BREEDING FOR DISEASE RESISTANCE: ISSUES AND OPPORTUNITIES

S.C. Bishop[1], J. Chesnais[2] and M.J. Stear[3]

[1]Roslin Institute, Roslin, Midlothian EH25 9PS, United Kingdom
[2]CCSI Inc., Central Exp. Farm, Bldg #54, Maple Drive, Ottawa, Ontario, Canada K1A 0C6
[3]Glasgow University Veterinary School, Bearsden Road, Glasgow G61 1QH United Kingdom

INTRODUCTION

Background. Infectious disease adversely affects livestock production and animal welfare. The costs of disease are estimated as 17% of turnover in the developed world (£1.7 billion in the UK) and 35-50% in the developing world. Disease resistance is often cited as the next great challenge facing animal geneticists. It is in fact a current major challenge, with large research efforts worldwide. Yet, with some exceptions, often there has been little tangible output in terms of implemented and successful breeding programmes.

There are many documented examples of genetic differences between animals in resistance to specific diseases or tolerance of infection. Examples exist in all major domestic species, and a summary is given in the appendix for chickens, cattle, pigs and sheep [most are reported in Axford *et al*. (2000) and OIE (1998)]. From this list we can draw two tentative conclusions: (1) wherever a disease is studied with sufficient detail, genetic differences between animals in resistance are usually found and, hence, (2) there are likely to be many more examples of genetic differences between host animals in disease resistance. This indicates that there is an excellent foundation upon which to base selection for disease resistance, although selection is but one of several ways in which genetics can be used to help control disease problems. The more generic term of genetic management of disease problems will be used to describe the use of genetics or selection to help control diseases. This paper critically evaluates the role of genetic management strategies in controlling disease.

Pressures on breeders to select for resistance. The need to develop sustainable production systems provides a compelling impetus for breeding to select for disease resistance. Foremost is the evolution of resistance in parasites to chemical or vaccine control measures. For example, anthelmintic resistance in nematode parasite problems is now prevalent in many countries with major sheep industries, requiring the development of alternative control measures, of which selection for resistance is an option. Likewise, antibiotic resistance places pressures on intensive livestock sectors to select animals for resistance, as do virulent pathogen strains resistant to current vaccines, as in Marek's disease (Witter, 1997). Required are integrated disease control strategies, of which genetic management is a component.

A recent phenomenon is that of Governments dictating breeding strategies to farmers. An example is the drive to remove scrapie from sheep flocks in Western Europe, using selection on PrP genotype. Additionally, legislation on antibiotic usage may push breeders to select for goals such as increased generalised immunity. Finally, most breeders will be motivated by increased profitability and a competitive edge for specific traits. Selection for disease resistance will help enable this. Moreover, when considered in an epidemiological context, as described below, there may well often be an epidemiological added value from selection, as the

enhanced resistance ultimately reduces the disease transmission and the degree of challenge faced by uninfected animals.

MECHANISMS OF DISEASE RESISTANCE

Disease resistance is usually mediated by innate and acquired immunity. Innate immunity is rapid, non-specific and acts as a first line of defence. It predominantly involves neutrophils, macrophages, natural killer cells and γδ cells, as well as factors such as acute phase proteins. Acquired immunity involves antigen specific responses via lymphocytes, i.e. T cells and B cells, which are produced through unique genetic mechanisms. A combination of gene segment re-arrangement, point mutation (occurring at about 10^7 times the normal rate), and gene conversion generate a very large repertoire of T cell and B cell receptors (approximately 10^{10} for each) with which to sample the antigenic environment. Without these mechanisms, such a repertoire would require more DNA than is present in the entire mammalian genome. An equally important genetic component of the immune system is the major histocompatibility complex (MHC), which derives its complexity from the large number of allelic variants at the more than 200 loci. There are significant differences in genetic mechanisms for acquired immunity amongst mammalian species and between birds and mammals, which have implications for disease resistance. In addition, there is considerable interplay between the innate and acquired systems. Resistance to many common infections is often the result of a combination of both.

Non-immune genes can also be involved in disease resistance. Obvious examples include genes coding for receptors such as those required for *E. coli* to bind to the gut, or the vitamin D receptor for resistance to tuberculosis. Genes controlling adaptative, behavioural, or physical characteristics, such as skin thickness for some vector-borne diseases, can also play a role.

