunity.

A second gene that had a significant effect on the immune response was *ODC*. Similar to the *GHR* genotype, it was associated with differences in the antibody response to NDV, but did not affect the DTH response to AEV (see Fig. 32.8). The distributions of the antibody response indicate an additive effect on the mean, and a marked effect on the standard deviations. The latter observation may indicate differential interactions of the *ODC* genotypes with other QTL affecting the antibody response. Again, it is possible that the primary effect of ODC is on viral propagation of the attenuated virus rather than antibody production.

Disease resistance

Disease resistance deals with the interplay of disease-causing agents and their host. As with other complex traits, it is determined by an interplay of many host pathogen genes and hence is expected to be strain and/or disease specific. When dissecting disease resistance, the first decision is to choose an end point to create a database. In the case of Marek's disease, obvious end points are viraemia at the initial lytic stage of infection or tumour formation and mortality. Furthermore, one has to decide on the route of infection: either injection (to ensure uniformity), or natural infection by intermingling healthy and infected birds (to be closer to the field situation). A further problem is to choose the right dose or virulency of the disease-causing agent in order to obtain a frequency of positive and negative responses that permits a meaningful statistical analysis. This is particularly

important when tumour formation or mortality are the end point.

An approach that has not yet been used systematically but may be promising is to search for markers in genes that are co-selected with disease resistance. Consistent co-selection with MD resistance has been shown for markers in the *GH*, mitochondria
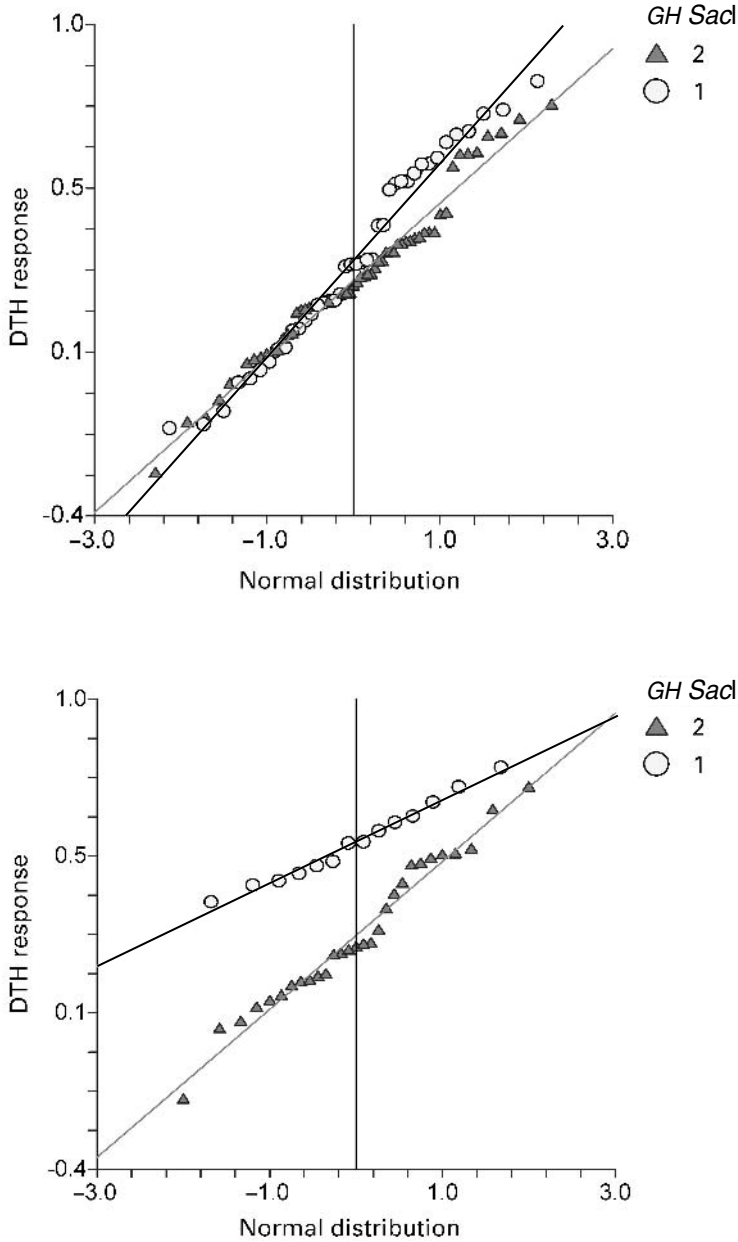
Fig. 32.7. Distribution of the delayed type hypersensitivity (DTH) to avian encephalomyelitis virus (AEV) for chickens with the *GH Sac*I and *IGF-1 Pst*I marker genotypes. The *GH Sac*I+/+ genotype is designated as 2 and the *GH Sac*I+/– genotype as 1. The *GH Sac*I genotype only influences the DTH response in the *IGF-1 Pst*I+/– background.
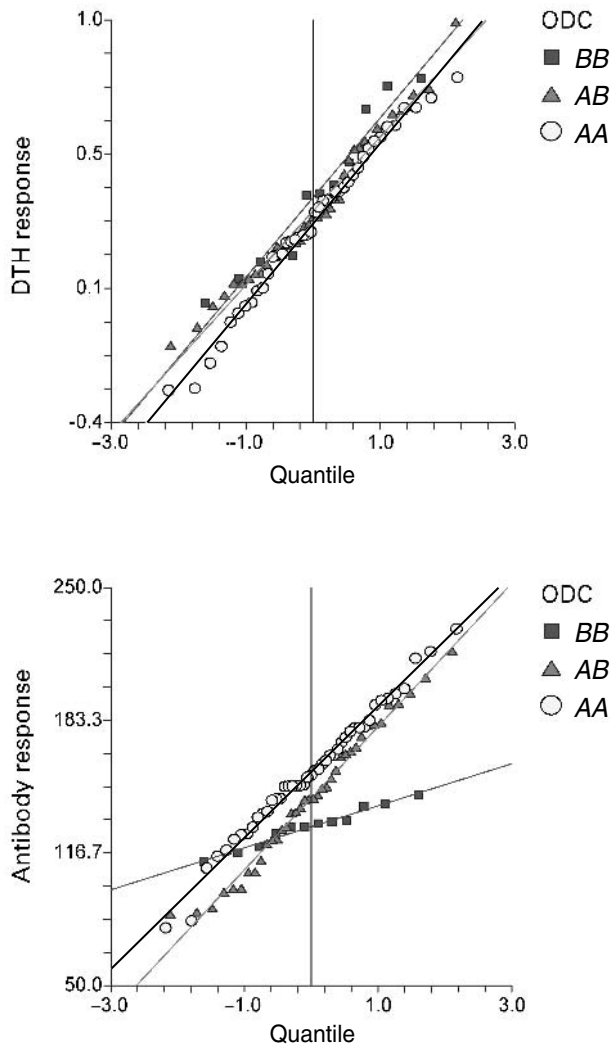
Fig. 32.8. Effect of *ODC* genotypes on the DTH response to AEV and the antibody response to Newcastle disease virus (NDV). Only the genotypes resulting from the most frequent haplotypes are indicated. They do not affect the DTH response. The effects of haplotype *A* and *B* on the antibody response is additive. It is noteworthy that the variances for the distribution of the antibody response for the three genotypes differ markedly.

and *PEPCK-M* genes (Kuhnlein *et al.*, 1997; Li *et al.*, 1998b). The latter two of these genes were initially identified by sequencing anonymous clones that harboured RFLPs that responded to selection for disease resistance. It may indicate that glucose metabolism is important in tumour growth and/or morbidity.

There are only a few direct association studies between DNA polymorphisms in functional genes and disease resistance. Liu *et al.* (2001) reported the association of the *GH Sac*I RFLP in White Leghorns with MD tumour formation in a commercial strain. The association was only significant in chickens of the MHC haplotype B^2/B^{15}, but not in chickens of the haplotype B^2/B^2. It may reflect interaction between the *GH* gene and the MHC haplotype. Together with their observation that GH forms a complex with

the MDV protein SORF2, it is strong evidence that GH is a determinant of disease resistance. We have also analysed marker/disease associations in two commercial strains (hybrids of several other strains) and found tissue- and strain-specific associations with tumour formation. However, both were hybrid strains and we expect extensive long-range linkage disequilibria between genes.

Based on co-selection analyses we had targeted the *GHR* and *GH* genes as candidates for disease resistance to MD. We consequently developed substrains homozygous for markers in the *GH* and *GHR* genes, a conducted an MD challenge test and measured viral titres during the initial period of the disease, which involves virus propagation in B and T cells. Virus was measured in the bursa, spleen and thymus. Rather than analysing the end point of a disease, which may reflect many different layers of genetic controls, one may concentrate on the analysis of early and possibly genetically less complex stages of the disease traits. An early stage in MD is the initial lytic proliferation of the virus in B and T cells. Six days after infection, the viral titres differed 1.5-fold in the thymus and sixfold in the spleen (Linher *et al.*, 2001). Comparisons at different time points indicated that the viral titre is time dependent and the differences between the two strains may reflect differences in the kinetics of viral infection, rather than in the overall virus production.

The strain associated with lower viraemia was the one that was homozygous for markers associated with a higher immune response. Hence, an increased immune response may indeed lead to lower viral titres. However, these same markers were co-selected with an increased susceptibility to MD. The same observation was made for a marker in *ODC*, where the marker associated with a lower antibody response was associated with a lower incidence of MD-induced tumour formation. This may reflect the fact that chickens with high immune responsiveness have a high number of activated T cells and hence a higher probability of developing transformed T cells and ultimately T-cell lymphomas. Hence, the course of MD may be a delicate interplay between the kinetics of the immune system and the time course of viral replication. Alternatively, the apparent association of the *GHR* and *ODC* markers with the antibody response to attenuated NDV described above may indeed reflect the association of these genes with viral proliferation.

Conclusion

This chapter is devoted to DNA polymorphisms in functional genes. It is apparent that genes that segregate for major QTL are relatively frequent and they are likely to be present in most genes coding for enzymes that are rate limiting. Such variants will be maintained in outbred populations, since their phenotypic effects are buffered by variants in other genes.

Evaluation of DNA polymorphisms for association with traits should not be restricted to analysing means, but should include the variance and trait correlations. Graphics are a useful tool for illustrating differences in trait distributions (means and variance) and trait associations (scatter blots). They are a very useful tool for visualizing differences before engaging statistical evaluations, which are often contingent on very specific assumptions.

Testing a single marker in a candidate gene is not sufficient. Several markers should be identified and haplotypes delineated in order to increase the resolution of trait association studies. Finally, QTL analysis is contingent on having strains and databases. Unfortunately the number of well-characterized strains is rapidly dwindling and databases may not be readily accessible.

It may be a solace that the estimated number of expressed genes may be as low as 30,000. Nevertheless, the number is still large and new methodologies such as expression analyses using DNA chips or proteomics are necessary to make a more educated guess as to what genes to analyse.

