FUTURE PERSPECTIVES FOR THE IMPLEMENTATION OF GENETIC MARKERS FOR PARASITE RESISTANCE IN SHEEP

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INTRODUCTION

Scope of this review

Gastrointestinal parasites, in particular nematodes of the order Strongylida cause significant losses of production and deaths in sheep and goats worldwide (Over *et al.*, 1992; Woolaston & Baker, 1996; Perry & Randolph, 1999; Nieuwhof & Bishop, 2005; Sackett *et al.*, 2006). The strongylid genera which are important include *Haemonchus*, *Trichostrongylus*, *Nematodirus*, *Teladorsagia*, *Cooperia, Bunostomum* and *Oesophagostomum*. These are related genera by both morphological and molecular criteria, but cause a diversity of pathologies in the host due to different tissue specificities, modes of feeding and varying propensity to undergo hypobiosis in the host at the L4 or L3 stages. Other livestock species are also infected with strongylid parasites in particular production systems and these sometimes are important economically, for example *Mecistocirrus digitatus*, *Ostertagia ostertagi* and *Haemonchus placei* in cattle, *Haemonchus contortus* in alpacas and *Oesophagostomum dentatum* in pigs. The development of productive lines of animals with resistance to these parasites is therefore an important objective for graziers, government organisations and research bodies throughout the world. This review will focus on these parasites and their sheep host, with occasional examples from other host-parasite systems. The information should be generally applicable to other situations, especially those involving helminth parasitism in ruminant mammals other than sheep. Because of the importance of strongylid parasites in human medicine (*Ancylostoma ceylanicum*, *Ancylostoma caninum* and *Necator americanus*), there have been various rodent models developed to study strongylid-mammal parasite interactions. The results of studies with these models may also be applicable to work with livestock species, so some information from these models, concentrating in particular on the *Heligmosomoides polygyrus* model in *Mus musculus* will be included here.

Focus on likelihood of success, possible issues and practical implementation

Emphasis will be placed on the future potential use of DNA-based tests for predicting parasite resistance. The use of these tests is advantageous because it obviates the need to conduct often unreliable diagnostic tests for parasitism. There are of course risks involved in adopting these new tests, and their limitations and caveats are highlighted. Finally, issues surrounding the practical implementation of tests in the livestock industries

are explored. Rather than be comprehensive emphasis is on examination of recent developments and exploration of issues of implementation of DNA tests in the field, along with key references for further reading.

BREEDING FOR PARASITE RESISTANCE IS A REALITY

Selection using FWEC in New Zealand and Australia

In both Australia and New Zealand, some sheep breeding enterprises have been selecting for parasite resistance using faecal worm egg counts (FWEC), for over a decade (McEwan *et al*., 1997; Bisset *et al.*, 2001; Khusro *et al*., 2004). In these enterprises, the need to use anthelmintics has been reduced through genetic progress toward more resistant sheep. In a number of instances, the use of drugs has been reduced from seven administrations in the first year of life down to two, even in years where conditions favoured the development of high parasite numbers on pasture. In New Zealand an antibody test has also been used as an alternative phenotypic measure for parasite resistance (McEwan *et al*., 1997). Clearly, the use of objective, phenotypic measurements for parasite burden can result in genetic progress towards more resistant animals. To improve opportunities for graziers to use these practices, both the Australian and New Zealand livestock databases (SGA and SIL) currently produce estimated breeding values (EBV) for parasite resistance, and these are available for all progeny for sale and publicly available for top ranked progeny tested sires. The use of link sires to enable comparison of EBV across environments is a key component necessary for successful use of this data. DNA-based markers for parasite resistance, therefore, can potentially be identified because parasite resistance has a significant genetic component which can be distinguished from environmental components of variance in this trait.

