# Chapter 1 The Characterisation and Utilisation of Genetic Resistance to Internal Parasites in Merino Sheep

The work in this thesis explores sources of genetic variation for parasite resistance in Merino sheep and assesses strategies for, and the implications of, including this trait in a Merino breeding objective.

## 1.1 Reasons for the focus on breeding for disease resistance

Improvement in disease resistance through genetic means is becoming increasingly attractive to animal breeders as conventional control strategies based on widespread drug usage begin to falter. The idea of breeding for a resistant host has been largely ignored while there were relatively cheap and effective chemical means of controlling disease. However, the outlook for the control of some livestock diseases, using current techniques, is not optimistic. Drug resistance in the disease organism is an unavoidable evolutionary consequence of the uses of therapeutic compounds. The rate at which new compounds can be discovered and developed is not sufficient, in some cases, to provide ongoing protection against disease. Therefore, the possibility of breeding for a resistant host needs to be considered thoroughly as a means of managing parasite populations in a sustainable manner.

In regions where there is chronic challenge to animals by disease, there is inevitably a detrimental impact on the welfare of the most susceptible individuals before the benefits of treatment outweigh the costs. Any shift in the general resistance of the flock or herd will result in a lower proportion of animals suffering the ill effects of the disease and will improve the general welfare of the group.

There is also increasing concern being shown by consumers of livestock products, towards the possible contamination of animal products with veterinary drugs. A

reduction in the reliance on therapeutic drugs will aid in the production of high quality livestock products, especially where the incidence of drug resistance has built up to the stage where the frequency and dose rate of drug administration have been multiplied in an attempt to gain effective disease control. The impact of veterinary drugs on non-target micro-organisms is another area of concern and a reduction in drug usage will lessen the risk of environmental damage.

## 1.2 The disease and its background

Gastrointestinal parasites have long been a significant production problem in the majority of sheep breeding areas of Australia (Anderson *et al.* 1978) and represent the greatest constraint on the productivity of animal grazing enterprises in Australia (McLeod 1995). Only in some of the pastoral areas of New South Wales, Western Australia, Queensland and South Australia are internal parasites of little significance. In the high rainfall areas of the New England tablelands and southern tablelands of New South Wales, most areas of Victoria and Tasmania, the south eastern region of South Australia and the south western region of Western Australia, internal parasites are a major constraint on sheep productivity, contributing heavily to the variable costs of a sheep enterprise as well as depressing both meat and wool production. Apart from the direct cost of keeping parasite infections at a low level, the constant need to monitor stock and adjust management procedures places a continuing demand on property managers and their resources.

With the development of new drugs during the last 25 years there have been very effective chemical measures against most diseases of sheep in Australia, with the exception of footrot. Consequently there has been little demand in the industry for animals that are naturally immune or resistant to certain parasites. However, during the same period the parasites have developed genetically based resistance to the drugs being used for disease control; for example, organo-phosphate resistance in the sheep blowfly, *Lucilia cuprina* (Hughes 1983), and anthelmintic resistance in gastrointestinal parasites (Prichard 1990). The advent of a new triazine drug alleviated many of the problems being experienced with blowfly control. A combination of new

drugs plus the development of strategic drenching programs (Dash 1986a) assisted in worm control, but these parasites have continued to evolve further anthelmintic resistance to the point where there are very limited management options for their control. This situation has predisposed breeders, and the industry as a whole, to consider alternative control strategies such as breeding for resistance to internal parasites.

Researchers anticipated this demand for alternative control strategies with an investigation of the heritability of worm resistance (Piper 1987) followed by the establishment of selection flocks to determine the rate at which progress can be made in breeding for disease resistance. Initial heritability estimates indicated that worm resistance has a significant genetic component ($h^2$=0.25) so that it should be possible to increase resistance by breeding. Selection lines were established in New South Wales at Armidale with *Haemonchus* selection lines (Woolaston 1990), in Victoria with *Ostertagia* selection lines (Cummins *et al.* 1991) and in New Zealand with lines selected after natural infection (Baker *et al.* 1991). In the course of immunological investigations (Dineen and Windon 980), *Trichostrongylus* selection lines were also established in Sydney and subsequently moved to Armidale.

Faecal egg count (FEC) was used as the measure of parasite resistance and in all cases the average FEC of the selection lines has diverged. Progress in all of these lines has been encouraging with realised heritability estimates for FEC of 0.2-0.4. How these gains should modify current parasite control strategies is yet unknown as in most cases the selection flocks have been run together to allow a valid comparison of the lines. As pasture contamination plays a major role in on-going parasite infections, it will not be possible to assess the real impact of reduced FEC on production and parasite populations until the flocks are run as separate management groups.

It is difficult to predict the likely impact of reducing FEC on the whole host-parasite system and subsequently the dollar output of the production unit. Therefore, it is difficult to put an economic weighting on reduced FEC in a selection index. An epidemiological model has been developed for *Trichostrongylus colubriformis*, a major sheep parasite in winter rainfall areas (Barnes and Dobson 1990b). Simulation

of host-parasite systems may shed some light on how resistant a flock must become before changes can be made to the management program. For instance, the model may assist in answering questions such as - by how much does FEC need to be reduced before chemical treatment with drenches can be modified either to reduce the number of drenches required or to remove them altogether? This type of information can then be used to attach a dollar value to reduced FEC allowing it to be formally incorporated in a selection index.

Using current breeding technology two possible options may be available to increase resistance to internal parasites. The first of these is to select within a flock for the most resistant animals. With heritability estimates for FEC about 0.2 to 0.3, progress is possible, its rate dependent on its phenotypic variance and other traits in the breeding objective and the genetic correlations between these traits and worm resistance. The second option is to choose between flocks, should differences exist in the Merino breed. Apart from one study in which there were no bloodline or strain effects detected (Gray and Raadsma 1992), there has been no work of this nature undertaken.