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33 Strategies for the Production of Transgenic Chickens

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Introduction

During the 1980s and 1990s, the concept of genetic engineering became a broad term used to describe the application of a new set of tools associated with advances in molecular genetics and specifically with transgenic technology. The field has recently expanded to include applications resulting from mapping the chicken genome, high-throughput genomics and proteomics, total genome sequencing and bioinformatics. With all of these competing technologies, interest in transgenic technology remains strong. One of the reasons for this has been the tangible success of transgenic mice as a tool to increase the understanding of almost every biological process. Hence, the application of transgenic technology to economically important species of livestock, poultry and fish has been a constant goal for many in animal agriculture. Many groups also see avian transgenics as the final means to test hypotheses concerning candidate quantitative trait loci gleaned from work on the avian genome. The huge economic value of the global poultry industry and applications in the pharmaceutical industry provide additional incentives for continuing efforts towards a transgenic approach for manipulation of the avian genome.

The goal of gene transfer for commercial poultry is simply to develop genetically better birds for the production of meat and eggs, and this has been the same goal of conventional selection programmes since the 1950s. The potential applications for gene transfer in poultry range from the rapid transfer of commercially important genes for disease resistance and higher production efficiency to using the chicken as a means for the large-scale production of therapeutic proteins for the pharmaceutical industry. The latter application stems from the anticipated demand for proteins for therapeutic use and the high efficiency of protein production in eggs found in commercial layer stocks, a feature that is a direct result of classical selection programmes over the last half of the 20th century. Therefore, it is not inconceivable that transgenic poultry could create an entirely new facet of the poultry industry in the future, where specialized farms are developed solely to produce eggs containing therapeutic proteins. To reach this end, the methods of developing transgenic poultry must move from the academic laboratory to the commercial sphere.

This chapter will highlight current approaches for developing transgenic poultry, including gene transfer methods under development and some practical considerations in developing a transgenic programme. It is not intended to be a comprehensive review of the literature but to provide an overview of the status of avian transgenics.

The Germ Cell Cycle: Entry Points for Gene Transfer

The goal in any transgenic programme is to introduce foreign DNA into the genome of the organism so that the gene is expressed and inherited in a Mendelian fashion. Because of this, the germ cell is the target cell type for inherited modifications to the chicken genome. Beginning with fertilization, the germ line is established in an extragonadal location early in embryonic development. Subsequently the germ cells migrate to the germinal ridge and proliferate during the first week or so of embryo development. For male germ cells, the diploid spermatogonia do not begin substantial levels of proliferation again until sexual maturity, when the process of spermatogenesis begins. For females, the oocytes undergo meiotic arrest shortly before or after hatch and remain quiescent until sexual maturity, when oocyte growth and maturation take place. The germ cell cycle is repeated with fertilization.

The events during the life of a germ cell immediately suggest several opportunities for facilitating the production of transgenic poultry. A summary of some of the various approaches for gene transfer at different time points in the germ cell life cycle in birds is listed in Table 33.1. Many of these approaches have yet to be proven, but they indicate the range of attempts to develop transgenic poultry.

The reproductive strategy of birds differs significantly from that of mammals and procedures for gene transfer in poultry have been adapted from the techniques used for mammals or have been developed specifically for poultry. Because of the characteristics of the avian ovum and embryo, the genome is rather difficult to access. Hence, it is worth reviewing some of the features of avian reproduction that have a bearing on transgenic technologies.

Table 33.1.   A summary of various entry points for gene transfer in birds.

Stage of development	Transfection methods	References
Developing oocyte	DNA microinjection and retroviral vectors	Cioffi *et al.* (1994)
Newly fertilized egg	DNA microinjection	Sang and Perry (1989)
		Love *et al.* (1994)
		Naito *et al.* (1994a)
		Sherman *et al.* (1998)
Newly laid egg– blastodermal cells	Liposomes	Brazolot *et al.* (1991)
		Fraser *et al.* (1993)
		Rosenblum and Chen (1995)
	Electorporation	Muramatsu *et al.* (1997)
		Wei *et al.* (2001)
	Replication-competent/replication-defective retrovirus	Salter *et al.* (1987)
		Bosselman *et al.* (1989)
		Thoraval *et al.* (1995)
PGCs – germinal crescent	Replication-defective retroviral vectors	Vick *et al.* (1993a)
	Ballistic transfection	Li *et al.* (1995)
PGCs – embryonic blood	Liposome	Watanabe *et al.* (1994)
		Vick *et al.* (1993a)
PGCs – early gonad	Replication-defective retrovirus	Allioli *et al.* (1994)
	Electroporation	Hong *et al.* (1998)
Embryonic stem cells	Liposomes	Pain *et al.* (1999)
	Electroporation	
Sperm	Irradiation	Pandey and Patchell (1982)
	Liposomes	Squires and Drake (1993)
		Nakanishi and Iritani (1993)
	REMI	Shemesh *et al.* (2000)
	Antibody	Chang *et al.* (2002)

One of the main characteristics of reproduction in birds is the oviposition of a calcified egg containing a large yolk-filled ovum surrounded by layers of albumen. After ovulation of the mature ovum, it is captured by the infundibulum of the oviduct, where fertilization takes place. Fertilization in birds is polyspermic and many sperm nuclei can be found after penetration of the vitelline membrane (Perry, 1987). Nevertheless, only one sperm nucleus will fuse with the nucleus of the ovum. Subsequently, the fertilized egg enters the magnum, where a firm albumen capsule encases the ovum. Later, as it transverses the isthmus, the outer and inner shell membranes are laid down in preparation for deposition of the eggshell. At this point the fertilized ovum has spent about 3.5–4 h in the oviduct. The first cleavage divisions occur upon entry of the ovum into the shell gland or uterus. The egg spends the longest time in the shell gland, taking an additional 20–22 h for complete formation of the eggshell. A considerable amount of cell division occurs during eggshell formation and the embryo acquires its polarity, e.g. anterior/posterior, yet visually it is radially symmetric. When the egg is laid, the disc-shaped embryo or blastoderm contains about 50,000–60,000 cells lying on the surface of the yolk. Generally the blastoderm can be divided into a peripheral ring of cells attached to the yolk, called the area opaca, and a central more translucent region, the area pellucida. The area pellucida is suspended above a non-yolky fluid deposited by the embryo. This arrangement is fortuitous and allows for the easy manipulation of the embryo required for most approaches for making transgenic poultry.

In general, the area opaca will contribute only to extraembryonic structures. Upon incubation, the area pellucida differentiates into two layers: an upper epiblast and a lower hypoblast. Only the epiblast will give rise to the embryo proper, while the hypoblast contributes to some extraembryonic tissues. This period of development, i.e. from fertilization through hypoblast formation, has been classified into a series of 14 stages by Eyal-Giladi and Kochav (1976) for the domestic hen and 11 stages by Bakst *et al.* (1997) for the turkey, indicated by roman numerals. Subsequent stages for both species are classified in the staging system of Hamburger and Hamilton (1951) using arabic numerals. All references to stages of development will be to those for the domestic hen.

The next period of embryo development that is significant for the production of transgenic poultry is during the establishment of the germ line. Tsunekawa *et al.* (2000) described the expression of the chicken homologue of the *Drosophila vasa* gene, an RNA binding protein associated with 'germ plasm'. Chicken vasa homologue protein is present in early cleavage embryos and in the stage X blastoderm, when presumptive primordial germ cells (PGCs) can be identified with immunological markers (Karagenc *et al.*, 1996). Definitive PGCs were identified almost a century ago, when Swift (1914) described primordial germ cells in an extraembryonic region called the germinal crescent. The germinal crescent lies in an anterior region formed during gastrulation as the hypoblast is displaced by the endoderm. Swift's identification of the germinal crescent was based on the morphological characteristics of PGCs. From the germinal crescent, the germ cells must find their way to the developing gonadal ridge. As the blood islands form and the intra- and extraembryonic vasculature develops, the PGCs are carried to the vicinity of the germinal ridge through the embryonic circulation (Swift, 1914; Meyer, 1964; Fujimoto *et al.*, 1976a,b). Subsequently, the blood-borne PGCs actively leave the vessels and migrate to the germinal epithelium through the dorsal mesentery. During this active phase of migration, chemokine signals released from the gonad (Dubois and Croisille, 1970), extracellular matrix components (Urven *et al.*, 1989) and the anatomical arrangement of the vascular system surrounding the gonadal epithelium (Nakamura *et al.*, 1988) work in concert to guide the germ cells to the gonadal anlage. Once the germ cells arrive in the primitive gonad, sexual differentiation begins.

Gene Transfer Methods as Applied to Poultry

All methods of producing transgenic poultry rely on techniques designed to insert novel genetic material into cells that will give rise to germ cells or the precursors of germ cells. Currently, two methods have been developed that have successfully produced transgenic poultry: retroviral-mediated transfection; and microinjection of DNA into the zygote. Other methods currently in development utilize fresh or cultured blastodermal cells or primordial germ cells to make a chimeric intermediate that it is hoped will harbour transgenic germ cells. Lastly, interest continues in the use of sperm as a vector for the development of transgenic poultry.

Retroviral-mediated transgenesis

Retroviral vectors for gene transfer are now commonly used and form the basis of transgenesis in laboratory and domestic animals as well as for gene therapy strategies in humans. Retroviral gene transfer of chick embryos is frequently employed for the induction of ectopic expression of exogenous genes and has become one of the techniques for examining gene function during chick embryo development (Iba, 2000). Its use in the development of transgenic poultry has been less widespread, mainly because of the need for a fully equipped research laboratory coupled with extensive hatching, rearing and egg production facilities. Nevertheless, retroviral gene transfer has remained the most successful method of producing transgenic poultry.

Retroviral vectors have significant advantages over other methods of gene delivery because of the viral life cycle (Fig. 33.1). All retroviruses have an RNA genome encased in a protein core that contains an integrase, reverse transcriptase and protease, surrounded by a protein envelope. During infection the viral envelope proteins bind to specific proteins on the host cell membrane and are internalized by receptor-mediated endocytosis. The envelope is removed by cellular enzymes, and viral reverse transcriptase copies the RNA into proviral DNA. The DNA translocates to the nucleus and is integrated into a chromosome of the host cell through the activity of the viral integrase and the long terminal repeats (LTRs) at each end. The proviral DNA replicates with the cellular chromosome and is inherited in a Mendelian manner; it is also capable of expressing the genes carried by the vector. What distinguishes retroviral methods of gene transfer from other methods is that the efficiency of integration is higher than if DNA constructs are transfected using electroporation or liposomes. It is this aspect of the retroviral life cycle that permits successful transgenesis in poultry. To complete the cycle, the proviral DNA can be transcribed into viral RNA for the synthesis of viral proteins. These RNAs encode three classes of proteins: Pol for polymerases, Gag for group-associated antigens and Env for envelope proteins. Once translated, Pol and Gag proteins associate with the specific packaging sequences on the viral RNA and assemble into new viral cores. The Env proteins are transported to the host cell membrane and the viral core buds from the cell and produces a new infectious particle.