EPIDEMIOLOGICAL CONTEXT

Pathways of infection. The value of using host genetic variability in the management of disease problems will depend not only on the nature of the genetic variation in the host population, but also upon the transmission attributes of the disease itself. Possible pathways of infection are shown, with great simplification, in figure 1. There are many diseases where pathway **a** is important and continual, and pathways **b** and **c** are non-existent or trivial.

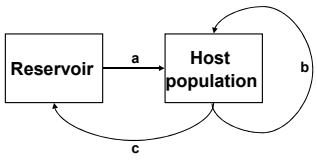

Figure 1. Summary of pathways of infection for diseases of domestic livestock

Examples include trypanosomosis and mastitis caused by environmental contamination. Subclinical disease challenges in intensive production systems, addressed using the concept of generalised immunity, may also fit into this framework. Diseases where pathway **b** is critical, with sporadic infection from the reservoir (pathway **a**) include viral diseases. Diseases where pathways **a** and **c** are critical, i.e. a continuous flow of infection between the host population and the reservoir, include nematode infections in ruminants, where the reservoir is the pasture, and some bacterial diseases. Successful genetic management of these diseases should decrease **c**, leading to a reduction in pasture or environmental contamination and, indirectly, to a further reduction in pathway **a**.

Resistance *vs.* tolerance. Resistance is best defined as the host's ability to moderate the

parasite lifecycle, and may comprise several components of the host-parasite interaction, e.g. increased resistance *per se* (i.e. the ability to lessen the probability of infection), altered latency or reduced infectivity. Improving resistance has the benefit of both enhancing animal health and reducing disease transmission, i.e. impacting upon disease epidemiology. Tolerance is the ability of animals to tolerate infection by the pathogen and withstand the effects of disease. In some cases it is an effective strategy for minimising the effects of the disease, an example being trypano-tolerance in cattle. However, trypanotolerant cattle breeds also control trypanosome growth better; tolerance and resistance may often be correlated. Breeding animals tolerant to a disease would be disadvantageous for non-selected animals in the same environment. Also, breeding animals tolerant to a disease would be disadvantageous when the aim is to reduce transmission of infection, e.g. for zoonoses the prevention of transmission to humans is essential.

Epidemiological consequences of selection. The pathways of pathogen transmission ensure that there will be epidemiological consequences of selection for resistance that often are not predictable by classical genetic theory – in many circumstances a combination of genetics and epidemiological theory is required. For example, for diseases transmitted by direct contact, the consequences of genetic management will be seen in terms of a reduced probability of an epidemic should the infectious agent be present, and/or a reduced epidemic severity should an epidemic take place (MacKenzie and Bishop, 2001). This conclusion has also been drawn from analysis of field data of disease incidence (Henryon *et al*., 2001). For infections where there is a continuous cycling of infection between the animal and a finite reservoir, such as nematode infections, improvements in the health, welfare and productivity of the host population can be expected (e.g. Bishop and Stear, 1999), as well as benefits for other populations in the same environment. Here, benefits of selection may be substantially greater than those predicted by genetic theory alone. Only when infection is from an essentially infinite reservoir (pathway **a**) is it not necessary to use epidemiological models to predict consequences of genetic management strategies. Such strategies may improve animals' resistance to the disease challenge, with an improvement in animal health, welfare and productivity, but have no detectable impact on the potential transmission from the reservoir. In contrast to resistance, tolerance should have little or no impact upon the transmission of the pathogen. Therefore, there will be no direct epidemiological consequences in terms of the prevalence of the pathogen, although the incidence of disease will be reduced.

Generalised immunity. In some circumstances there is a desire to genetically improve the health status and performance of groups of animals, or provide them with protection against accidental infection from existing or new diseases. Additionally, the breeder may not wish to base a genetic management strategy upon a single mode of resistance, as selection for one mode has sometimes been shown to adversely affect the other. In such circumstances it may be appropriate to select for generalised immunity, based upon a combination of immune response variables. Such variables need not be related to specific disease challenges. The feasibility of such an approach has been explored in pigs by Mallard *et al.* (1998) and in chickens by Pinard *et al*. (1992) and Sarker *et al*. (1999). In addition to these studies, the genetic control of various immune measurements is well demonstrated (e.g. Edfors-Lilja *et al*., 1998 and 2000). Effective selection for generalized immunity, however, entails the challenging task of finding a limited number of indicator traits,

genes or markers that reflect resistance to a wide range of diseases.