Selection against high susceptibility using FAMACHA in South Africa and Brazil

The FAMACHA© system, developed in South Africa (Malan *et al.*, 2001) is a method for estimating haematocrit (packed volume of red blood cells, also called PCV), based on the colour of the mucosae of the inside lower eyelid. Animal's eyes are compared to a colour chart and scored from 1 to 5 according to the degree of redness seen. This system can identify animals suffering from anaemia due primarily to infection with *H. contortus*, a parasite common in sheep breeding areas over the warm temperate and tropical regions of the world. Animals with a high score (pale coloured mucosae) are likely those with a particular susceptibility to parasitism, and can be removed from the breeding flock resulting in a decrease of susceptible animals in the following generation. FAMACHA© has been used for selective breeding in Brazil (Molento *et al.*, 2007) and the system is being used for selective treatment in South Africa, Australia and the United States of America (van Wyk *et al.*, 2006), where the extension to selective breeding has begun to occur. This process of removing susceptible animals using a phenotypic marker has been successful, so the potential for DNA-based genetic markers for identifying and eliminating susceptible genotypes exists as an alternative or complementary approach to identifying and retaining resistant genotypes.

GENETIC MARKERS WILL MAKE BREEDING FOR PARASITE RESISTANCE MORE PRACTICAL

Unpredictable and changing parasite incidence leads to an unequal ability to assess phenotype across environments and across generations

There are few environments which exhibit uniform temperature and rainfall from one year to the next. As a result, the quality of pasture and the development of free-living stages of parasites also varies from year to year. This causes some difficulties in measuring parasite burden for the calculation of EBVs for use in breeding programs. Sires used only in one year cannot be directly comparable to those used only in other years, so at least some animals will have to be tested in multiple years to enable EBVs to be linked. Furthermore in some environments (for example the Riverina district of New South Wales, Australia) runs of

three or more years can occur where drought conditions prevent parasite development on pasture, and so measurement of FWEC-EBV is impossible. Some graziers have resorted to the use of artificial infections to ensure a FWEC measurement can occur, but this is a very labour intensive and therefore costly exercise, and brings with it the risk of introducing novel parasite genotypes which may be drug resistant or may be more virulent than those endemic to the property.

Another factor to consider is that different parasites exist in different environments, and that environment by parasite infection level interactions commonly called genotype by environment, or GxE, have been shown to be significant (McEwan *et al.*, 1997). However, in studies conducted to date in wet temperate climates over multiple years to ensure parasite numbers are measurable, the majority of genetic variation is consistent across environments, parasite species and challenge length and ages, if appropriate statistical transformations are undertaken. This commonality underpins the use of BLUP (Best Linear Unbiased Prediction) evaluations and sire referencing by sectors of the livestock industries in some countries. However, it would be an improvement if the remaining differences between environments could be better predicted. An example is scouring in response to *Trichostrongylus* spp., where resistance in one environment can be favourable, but these same animals respond inappropriately to infection in another environment. This occurred when animals bred for nematode resistance in the Northern Tablelands of New South Wales were taken to South West Western Australia. The sheep continued to have low parasite burdens in the new environment, but exhibited scouring which can reduce weight gain and spoil wool (Greeff & Karlsson, 1998; Karlsson *et al.*, 2004).

These observations suggest that DNA-based tests would be advantageous, as they avoid the problems of years when no challenge is present and offer the potential to better segregate significant GxE effects. A cautionary note must also be added however, as genotypes selected by these DNA tests will have to be either limited to environments where their impact is well understood, or have been developed with a cross-environment perspective specifically in mind. This will be addressed later.

Good quality phenotypic measurements are costly

i – Current methodology is labour intensive

The process of obtaining an individual faecal sample from an animal, packaging it and sending away to be tested is not onerous. It is the challenge protocol which is the problem, because it constrains overall farm management. However, when considered amongst the myriad of other tasks graziers must perform in modern grazing enterprises, and when considering the effort is for just one trait in a genetic selection index and that both labour costs and labour supply is also very limited in many agricultural industries, the gathering of faecal samples is considered by many graziers to be too difficult. In contrast, a blood smear obtained at lamb marking for DNA testing is easier to obtain, is conducted as part of another necessary operation and can also be used to test for multiple traits. For example, Catapult Genetics (http://www.catapultgenetics.com/) have already released combined DNA tests for parentage, meat yield and parasite resistance. A similar argument can be used to show that a DNA test would be preferable to measurement of blood antibody levels.