Based on the features of the retroviral life cycle, vectors can be classified as replication-competent, where new viral particles are produced in the host cell, and replication-defective, which allow for integration of the proviral DNA but are unable to replicate after infection of the host cell. Replication-competent vectors contain the exogenous gene to be expressed as well as all of the viral sequences needed to produce viral particles. Replication-incompetent vectors are missing all or a portion of the *gag*, *pol* and *env* genes but retain the encapsidation site required for packaging. Since defective vectors cannot replicate on their own, viral particles are generated *in vitro* using helper cell lines that express all of the proteins needed to package the virus. There are some disadvantages to retroviral vectors. These include limits to the size of the exogenous DNA that can be packaged into a virion, which often results in deletions or
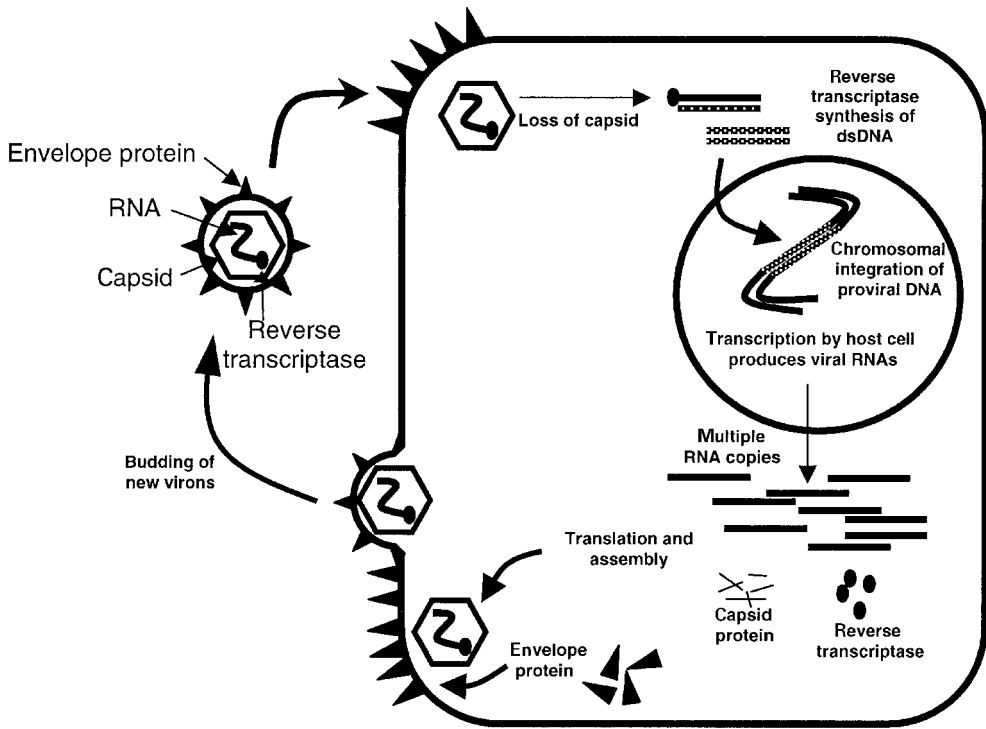
Fig. 33.1. A diagram of the life cycle of a retrovirus. The major feature that makes retroviral vectors attractive is that the RNA viral genome is reverse transcribed and the proviral DNA becomes integrated into the genome. Replication-competent vectors contain an intact viral genome that allows expression and replication of new viral particles. Replication-defective vectors are missing a large portion of the viral genome and cannot produce infectious virus. In this case, helper cells lines are needed to produce the initial viral particles (see Leber *et al.*, 1996; Morgan and Fekete, 1996).

rearrangements, production of low viral titres, and the possibility that exogenous viral inserts will become replication-competent through recombination with endogenous viral sequences, thereby possibility becoming pathogenic. While the latter feature has a low probability, it does preclude their use on a commercial scale where several billion birds are produced each year. Finally, it is now apparent that many retroviral vectors undergo transcriptional silencing, making the design of the vector much more critical to achieve the desired outcome (Lund *et al.*, 1996; Pannell and Ellis, 2001).

Efforts to produce transgenic birds using replication-competent vectors began with Souza *et al.* (1984), who were the first to introduce foreign genes into the newly laid egg using a replication-competent Rous

sarcoma virus (RSV) vector. In this case, chicken growth hormone was overexpressed and resulted in measurable increases of growth hormone in the circulation but did not affect the growth of the birds. In this approach, the birds were mostly likely mosaic for the transgene and no attempt was made to assess transmission of the gene to the next generation. Salter *et al.* (1986, 1987) injected the blastoderm or the abdomen of newly hatched chicks with a replication-competent wild-type, and a recombinant avian leukosis virus (ALV), or a reticuloendotheliosis virus (REV) vector, and produced viraemic founders that also transmitted the integrated provirus. Subsequent generations transmitted the proviral DNA in a Mendelian fashion, thereby demonstrating the ability of viral vectors to develop transgenic chickens. Interestingly,

of the 23 proviral inserts generated, two lines expressed envelope proteins of ALV subgroup A but not complete infectious virus making the birds resistant to subgroup A ALV infection (Crittenden and Salter, 1990; Salter and Crittenden, 1991; Salter *et al.*, 1998). Subsequently, Chen *et al.* (1990) reported that a modified form of the RSV containing bovine growth hormone could enter the germ line in chickens and yielded measurable levels of bovine growth hormone in two males. Since that time, little has been reported on the generation of transgenic poultry using replication-competent vectors, because of the risk of pathogenicity. In the following 10 years or so, replication-defective vectors became the method of choice when using a retrovirus to generate transgenic chickens.

With replication-defective vectors, deletions are made in the genes essential for replication (*pol*, *gag*, *env*). To produce infectious particles for transgenesis, helper cell lines are generated using a proviral vector that is missing the encapsidation site but contains the *gag*, *pol* and *env* regions of the virus. When this cell line is transfected with a replication-defective viral vector containing the exogenous gene of interest, the helper cells can package the recombinant viral RNA into infectious particles that do not produce progeny virus. Since replication-defective vectors do not carry the original viral genome, cDNA of a larger size can be packaged and expressed compared with replication-competent vectors. This often allows the expression of more than one gene. Several packaging cell lines have been produced for avian retroviral vectors, including those for ALV (Savatier *et al.*, 1989; Cosset *et al.*, 1990), REV and spleen necrosis virus (SNV) (Watenabe and Temin, 1983; Hu *et al.*, 1987; Meyers *et al.*, 1991), and RSV (Reddy *et al.*, 1991; Boerkoel *et al.*, 1993). One of the critical features of a helper cell line for transgenics is the absence of any replication-competent helper virus. The need for packaging cell lines makes the construction of replication-defective retroviral vectors more difficult and time consuming than replication-competent systems. In addition, viral titres are reduced, which often requires considerable concentration to yield sufficient material for infection.

Transgenic chickens have been produced using replication-defective REV vectors when injected beneath the blastoderm (Bosselman *et al.*, 1989). About 8% of the male birds that hatched carried the neomycin resistance gene and transmitted the vector to progeny at about 2–8%. The same REV vector was used to express chicken growth hormone constitutively in embryos. About 50% of the embryos had elevated concentrations of growth hormone but none hatched. Defective ALV vectors have also been used to generate transgenic chickens. Thoraval *et al.* (1995) used a vector carrying the neomycin resistance gene and *Escherichia coli* β-galactosidase (*lacZ*). Out of 36 individuals that hatched after injection of virus, one male produced G_1 transgenic progeny at a frequency of 2.2%. Expression of *lacZ* was detected in embryonic fibroblasts, but the status of other tissues was not reported. In addition to injection of viral stocks into the blastoderm prior to incubation, Vick *et al.* (1993a) demonstrated that replication-defective vectors could be used to infect primordial germ cells from the germinal crescent or blood to produce transgenic chickens. Mizuarai *et al.* (2001) used a Moloney murine leukaemia virus (MoMLV) pseudotyped with vesicular stomatitis virus G protein (VSV-G) to express green fluorescent protein and the neomycin resistance gene and obtained 80% germline transmission from the founder birds. The neomycin resistance gene was driven by an RSV promoter and was expressed in some tissues in subsequent generations; however, green fluorescent protein (GFP) expression was not detected even using reverse transcriptase (RT)-PCR. Nevertheless, this is an important result since ALV vectors have been found to be of limited use for the generation of transgenic quail (Salter *et al.*, 1999). In addition, the high rate of germline transmission associated with using a VSV-G pseudotyped vector could lend a boost to the efficiency of producing transgenic chickens where germline transmission is often low. Harvey *et al.* (2002a) overcame low germline transmission with ALV vectors by using

high-throughput screening of large numbers of offspring. The same group generated transgenic chickens that constitutively expressed the β-lactamase gene responsible for bacterial ampicillin resistance and reported the consistent production of β-lactamase in egg white during 16 months of egg production and over four generations (Harvey *et al.*, 2002b). This work confirms that the production of novel proteins in eggs is biologically feasible.

While retroviral vectors can be used to generate transgenic birds, there are significant problems that need to be overcome before the technology moves from the laboratory to the industrial arena for the genetic improvement of commercial stock. Besides the limitations of the size of the gene that can be packaged and difficulties in obtaining high viral titres, the integration of the provirus occurs at random in the host chromosome. In addition, retroviruses are susceptible to transcriptional silencing even with multiple insertion events (Mizuarai *et al.*, 2001). The use of chromatin insulators and matrix attachment regions may help to prevent gene silencing (McKnight *et al.*, 1996; Emery *et al.*, 2000; Rivella *et al.*, 2000) but this remains to be tested. Overcoming these disadvantages is the main reason for the development of non-viral technologies for gene transfer in poultry. Nevertheless, the use of replication-competent and replication-defective viral vectors has expanded to the point where they have become tools available for answering questions regarding the molecular basis of avian biology. Foremost in this regard is their use in developmental biology, where the avian embryo is a major system for the study of cell lineage analysis, cell migration and the *in vivo* action of expressed proteins.

DNA microinjection

In addition to retroviral vectors, the micro-injection of DNA is the only other means demonstrated to produce transgenic poultry. The generation of transgenic mice and commercial livestock through the injection of DNA into the pronucleus of the newly fertilized mammalian egg provided the stimulus for attempting a similar procedure in the chicken. Unfortunately, the procedures for mammalian embryo manipulation such as superovulation, embryo transfer and *in vitro* fertilization were not readily adaptable to the chicken and standard microinjection facilities could not be used to inject the large avian egg. Before DNA microinjection could be attempted in birds, a complete *ex ovo* culture system from fertilization to hatch needed to be developed that would yield sufficient numbers of hatchlings to screen for gene integration. The basic method currently in use is a three-stage system develop by Perry (1988) using a combination of methods employed by Ono and Wakasugi (1984) and Rowlett and Simkiss (1987) for post-ovipositional stages of development (Fig. 33.2). Since cleavage usually begins once the egg has reached the shell gland, it is often necessary to sacrifice a hen to obtain one egg for microinjection. Ideally, newly fertilized eggs, surrounded with a capsule of albumen, are removed from the magnum. In the first stage, these are cultured for about 18–24 h in synthetic oviductal fluid without a shell. The second stage requires transfer of the egg to an eggshell, completely sealed with no simulated air space for 2–4 days. In the third and final stage, the embryo is transferred to a larger shell to create an air space for the remaining period of incubation. Such procedures have also been adapted to quail embryos, using chicken eggshells (Ono *et al.*, 1994).