Pathogen evolution. Selection for resistance has the drawback, under some circumstances, of imposing selective pressure upon the pathogen. In common with strategies based on vaccines, antibiotics and anthelmintics, hosts with enhanced resistance that compromises the survival of the pathogen run the risk of providing a selective advantage for new forms of pathogens – i.e. parasite evolution (Gandon *et al*., 2001). Tolerance, on the other hand, is an evolutionary stable strategy that does not necessarily place selective pressure upon the pathogen.
Risks of pathogen evolution with selection for resistance are complex and depend upon many factors. Major factors influencing risk include: (1) selection intensity placed upon the pathogen – situations where only the pathogen that survives is the one with a mutation conferring a selective advantage against the treatment will ensure proliferation of that pathogen genotype, hence co-evolution; (2) relative generation intervals of host and pathogen - these may be large for viral infections but much smaller for (e.g.) nematode infections; (3) genetic heterogeneity of host resistance – the more complex the mechanisms of resistance the greater the challenge to the pathogen and the less the risk of pathogen evolution.

GENETIC VARIATION IN DISEASE RESISTANCE: RESEARCH APPROACHES

Quantifying genetic variation in resistance. The appendix summarises many of the diseases for which there is genetic variation in resistance. The first indication of genetic variation in disease resistance often comes from breed comparisons, especially the ability of some breeds to tolerate endemic diseases or the ability of specific breeds to withstand an epidemic that sweeps through a population. A well-documented example is gastrointestinal nematode resistance in ruminant livestock (Gasbarre and Miller, 2000), which has been quantified under both temperate and tropical conditions for a variety of species of parasite. For example, in UK conditions the generic nature of genetic variation in resistance has been demonstrated for a variety of parasite types, sheep breeds and physiological status of host (Bishop *et al*., 1996; Bishop and Stear, 2001; Bishop *et al.*, unpublished). Another well-documented example of disease resistance is for mastitis in both dairy cattle (e.g. Heringstad *et al*., 2000) and sheep (e.g. Barillet *et al*., 2001). Both diseases share features that make them particularly amenable to quantitative genetic studies, *viz*. 1) they are endemic and hence predictable in their occurrence and 2) indicator traits of host response to infection exist, i.e. faecal egg count and somatic cell count, respectively. However, care must always be taken to ensure that indicator traits reflect differences in host response rather than disease challenge. In general, well-understood endemic diseases are more likely to be tractable than epidemic diseases, which require specific challenges.
It is not only studies of specific diseases or conditions that demonstrate genetic variation amongst animals in disease resistance. Recent analyses of field data in which diseases are classified into broad categories have also revealed genetic variation in disease incidence (Henryon *et al.*, 2001; Janss *et al.*, 2001). It appears that the heritability tends to rise as one goes from general disease category to specific disease resistance to specific immune response, with antibody responses sometimes being highly heritable (e.g. Mallard *et al*., 1998; Sarker *et al*., 1999; Stear *et al*., 2001). Analysis of responses to selection for resistance to specific diseases indicates that these are similar to selection for production traits (Morris, 1998).