FAMACHA© is most often used in developing countries where labour shortages can be less of a problem, and importantly the test provides an instant answer. So, from a labour point of view a DNA test might not be regarded as superior, though it must be remembered that FAMACHA© only detects conditions that result in reduced haematocrit.

ii – The need for repeated measures

The use of the phrase 'good quality measurements of parasitism' might be regarded as an oxymoron by many researchers and laboratory technicians due to the high level of variation between subsequent tests on a single animal. FWEC varies between sampling times even within one day with a repeatability over consecutive days of around 0.6 using standard

McMaster techniques and low to moderate infection levels. Multiple tests provide better data and result in faster genetic progress. More labour is required for multiple testing which might be applied by taking multiple samples per animal, or by using larger progeny groups, though there are statistical differences between these two approaches. With FAMACHA©, the recommended procedure also entails identifying an animal as a score 4 or 5 on multiple occasions before the animal is removed from the breeding pool. DNA-tests are only required to be undertaken once in an animal's entire life, again making them superior to phenotypic measures.

Successful breeding for resistance will reduce the ability to continue selection based on field infection

Over a long period of time an irony of selecting for parasite resistance is that the subsequent reduced exposure to parasitism can make further observations of phenotype and therefore further selection more difficult. Based on research results this is not a major constraint within the first 20-30 years of selection and the result is also consistent with reduced selection pressure being placed on this trait as the economic benefits of further reduction decline. However, DNA tests enable continued genetic progress even when good genetic progress has already had a limiting impact on parasitism. This is especially valuable when climatic conditions are variable with wildly different challenge levels from year to year.

WILL GENETIC MARKERS BE A RELIABLE REPLACEMENT FOR PHENOTYPIC SELECTION?

Phenotypic selection can lead to multi-species resistance whilst QTL can be species-specific

Long term selection studies have shown that, at least amongst parasites from the order Strongylida, deliberate selection for resistance to one parasite results in significant cross resistance to other parasites. For example, two selection flocks maintained by CSIRO from the 1970s till the mid 1990s, were selected for resistance to *Trichostrongylus colubriformis* (Woolaston & Windon, 2001) or *H. contortus* (Woolaston & Eady, 1995). Despite these parasites being located in different gut compartments (jejunum and abomasum respectively) and having very different feeding mechanisms, resistance to both parasites was evident in both flocks (Ingham *et al.*, 2007). In contrast, when multiple species of parasites are used to generate phenotypic information for QTL mapping experiments, QTL are often found which explain a proportion of genetic variance in response toward one parasite, but not another. For example Dominik & Hunt (unpublished data) identified two significant QTL on separate chromosomes in an interbreed backcross population. Neither QTL explained differences in FWEC for both a secondary *T. colubriformis* and a tertiary *H. contortus* infection. Similarly, for a field infection QTL study of Scottish blackface sheep (Davies *et al*. 2006), separate parasite resistance QTL were discovered for "strongyle eggs", mostly *Teladorsagia circumcincta* and *Nematodirus* spp. eggs. Other studies have measured multiple species, but report QTL only for a subset of those observed (Crawford *et al.*, 2006; Beraldi *et al*., 2007) this may also be an indication of locus specific resistance to some parasites, but could also be due to a small sample size of animals with detectable infections.

The biological mechanisms of resistance are complex

i – assessment of immunity is temporally sensitive

There is ample evidence both that immune responses have a heritable component and also that effective immune responses against parasites can be a key resistance mechanism. The timing of an effective immune response is however variable. Using two separate populations of Merino sheep it has been shown that resistance can be manifest predominantly upon secondary exposure to parasites (HSF flock), or can be observed during both primary and secondary infections (TSF flock) (Ingham *et al*., 2007). The logical extension is that not all QTL will influence resistance at the same time relative to parasite exposure.

The timing of immune responses is difficult if not impossible to observe in field infection regimes, which constitute many of the studies seeking QTL on the pathway to developing markers for resistance (Crawford *et al.*, 2006; Davies *et al*., 2006; Beraldi *et al.*, 2007). Therefore, knowledge of the immune mechanisms behind genetic variants will not be possible if only linked, but not causative, markers are identified for selection rather than the actual allelic variants responsible for the QTL effect (called 'perfect' markers in plant genetics). Arguably, even after the identification of a perfect marker substantial further investment in research is required to elucidate the mechanism. Major histocompatability complex (MHC) class II genes are one class of resistance genes where we do have some knowledge of the resistance mechanism, however, the majority of other sources of genetic variation presented publicly are currently unknown. The selection for resistance based on MHC alleles remains controversial because it is known that a loss of heterozygosity in the MHC regions can result in immunological deficits. This effect could be evaluated as part of a properly organised DNA test evaluation program, but any such program would need to encompass a very large number of individuals over many years to allow observation of the effects under rare but economically significant disease challenges.