As pressure to find replacement control strategies increases, the potential market for resistant animals will develop, especially in areas where internal parasites are a chronic problem. A number of Merino studs across Australia are now including worm resistance in their breeding objective as a necessary step to ensure their survival in an environment where running sheep is becoming increasingly difficult through rampant anthelmintic resistance in the parasite population. To assist breeders wishing to improve the internal parasite resistance of their sheep, scientists have seen the need to provide information on the genetic parameters involved, the practicalities of selecting for disease resistance, techniques to incorporate disease resistance in their breeding objective and the likely benefits of such a procedure to their worm control programmes.

## 1.3 Objectives of the project

The objectives of this research project are i) the estimation of genetic parameters of variation for internal parasite resistance between strains of Merinos and within Merino flocks; ii) the estimation of genetic associations with important production traits; iii) an assessment of the sensitivity of genetic response to errors in the genetic correlations between production traits and parasite resistance; and iv) the development of breeding strategies to utilise genetic variation in resistance to internal parasites.

The first objective of the project uses Merino resource flocks, in which strain comparisons are conducted, to obtain data on genetic variation for internal parasite resistance. The flocks studied are the CSIRO Finewool Flock at Armidale, the South Australian Department Agriculture Turretfield Merino Resource Flock, the New South Wales Department of Agriculture D Flock and single trait selection line flocks, the University of Sydney JB Pye Flock and the Western Australian Department of Agriculture Katanning Base Flock. Between-sire variation for worm resistance is also examined in the New England Sire Evaluation Scheme. Practical techniques for assessing resistance and data analysis are developed as an integral part of the project.

The second objective is to determine the genetic associations between worm resistance and important Merino production traits. It is not until fairly robust estimates of these parameters are available that assessment can be made of the impact of selection for worm resistance on the breeding objectives of Merino flocks. The genetic correlations between FEC and clean fleece weight, fibre diameter and live weight are estimated in the larger resource flocks.

The third objective is to assess the sensitivity of genetic response in production traits to errors in the genetic correlations between production traits and parasite resistance. This will provide a guide as to the need to determine genetic correlations with improved precision. The consequences of the current industry practice, of using zero genetic correlations between resistance and production traits, are examined to determine if this should continue.

The fourth objective addresses methods of incorporating resistance to internal parasites into sheep breeding programs. Various measurement strategies are investigated and recommendations are developed to assist Merino breeders in the improvement of the disease resistance of their flocks.

# Chapter 2 Literature Review

The literature reviewed in this chapter relates to the assessment of current worm control strategies and the selection of a suitable criterion to assess resistance. Literature relating to the specific issues addressed in each experimental chapter is reviewed in the introduction to each chapter.

## 2.1 The future effectiveness of strategies currently used to control internal nematode parasites in sheep

The last 50 years have seen an evolution of worm control strategies from the largely ineffective use of copper sulphate and arsenic, to the nearly complete reliance on therapeutic drenching with modern anthelmintic drugs. With the development of parasite resistance to these drugs, an integrated approach is recommended which encompasses strategic drenching, drug rotation and grazing management. The further development of anthelmintic resistance in worm populations continues to push control strategies to their limit and fuels interest in alternate avenues of worm control such as breeding for parasite resistance. The following review examines the major parasites, the development and control of anthelmintic resistance, factors influencing the build up of resistance and the current approaches to parasite control.

### 2.1.1 Economically important parasite species and the development of anthelmintic resistance in these species

Internal parasite control is important and often essential to the profitable production of sheep and wool in many regions of Australia. In 1985 approximately 63% of the farm-level costs of parasites were attributed to internal parasites (Beck *et al.* 1985). There are a range of parasites that infect sheep, their distribution primarily influenced by climate. *Haemonchus contortus* and *Trichostrongylus colubriformis* are the most serious nematode parasites of sheep where there is significant summer rainfall such as on the Northern Tablelands of NSW. *H. contortus* is the most pathogenic species,

infecting mainly young sheep from 3 to 7 months of age during the summer and autumn. *H. contortus* is a blood sucking parasite which if uncontrolled can cause high levels of mortality. *T. colubriformis* causes ill-thrift and scouring in young stock. In the southern winter rainfall regions *T. colubriformis*, *T. vitrinus* and *Ostertagia* spp. (mainly *Ostertagia circumcincta*) are the most common species, all causing gastro-enteritis in young sheep (Anderson *et al.* 1978; Beveridge and Ford 1982).

Since the introduction of the drug thiabendazole in 1962, internal parasite control has relied largely on the frequent dosing of sheep with anthelmintic compounds. The first reports of problems with worm control using thiabendazole appeared in the summer of 1966-67 when failure of the drugs to control *H. contortus* resulted in acute haemonchosis in three commercial flocks in the Tenterfield district of northern NSW (Smeal *et al.* 1968). During the same summer suspected anthelmintic failure to control *T. colubriformis* was reported in experimental sheep at Badgery's Creek, near Sydney (Hotson *et al.* 1970). Many reports of anthelmintic failure have been documented since that time (Taylor and Hunt 1989; Prichard 1990).

The term "anthelmintic drug resistance" has been used to describe the ability of some nematode parasites to survive treatment with drenches at the recommended therapeutic dose levels. "Resistance" is deemed to be present when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species (Prichard *et al.* 1980). In the field resistance to a certain anthelmintic is defined as failure to reduce the FEC of sheep by more than 90%, or in some instances 95%, after recommended therapeutic doses of the drug have been given to the animals (Love *et al.* 1992). Resistant worms have physiological characteristics which render them resistant to the toxic effects of the drugs. These characteristics are heritable so repeat treatment across generations can select for increasingly resistant individuals. Currently there are four main chemical groups used for worm control.

### 2.1.1.1 Benzimidazoles and pro-benzimidazoles

These compounds inhibit the fumarate reductase system in the fermentation pathways of nematodes. The resistant forms are able to counteract this energy-depleting effect by increasing ethanol production which does not involve fumarate reductase activity (Prichard *et al.* 1980).

### 2.1.1.2 Levamisole and morantel

Although chemically unrelated, both of these compounds act as cholinergic agonists which induce outflow of Na+ causing depolarisation of muscle bag membranes and muscle contraction. Resistance to levamisole is brought about by a reduction in the number of cholinergic receptors or in the affinity of these receptors for levamisole in resistant strains (Prichard 1990).