QTL and major gene detection. There are compelling reasons for attempting to detect QTL or genes underlying disease resistance. Most importantly, QTL or genes allow selection for disease resistance in the absence of disease challenge. Such challenges can be expensive, wasteful of animals or, for field data, unreliable. Field data requires knowledge of disease epidemiology, so that the challenge faced by the animal can be factored into the calculations. Whilst this may not be an issue when disease challenge is guaranteed, it becomes a problem with sporadic disease conditions. Genetic architecture of disease resistance varies from an implied large number of genes, e.g. nematode resistance or mastitis, to single gene effects for several diseases including the PrP conferring resistance to scrapie (Hunter, 2000) and genes on pig chromosomes 13 and 6 conferring complete resistance or susceptibility to neonatal and post-weaning *E. coli* diarrhoea, respectively (Edfors-Lilja and Wallgren, 2000).
Much of the emphasis in mapping resistance QTL is currently based on nematode resistance and mastitis, where several large programmes exist worldwide. Although these have generally been successful (e.g. Klungland *et al*., 2001; Coltman *et al*., 2001), these are diseases where indicator traits are reliable and available and, hence, QTL are not vital. Other diseases where considerable success has been found in identifying QTL include Marek's disease (Bumstead, 1998; Yonash *et al*., 1999), both within and outwith the MHC region, and trypanotolerance (e.g. van der Waaij, 2001). In addition to these examples there are current QTL and gene detection studies for a variety of other diseases worldwide including scrapie (apart from PrP), salmonellosis, fleece rot and liver fluke in sheep, PRRS in pigs, salmonellosis in chickens, as well as general health status, immunity and vaccine response in various species. It will remain the case that the utility of QTL will be greatest for diseases that are not predictable in their occurrence or are difficult to diagnose. However, such QTL will be the most difficult to find. Additionally, known genes will be of much greater utility than QTL for which knowledge of within-family linkage phase is usually required. Only when markers are in population-wide LD with the gene, will within-family phase not need to be determined - this is almost equivalent to directly using the gene.

Functional genomics. Expression profiling by means of cDNA microarrays, often referred to as functional genomics, is currently a popular technology for gene discovery. The aim is to detect genes that are expressed in specific tissues at particular time points. Thus, it lends itself to disease response studies where the aim is to detect genes that are expressed in response to a pathogen challenge, in well-defined cell types. Knowledge of such genes has many applications in understanding disease pathology, genetic control of resistance and determining vaccine targets. However, an expressed gene will only be a QTL candidate under some circumstances, as (1) it may not differ between animals, (2) it may be metabolically downstream from causative mutations and (3) resistance QTL may be due to differences in protein form rather than quantity. Nevertheless, comparison of uninfected *vs*. infected animals and resistance *vs*. susceptible will be informative in understanding genetic control of disease resistance. Current microarray studies at Roslin Institute include a comparison of cattle genetic resistant or susceptible to theileria, immune response to vaccination in chickens and response to PPRS infection in pigs.

PRACTICAL IMPLEMENTATION

Current examples. Despite the large number of well-documented examples of genetic

variation in resistance, there are surprisingly few cases of structured commercial breeding programmes for disease resistance. The best-known examples are for nematode resistance in sheep, tick resistance, mastitis and Marek's disease. For nematodes there are breeding programmes underway in New Zealand, Australia and the UK; tick resistance is an integral part of cattle breeding programmes in subtropical Australia; mastitis resistance, either in terms of clinical cases of mastitis or reduced somatic counts, is incorporated into many dairy cow and sheep breeding programmes; and Marek's disease resistance is a feature of modern chicken breeding. Breeding for resistance to post-weaning *E. coli* diarrhoea in pigs, where the causal mutation or closely linked markers are known, has begun in some selection programmes. Additionally, scrapie resistance selection is now underway in several countries in Western Europe, although arguably this is imposed rather than a choice by breeders. Although this list is not exhaustive, it must be concluded that there are limited convincing examples of breeding schemes for disease resistance. Possible reasons are outlined below.

Opportunities. There are many unexploited opportunities for breeding for disease resistance, especially in cases such as neonatal *E. coli* diarrhoea in pigs where markers closely linked to the causal mutation are known. Moreover, the recent evidence from Henryon *et al*. (2001) demonstrates that within the context of well-designed breeding programmes there is ample opportunity to select for decreased disease incidence. This raises several questions: why there are not more examples of breeding for resistance in practice; under what circumstances will breeding be implemented and what information is needed for successful breeding schemes to be designed. In addition to those mentioned above, specific temperate climate diseases that could conceivably and relatively easily be selected against include PRRS in pigs, paratuberculosis and nematodiasis in cattle and footrot, paratuberculosis, and flystroke in sheep and goats. In a tropical context there are a number of potential target diseases, for which natural selection often acts when temperate species are introduced into tropical production systems.