This problem of timing therefore means that the practical outcome of adopting any DNA test for parasite resistance/susceptibility will be hard to predict. Will most benefit be gained in adult sheep, lambs, weaners or lactating ewes? How should sheep bred using the test be best managed? The bottom line is that QTL and subsequently markers can be identified with carefully constructed infection regimes which are costly and complicated to analyse, or they can be discovered using simple observations of parasitism in the field; in the former case better knowledge of immunological specificity is at hand through the whole procedure, but in the latter a significant investment in time and money will be necessary at the other end of the marker discovery pipeline. There are no short cuts, but a significant investment in understanding parasite immunity in sheep and other livestock would help, by pinpointing circumstances which differ significantly between environments and which therefore will require separate rounds of validating DNA tests. Luckily, there are a dedicated body of researchers who do have immunity (as opposed to genetics) as their main focus. Recent references for sheep include (Amarante *et al.*, 1999a; Strain *et al*., 2002; Peña *et al*., 2004; Amarante *et al.*, 2005; Shakya, 2007; Terefe *et al.*, 2007), for goats (Macaldowie *et al*., 2003) and for cattle (Li *et al*., 2007). A further source of information which will help elucidate links between immune mechanisms and genetic mechanisms are gene expression studies, a few of which are now published in sheep (Diez-Tascon *et al*., 2006; Ingham *et al.*, 2007; Keane *et al.*, 2007).

ii – immunological mechanisms follow multiple pathways

Timing is not the only issue which makes immune mechanisms complex. The immune system is our (as mammals) chief defence mechanism and therefore contains many overlapping and complementary mechanisms. A genetic variant which changes an immune response will likely have a small effect, or perhaps a situation-dependent effect. This is the best explanation for the observed quantitative behaviour of parasite resistance in sheep where heritability is low, genetic progress is additive and no major loci have (so far) been identified.

In this complex immune system, the potential for epistatic interactions between loci is very possible. To date, studies undertaken have insufficient marker density statistical power for the full potential of epistasis to be explored for parasite resistance in sheep. The advent of screening large populations with high density single nucleotide polymorphisms (SNP) methods may reveal the true extent of epistasis in the next decade. Because of the known complexity of immune reactions, it might be expected that these future studies will also identify some combinations of DNA alleles which are not compatible, or which in combination are superior.

iii – the immune system is not an island

A third important point about the immune 'system' is that biological systems are not separate from each other as genes involved in one system are often involved in other processes. There are many examples, one is the TGF-β family of genes which include genes involved in immune responses, embryonic and adult tissue development, ovulation and more. A cautionary note regarding DNA tests for immune responses therefore is that these might have unpredictable effects not only on other aspects of animal health, but also in seemingly unrelated aspects of physiology. A hypothetical example might be an allelic variant of the gene encoding vasoactive intestinal peptide (VIP), which responds to immune signalling and can increase gut motility. Despite its name, this peptide has crucial roles in the immune system and the neuro-endocrine system in addition to its role in the gut. Before developing a test for parasite resistance based on a particular allele of VIP, it would be wise to investigate the consequences of that allelic variant for nervous and endocrine functions. An evaluation program for a marker therefore, should pay particular attention to behavioural, disease and reproduction traits because of this prior knowledge. Such a strategy will not be possible where there is no knowledge of the genes associated with the DNA test.

An alternate argument is to say that we should measure all significant traits in any evaluation program, but this can be limited in actuality by technical, financial, environmental and regulatory constraints.

The application of "whole genome selection" to animal health traits

A potential solution for the problems of DNA tests explained above would be to use multiple DNA tests simultaneously, and select animals with any favourable combination to contribute to the next generation of breeding, thus combining favourable alleles at many loci, negating the problems of partial and small effects inherent in single locus tests (Meuwissen *et al.*, 2001). This approach has received much recent attention in the literature and has become known as whole genome selection (WGS). It is not, of course, really the whole genome which is analysed, but rather a collection of thousands of small allelic variants (SNPs) which are spaced throughout the genome at a high density. This density is important because to work well, adjacent SNPs throughout the genome are sufficiently close to form historically conserved associations or haplotypes. These blocks are very rarely disrupted by recombination so can serve as good markers for QTL located within or close to them.