### 2.1.1.3 Salicylanilides and substituted nitrophenols

These chemicals cause uncoupling oxidative phosphorylation which interferes with energy metabolism. Their affinity for plasma proteins explains the restriction of their activity to blood ingesting parasites (Prichard *et al.* 1980). Resistance mechanisms are not known.

### 2.1.1.4 Avermectins

These drugs act on the mediation of neuro-transmission by γ-aminobutyric acid (GABA). Avermectin B1 (ivermectin) probably acts by blocking signal transmission from inter-neurones to excitory motor-neurones, inducing neuromuscular paralysis of target tissues via an increase in chloride permeability (Arena 1994). Resistance mechanisms have not been elucidated.

### 2.1.2 Genetic control of anthelmintic resistance

Research efforts have concentrated on identifying ways to manage anthelmintic resistance and reduce the rate at which it builds up. As a pre-requisite to this research the genetic control of resistance in the parasite has been studied for both thiabendazole and levamisole resistance. Thiabendazole resistance appears to be controlled by a β-tubulin gene called *tcb-1* in *T. colubriformis* and *gru-1* in *H. contortus*. Resistance is inherited as an incomplete recessive character with a strong maternal effect (Grant 1994). This maternal influence was found to be strongest in egg hatch assays but was also detected in the parasitic stages indicating a more persistent effect than the females' contribution to the egg cytoplasm and shell alone.

Martin and MacKenzie (1990) demonstrated levamisole resistance being inherited as a recessive character, consistent with resistance being controlled by a single gene or a tightly linked group of genes. The effect of this single gene appeared to be slightly modified by other autosomal loci. Once again there is a strong maternal effect on the phenotypic expression of resistance plus evidence of sex-linked inheritance. Male heterozygotes show a greater resistance when this trait is originally inherited from the female parent compared to the male parent, while heterozygous females, regardless of the origin of resistance alleles, are relatively susceptible to levamisole. There is evidence that resistance to the two chemically unrelated drugs levamisole and morantel is co-inherited (Sangster *et al.* 1979), perhaps because of the similar mode of action of these two chemicals. Similar levels of resistance developed to both compounds even though strains were selected only with levamisole. However, selection with morantel does not necessarily confer resistance to levamisole (Waller *et al.* 1986).

Little is published on the genetic control of closantel or ivermectin resistance. However, in Australia *H. contortus* resistance to ivermectin is controlled by an autosomal dominant gene (le Jambre unpublished data).

Genes for resistance are thought to be present at low levels in the population with no survival advantage (Wood 1981) as well as appearing as mutations which may be induced by chemical exposure. The first exposure to drugs results in a very high mortality rate with selection from the upper extreme of the normal distribution of anthelmintic tolerances. Selection acts via the resistant phenotype for alleles that confer resistance. Survivors of the chemical treatment make a greater contribution to the succeeding generations than they would have if they had remained unselected. The frequency of resistance alleles builds up to a level where their effect can be detected in the field by drench failure.

The rate of change in frequency of resistance genes will depend on the genetic variation in the population, the extent to which fitness characteristics are co-adapted with resistance alleles, the intensity of selection by anthelmintic treatment and the generation interval of the parasite (Prichard *et al.* 1980). There is a latent period when an increase in the frequency of resistant individuals is not apparent. A point is then reached where a rapid exponential increase occurs. The latent period will depend on the dominance model operating. If the allele for resistance is completely recessive the latent period will be longer than if the allele is co-dominant or dominant (Falconer 1989).

### 2.1.3 Types and incidence of anthelmintic resistance

Different types of anthelmintic resistance have developed since the first reports in the early 1970s. Many cases of anthelmintic resistance have been documented in Australia and overseas (See review by Prichard (1990) for an extensive listing of reports of anthelmintic resistance.) Cross resistance or side resistance has become common within families of chemicals to the extent that each group can only be considered as the one effective drug. The initial reports of resistance were for a particular parasite being resistant to a particular drug group. Anthelmintic resistance has now developed to the stage where there is multiple resistance. Multiple resistance can develop on the one farm with many species of nematodes resistant to one type of anthelmintic. Benzimidazole resistance was reported in a range of parasites where the recommended

dose rate of 12.5 mg/kg mebendazole had efficacies of 0, 60, 66, 90, 54 and 38% against *H. contortus*, *Ostertagia* spp., *Nematodirus* spp., intestinal *Trichostrongylus* spp., *Strongyloides* spp. and *Oesophagostomum venulosum* respectively (McKenna 1989). Alternatively multiple resistance can be in the one worm species to many types of anthelmintic. Multiple resistance to levamisole and oxfendazole was confirmed in field strains of *T. colubriformis* at Armidale (Dash 1986b).

Where there is multiple resistance within a worm species it may be that a proportion of the population is resistant to one drug and a proportion to the other. However, if selection continues and the populations become highly resistant it is possible for individual worms to be simultaneously resistant to both drugs. This was demonstrated by Dash (1986b) where 32% of a *T. colubriformis* population was resistant to levamisole, 19% to oxfendazole and 12% to both drugs.

Resistance to two other important anthelmintics, closantel (Rolfe *et al.* 1990) and ivermectin (le Jambre 1994) has been reported in Australia. The ability of closantel (salicylanilide anthelmintic) to control *H. contortus* is an integral part of the strategic worm control program Wormkill (Dash 1986a). This chemical's long acting nature allows control of *H. contortus* and reduces the frequency of broad-spectrum drench use. Ivermectin comes from a family of drugs that constitute a new chemical class to which there was no cross resistance from previous anthelmintic selection. Ivermectin is used as a broad-spectrum anthelmintic and in many areas of Australia is the only effective broad spectrum anthelmintic available. The first report of ivermectin resistance in sheep parasites came from South Africa after the drug had been available for three years. The relatively rapid evolution of resistance to ivermectin may be a consequence of the high efficacy and therefore high selection pressure of this drug (van Wyk and Malan 1988) or its frequent use in intensive management systems based on irrigated pastures. In comparable regions of Australia, where *H. contortus* is a major parasite, sheep are run more extensively on dry-land pastures. Thus, the development of widespread ivermectin resistance in Australia may not be as rapid, but it is still inevitable (Barger 1993). One case each of resistance to ivermectin in field strains of *H. contortus* (le Jambre 1994) and *T. colubriformis* (le Jambre, unpublished

data) has been detected at Armidale NSW, and *Ostertagia* spp. in WA (Besier and Wroth 1993).