Prioritising target diseases. With the exception of the well-known diseases, much of the convincing evidence regarding possibilities for selecting for disease resistance is either relatively recent or poorly documented. Therefore, industries have had insufficient time or evidence to consider breeding. In addition to this there is the problem of prioritising diseases. To forego selection pressure on production traits and focus on a specific disease, the disease must be one of overriding economic importance, for which other control measures are in some way inadequate or have harmful consequences (e.g. overuse of antibiotics). These will tend to be widespread endemic diseases - there are usually examples in each livestock production system. Prioritising diseases may not be straightforward, and often there will be disagreement over which are the critical diseases. Moreover, recording disease resistance will entail extra effort in industries that are generally already under pressure, and effective strategies for incorporating resistance traits into breeding goals are not necessarily straightforward, as described below.

In situations where there is no specific disease of overriding importance, then the focus should change to generalised resistance, to a variety of disease conditions, or generalised immunity. Evidence in support of this concept has been discussed above. Approaches include selecting on combinations of immune measures, which cover several axes of immune response, or simply selecting upon disease categories that appear to be heritable under the production system of

relevance. This approach will generally be more applicable under intensive than extensive conditions, e.g. for chicken or pig breeders, as specific endemic problems will be more likely and more easily identified in extensive production systems.

Incorporation into breeding goals. It is unlikely that disease resistance will be the sole selection criterion, thus resistance traits will need to be included in the overall breeding goal. This presents a challenge, and requires the breeder to reconsider the epidemiological context of the disease. The question to be addressed is what are the benefits of increasing resistance? These are best answered using genetic epidemiological models. For example, for nematode resistance the benefit may be calculated as the improvement in performance arising from the decreased pasture larval contamination (Bishop and Stear, 1999), or from the decreased treatment requirements. Likewise, for mastitis benefits come from decreased mastitis incidence but also from improved milk quality arising from reduced somatic cell counts. In both cases, there is the issue of "invisible gains", i.e. epidemiological gains that benefit all animals, not just genetically improved animals. Invisible gains are a technology transfer challenge. For sporadic diseases the problem is harder still. The framework for estimating benefits in terms of decreased probabilities and severities of epidemics is established (MacKenzie and Bishop, 2001), but appropriate incorporation into breeding programmes remains a challenge and will be dependent upon the genetic architecture of resistance, i.e. one or many genes. Disease management is successful by the absence of disease, hence success is less visible than failure. Only in the case of continuous challenge diseases from essentially-infinite reservoirs (van der Waaij *et al*., 2000), can the problem be answered without epidemiological models.

Pathogen evolution risks. As described above, pathogen evolution risks following genetic change in the host are inestimable. They are probably low in the short to medium term (i.e. decades), but high in the long term. Genetic heterogeneity of host will be the primary risk factor – the fewer the genes underlying resistance, the less the obstacle to the pathogen and the greater the risk. Risks will arise when the pathogen has a limited (but not zero) chance of invading the population, in which case a mutated pathogen which can avoid the host defence mechanism will have a large selective advantage (e.g. Gandon *et al*., 2001). The scenario most appealing to breeders, *viz*. a single gene conferring resistance, will be the most dangerous in terms of evolution risks, especially if this gene confers incomplete resistance. If selection is based on an indicator trait measured in infected animals, then the breeder is in a more favourable situation, as selection will always be against the current predominant strain of pathogen.

LOOKING FORWARD

Disease resistance is likely to become an ever-greater focus of breeders. However, success in implementing genetic control strategies will require an integrated multi-disciplinary approach. Naïve approaches, e.g. studies that ignore the disease biology, the epidemiological context of the disease or underlying immune mechanisms of resistance, run the risk of failure. We predict ever-greater use of genetics to control many, but not all diseases. Critically, as genetic control strategies are often slower than other strategies, they must be demonstrated as being cost-effective in reducing disease incidence and improving animal health in a reasonable time period.

ACKNOWLEDGEMENTS
SCB was supported by BBSRC. Jack Dekkers is thanked for helpful comments.

REFERENCES

Axford, R.F.E., Bishop, S.C., Nicholas, F.W. and Owen, J.B. (2000) Breeding for Disease Resistance in Farm Animals, 2nd Edition. CABI Publishing

Barillet, F., Rupp, R., Mignon-Grasteau, S., *et al*. (2001) *Genet. Sel. Evol*. **33** : 397-415.

Coltman, D.W., Wilson, K., Pilkington, J.G., *et al.* (2001). *Parasit*. **122** : 571-582.