A common misinterpretation of the potential for WGS is that measurement of phenotypes will no longer be necessary, saving enormous amounts of time and money whilst dramatically increasing genetic progress for difficult to measure traits. Unfortunately, this panacea is not truly achievable for both researchers and livestock producers. The successful implementation of WGS does not rely solely upon the availability of a great set of SNPs! Just as earlier BLUP based methods relied upon good phenotypic measurements in concert with good estimates of genotype (as pedigrees), so too does WGS. Many of the caveats listed above for single marker DNA tests, also have importance for WGS. Good phenotypic measurements which take into consideration the timing and type of parasite infection, correlates with production and other animal health traits will be necessary in order that research delivers truly useful WGS-type DNA tests for parasite resistance which can be used in the livestock industries. Initially, these tests will be based on the assumption that the effects detected are co-dominant and additive: the same assumption that underlies existing BLUP genetic evaluations that have served agriculture well for the last 100 years. In practice, WGS offers the potential to offer further improvements because, dominance, parent of origin commonly called imprinting, genotype by genotype and genotype by environment interactions, can be identified and explicitly accounted for. This will speed genetic improvement and more importantly provide better predictors of animal performance in a given environment.

GENETIC MARKERS WILL BE AVAILABLE SOON

QTL mapping projects for nematode resistance using sheep

There have been a number of published reports considering QTL for parasite resistance in sheep. Some studies, driven by the economic importance of strongylid parasitism in sheep, were undertaken before the statistical, molecular and immunological tools were available in their current state, so they need to be re-evaluated in the light of current knowledge. Meta analysis has been suggested as a way of evaluating multiple data sets using a common analytical method, and a study is underway to do this (J. Maddox, pers comm.). In the interim, a qualitative comparison of studies rather than a truly quantitative synthesis of the work is provided here.

The MHC class II genes have been implicated in parasite resistance for some time (Schwaiger *et al*., 1995) and in some populations of sheep are responsible for a large amount of the genetic variation in response to some parasites (Stear *et al.*, 2005). An issue with the use of DNA tests for MHC genes, however, is the nature of resistance as conferred by alleles at MHC loci. The G2 allele of DRB1 confers significant resistance to parasitism in Scottish Blackface sheep, but only in the heterozygous state, as homozygotes suffer from increased susceptibility (Stear *et al*., 2005). There are many situations therefore when it would be difficult to implement selection for the G2 allele in a breeding objective, however, it could be used to impart resistance to crossbred lambs where the allele was absent from the dam flock, but homozygous in terminal sires. In some production systems (Australia and New Zealand meat sheep), the value of increased resistance in crossbred lambs would outweigh the negative impact of decreased resistance in the terminal sire breeding flock, but the situation should be re-evaluated if terminal sire progeny are retained widely in the commercial tier as breeding ewes. There are other MHC alleles which have been reported as deleterious under an additive hypothesis (Paterson *et al.*, 1998). More recently, Keane *et al.* (2007) have identified that host resistance in some populations is tightly linked to the presence or absence of DQA1 allele differences in two selection lines of different breeds, but results were unable to be replicated in separate industry populations. The use of these for selection, based on DNA tests, would seem to be less complex because selection against these alleles would not be expected to drive toward homozygosity. A population wide analysis of any locus to be targeted in this way would be wise however, as a high frequency of more than two other alleles would be needed to avoid homozygosity-induced susceptibility as a result of selection using the test.

Apart from the MHC loci, there are many other QTL candidate regions which have been identified using sheep populations (Dominik 2005; Bishop & Morris, 2007). Some studies have utilised inherent differences between breeds (Raadsma *et al*., 2002; Miller *et al.*, 2006; Hadfield *et al*., 2007) or between selection lines (Beh *et al.*, 2002; Crawford *et al*., 2006), whilst others have concentrated on the variation within breeds (Davies *et al.*, 2006; Beraldi *et al*., 2007). Chromosomal locations are variable, indicating that genetic and/or environmental variation between studies has resulted in different loci being significant in different situations, though a lack of statistical power in all studies may mean that some loci were not detectable. The results of multiple studies support the hypothesis that resistance to parasitism is a quantitative trait which behaves additively. There are likely to be many loci affecting host resistance and this has implications for the development of resistance tests.