Surveys of anthelmintic resistance (Webb *et al.* 1979; Beveridge *et al.* 1990; Love *et al.* 1992) have indicated that the incidence is increasing in Australia and that anthelmintic resistance in the important sheep nematodes is very common, most flocks demonstrating resistance in at least one of the more important species. The most recent published study (Overend *et al.* 1994) indicated that in excess of 90% of farms had sheep infected with nematodes resistant to either benzimidazole, levamisole or a combination product (benzimidazole+levamisole). Resistant parasites are spread by the purchase and transport of sheep harbouring resistant worms. However, in many instances resistance has developed on farm from anthelmintic usage. Routine monitoring of drench efficiency, by the use of FEC reduction tests, is only just becoming common (Love *et al.* 1992).

The conclusion to be drawn at this stage is that parasite resistance to all four groups of chemicals exists to varying degrees. With repeated use of the remaining effective chemicals, resistance to these drugs will increase at a similar or possibly faster rate to that observed for the benzimidazoles and levamisole, the first two families of drugs to become ineffective due to anthelmintic resistance in worms.

### 2.1.4 Factors influencing rate of resistance build-up

An understanding of the factors contributing to anthelmintic resistance is important if the rate at which drug resistance is developing is to be reduced to a level that will ensure a long enough period for either new drugs or alternate control strategies to become effective.

### 2.1.4.1 Frequency of drenching

Australian studies have shown clearly that the more frequent the treatment, the higher the rate of selection for resistance (Martin *et al.* 1984). Surveys of sheep properties have shown that the number of treatments for worm control ranges from 3-6 drenches for weaners in the south western districts of Victoria (Riffkin *et al.* 1984) to 6-9 drenches for weaners on the Northern Tablelands of NSW (Newman 1984). Barton (1983) demonstrated a rapid increase in anthelmintic resistance where groups of weaner sheep were treated nine or more times with thiabendazole in the one year. Monthly treatment with levamisole was sufficient to convert a largely susceptible *Ostertagia* population to one with a very high degree of resistance. Even anthelmintic treatment every two months markedly increased the degree of resistance. The regime of drenching 9-12 times gave the best economic outcome in terms of wool production and live weight gain compared to more or less frequent drenching. However, these anthelmintic frequencies also selected strongly for resistant strains of parasites. From a survey of drenching frequency in northern NSW (Newman 1984) it could have been predicted that anthelmintic resistance would become widespread in this region. Recent surveys have confirmed this outcome with most flocks having resistance in at least one of the more important worm species (Love *et al.* 1992).

A high use of anthelmintics increases the selection intensity for resistant worm phenotypes. The overall proportion of the population exposed to the drug will depend on the proportion of larvae that are resident in the sheep at the time of treatment. Larvae on the pasture are said to be in "refugia" and when none, or a small proportion, are in refugia a rapid increase in resistance can be expected. These results fit with the anecdotal evidence of massive crashes in anthelmintic efficacy after periods of drought when larval survival at pasture is poor. The "drench and move" strategies of integrated control programs have been questioned as they effectively increase the proportion of larvae contributing to the next generation that have been exposed to a drench. Barnes and Dobson (1990b) investigated the "drench and move" strategy using an epidemiological model that included anthelmintic resistance and predicted that this strategy could be used for many years without serious problems, although

pasture contamination will come from worms which have survived anthelmintic treatment. The model predicts a trade-off between exposure to worm burdens and the proportion of susceptible alleles in the worm population.

### 2.1.4.2 Anthelmintic under-dosing

Under-dosing has also been implicated as a factor contributing to rapid build up of resistance. Because of the co-dominance of the resistance alleles, the heterozygous worm shows more resistance to the anthelmintic than susceptible worms but is not wholly resistant (le Jambre *et al.* 1979; Martin and McKenzie 1990). Administering less than the therapeutic dose of anthelmintic can allow the survival of heterozygotes which will increase the frequency of the resistant allele in the worm population. However, if there is severe under-dosing some susceptible worms may survive and reduce the rate of build-up of resistant alleles (Barger *et al.* 1994). Under-dosing is usually a result of an underestimate of the live weight of the sheep to be drenched. Besier and Hopkins (1988) found that 86% of estimates of live weight were too low when farmers were asked to estimate the live weight of sheep. In addition to this many farmers made arithmetical errors in calculating the volume of anthelmintic required even when the liveweight of the sheep was known. Incorrect calibration of drench guns can also result in under-dosing but checking of drench gun calibration is not a routine practice.

### 2.1.4.3 Rate of reversion of resistance

Given that there already is considerable resistance to the benzimidazole family of drugs and increasing resistance to the levamisole family, the usefulness of these compounds will depend on the level of reversion to susceptibility that may occur if their use is discontinued (Waller *et al.* 1985). Reversion is a decrease in the frequency of resistant individuals in a population following removal of the selecting agent.

Factors affecting the rate of reversion are largely related to the fitness of homozygotes and heterozygotes carrying the resistance alleles. In some instances the resistant

worms have demonstrated increased fitness (Maingi *et al.* 1990) in which case there would be no reversion. Coles and Roush (1992) cite evidence for and against reversion, the general conclusion being that should reversion occur it is unlikely to return gene frequencies to their original levels and that reintroduction and continued use of the agent may lead to a resistant population more rapidly than originally occurred.

Experimental evidence certainly has shown that resistance alleles do persist at low but appreciable levels from previous exposure (Waller *et al.* 1985) resulting in a faster build up in resistance than seen in a susceptible line. However, this may not preclude the use of the original drug particularly if alternation with one or more effective drugs commences while the frequency of resistance genes is still relatively low. The problem is being able to detect the presence of resistance genes prior to drug failure under field conditions.