Bishop, S.C., Bairden, K., McKellar, Q.A. and Stear, M.J. (1996) *Anim. Sci.* **63** : 423-428.

Bishop, S.C. and Stear, M.J. (1999) *Anim. Sci*. **69** : 515-525.

Bishop, S.C. and Stear, M.J. (2001). *Anim. Sci.* **73** : 389-395.

Bumstead, N. (1998). *OIE Rev. Scien. Tech*. **17** : 249-255.

Edfors-Lilja, I. And Wallgren, P. (2000) In "Breeding for Disease Resistance in Farm Animals, 2nd Edition", 253-267. Ed. R.F.E. Axford *et al*. CABI

Edfors-Lilja, I., Wattrang, E., Andersson, L. and Fossum, C. (2000) *Anim. Genet*. **31** : 186-193.

Edfors-Lilja, I., Wattrang, E., Marklund, L., *et al*. (1998) *J. Immun*. **161** : 829-835.

Gandon, S., MacKinnon, M.J., Nee, S., and Read, A.F. (2001) *Nature* **414** : 751-756.

Gasbarre, L.C. and Miller, J.E. (2000) In "Breeding for Disease Resistance in Farm Animals, 2nd Edition", 129-152. Ed. R.F.E. Axford *et al*. CABI

Henryon, M., Berg, P., Jensen, J. and Sorensen, S. (2001) *Anim. Sci.* **73** : 375-387.

Hunter, N. (2000) In "Breeding for Disease Resistance in Farm Animals", 325-339. CABI.

Janss, L.L.G., de Greef, K.H., *et al.* (2001) *Proc. 52nd EAAP*, 22.

Klungland, H., Sabry, A., Heringstad, B. et al. (2001). *Mam. Genome* **12** : 837-842.

MacKenzie, Katrin and Bishop, S.C. (2001) *J. Anim. Sci*. **79** : 2057-2065.

Mallard, B.A., Wilkie, B.N., Kennedy, B.W., *et al*. (1998) *Proc. 6th WCGALP* **27** : 257-264.

Morris, C.A. (1998) *Proc. 6th WCGALP* **27** : 295-302.

Heringstad, B., Klemetsdal, G. and Ruane, J. (2000). *Livest. Prod. Sci*. **64** : 95-106.

Office International des Epizooties (1998). *Rev. Scien. Tech*. **17.** OIE

Pinard, M.H., van Arendonk, J.A.M., *et al.* (1992) *J. Anim. Sci*. **70** : 2986-2993.

Sarker, N., Tsudzuki, M., Nishibori, M., and Yamamoto, Y. (1999) *Poult. Sci.* **78** : 1-7.

Stear, M.J., Bishop, S.C., Mallard B.A., and Raadsma, H. (2001). *Res. Vet. Sci.* **71** : 1-7.

van der Waaij, E.H., Pijma, P., *et al.* (2000) *J. Anim. Sci.* **78** : 2809-2820.

van der Waaij, E.H. (2001). Ph.D thesis, Wageningen University.

Witter, R.L. (1997). *Acta. Vet. Hung*. **46** : 251-266.

Yonash, N., Bacon, L.D., Witter, R.L. and Cheng, H.H. (1999) *Anim. Genet*. **30** : 126-135.

Appendix. Examples of diseases where there is genetic variation in host resistance.
Chickens: Marek's disease, Infectious laryngitracheitis, Avian leucosis, Infectious bursal disease, Avian infectious bronchitis, Rous sarcoma, Newcastle disease, Pullorum, Fowl typhoid, Salmonell-osis, Coccidiosis, Ascaris. **Pigs:** African swine fever, FMD, Atrophic rhinitis, Neonatal and post-weaning diarrhoea. **Cattle:** FMD, Bovine leukaemia, Paratuberculosis, Mastitis, Tuberculosis, Bru-cellosis, Salmonellosis, Dermatophilosis, Cowdriosis, Trypanosomosis, Theileria (*T.annulata*), East Coast Fever (*T. parva*), Babesia, Nematodiasis, Ticks. **Sheep:** Scrapie, Footrot, Mastitis, ParaTB, Dermatophilosis, Salmonellosis, Cowdriosis, Trypanosomosis, Nematodiasis, Liver fluke, Flystrike.