The interesting aspect is that a large number of QTL for parasite resistance have been mapped and continue to be studied. The progress of mapping has increased because of an enormous increase in the amount of genomic data for sheep including the discovery of many SNPs. The first DNA test for parasite resistance has been released commercially in New Zealand (http://www.catapultsystems.co.nz/), and there are likely to be more tests available for parasite resistance in at least some countries by the end of the decade. There continue to be many studies revealing interesting genetic variation in parasite resistance between and within breeds of sheep

(Amarante *et al.,* 1999b; Gauly & Erhardt, 2001; Li *et al*., 2001; Gauly *et al*., 2002; Gruner *et al*., 2003; Vanimisetti *et al*., 2004a; Vanimisetti *et al*., 2004b; Good *et al*., 2006;; Hielscher *et al.*, 2006) and so there may well be many more QTL mapped and markers available than would be indicated by reading only the QTL mapping literature.

The proportion of the variance in a particular measure (often FWEC) accounted for by a particular locus, is often discussed in respect to particular predicted QTL. Although this concept is valid in the context of a particular study wherein a particular parasite exposure regime has been implemented, it is difficult to extrapolate beyond the study population or beyond the particular parasite challenge experienced by that population during the study period. The first reason is that the amount of variation depends on the allele frequency in the population concerned. For other DNA tests this has ranged between 0 and close to 100% in different breeds. The second reason is that the effects of a single locus may depend on the genetic background or the disease challenge involved. For this reason it is important, particularly for associated marker or haplotype tests, that prior to release it has been validated both for its likely frequency across a wide variety of breeds and its effects across many breeds and environments. In the case of the commercial New Zealand test, this involved genotyping the sires of over a 100,000 progeny from several breeds, many flocks and years which had been measured for a wide variety of traits including FWEC. Similar evaluation procedures will be needed in any farming system in which this DNA test and other future tests are used.

In addition to variation introduced by the physical environment and the genetic background of the stock, the parasites and other pathogens also vary considerably between farming systems in different countries. The result of parasite exposure cannot always be easily predicted between different situations because parasite establishment rates are not linearly related to parasite exposure but are subject to thresholds (Dobson *et al.*, 1990), parasite genotypes can differ resulting in different infectivity rates (Hunt *et al*., 2007) and because the mix of parasites differs between locations and seasons. These factors need to be considered at the beginning of a DNA test evaluation program.

QTL mapping projects for nematode resistance using the mouse model

Although QTL mapping in sheep is now simpler than in the past, the whole procedure is much more precise in model organisms such as the mouse. A number of key mouse QTL have been described (Behnke *et al*., 2006) for various aspects of parasite resistance and immune response to *H. polygyrus*. Because of the superior genome resources and far superior immunological techniques available to mouse researchers, much more will soon be understood about parasite resistance in this model. A possible caveat is the preference for using inbred strains of mice which may have accumulated alleles deleterious in wild populations; therefore, it would be advantageous if some mouse parasite resistance studies used wild populations. This information might be used in a number of ways to accelerate progress in development of DNA tests for parasite resistance in sheep. First, the genes or genomic regions identified in the mouse may also be genetically variable and responsible for resistance in the sheep. If this were the case, a study of synteny between sheep and mouse will reveal markers for these loci from sheep. Second, the identification of key mouse genes will elucidate pathways in which these genes act, and provide a number of candidates for those seeking to fine map QTL to discover perfect DNA markers for parasite resistance in sheep. Third, although there might not be QTL mapped near the sheep homologs of these mouse genes, there may be allelic variation evident from genomic studies, for example amino acid changing SNPs in the coding regions of the gene or indels in the promoter region which may change the gene's expression level. In these cases, a reverse genetics approach could be used to discover if these naturally occurring sheep variants have any statistically significant effect on parasite resistance/susceptibility.