DNA expression cassettes are injected into the cytoplasm of the germinal disc of the ovum upon recovery from the magnum prior to culture. In early attempts to inject DNA into the avian egg, the DNA formed concatemers and remained episomal, an observation similar to that seen after micro-injection of mammalian pronuclei (Sang and Perry, 1989; Naito *et al.*, 1991, 1994a). In a subsequent study, however, one mosaic rooster was produced that transmitted the β-galactosidase gene from *E. coli* to about 3.4% of its offspring (Love *et al.*, 1994). Transgene copy number averaged about 6, apparently in a single chromosomal

Stage I (before oviposition)

Ovum with
albumen capsule

Beaker or
other vessel

Artificial oviductal
fluid

Stage II (0–3 days)

Filled with thin
albumen

Ovum

Plastic film

Eggshell

Stage III (3–21 days)

Air space

Larger
eggshell

Plastic film

Developing embryo

Thin albumen
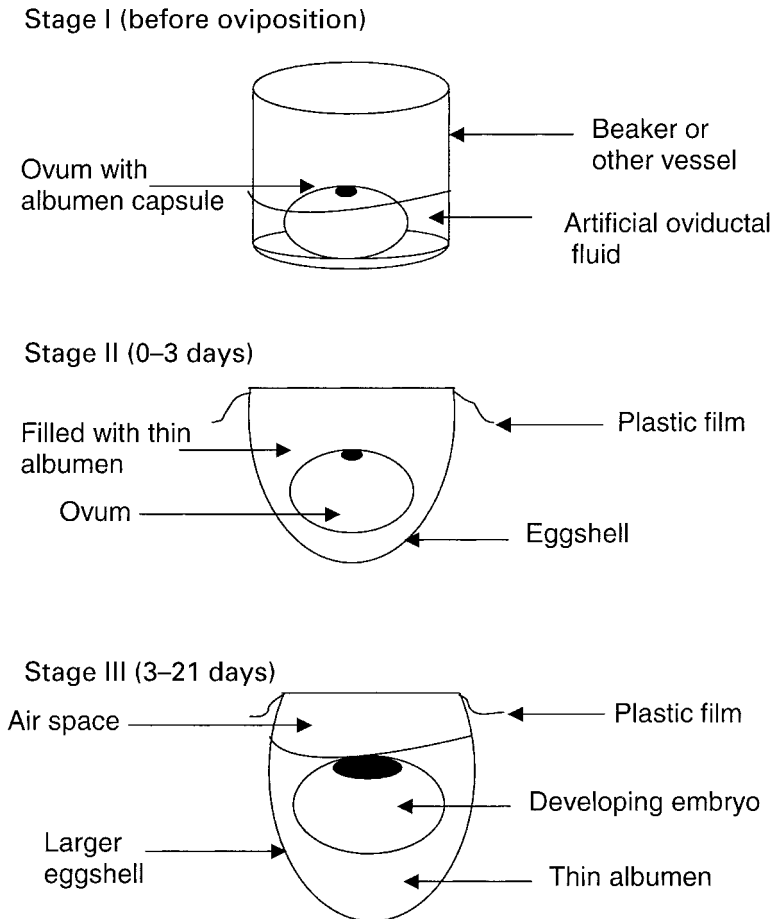
Fig. 33.2. *Ex ovo* culture after the microinjection of DNA at 22 h before oviposition through to hatch. The process requires three stages. Stage I: after the injection of DNA into the cytoplasm of the germinal disc, the ovum is cultured in a vessel with an artificial oviductal fluid for 24 h. Stage II: the contents from Stage I are transferred to a host eggshell, which is filled with thin albumen and sealed with plastic film. The sealed egg is laid on its side and rocked for 3 days. Stage III: the contents of the Stage II composite egg are transferred to a larger eggshell (e.g. in the case of a chicken embryo this can be a turkey eggshell). For this final period, an air space is created over the embryo and the composite egg is sealed with cling film. The egg is rotated only about 30 degrees to prevent contact with the plastic film. After about day 21 the eggs are transferred to a hatcher and the plastic film is removed when the embryo has reached internal pipping. For the injection of retroviral constructs or the development of chimeras, Stage I can be omitted. (Rowlett and Simkiss, 1987; Perry, 1988.)

location, but with considerable rearrangements. Test mating of one transgenic rooster showed predictable Mendelian inheritance of the gene although no expression was reported. Nevertheless, this demonstrated that it was possible to produce transgenic poultry using DNA injection.

Sherman *et al.* (1998) tested whether the *Drosophila* transposable element *mariner*

would transpose into the chicken genome after injection into the newly fertilized egg. After *ex ovo* culture, germline transmission of *mariner* from one of three surviving chicks was observed. Subsequent test mating showed that the gene construct was inherited in a stable Mendelian fashion. The frequency of transposition into the genome and the rate of germline transmission was

higher than that observed without a transposable element (Love *et al.*, 1994). This suggests that the addition of a transposable element in the design of plasmid vectors for DNA microinjection could lead to a significant increase in efficiency.

The use of DNA microinjection into avian ova overcomes some of the disadvantages of retroviral vectors, such as the need to generate replication-defective vectors and the limits on the size of the transgene that can be used. Success with the production of transgenic mice via microinjection of 250 kb yeast artificial chromosomes suggests that a similar-sized construct could be used to produce transgenic birds. The efficiency of transgene integration is often a limiting variable for the production of transgenic animals (mammalian or otherwise). However, in the case of the chicken, it appears to be low but not lower than that for other agricultural livestock.

Other Approaches Towards Avian Transgenics

Despite the success of using retroviral vectors and microinjection of DNA, the generation of transgenic poultry has yet to become widespread. Because of this and the fact that integration of the transgene into the genome occurs at random, other approaches are currently under development in the hopes of filling the gap and providing a repertoire of techniques for the generation of transgenic birds. Two of these approaches utilize an intermediate step where blastodermal cells or primordial germ cells are removed from a donor embryo, cultured, transfected and returned to a recipient embryo to generate transgenic/chimeric birds (Fig. 33.3). This approach is based upon the work with embryonic stem cells that began in the mid 1980s in the mouse. Other approaches in the pipline include the use of sperm as a vector for gene transfer and, with the recent ability to clone mammals using nuclei from cultured tissues, efforts have begun to investigate the possibility of nuclear cloning in birds.

Primordial germ cells and embryonic stem cells

Figure 33.3 illustrates the basic approach to generating transgenic poultry using either blastodermal cells or primordial germ cells. In this approach, three techniques must come into play. The first is the ability to culture early blastodermal cells or PGCs in such a way that they retain or acquire the ability to give rise to somatic cells and germ cells – in other words, to act like embryonic stem cells. The second is the ability to transfect these cultured cells so that selection of stable integration events can be identified and those cells expanded. This step has the potential to take advantage of gene constructs that can undergo homologous recombination to generate specific changes to the avian genome. The final step is the ability to inject these cells back into a recipient embryo to generate a transgenic/chimeric chicken. Several groups have investigated various parts of this multi-step technical scheme. In addition, much of the work has been intertwined with work on the origin and the early life of avian primordial germ cells in the embryo. However, for the sake of brevity, the discussion will be limited to the production of germline chimeras and the culture of embryonic stem cells and primordial germ cells. Those requiring a more detailed discussion of the literature on primordial germ cells should consult Wentworth and Wentworth (2000).

Of the three technological steps, procedures for the generation of germline chimeras were investigated first. Marzullo (1970) showed that pieces of blastoderm from one unincubated embryo could be transplanted to another embryo *in ovo*. No chicks hatched at the time, but feather markers indicated that some cells were derived from the donor embryos. Two decades later, Petitte *et al.* (1990) injected dispersed stage X blastodermal cells into the subgerminal cavity of unincubated embryos and obtained a rooster that was both a somatic and germ cell chimera. While the efficiency of germline transmission was about 0.3%, this stimulated a considerable amount of work related

Step 1

Isolated blastodermal   Culture cells from
cells or PGCs   donor embryo

Step 2

Transfection   Select cells for integration
                               of transgene

Step 3

Inject into recipient embryos   Produce germline
and culture to hatch   chimeras and breed
                                     and screen transgenics
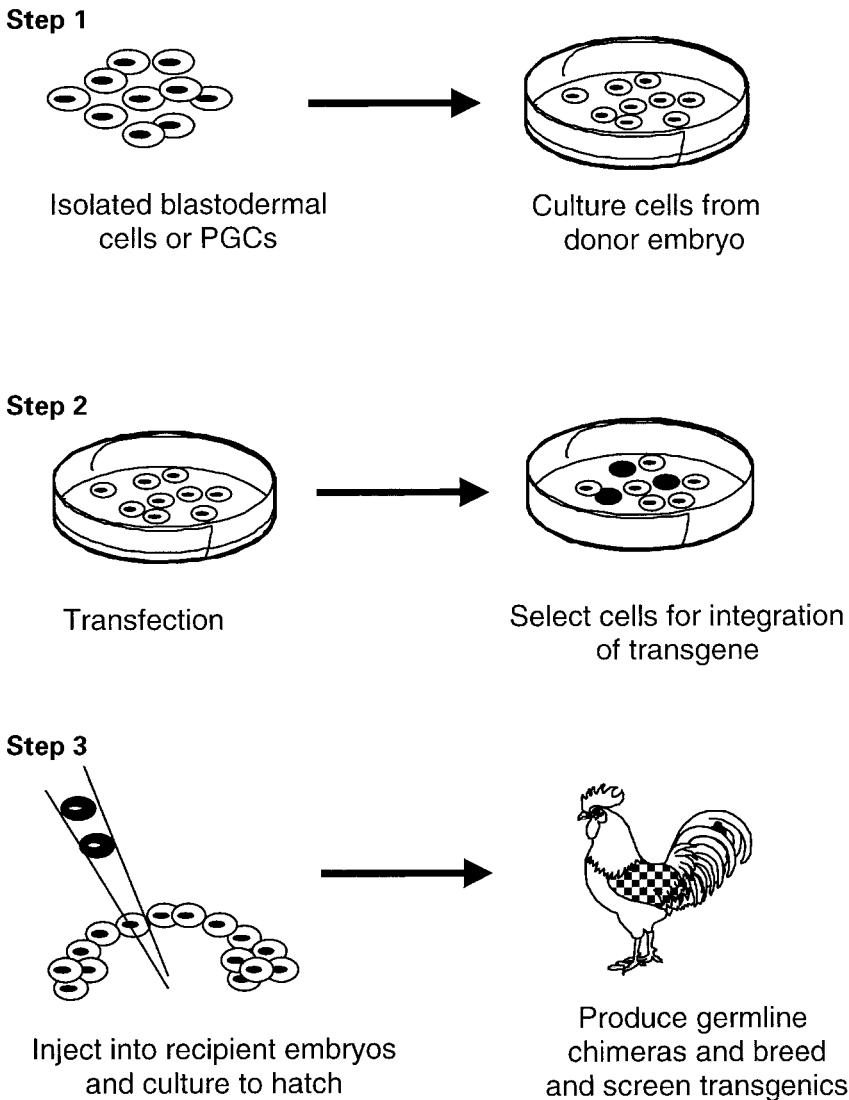
Fig. 33.3. The three major steps in the production of transgenic poultry using chimeric intermediates. Step I: blastodermal cells from stage X embryos or PGCs are isolated and cultured using conditions that allow proliferation without differentiation, e.g. the formation of embryonic stem cells. This has been the most difficult aspect of the process. Step II: the cultures are transfected with DNA using either liposomes or electroporation. Cells with the correct integration event are expanding, using the appropriate selectable markers. It is also at this stage that selection of homologous recombination events could be employed. Step III: selected cells are transferred to recipient embryos and cultured to hatch using *ex ovo* culture or through various windowing techniques. The resulting chimeras are bred and the offspring screened for the presence of the transgene.

to the development of chimeras using fresh blastodermal cells or primordial germ cells. Today, the efficiency of producing germline chimeras using blastodermal cells has increased substantially by compromising donor embryos with a sublethal dose of 500–600 rad of gamma radiation (Carsience *et al.*, 1993; Fraser *et al.*, 1993) or through the

removal of about 700 cells from the central part of the area pellucida of recipient embryos and replacing the same location with donor cells (Kagami *et al.*, 1997). Today it is fairly routine to generate germline chimeras using blastodermal cells. When this is coupled with *ex ovo* culture or modifications to standard windowing procedures, embryo survival to hatching can be increased to 30–70% (Borwornpinyo, 2000; Speksnijder and Ivarie, 2000).