### 2.1.4.4 Duration of exposure to a chemical group

Drench rotation, that is alternating between drenches from different groups of chemicals, may delay very high levels of resistance from developing thus prolonging the practical usefulness of drenches (Waller *et al.* 1989). It has been suggested that to reduce the rate at which anthelmintic resistance develops, alternation of anthelmintic types needs to be timed so that one generation of parasites only is exposed to each chemical group (Prichard *et al.* 1980). This practice assumes there is some reversion to susceptibility for the drug not in current use.

Another practice that has been adopted is to give combination drenches. Some results suggest that this simultaneous strategy is a more effective method of resistance management than the strict rotation of drugs because each treatment kills a greater proportion of the target population (Smith 1990). Combination drenches will be effective when individual worms are resistant to one drug only (le Jambre *et al.* 1978; Smith 1990). However, a mixture may neither work nor delay increases in resistance if

resistance is already high (Waller *et al.* 1990) and multiple resistance within the one individual worm has been demonstrated (Smith 1990).

### 2.1.5 Current approaches to parasite control

By the early 1980s it became obvious that methods relying on chemical means alone to control worms had a very limited future. Programs such as Wormkill were developed (Dash 1986a) using information on anthelmintic efficacy and epidemiology of the parasite (such as the peri-parturient rise in FEC in ewes), along with the rotation of chemical groups and the strategic use of narrow spectrum drugs to control *H. contortus*. Wormkill is a preventative treatment program designed to control haemonchosis and trichostrongylosis, while at the same time reducing the frequency of treatment with broad-spectrum anthelmintics, for lambs reared on contaminated pastures under set stock conditions. The key to this program was the effective control of *H. contortus* with the narrow spectrum drug, closantel. Reports of resistance to this drug have been made (Rolfe *et al.* 1990), but since the introduction of Wormkill, closantel's persistent action against *H. contortus* for 6-8 weeks has resulted in *H. contortus* becoming rare on some farms (Barger *et al.* 1991). One other key to the success of Wormkill was the availability of an effective broad-spectrum drench and for properties exhibiting both benzimidazole and levamisole resistance this has been provided by the introduction of ivermectin.

Anthelmintic resistance can be managed by adopting the strategies discussed above, but an integrated control program is not straight forward for farmers to adopt. The method of worm control most favoured by sheep producers is frequent anthelmintic treatment (Dash 1986a). The adoption of a strategic drench program relies on assessing which drenches are effective by a FEC reduction test, following the prescribed drenching schedule closely and providing "clean" pastures for susceptible classes of stock such as weaners. Compared to drenching alone, this program requires expertise outside the scope of the farm manager and imposes restrictions on stock movements on a property. Consequently worm control is still largely dependent on regular therapeutic anthelmintic treatment. The management of resistance also relies

on early detection so that resistance alleles do not get to the stage of re-association with other fitness characteristics. This may happen after continuous intense selection such as using a drug until it fails. However, it usually takes control failure manifested as stock losses before anthelmintic resistance is diagnosed in the field. More sensitive tests are required to detect the effect of resistance alleles at a low frequency to conserve the life of currently effective drugs.

Currently ivermectin is highly effective, but its useful life will be determined by the rate to which resistance development can be slowed. The likelihood that new drugs can be discovered and developed at a rate to keep pace with parasite resistance is small. A downward trend in innovative activity in the pharmaceutical industry strongly indicates that the problems caused by anthelmintic resistance cannot be avoided merely by replacing old drugs with new ones (Smith 1990). The search for effective drugs has become increasingly expensive. In 1956 the screening of 1800 compounds produced one effective insecticide, in 1965 the average number screened was 3600, in 1969 this had increased further to 5040 and in 1975 10,000 compounds were screened to find a successful commercial drug (Metcalf 1980). The avermectins were discovered during an intensive search for natural anthelmintic compounds in which over 40,000 cultures were screened (Campbell *et al.* 1983). Adding to the cost of screening is the complexity of the synthetic process required to manufacture new drugs. For example the production of DDT is a one step synthesis, dieldrin takes three steps and the production of the synthetic pyrethroid allethrin is a 13 step synthesis.

The amount of screening to find new drugs and the complexity of their production has led to a dramatic increase in the cost of research and development. The conclusion is inevitable that it will be more difficult and increasingly expensive to discover and develop new drugs. Also increased awareness of the hazards of pesticide usage, chemical mutagenesis and carcinogenesis will increasingly bring public health regulations into force to limit the use of chemicals in the production of food and fibre products (Metcalf 1980).

Other possible avenues for nematode control include the development of an effective vaccine (Miller 1996), biological control of free-living stages of the parasite (Waller

and Faedo 1996) and the manipulation of host nutritional status to boost immune response (Coop and Holmes 1996). All of these avenues show some promise but are unlikely to provide complete protection equivalent to anthelmintic treatment. In this sense they may be considered to be similar to host resistance and may play an important role in an integrated control system in the future.

### 2.1.6 Conclusion

The idea of breeding for a resistant host has largely been ignored while there have been relatively cheap and effective chemical means of controlling sheep parasites. However, the outlook for parasite control, using current control techniques, is not optimistic. Anthelmintic resistance is an unavoidable evolutionary consequence of drug use. The rate to which build up in anthelmintic resistance can be slowed is not sufficient to provide the time for the progressive discovery and development of new drugs for parasite control. Therefore, the possibility of breeding for a resistant host needs to be considered thoroughly as a means of assisting in the management of parasite populations.

## 2.2 Phenotypic traits associated with resistance to internal nematode parasites

### 2.2.1 Introduction

Genetic resistance to disease is an important method of prevention and control for a small number of livestock diseases. Mastitis and ketosis are included in the Norwegian commercial dairy cattle breeding program and have resulted in a valuable reduction in the incidence of these diseases (Solbu and Lie 1990). An increasing number of New Zealand sheep breeders are using genetic resistance to reduce the debilitating effect of facial eczema, a disease caused by a fungal toxin in pasture (Towers *et al.* 1990). Host resistance to a range of commercially important diseases such as bovine leukemia, scrapie, fleece rot, trypanosomiasis, gastro-intestinal nematodes and cattle ticks has a genetic component (Gavora 1984) indicating the potential to use selection to reduce economic losses from these diseases. This section will discuss phenotypic traits that may be used to assess the resistance of sheep to parasitic nematodes and the requirements of a good indicator trait for disease resistance.