QTL identified in other species

The approach of using synteny or gene pathways to link information from mouse to

sheep can also be used to link other mammalian and perhaps other chordate species. Mapping of parasite resistance QTL is underway in many species, for example pigs (Reiner *et al*., 2007) and salmon (Gilbey *et al*., 2006). Candidate genes are being analysed in others, for example stickleback fish (Kurtz *et al.*, 2004). In addition, recent studies assessing genetic variation which could be used for mapping in future studies have also been undertaken using goats (Chiejina *et al.*, 2002), cattle (Bricarello *et al.*, 2007) reindeer (Côté *et al.*, 2005) and hens (Gauly *et al*., 2002). Immunological studies of the mechanisms of parasite resistance, though most common in rodent models and sheep have also been undertaken using other livestock species such as cattle (Li *et al.*, 2007) and goats (Macaldowie *et al.,* 2003). This information from other livestock species will be useful to some extent to those seeking to develop DNA tests for parasite resistance/susceptibility in sheep, and also the sheep information will be useful for those seeking parasite resistance genes in these other species.

HOW SHOULD GENETIC MARKERS BE USED?

As a component of the breeding objective?

Three approaches to the use of DNA test information are used in livestock breeding. In the first, DNA test results are considered separately from the quantitative EBV data, and selection decisions are made using independent culling thresholds on both criteria separately. For example, DNA test results could be used to cull a proportion of undesirable animals, with the subsequent evaluation made on those remaining animals using EBV information in a selection index. The second and third approaches combine DNA test information with quantitative data to calculate a combined EBV. The second approach takes BLUP EBVs and their reliabilities and the genotype results and their known effects on production and "blends" them using selection index theory (Goddard, 1999). This has the advantage that sporadically collected genotype information can be simply combined with information from existing genetic evaluations. This second approach will become increasingly important as the number of DNA tests available increases (with or without WGS), as the first approach is not amenable for high numbers of markers. Genetic evaluation services (eg SGA in Australia and SIL in New Zealand) are working hard to provide EBV-type information which will use both phenotypic and genetic sources of data. The third method is to simultaneously use phenotypes, pedigrees and genotype data to estimate breeding values. Numerous methods have been proposed but the only commercial use in sheep known to the authors is where this is done in New Zealand for animals that are typically all DNA parentage tested and so genotypes are available or can be inferred on the majority of progeny. This simplifies the problem considerably. In these cases, the genotypes (typically haplotype probabilities obtained via the EM algorithm) are fitted as fixed effects given the known mode of inheritance with the BLUP analysis and the results estimated as a residual polygenetic breeding value and a locus breeding value (McEwan & Dodds, pers. comm.). Whether the two results are combined or reported separately depends on the nature of the inheritance and their use (Amer, 2007). However, this approach is also limited to low numbers of QTL perhaps 4-5 per major trait group. For the mathematically inclined, there are excellent discussions of the way in which genotype information can be used in combination with quantitative data (Walsh, 2001) and, with respect to WGS (Meuwissen *et al*., 2001).

The need for continual re-evaluation of markers

i – progress toward homozygosity

Genetic progress using DNA tests can be very efficient where there are alleles which are desirable and at moderate frequency in the selected flock or alternatively are being introgressed from another source or breed. If selection pressure is great enough, a whole flock could be driven to homozygosity at a single locus in one or a few generations. In reality, the progress will be slower as in most production systems DNA tests are used only with males (except in New Zealand), and the

need to select for multiple traits lowers selection pressure for any one locus. Nevertheless, the use of any single marker within a closed flock will have a finite time span after which no further selection will be possible. In this case, further selection via DNA tests will have to be for either a different superior allele at the same locus (via introgression of genetic material from outside the flock) or for a favourable allele at another locus altogether. A full WGS approach is much less prone to this scenario as multiple loci (typically around 30,000-300,000) will be used simultaneously. Even so, some loci will be subject to more selection pressure than others and the same effect will occur, but at a much slower rate. However, if WGS is delivered in industry as a more cost effective boutique SNP set (commonly called a "SNP key"), which simplistically reflects those markers associated with larger effects, then progress toward homozygosity in the selected regions will be faster with the rate depending on selection pressure and number of regions.