With the successful generation of germline chimeras using blastodermal cells, attempts were made to develop transgenic birds through the transfection of uncultured blastodermal cells through either liposome-mediated transfection (Brazolot *et al.*, 1991; Fraser *et al.*, 1993), fluorescent activated cell sorting of *lacZ*-expressing blastodermal cells (Speksnijder, 1996) or electroporation (Etches *et al.*, 1996). Some attempts have been made to transfect the unincubated blastoderm *in situ* using the injection of DNA and liposomes in the subgerminal cavity (Rosenblum and Chen, 1995; Inada *et al.*, 1997). In all the above cases, evaluation of transgene expression in early embryos or the screening of hatched chicks indicated that the efficiency of DNA integration was too low to expect germline transmission to yield transgenic offspring.

Like blastodermal cell transfer, efforts to produce germline chimeras from primordial germ cell transfer as a means of accessing the avian genome was viewed as another possibility. Three sources of PGCs have been used: (i) germinal crescent; (ii) circulating PGCs between stages 14 and 17 of development; and (iii) after the PGCs have taken up residence in the primitive gonad. Reynaud (1969)was the first to show that PGCs from the germinal crescent could be injected into the blood vessels of recipient embryos and settle in the hosta gonad. Wentworth *et al.* (1989) reported that quail germinal crescent PGCs could be used to generate germline chimeras. Simkiss *et al.* (1989) demonstrated that circulating chick PGCs could be used as donor cells. Subsequently, Tajima *et al.* (1993) successfully hatched germline chimeras after the transfer of blood PGCs to recipients at the same stage of

development. When endogenous PGCs were removed from the embryonic circulation prior to injecting donor PGCs, the level of germline transmission increased (Naito *et al.*, 1994b). Similar results were obtained with quail (Ono *et al.*, 1998). The number of endogenous PGCs can also be reduced using an injection of busulphan (Aige-Gil and Simkiss, 1991) and can also lead to relatively high germline transmission rates (Vick *et al.*, 1993b). Along with reducing the number of endogenous PGCs, attempts have been made to concentrate the number of donor PGCs obtained from embryonic blood using Ficoll density gradient centrifugation (Yasuda *et al.*, 1992) and immunomagnetic cell separation (Ono and Machida, 1999; Wei *et al.*, 2001). In addition to PGCs from the germinal crescent and the blood, gonadal PGCs can also be used to generate germline chimeras. For the most part it is now accepted that PGCs obtained from the gonad of 5–7-day-old embryos can actively migrate to the gonadal ridge in embryos during the period of circulation in the blood, i.e. stages 14–17 (Tajima *et al.*, 1998). Wentworth and Wentworth (2000) reported a similar phenomenon in quail. Tajima *et al.* (1998) also demonstrated that cryopreserved gonadal PGCs retained the ability to generate germline chimeras. In total, it is clear that the source of donor PGCs can be from the germinal crescent, embryonic blood, or early gonad, but the time to inject them into the host is prior to the period of active migration to the gonad, i.e. when the PGCs begin to circulate in the embryonic blood.

Transfection of germinal crescent PGCs has been attempted using retroviral vectors (Simkiss *et al.*, 1989; Savva *et al.*, 1991), ballistic transfection (Li *et al.*, 1995) and liposomes and electroporation (Hong *et al.*, 1998). Transfection of blood-derived PGCs has been attempted using liposomes (Naito *et al.*, 1998). Although the transgene was detected in two out of 18 hatched chicks, none transmitted the gene through the germline (Naito *et al.*, 1998). However, as cited above, infection of blood-borne PGCs with a retroviral vector has successfully generated transgenic chickens (Vick *et al.*, 1993a). Since more PGCs can be obtained

from gonadal preparations than from other sources, some investigators have attempted to transfect gonadal PGCs before making chimeras. Hong *et al.* (1998) transfected dispersed gonadal cells using electroporation or liposomes, then purified the cells using Ficoll density separation. In general, however, most of these studies have evaluated embryonic gonads for the presence of the transgene. Like the work with the transfection of blastodermal cells, the transfection of PGCs suffers from the fact that the frequency of transgene integration using liposomes or electroporation is very low and does not allow for the amplification of cells that have incorporated exogenous DNA into the genome.

Given the low efficiency of transgene integration, it is clear that the major hurdle has been the need to culture blastodermal cells or PGCs so that transfection and selection of stable integration events could be performed *in vitro*. The tricky part in this scenario is to culture the cells in such a way that they retain their ability to give rise to germ cells once introduced into a host embryo. In other words, what was needed is a means to culture the avian equivalent of embryonic stem cells.

Evans and Kaufman (1981) and Martin (1981) were the first to describe cells derived from the mouse blastocyst that could be cultured relatively indefinitely and retain an undifferentiated state. These came to be known as embryonic stem cells. The most useful embryonic stem cell lines were capable of contributing to both somatic and germ cells when injected into a recipient blastocyst and paved the way for approaches to develop transgenic mice with specific changes to the genome using constructs designed to undergo homologous recombination with the endogenous locus (Doetschman *et al.*, 1987; Hooper *et al.*, 1987; Capecchi, 1989, 2001; Thomas and Capecchi, 1990; Thomas *et al.*, 1992). In addition to the blastocyst, embryonic stem cells can also be derived from primordial germ cells (Matsui *et al.*, 1992; Resnick *et al.*, 1992). These are usually referred to as embryonic germ cells to distinguish their source of derivation, even though embryonic germ cells express a cellular phenotype and function exactly as embryonic stem cells derived from the inner cell mass of the blastocyst (Stewart *et al.*, 1994; Labosky *et al.*, 1994). Embryonic stem cells have now been described for a number of livestock species (Piedrahita *et al.*, 1998) as well the controversial development of human embryonic stem cells (Pera *et al.*, 2000; Thomson and Odorico, 2000).

Since the early 1990s, efforts to develop avian embryonic stem cells have been slow and rather tedious. Much of this stems from the fact that the culture conditions required for the production of avian embryonic stem cells are unknown and from the lack of homology and biological activity of growth factors and cytokines between mammals and birds. Nevertheless, various attempts have been made to culture blastodermal cells or PGCs so that they could be used for the production of transgenic poultry. Etches *et al.* (1996) tested the culture of blastodermal cells as intact blastoderms, dispersed blastodermal cells cultured in a monolayer or with a confluent layer of mouse fibroblasts. After 48 h of culture, the cells were injected into stage X embryos to generate somatic and germline chimeras. More somatic chimeras were generated when the blastodermal cells were cultured on the feeder layer but contributions to the germline did not differ based upon the culture treatment. Petitte and Yang (1994) described the culture of cells from the stage X embryo using methods similar to that for the mouse where media conditioned with buffalo rat liver (BRL) cells and a feederlayer of mouse embryonic fibroblasts successfully cultured early blastodermal cells that exhibited a typical embryonic stem cell phenotype. The cells could be cultured for 2 months and expressed embryonic stem cell markers such as SSEA-1 and EMA-1. Immortal cell lines derived from the unincubated chicken blastoderm have been derived without a feeder layer but the ability of this to form functional chimeras has not been documented (Tsai, 1995). Pain *et al.* (1996) reported on the long-term culture of embryonic stem cells from stage X chicken embryos using a combination of growth factors and an antibody to retinoic acid. The cells were capable of differentiation into

various cell types *in vitro* and could be used to produce somatic chimeras. To generate cultures of transgenic cells, it may be possible to enrich the starting cultures with cells that express the gene of interest (Wei *et al.*, 2001) or transfect established blastoderm-derived stem cells (Pain *et al.*, 1999).

In addition to blastodermal cells, the culture of embryonic stem cells from PGCs for various time periods has been tried. For the most part, PGCs have been cultured for less than 1 week. Allioli *et al.* (1994) cultured gonadal PGCs harvested from day 5 embryos for 4 days. Initially, some cell death was observed followed by a period of proliferation. Furthermore, these cultured PGCs could be infected with a retroviral vector that expressed *lacZ*. Chang *et al.* (1995a) isolated blood PGCs and cultured them on a feeder layer of stromal cells obtained from 5-day gonadal ridges and noted an increase in the number of germ cells over 4 days. The cultures were supplemented with leukaemia inhibitory factor (LIF), insulin-like growth factor I (IGF-I) and basic fibroblast growth factor (bFGF). Similarly, Chang *et al.* (1995b) cultured PGCs from stage 27 germinal ridges for 5 days and proliferated on the stromal cells that arose from the germinal ridge. In this case, the PGCs were shown to retain their ability to migrate back to the gonad when injected into the bloodstream of 2.5-day embryos. In a subsequent study, Chang *et al.* (1997) demonstrated that gonadal PGCs cultured for 5 days and injected into recipient embryos gave rise to chickens that transmitted the donor genotype to about 3% of the offspring. Park and Han (2000) described the establishment of embryonic stem cells from gonadal primordial germ cells that could be cultured for several months. Interestingly, the embryonic stem cells could only be established using chicken feeder layers that were not mitotically inactivated and did not attach to the feeder layer like other embryonic stem cell cultures. The reasons for this remain unknown, but the cultures were also supplemented with stem cell factor (SCF), LIF, bFGF, interleukin-11 (IL-11) and IGF-I. With these reports on the culture of embryonic

stem cells from blastodermal cells or PGCs, expectations are high that it will be possible to develop transgenic chickens using cultured cells from either source.