### 2.2.2 Defining resistance

Animals can minimise the detrimental impact of parasitic organisms in two ways, firstly by limiting the number of parasites they are host to, and secondly by reducing the impact of the disease organisms on production. These two attributes have been termed "resistance" and "resilience" respectively (Albers *et al.* 1987). Phenotypic traits will be discussed in reference to disease resistance rather than resilience. Increased resistance to internal parasites is achieved by the host through a number of avenues - reduced establishment of incoming larvae, arrested or delayed development, accelerated expulsion of adult worms and reduced fecundity of female worms, all achieved through a more rapid onset of the immune response to parasite challenge (Dineen 1978).

## 2.2.3 Direct and indirect selection

The direct measure of worm resistance is the total number of parasites carried by the animal. This is a measurement that can only be obtained by slaughter and collection of worms from the relevant section of gastro-intestinal tract. Therefore, an indirect measurement of resistance is required for the selection of breeding stock. Indirect selection traits or indicator traits can be either traits associated with the parasite infection or traits that are independent of infection and predict the animal's response to infection when challenged. The latter category of indicator traits has the distinct advantage of not having to challenge the host animal with the virulent parasite in the process of resistance assessment. Indicator traits associated with infection differentiate between animals on the basis of their response to infection and for a period of time the host will suffer the adverse effects of the disease.

## 2.2.4 Candidate indicator traits

A range of indicator traits for nematode resistance have been studied and are summarised in Table 2.1. Because of the strong influence of the immune system on nematode resistance, prime candidates for investigation as possible predictor traits have been processes associated with the immune response and the products of the immune response. Studies with sheep selected for responsiveness to vaccination against *T. colubriformis* have shown that the high responders have increased reactivity across a broad range of immunological functions (Windon 1991).

### 2.2.4.1 Faecal egg count (FEC)

To date FEC has been the most direct measure of worm burden or parasite number. This measurement is defined as an indicator trait because the definition of resistance is described in terms of nematode numbers in the sheep. However, FEC also has some value of its own as a selection objective, as it provides a direct measure of pasture contamination.

Table 2.1 Phenotypic indicator traits associated with resistance to nematode infection in sheep

| Indicator Trait | Predictive or Disease Trait | Reference |
|---|---|---|
| FEC | Disease | McKenna 1981 |
| Blood eosinophil count | Disease | Dawkins *et al.* 1989<br>Rothwell *et al.* 1993<br>Woolaston *et al.* 1996 |
| Resilience | Disease | Albers *et al.* 1987<br>Morris *et al.* 1995 |
| Whole blood lymphocyte culture stimulation index | Predictive | Riffkin and Yong 1984<br>Cummins *et al.* 1991 |
| Ovine lymphocyte antigen type | Predictive | Outteridge *et al.* 1985<br>Outteridge *et al.* 1988 |
| Haemoglobin type | Predictive | Agar *et al.* 1972<br>Courtney *et al.* 1985 |
| Enzyme linked immunosorbent assay of blood larval antigens | Predictive and/or disease | Douch *et al.* 1995<br>McEwan *et al.* 1995b |

FEC has been established as a good indicator of strongyle worm burden (*Nematodirus* spp excluded), with a correlation coefficient of 0.74 between individual egg counts and worm counts in sheep up to 12 months of age (McKenna 1981). This correlation was lower in adult sheep (0.23), presumably due to suppression of egg production as a result of a strong immune response The FEC and worm counts were measured on sheep drawn from a number of flocks with natural infections at time of slaughter, with no control over the duration or stage of infection. A closer examination of the relationship between FEC and worm count, within and between selected resistant and susceptible Romney sire progeny groups, has verified this association. The correlations between FEC and total worm burden were high in both resistant and susceptible groups being 0.83 and 0.75 respectively (Bisset *et al.* 1991). The heritability of FEC has been estimated in a number of studies for different breeds, types of infections and age of animals (See Table 2.2).

Table 2.2 Heritability (±standard error) estimates for transformed FEC after natural and artificial nematode infection in a range of sheep breeds

| Breed | Age of sheep | Infection type and number of FECs | Heritability | Reference |
|---|---|---|---|---|
| Merino | 4-5 months | Artificial *H. contortus* 1 count, max. of 1-3 weekly measurements | 0.29±0.03 | Woolaston and Piper 1996 |
| Romney and Romney cross | 5-8 months | Natural (*T. colubriformis* and *Ostertagia* spp), 1 count | 0.34±0.19 | Watson *et al.* 1986 |
| Merino | 18 months | Artificial *H. contortus*, 1 count | 0.23±0.13 | Piper 1987 |
| Merino | 4-5 months | Artificial *T. colubriformis* after vaccination with irradiated *T. colubriformis*, mean of 5 counts made at weeks 3-7 p.i.[A] | 0.41±0.04 | Woolaston *et al.* 1991 |
| Merino | 6-12 months | Natural, 2 counts in different infection cycles | 0.42±0.14 | Cummins *et al.* 1991 |
| Romney | 5-8 months | Natural, 2 counts in different infection cycles | 0.53±0.15 | Baker *et al.* 1991 |
| Romney | 5-8 months | Natural, 1 count | 0.27±0.07 | Bisset *et al.* 1992 |
| Romney | 5 months | Natural, 1 count | 0.13±0.07 | McEwan *et al.* 1992 |
| Merino | 4-5 months | Artificial *H. contortus*, 1 count at 4 weeks p.i.<br>1 count at 5 weeks p.i. | 0.34±0.10<br>0.26±0.09 | Albers *et al.* 1987 |
| Red Maasai and Dorper | 10 months | Natural, average of 2 counts 2 days apart | 0.20±0.08 | Baker *et al.* 1994 |
| Romney | 4 months<br>6 months | Natural infection, 1 count<br>1 count | 0.39±0.13<br>0.46±0.14 | Morris *et al.* 1993 |
| Romanov | 6-10 months | Artificial infection, mean of six counts | 0.55 | Gruner and Lantier 1995 |
| Polish Long Wool | 6-8 months | Natural, average of 2 counts 2 months apart | 0.28±0.16 | Gruner and Lantier 1995 |
| Hungarian Merino | 6-7 months | Artificial *H. contortus*, average of 4 counts with second infection imposed | 0.49±0.17 | Sreter *et al.* 1994 |

[A] Post infection.