Progress toward homozygosity is a sign of a successful breeding program and should not be regarded as a problem in itself. It presumably occurs, and has occurred frequently in the course of traditional quantitative selection and DNA tests have just made the process visible. For example, there is near homozygosity in Texels around the GDF8 muscling mutant after intensive selection for muscling in this breed (Clop *et al.*, 2006). A more pressing issue is that the density of even the largest SNP chips in farmed animals at around 60,000 SNPs is insufficient to reliably predict molecular genetic breeding values across breeds, and this density also means there is more uncertainty about the stability of the predictions within breed over time. Thus, periodic or continuous calibration of the SNP associations will be required *(*Hayes *et al.*, 2007).

ii – realities of diverse environments

For animal health traits more than many production traits, the influence of environment is highly significant. For example, resistance to filarial parasites in Australian sheep will not be considered important, whereas in areas of India these parasites are a significant problem. So too, many viral, bacterial and parasitic diseases have incomplete distributions worldwide and within individual nations. The production system provides another source of environmental variation which can be highly significant. For example, compare continuously housed pigs with those kept in an extensive system. Nutrition, for gastro-intestinal parasitism has a large influence (Kahn *et al*., 2003; Bricarello *et al.*, 2005; Jackson & Miller, 2006; Vagenas *et al.*, 2007) and can vary between production systems, environments, seasons, years and with soil type.

DNA tests will have to be used carefully in environments different to those in which correlations between genotype and phenotype have been discovered. Another way of describing this is to state that the component of genetic variance attributable to particular loci are potentially functions of the background genotypes and environments (Walsh, 2001). Undoubtedly the next twenty years will see at least one case where a DNA test has an unexpected deleterious effect because it has been implemented in a situation which differs from those over which it was first evaluated. The likelihood of such incidents will be minimised by firstly ensuring that tests are developed and validated over a broad and documented range of conditions, secondly that such tests are sold with these conditions explained and thirdly that producers are educated to understand the advantages and limitations of the use of DNA tests in breeding systems.

iii – unpredicted outcomes

Biology is extremely complex, especially when it is analysed in a complex environment such as that experienced by grazing animals. Unexpected occurrences will occur, and these will sometimes enhance and other times impede progress toward more profitable livestock farming. There is no reason to suspect that genetic selection for parasite resistance using DNA tests will be any more or less prone to unexpected outcomes.

There are some surprising examples in the broader literature regarding parasite resistance which might help illustrate the point. Salice & Roesijadi (2002) studied *Schistosoma*

mansoni (human blood fluke) resistance in *Biomphalaria glabrata* (a snail) and found that resistant snails were more susceptible to Cadmium. *Tribolium castaneum* (a beetle) resistant to *Hymenolepis diminuta* (a tapeworm) are more susceptible to death during larval development, and are less fecund, and some QTL for these traits are located in the same regions as those for resistance (Zhong *et al.*, 2005). Male *Rutilus rutilus* (a fish) resistant to *Rhipidocotyle campanula* (a digenean flatworm) have more ornamentation, but their progeny are less likely to survive till adulthood (Kortet *et al.,* 2004).

In the livestock literature there are other examples, both those where resistance is positively correlated with another trait, such as live weight in sheep (Coltman *et al*., 2001), *Bovicola ovis* (louse) resistance in sheep (James *et al.*, 2002), trypanotolerance in goats (Chiejina *et al.*, 2005) and those in which the correlation is not favourable, for example fleece weight and fibre diameter in Australian Merinos (Khusro *et al.*, 2004).

The need to evaluate DNA tests in farming systems similar to those in which the tests will be used cannot be over-emphasised. Some knowledge of genetic characteristics and correlations in other farming systems and/or some knowledge of the mechanisms by which loci affect the trait will enable the prioritisation of tests for evaluation in a new situation. Further, the evaluation process itself needs to consider all traits which are locally economically important and results analysed using appropriate statistical methodology.

The need for an objective measurement culture in animal health-care

In conclusion, it is stressed that the success of DNA tests for parasite resistance will depend on the desire of livestock owners to increase the health of their animals. Stud breeders will only use the tests in selective breeding programs if commercial farms are demanding sires superior for these traits. Similarly, the success of breeding programs can only be measured if commercial grazing and feedlot operators and meat processors make objective assessments of the health of their animals (or meat products) and communicate the results with stud breeders, other farmers, extension officers and researchers. Many of the potential problems of implementing advanced breeding programs for parasite resistance would be lessened or at least be made less risky, if better and cheaper disease diagnostic systems were available and were used by livestock farmers. Clearly there is a need for more research in this area (see Colditz, this issue), but there is also a very important need for education/extension. An objective measurement culture has been successful in remodelling livestock breeding for production traits such as milk yield, growth rate and wool fibre diameter; it is time that a similar approach be taken with animal health issues including parasitism.

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