Sperm-mediated transfection

The transfer of DNA utilizing sperm is viewed as intuitively simple for transgenics based on the early work on generating transgenic mice (Lavitrano *et al.*, 1989) and pigs (Lavitrano *et al.*, 1997). The enthusiasm for this approach was subsequently tempered when other laboratories failed to repeat these experiments in mammals (Gandolfi, 2000). Sperm-mediated gene transfer is successful in fish and some invertebrates but the sperm must be electroporated or microinjected into the egg cytoplasm (Wentworth and Wentworth, 2000). So far, sperm-mediated gene transfer in the chicken has been ambiguous at best. It is known that DNA can specifically bind to rooster sperm using a variety of liposome preparations (Squires and Drake, 1993). Attempts at generating transgenic chickens using sperm have not been shown to be consonant with Mendelian inheritance and in some cases the DNA is not integrated into the genome (Rottmann *et al.*, 1992; Nakanishi and Iritani, 1993). Transgenic mice and pigs have now been produced by binding a monoclonal antibody to the sperm and then binding the DNA to the antibody-coated sperm (Chang *et al.*, 2002). The antibody recognized sperm from various species, including chicken, and offers the hope that this approach will work with the domestic fowl.

Cloning the domestic fowl?

Since cloning of the sheep 'Dolly' using nuclear transfer (Wilmut *et al.*, 1997) and the subsequent production of transgenic livestock using cloned transgenic cells (Schnieke *et al.*, 1997), cloning now provides another option for generating transgenic mammals. This has spawned

some speculation as to whether cloning through nuclear transfer is possible in birds. The task seems daunting for the same reasons that have plagued avian transgenics from the beginning. Is it possible to adapt, once again, the techniques used in mammalian cloning to the avian egg, i.e. enucleation, nuclear transfer and normal development of the embryo? Many unknowns currently exist in this regard, yet the potential for the pharmaceutical production of proteins in eggs has stimulated commercial laboratories (rather than academic or government institutions) to examine this possibility. Certainly the large capital investment required for such a task seems to be best met in the commercial sphere. Only time will tell whether cloning, avian embryonic stem cells, or sperm-mediated transfection will become part of the short menu of options available for those who would like to use transgenics as a basic tool for studying avian biology.

Some Practical Considerations in Developing Transgenic Birds and Future Issues

The effort to develop transgenic poultry is substantial and requires a significant investment of time, laboratory facilities and bird facilities. Before venturing into transgenics, it is important to have an understanding of the contribution that various genes make towards the phenotype of interest so that a more predictable result can be expected. For single-gene traits, this can simply mean the cloning and isolation of the gene and associated regulatory sequences. However, to influence complex traits such as feed efficiency, egg production or growth, several key genes will probably need to be identified. As the work in avian genomics proceeds, the evaluation of global gene expression and its association with economically important traits will help to make better choices towards manipulating the avian genome. Once a specific locus is identified for modification or a candidate gene is selected, a choice must be made regarding

how to develop the transgenic. This choice will influence the make-up of the vector used, the screening procedures, and the method of evaluating expression. The generation of founder transgenic birds (G_0), which are often mosaics, requires extensive mating and screening for the transgene in G_1 individuals. Once these G_1 birds have been produced, it is usually only possible to begin the evaluation of gene expression and the specific phenotype in the hemizygotes. However, it is also necessary to determine whether the insertion of the transgene results in a lethal phenotype; hence, homozygous individuals must be generated. This often carries the analysis to the third generation. Subsequent steps require the production of transgenic breeding stock. This could be accomplished through crossing the selected transgenic birds to selected non-transgenics, then crossing the hemizygotes to produce homozygotes. In doing this, inbreeding must be monitored carefully. This can be alleviated somewhat by producing more than one transgenic founder so that inbreeding can be kept to a minimum; however, given the technology as it exists today, it is unlikely that several transgenic founders with the desired phenotype can be generated.

The above considerations are mild compared with the next steps required to develop the transgenic birds for the marketplace. Given the controversy over genetically modified plants in recent years, entry of genetically modified animal products for human consumption will certainly have its share of government regulatory hurdles and critics from certain segments of society. In the Unites States, transgenic foods from animals are regulated by the Food and Drug Administration (FDA). Interestingly, the FDA anticipates that most of the gene-based modifications of animals for food production will be regulated as new animal drugs. This best fits those transgenics that have an agricultural benefit, such as improved disease resistance or increased production performance. The apparent view is that expression of a protein through transgenesis is no different than the administration of the protein to the animal in question. Products

regulated as 'new drugs' will be evaluated for animal safety, safety to the environment, and safety for consumers in eating the foods.

The regulation of transgenic animals to produce pharmaceutical proteins, such as the production of proteins in eggs, is apt to be slightly more complex. First would be the regulation of the transgenic birds themselves. It would seem unlikely that birds created to produce therapeutics would be allowed to enter the food chain. There is no doubt that the housing, biosecurity and husbandry standards for transgenic chickens designed to make human therapeutics will be very different from those used for the production of table eggs. The second regulatory question is the efficacy and safety of the proteins produced for therapeutic use. This adds another tier of regulation into the process of producing a marketable therapeutic product using transgenic chickens even though the same product is produced using another process, e.g. recombinant protein production through fermentation.

With the lessons learned from the genetic engineering of plants, the regulatory route to commercial products via transgenic animals will certainly evolve as society grapples with the issues. Social fears of 'unnatural' manipulation, 'Franken-food' and unsafe foods will have to be addressed before any transgenic animal becomes a consumer product. In this regard, cooperation between the scientific community, government regulatory agencies and the industrial sector will go a long way to allay the fears associated with genetically modified products. Social acceptance of the production of biologicals such as antibodies and therapeutic proteins from transgenic animals seems less problematic on the surface. This, and the fact that the pharmaceutical industry is facing a shortage in protein production manufacturing capacity, might explain the recent commercial interest in using chickens as 'bioreactors'. Whether the first product developed from transgenic poultry will be a food commodity or a biological therapeutic is unknown at this time. What is certain is that as research continues and the knowledge base on the molecular genetics of the domestic fowl expands, transgenic technology will continue to be one of the means that has the potential to yield new products and a new facet to the poultry industry.

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34 The Future of Molecular Genetics in Poultry Breeding

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Introduction

It is a daunting prospect indeed to be asked to speculate on the future of any field of biology, especially one as volatile and market-driven as molecular genetics[1] in poultry breeding. For example, as recently as the 1980s, it would have been nearly ludicrous to speculate on obtaining the complete DNA sequence of the chicken genome, but today one can expect this to be available by 2004 (estimated 95% confidence interval: ± 2 years). However, I take comfort in Lincoln's words, 'The world will little note, nor long remember what we say here,' in the certain knowledge that his statement will apply with far greater accuracy to my comments than it did to his.

Some might say that being asked to comment on the future of molecular genetics in poultry breeding is like being asked to comment on the future of the string quartet on MTV™. Certainly both molecular genetics and poultry breeding have exciting prospects, but is there any intersection between their two separate paths? Indeed, one of the editors of this volume (WMM) annually reminds me of the limitations of applying molecular information to poultry, but I suspect that I find his simulations more persuasive than most. The title assigned to this chapter (not by me) implies that most of us believe that there *is* a future for molecular genetics in poultry breeding, but just what might it be? Below, I attempt to deal with my assigned task, but I would also point the interested reader to other discussions of this topic (not all focused on poultry) that they might find more edifying (Haley and Visscher, 1998; Dodgson and Cheng, 1999; Dodgson *et al.*, 2000; Hill, 2000; Georges, 2001).

BLUPers and GENEees: the Odd Couple?

As pointed out elsewhere (Dodgson *et al.*, 2000), poultry genetics can be viewed as distributed along a sliding scale that at the extremes involves a totally quantitative or totally molecular approach. However, neither the science nor the scientists are normally distributed along this scale; indeed, they tend to cluster at its ends. At the quantitative extreme are those only interested in phenotypic effects; for the purpose of discussion, we shall call them the BLUPers. At the molecular extreme are those (the GENEees) solely focused on the mechanisms through which relevant genes/alleles

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[1]In this chapter I use molecular genetics and the somewhat more restrictive term 'genomics' interchangeably. See Kuska (1998) for the etymological origin of genomics.

induce an effect. Of course, at least in the agricultural domain, both camps are interested in economic traits, whose hereditary aspects are generally ascribed to so-called quantitative trait loci or QTL,[2] but they often approach QTL in very different ways. Indeed, one sociologist has even made a study of the contrasting attitudes of (rice) breeders and molecular biologists and suggested that 'cognitive empathy' was called for to promote an interdisciplinary rapprochement (Haribabu, 2000). So I will try to apply cognitive empathy in the discussion that follows.

At least in the poultry industry, the BLUPers presently have the upper hand. Purely phenotypic selection has made, and continues to make, extraordinary progress, at least for major production traits. In addition, the BLUPers are generally more in tune with the real challenges that confront the industry. As pointed out elsewhere (Dodgson and Cheng, 1999; see also Muir, Chapter 28), the structure of the worldwide poultry breeding industry and the very low profit margin per animal make it more difficult to justify molecular interventions on an economic basis. Among the most compelling of the economic cases against use of molecular information is the fact that modern poultry breeders are truly caught in the classical 'Red Queen' dilemma: they are running as hard as they can just to stay in the same place (not in real terms, of course, but with respect to competitors in the marketplace). There is little opportunity to slow down the pace in order to introgress desirable single-gene or multi-gene QTL from inferior background germplasm into commercial lines.

However, the cry of the GENEee is also heard upon the land. First and foremost, the rate of progress in genomic technology has been truly phenomenal, and the success of molecular approaches to medical and plant genetics is undeniable. Second, what we have learned about the human genome and those of model organisms refutes many of the classic assumptions of quantitative genetics, i.e. the infinitesimal model, the preponderance of additive genetic variation, and the relative unimportance of epistasis. Despite the proven results, it is difficult to believe that a system based on such fantasies cannot be improved. The question that remains unanswered is: how?

One thing seems certain: the worldwide demand for poultry products will only continue to grow, thereby providing economic impetus to apply new technology. A recent study by the International Food Policy Research Institute (Rosegrant *et al.*, 2001) estimates that poultry will account for 40% of the total increase in meat demand of 119 million tonnes from 1997 to 2020 (equivalent to about a 60% increase over 1997 meat production). While poultry, of course, is not immune from food safety concerns and other disease problems, foot-and-mouth disease and bovine spongiform encephalopathy (BSE) have the potential to make poultry even more predominant in this picture. Surely, in the long run, some mix of phenotypic selection and molecular genetics will emerge to help in meeting the expanding international demand for high-quality animal protein. One suspects that the balance in poultry will be more towards the BLUPers than in cattle or even pigs, but future developments in chicken genomics might prove otherwise. The remainder of this chapter will consider some possible outcomes of the mating ritual between BLUPers and GENEees that is now under way. (For more humorous and insightful treatments of the flirtation/marriage between molecular and quantitative genetics, the reader is referred to Gibson, 1999, and Soller and Medjugorac, 1999.)