### 2.2.4.2 Blood eosinophil count

Studies with sheep selected for responsiveness to vaccination against *T. colubriformis* have shown that high responders have increased blood eosinophil count (Rothwell *et al.* 1993), the difference between the high and low responding lines being significant only after *T. colubriformis* infection, when the immune response was having its greatest impact on the parasite burden. Eosinophil count had for many years been considered an important indicator of helminthiasis. The relatively low level of circulating eosinophils in the low responders during parasite infection suggests that eosinophil response was more a measure of immune mediated response rather than level of worm burden (Dawkins *et al.* 1989).

A study with Australian Merinos gave a heritability estimate for blood eosinophil count, 4 weeks after artificial infection with *T. colubriformis* larvae, of 0.20±0.08, and a genetic correlation with FEC of -0.56±0.27 (Woolaston *et al.* 1996). In this instance the heritability of FEC was 0.39±0.10 and the use of eosinophil count as an indirect selection trait for FEC would have been 40% as efficient as using FEC itself (biologically the closest indicator trait we have for resistance). Blood eosinophil count has also been measured in a flock of Fijian sheep and in this flock the sire component of variance was negative precluding any estimate of heritability or genetic correlation with FEC (Woolaston *et al.* 1996). The phenotypic correlation between the two traits was -0.02. In this instance the parasite infection was natural from grazing infected pastures and the measurement was made 4-5 weeks post-drenching. Based on the available estimates for genetic parameters for blood eosinophil count, it appears that this trait alone would not be a useful indicator of parasite resistance.

### 2.2.4.3 Resilience

In the area of breeding for disease resistance the term resilience had been used to describe the effect of the parasite on the host and has been suggested as an alternative selection trait to the actual number of parasitic organisms infecting the host. In two separate studies with sheep resilience has been defined in different ways. Albers *et al.*

(1987) quantified resilience by measuring wool growth and liveweight gain on individual sheep in a parasitised then unparasitised state. Sheep with the highest resilience, in terms of wool growth, showed the smallest depression in production and resilience was moderately to highly correlated (0.59±0.36) with resistance (FEC), but lowly heritable (0.08±0.07). The heritability estimate for FEC (0.34±0.10) and genetic correlation between the two traits were such that the most efficient way to improve resilience was through selection for resistance rather than direct selection for resilience. The low heritability estimate for resilience precluded the reverse scenario, that is, selection for resilience being a more efficient way of decreasing FEC than direct selection for FEC itself.

In NZ work (Morris *et al.* 1995) resilience is defined in terms of the age at first drench and number of drenches that sheep require to gain liveweight and show minimal dags in spite of a nematode challenge. This definition of resilience, ability to produce at an "acceptable" level while infected, is not the same as that used by Albers *et al.* (1987) where production was measured in both the diseased and disease free state and resilience was defined as the ability to minimise the depression in production due to infection. The assessment of when an animal needs drenching is subjective and the repeatability of this trait is unknown. The heritability estimates for age at first drench were variable (0.06±0.03 to 0.14±0.03), as were the correlations with resistance (0.01±0.23 to 0.16±0.18) measured in half-sib groups that were routinely drenched. Given the low heritability of resilience this trait would not be open to significant improvement by performance testing but could possibly be improved by progeny testing. However, this would occur without a corresponding gain in resistance and if both traits were to be improved they would need to be selected for independently in an index.

#### 2.2.4.4 Whole blood lymphocyte culture (WBLC)

Lymphocyte responsiveness to nematodes develops early in the life of the sheep in the absence of the parasite, possibly as a genetically controlled response to heterophile antigens (Riffkin and Dobson 1979). Pre-infection lymphocyte stimulation index (SI)

levels gave the best single predictior of the potential of sheep to resist *Haemonchus contortus*. Riffkin and Yong (1984) demonstrated a phenotypic relationship between the SI and FEC in response to trichostrongylid antigens. In subsequent heritability studies (Cummins *et al.* 1991) the lymphocyte SI was moderately heritable (0.29±0.13) but not as heritable as FEC (0.42±0.14). The efficiency of WBLC is variable depending on the effects of a nematode challenge at the time of testing. Ideally the sheep should be worm free and under minimal challenge from pasture for the base level of intrinsic response to be measured accurately.

Selection lines were established to gain some measure of the impact of both WBLC and FEC on nematode resistance (Cummins *et al.* 1991). In the high resistance line selected for low FEC and high WBLC, FEC made the most contribution to resistance and this flock has continued to be selected for FEC alone (Cummins, pers comm.).

### 2.2.4.5 Ovine lymphocyte antigen type

Ovine lymphocyte antigens (OLA) are glycoproteins and are a sub-group of the major histocompatibility complex (MHC) antigens (Cullen *et al.* 1982). OLA type can be determined by serological testing and it is polymorphic, controlled by a number of loci (Outteridge *et al.* 1984). An examination of OLA type in sheep selected for responsiveness to vaccination against *Trichostrongylus colubriformis* has shown a different incidence of OLA type between the high and low responders, the high responders having a greater incidence of SY 1 type (Outteridge *et al.* 1985). There has been significant divergence in FEC of the two lines (Windon *et al.* 1987) and this may indicate an association between FEC and OLA type. However, the difference in OLA type may be a founder effect resulting from the selection and mating of particular sires in each selection line. A consistent relationship between FEC and OLA has not been demonstrated within selection lines.