Expected Progress in Poultry Molecular Genetics

It seems likely that molecular and quantitative genetics will, in the near term, continue

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[2]For purposes of this discussion, a QTL is defined as any site in the genome at which (at least) two *alleles* of different effect exist that are segregating in the population in question. Note that this is distinct from the gene in which the QTL alleles reside.

on parallel tracks but with increasing dialogue (or dalliance?) and, hopefully, increasing relevance between the two. One can predict the course of poultry molecular genetics with some confidence, despite the rapid technological developments that have characterized genomics during its brief lifetime. This is because chicken genome research necessarily travels in the wake of human and model organism genomics, mimicking their successes, albeit with lower volume and a much lower price tag (Dodgson *et al.*, 1997; Dodgson and Cheng, 1999). The discussion below will focus on the chicken, but most of the commentary applies to (at least) turkey and quail, although other poultry (and avian) species stand with respect to the chicken in much the same situation as chicken stands with respect to human. Each of the sections below has been covered in greater detail earlier in this volume. Here I only provide my estimates of likely progress in each domain and a personal assessment of the possible impact on poultry breeding. My estimates (Table 34.1) may or may not agree with those lucky enough to get a less global chapter assignment than I did.

Genetic linkage map
(See Aggrey and Okimoto, Chapter 23; Groenen and Crooijmans, Chapter 26)

The present consensus chicken reference linkage map (Groenen *et al.*, 2000; Schmid *et al.*, 2000) consists of about 2000 markers mapped with varying precision in one or more of three separate crosses. The map is fairly inclusive (i.e. any new marker has a > 95% chance of being linked to an existing marker), and it provides a suitable tool for low-resolution (10–20 cM) QTL searches (e.g. Vallejo *et al.*, 1998; van Kaam *et al.*, 1999a,b; Yonash *et al.*, 1999). However, the map also has several deficiencies in its present state (Dodgson and Cheng, 1999). Most notably, there exist several regions of low marker density and only about 40% of the markers are polymorphic microsatellites that can be used in many different populations. Secondly, many of the markers have been mapped using only approximately 50 meioses, so distances and, to a lesser extent, marker order are often uncertain. Thus, the present chicken linkage map can only claim 'moderate' resolution, at best, by today's standards. Thirdly, only about 10% of the markers are in known (evolutionarily conserved) genes, so the comparative chicken–human map has low resolution (approximately 20 cM average marker-to-marker distance, but there are many gaps that are much larger). While nothing but hard work and/or high throughput stands in the way of addressing all of these deficiencies, progress has slowed in recent years, due in part to the mystique of the QTL (Gibson, 1999) that has lured genome mappers away from the tedious business of cloning random markers. Data from the only recent major

Table 34.1. Great expectations.

Benchmarks of progress in chicken genomics	Year[a]
Av. 2 cM resolution reference linkage map (2000 markers)	2000
Av. 0.2 cM resolution reference linkage map (20,000 markers)	Post-sequence
Low-resolution chicken–human comparative map (~200 loci)	2000
High-resolution chicken–human comparative map (> 1000 loci)	Post-sequence
BAC contig physical map (av. contig ≥ 2 Mb)	2002
Full genome sequence (draft quality)	2004
500,000 chicken ESTs in public databases	2004
Moderate quality array data (> 1000 genes) for all major tissues/stages	2003
High-resolution (0.1–0.3 cM) SNP map	Post-sequence
Cost-effective transgenic chicken technology (random gene insertion)	2003
Cost-effective transgenic chicken technology (targeted gene insertion)	2006
Routine use of molecular information in commercial breeding	?

[a]Guesstimates of the author, except when in hindsight.

effort (to my knowledge) to add chicken microsatellite markers (Takahashi *et al.*, 2000) have not been integrated with the consensus map, nor are they available in the public sector. It seems clear that substantive improvement of the chicken linkage map will now await the availability of an integrated physical map and, especially, a full genome sequence (see below). In theory, at least the order and spacing of existing markers could be improved by integrating data from QTL searches into the consensus map, but many of these results also are not public, and it is still unclear to what extent resource and reference population data can be combined for the chicken.

Comparative chicken–human map
(See Burt, Chapter 29)

One of the most pleasant surprises in recent years has been the extent to which synteny and, to a lesser extent, gene order have been conserved between chicken and mammalian (especially human) genomes (e.g. Burt *et al.*, 1999; Groenen *et al.*, 2000; Waddington *et al.*, 2000; Suchyta *et al.*, 2001). At least in part, this happy circumstance is probably an overly optimistic interpretation of 'the early returns', since only poor resolution is now available on the chicken side (Table 34.1), but continuing research will provide a high-quality comparative map that links regions of the chicken genome with the corresponding intervals of the human genome (especially in regions containing 'hot' QTL). Just as with the linkage map, high-resolution comparative mapping will await the chicken genome sequence. Once available, we will surely find the map to be densely peppered with discontinuities (mostly retrotransposon insertion/deletion events) that will make exact alignment rather more difficult than we might now expect (Gottgens *et al.*, 2000; Suchyta *et al.*, 2001).

Physical map
(See Groenen and Crooijmans, Chapter 26)

A long-term problem with the chicken genome map has been lack of full correspondence between linkage groups and cytologically identifiable metaphase chromosomes, especially the microchromosomes. Recently, major progress has been made in this area, leaving only a handful of microchromosomes yet to be assigned (Schmid *et al.*, 2000). One can reasonably expect this effort to be complete very soon (probably by the publication date of this volume). A much more useful physical map is one in which most, if not all, of the genome can be assigned to one or more identified (overlapping) recombinant DNA inserts, typically chicken DNA fragments of about 150,000 base pairs (bp) carried in bacterial artificial chromosome (BAC) vectors. Each contiguous set of overlapping BAC inserts is a 'contig', and the sum of all the contigs is a BAC contig physical map. At least two programmes to generate chicken BAC contig maps are now under way (Lee *et al.*, 2001; Crooijmans *et al.*, 2001), and one can reasonably expect to have at least one or more first-generation BAC contig maps by the end of 2002. At least initially, such maps can be expected to divide the genome into about 500–1000 contigs (average size: 1–2 million bp). Considerable effort will still be required to close the remaining gaps in these maps and to align the genetic linkage map exactly with the BAC contig physical map.

Complete genome sequence
(See Groenen and Crooijmans, Chapter 26)

The ultimate physical map is the complete DNA sequence of the genome. For chicken, this would correspond to about 1.2×10^9 bp of sequence information. The BAC contig maps described above are a logical, but not essential, prelude to a full genome sequence. As the large human genome sequencing centres begin to complete the finishing phase of the human sequence, they require fodder for their sequencing machines. The chicken genome is an attractive target for both agricultural and evolutionary reasons, as well as its central importance as a model organism for developmental biology and virology. The present resources available for animal agriculture

genomics at the US Department of Agriculture (USDA) are not sufficient to complete such a project (estimated cost: $35 million), but the US National Institutes of Health (NIH) and several foreign nations have shown significant interest (Yang, 2001; J. McPherson, Washington University; M. Guyer, NIH; and R. Davis, Stanford; personal communications). It seems reasonable to expect that a full genome sequence of the chicken will be in hand no later than 2005. (It is unclear, however, whether the quality of the chicken sequence will be taken much beyond the present 'draft' quality of the human genome; e.g. International Human Genome Sequencing Consortium, 2001.)

Genes/ESTs/arrays
(See Cogburn *et al.*, Chapter 31)

Expressed sequence tags (ESTs) are single-pass sequences derived from either end of random cDNA clones (Adams *et al.*, 1991). Each EST corresponds to a portion of a particular expressed gene, but a single gene may generate numerous ESTs that, initially at least, may or may not overlap. The largest contributor to the approximately 34,000 chicken ESTs now present in GenBank has been the Delaware Chicken EST sequencing project (Tirunagaru *et al.*, 2000; www.chickest.udel.edu), but several other large-scale poultry EST projects are under way in Europe and the US (see Cogburn *et al.*, Chapter 31, for a more detailed discussion). Together, it is reasonable to expect that 100,000–200,000 chicken ESTs will be deposited in public databases by 2004. Chapter 31 also discusses the application of ESTs to generate microarrays for the global measurement of gene expression in various tissues, at different developmental time points, in different chicken lines, and in response to various environmental challenges (e.g. viral infection; Dodgson *et al.*, 2000). Thus, one can safely predict that by 2003, data will be forthcoming that indicate the identity of at least the predominant > 1000 transcripts in most chicken tissues at most key developmental stages. However, it is important to note that even a complete catalogue of gene expression in a given tissue has at least two major limitations. Firstly, the exact functions of most genes in the EST collections remain unknown (filling in this enormous gap will be the heavy burden of 'functional' genomics) and, secondly, most genetic variation (e.g. QTL) probably exerts direct effects not as changes in transcript levels, but as differences in protein function (e.g. missense mutations). EST arrays can detect only a subset of the latter (and then only via their secondary effects on downstream transcript levels).

SNPs
(See Kuhnlein *et al.*, Chapter 32)

Single nucleotide polymorphisms (SNPs) account for the bulk of genetic variation in humans (e.g. Cargill *et al.*, 1999) and can reasonably be expected to account for most QTL alleles in poultry. In theory, SNPs can provide the highest resolution possible in linkage (or linkage disequilibrium) maps. In addition to direct efforts to generate chicken SNP collections (Smith *et al.*, 2001), the genome and EST sequencing efforts described above will generate large collections of candidate SNPs (re-sequencing or other verification will be required to separate real SNPs from sequencing errors). Although SNPs are much less polymorphic than most microsatellites, these SNP collections should be large enough to provide fine structure mapping markers in most populations of interest. Will these collections also include the SNP alleles that probably account for most QTL? To date, SNP searches in panels of candidate genes have not been very productive in generating likely alleles that predispose to complex traits in humans (such as cardiovascular disease; Cargill *et al.*, 1999; Halushka *et al.*, 1999). As poultry QTL-encoding alleles may have even more obscure connections to nucleotide sequence than in the case of heart disease, considerable scepticism is warranted as to whether the actual QTL alleles will be found by random SNP searches.

Transgenic chickens
(See Petitte, Chapter 33)

When it comes to predicting the future of transgenic chickens, one steps into 'the heart of darkness'. Had my predictions of 15 or even 10 years ago in this regard been made in public, I surely would not have been asked to write this chapter. What both industry and scientists need is an efficient method that allows for gene insertion, deletion and replacement in live birds. I think I can safely say that almost all of the component parts of such a process are now functional in one or more individual systems or laboratories (see Petitte, Chapter 33). However, estimating when all the parts will be put together such that we shall be able *routinely* to generate desired transgenic birds in a *cost-effective* manner is still difficult, not the least because so much of the research is industry-supported and not publicly available. Soller and Medjugorac (1999) suggested that, 'In a somewhat mysterious way, needs have a way of inducing their fulfillments.' I generally agree, but this has yet to be the case in transgenic chicken research. Hopefully, by 2003 the picture will have changed for the better. The financial futures of several companies depend upon it. Perhaps more important, the search for the elusive QTL depends upon it. As pointed out elsewhere (Gibson, 1999; Dodgson *et al.*, 2000), final verification of a suspected QTL allele is the worst nightmare of the GENEee. Perhaps the only rock and sling capable of slaying this Goliath will be effective (gene replacement) transgenic chicken technology.

Incorporating molecular information into breeding
(See Fernando and Totir, Chapter 27; Muir, Chapter 28)

This area is distant from my personal experience and expertise, so I am sure the distinguished authors of the earlier chapters cited can estimate its future better than I, who possesses only the advantage of naïveté.

The following section is thus a PLUP (pretty lame unbiased prediction). I will not cite or try to summarize the numerous theoretical treatments of the possible prospects for marker-assisted selection (MAS), other than to say that the outcomes are clearly model dependent. At this point, the most critical factor appears to be the *cost* of obtaining molecular information (again I quote Soller and Medjugorac, 1999: 'What this country needs is a good 5 cent data point.'). For those cases where random selection is now being practiced, 'what could it hurt' to use molecular information to influence that selection (as long as it does not cost too much)? Clearly selection on traits that require costly progeny testing (egg production in sires, disease resistance, etc.) and that is rarely now in practice offers the most attractive targets, if QTL alleles of significant effect can be identified and localized within a well-marked interval. The per marker cost of genotyping seems likely to decrease to the point that the incorporation of marker information for one or a few individual QTL alleles will become attractive. The ideal situation is exemplified by the halothane locus in swine: one QTL of large effect on an important trait, ability to genotype the QTL allele directly (rather than requiring linked markers), and the desirable allele already existing in commercially relevant stock (reviewed in Webb, 1998). In such a situation, the use of a single marker would allow one to drive the preferred allele to fixation in a population large enough that one might lose a minimal amount of potentially desirable linked genetic variation. Large-scale genome information (as is needed for marker-assisted introgression or to fully incorporate genetic relatedness into BLUP; Haley and Visscher, 1998) is, of course, much more costly. While theoretically attractive, it seems likely that these more global MAS programmes will take significantly longer to come into wide use in poultry breeding.

Whereas molecular approaches have been used to fix the advantageous halothane allele in pigs and there are serious efforts being made to introgress Meishan litter-size QTL into commercial herds (Webb, 1998), to

my knowledge the equivalent has yet to occur in poultry. Is it because, by chance, we have yet to come upon such obviously advantageous targets or because the financial structure of the industry and the cost per animal cannot justify it? Or is it that the rate of progress still available in poultry makes any delay needed to incorporate marked alleles untenable (the Red Queen conundrum, mentioned above)? Time will tell. However, one can be certain that the prospects for using marker information can only improve as more science gets done and the genotyping cost decreases.

2020 Vision, Building the 'Ideal' Chicken

To get a better handle on this problem, let us consider it from the hypothetical perspective of a 'super geneticist' who knows all the genes in the chicken genome (approximately 30,000–40,000 genes,[3] within a factor of 2), has a good idea of what each gene does, and wishes to produce the 'ideal' chicken (at least for a given trait of interest, e.g. egg production). I am not suggesting that this knowledge base will exist for the chicken by 2020, but it is a realistic goal for many model species, now being actively pursued. First one might ask: how many of these 30,000 genes could *possibly* influence a complex trait like egg production? Both human disease mutations and transgenic mouse experiments suggest that allelic diversity in one gene can have very wide pleiotropic effects. In addition, where detailed biochemical analysis of (especially) vertebrate proteins has been done, it is often observed that a single gene product contains numerous domains that perform separate functions and interact with multiple pathways (Henikoff *et al.*, 1997). Thus,

it would not seem unreasonable to guess that allelic diversity in 1000 or more genes might *somehow* affect a particular trait value under consideration. However, as discussed elsewhere (Dodgson *et al.*, 2000) and inherent in my definition of QTL, we need to think not about genes but about QTL *alleles*. Each of the approximately 1000 genes could harbour on the order of 10 or more sites[4] of allelic variation that generate a non-null phenotype (trait value). Furthermore, the effects of the alleles at each of these loci need not be independent (i.e. there may well be epistasis). Thus, our 'super geneticist' would have many thousands[5] of effectively different allele combinations (genomes) to try out in search of the ideal bird. Even if she or he had this vast knowledge of the genome, how could our super geneticist then create this ideal chicken? Note that even in a large commercial population of birds, a generous estimate (Hayes and Goddard, 2001) might be that 100 QTL of significant effect are actually segregating. Thus, *in absence of new mutations*, breeding within this family could 'explore' only a small percentage of the possible useful combinations. This is the fundamental problem facing all poultry geneticists, super or otherwise.

How might the BLUPer or GENEee help us in our search for the ideal chicken? I have argued that the existing genetic variation in even a large commercial population is insufficient, in and of itself, to come close to the ideal. On numerical grounds alone, it seems highly unlikely that all (or almost all) important QTL alleles are already fixed in these populations, especially when most of the traits 'desirable' for animal agriculture differ from those that nature selected over millions of years in the jungle fowl progenitor genome.[6] Inbreeding during domestication

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[3]Here, I use 'gene' in the broad sense to include not only an individual coding sequence but also flanking control regions in which allelic diversity might influence gene and trait expression.

[4]Recall that, inherent in the definition of the gene being used here, this allelic variation may reside both within the coding sequence of the gene in question or within flanking regulatory sequences.

[5]Obviously, no useful trait can be so accurately assessed that one could measure many thousands of different effective trait values that might be generated by all possible allele combinations in the genome, but my focus here is to estimate the complexity of the genotype, not that of the phenotype.

[6]In addition, were most major QTL alleles already fixed, it seems likely that genetic progress would have slowed, if not stopped altogether, much more quickly than has actually been observed (e.g. Pollock, 1999).

may have further reduced the available useful variation in the genome of commercial chickens. For example, de Vicente and Tanksley (1993) found desirable QTL alleles in wild relatives of the domestic tomato, even when the phenotype of the wild species was far inferior, overall, to that of the domestic variety. Thus, breeding alone within the existing genetic base will probably not do the trick.

On the other hand, this analysis demonstrates the fundamental *advantages* of phenotypic selection: (i) it works with the full (but comparatively narrow) range of existing genetic variation to seek a local maximum among hundreds to thousands of relevant QTL allele combinations (i.e. the infinitesimal model); and (ii) as most widely advocated by Hill (e.g. 1982), new mutations are likely to arise, even in finite commercial populations, and phenotypic selection is the *only* way to retain and potentially fix favourable new spontaneous mutations. The BLUPer is clearly the person who does not know that much about any one thing, but knows a little bit about a whole lot of things. More important, the BLUPer's mind is open to change.

Can the GENEee help us at all in our search for the ideal chicken? The GENEee is working with, at best, a handful of QTL alleles. They may well be the most important alleles (at least until they get fixed), but the GENEee is clearly outnumbered by the BLUPer, who has thousands (or even hundreds of thousands) of combinations available (allowing for mutation). However, in the near future, the GENEee will be able to know not only where her/his QTL maps, but in many, if not most, cases a convincing argument will exist to identify the relevant candidate gene itself. This then will open up a cornucopia of additional valuable information: biochemical studies and proteomics, microarray results, comparative analyses in other species, phenotypes of transgenic animals in model organisms, etc. Indeed, as argued elsewhere (Dodgson *et al.*, 2000), normally it will be *only* with the help of

these additional approaches that candidate QTL alleles can be identified. The GENEee, then, is the person who knows only one thing (or a few), but knows it very, very well. All this additional information will open up a variety of approaches to improving poultry productivity, only some of which will employ breeding strategies.

As we look 20 years down the road, it appears to me that the single most critical factor in determining the ideal balance between phenotypic selection and molecular biology is the feasibility of transgenic approaches in animal improvement. The BLUPer has considerable genetic variation available but must rely on two cumbersome processes: mutation and recombination. Mutations, especially beneficial mutations, are rare and often difficult to fix, unless they are of very high selective value. Recombination events likewise can be rare, especially if one seeks new combinations of closely linked alleles (e.g. within the same gene). 'Directed evolution' studies (e.g. Kolkman and Stemmer, 2001) that speed intergenic allele rearrangement using PCR suggest that such *in vitro* recombination can outdo millions of years of natural selection in generating desirable traits[7] (especially when those traits relate to human rather than 'nature's' desires). Transgenic technology would allow the GENEee to employ *in vitro* recombination and *in vitro* mutagenesis to outpace the 'old-fashioned' natural selection processes used by the BLUPer. In the short time frames most relevant to commercial interests, this is probably the only way to go much beyond the local maximum trait value that can effectively be reached in commercial populations and explore the full potential of the chicken genome.

To appreciate realistically the potential for transgenics in poultry breeding, one needs only to look at crop species. For a variety of reasons, both quantitative and molecular genetics tend to run several years ahead in major crops vs. agricultural animals. Although significant progress has been made in crops with MAS and related

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[7]Note that high-throughput assays of, or selection for, the desired effect are also required for this to work.

breeding techniques, this pales in comparison with transgenic technology. At least in North America, the majority of some major crops are grown from transgenic seed, and there have been field trials of transgenics of at least 52 different species (Dunwell, 2000). A wide variety of traits is being explored with transgenics for eventual use in the field (Cushman and Bohnert, 2000; Melchers and Stuiver, 2000; Richards, 2000). The present status of transgenic chicken technology may be as much as a decade behind that of crop plants (perhaps more important, the number of scientists addressing this challenge is many fewer). However, even in crops, the time between initial planning and final release of a new variety is 10–12 years (Dunwell, 2000), so the productivity and widespread field trials we see today are in many cases the products of transgenic plant technology as it existed many years ago.[8] Some might argue that the public is already suspicious of genetically modified crops and will never accept transgenic animal products. My guess is that, in the long run, consumers will make fairly rational cost/benefit decisions. For example, it is quite conceivable that we will have transgenic cattle that are resistant to BSE in the not-too-distant future, and these might open the door to wider consumer acceptance of the products of this technology.

In summary, it is reasonable to expect that phenotypic selection will continue to be the workhorse of poultry breeding, and it seems certain that continued genetic progress in the chicken will be made (i.e. we are still a long way off from the 'ideal' chicken). The real question is whether continued progress in quantitative genetic theory will be made. Certainly computer technology will make it possible to collect and process more data and to employ more sophisticated models, but how far can one go with a technology based on *not knowing* what the genes/alleles are actually doing? Meanwhile, molecular genetics (genomics) technology continues to

move forward at breathtaking speed. Had I been asked in 1990[9] to make similar estimates for the human or mouse genomes to those in Table 34.1 for the chicken, I surely would have been at least a decade too conservative in almost all cases. Despite the fundamental problems in applying genomics to poultry breeding that I have discussed, it seems difficult to believe that it will not soon reach the stage where it can make significant contributions. Furthermore, I have argued that possibly the greatest impact of molecular genetics on poultry breeding in the long run could arise from the development of robust transgenic chicken technology.

Acknowledgements

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[8]Alternatively, consider the opportunities for transgenesis from the perspective of the cat. Its meat source of choice, the mouse, can already have its genotype engineered to fit almost any conceivable feline desire.
[9]I choose 1990 because this is roughly when human genomics (or mouse with regard to transgenics) was in a similar state to that in which chicken genomics now finds itself.

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