An independent study of sheep bred for a particular OLA type (Outteridge *et al.* 1988) has also indicated a relationship between FEC and OLA type but once again the association was not consistent within sire groups and the chance effect of founder

sires (5) on FEC in each selection line cannot be ignored. This relationship has not been confirmed in *H. contortus* selection lines where there was no association between OLA type and susceptibility to *H. contortus* infection (Cooper *et al.* 1989). Once again the number of sires was small (6) but the hypothesis that OLA type affects resistance cannot be dismissed.

An analysis of OLA type and FEC in a large random bred population and within a central test sire evaluation (Woolaston and Gray, unpublished data) has not been able to confirm any significant relationship between OLA type and FEC. Given these results it is unlikely that OLA type will be a useful indicator trait of nematode resistance.

### 2.2.4.6 Haemoglobin type

Initial studies with sheep of different haemoglobin (Hb) types suggested that sheep of Hb type A may harbour fewer worms than sheep with Hb type AB or type B following *H. contortus* infection (Evans *et al.* 1963; Allonby and Urquhart 1976). The lower FEC of the type A sheep suggested these animals were more resistant to the establishment of infection than the type AB. However there were no significant differences in the haematological changes between type A and AB sheep (Evans *et al.* 1963). There appears to be a sound relationship between Hb type and Hb concentration, with type A sheep having a higher haematocrit (PCV) than type AB or B in an uninfected state (Agar *et al.* 1972).

The suggestion has been made that the relationship between Hb genotype and PCV during *H. contortus* infection may be a result of the initial relationship between Hb type and PCV in the uninfected sheep (Gray 1989). In a comparison of resistant sheep breeds (St Croix, Barbados Blackbelly, Florida Native) with relatively susceptible sheep (Dorset) there was no effect of Hb type on egg count, PCV or worm count in two experiments; in a third experiment type AB animals were superior for egg count and PCV but not worm count (Courtney *et al.* 1985). With *T. colubriformis* infection

there was no significant correlation between Hb type and egg counts during either primary or secondary infection (Wincon and Dineen 1980).

Using Hb type as a possible selection criteria for parasite resistance is unlikely to be successful due to the inconsistent effect of Hb on resistance and the fact that it has only been linked to *H. contortus* infection, whereas most field infections are a combination of parasites which often will not include *H. contortus* in the temperate winter rainfall regions. Breeds that are reputed to be resistant to *H. contortus* vary greatly in their gene frequency for Hb A, with some resistant breeds showing no incidence of type A (Agar *et al.* 1972) so that selection for a particular Hb type may give varying responses within different breeds. However, with the majority of studies reporting Hb influences on parasite resistance there often has been small numbers per experimental group and comparisons have not been statistically powerful.

### 2.2.4.7 Enzyme linked immunosorbent assay (ELISA)

Recent reports from New Zealand suggest that resistant sheep can be identified by their antibody response (Ab and $IgG_1$) to *T. colubriformis*, as measured by ELISA techniques (Douch *et al.* 1995). Animals were sampled as weaners (4-10 months of age) at various times relative to infection and anthelmintic treatment. The estimates of heritability for $log_eAb$, $log_eIgG_1$ and $log_e(FEC+100)$ at 6 months of age (March) were 0.29, 0.43 and 0.20 respectively. The genetic correlations between $log_eAb$ and FEC and $log_eIgG_1$ and FEC were -0.56±0.18 and -0.35±0.19 respectively. Using these results, selection on serum antibody levels at 6 months of age would result in 51-67% of the genetic gain possible from directly selecting for FEC. A similar response was predicted for FEC (45%) by selecting for level of antibody using data from Coopworth sheep (McEwan *et al.* 1995a), but in the same trial there was no genetic correlation between antibody level and FEC in Romney sheep.

In the situation where there is a correlated response the ELISA test for antibody response would need to have benefits far greater than FEC, such as ease of sample collection, transportation and cost of testing, to be the preferred method of testing for

resistance. It does appear that an antibody response is not sensitive to the level of infection at time of measurement but there is still an obvious requirement for sheep to have been exposed to the parasite at some stage prior to testing.

## 2.2.5 Characteristics required of indicator traits

For an indicator trait to be usefully included in a selection program it must meet a number of criteria from a genetic and practical point of view.

### 2.2.5.1 Genetic variation

The variation observed for the indirect trait must have a genetic component, which will be indicated by its heritability estimate. Heritability estimates are available for some of the indicator traits discussed in this chapter. OLA type and Hb type are the result of single gene action and display a Mendelian inheritance. Heritability estimates for WBLC (0.29±0.13, Cummins *et al.* 1991), blood eosinophil count (0.20±0.08, Woolaston *et al.* 1996) and ELISA (0.27 to 0.43, Douch *et al.* 1995) are similar to, or lower than for FEC. As the genetic correlation between the traits listed above and FEC is likely to be substantially less than perfect (-0.10±0.00 for WBLC, -0.56±0.27 for eosinophil count, -0.35 to -0.56 for ELISA), progress in reducing FEC and worm numbers by the use of these indicator traits will be slow.

The indirect trait must also be well correlated with the disease trait and this relationship must be genetic in nature. If the disease component of the breeding objective is to increase parasite resistance throughout the life of the animal, it is also important that the indirect trait be correlated with worm burden at all ages, or in all classes of susceptible stock, as well as worm burden at the point in time that it is measured. Total worm burden is not a practicable measurement because it requires the slaughter of the animal, so there have been no estimates published for genetic correlations between indirect traits of resistance and worm burden. Many studies have reported phenotypic relationships between indirect traits and worm counts.

To date FEC has been the most direct measurement of parasite burden and its phenotypic relationship with worm number has been well established (McKenna 1981; Bisset *et al.* 1991). Candidates for indirect measurement of resistance have often been assessed in terms of their performance relative to FEC. If a phenotypic marker has a heritability of 0.3, which is consistent with estimates for immunological traits, the genetic correlation with FEC would need to be 1 for an equal response to selection. As this is unlikely to be the case in practice, the use of a phenotypic indicator will result in a loss of response relative to selection for FEC. However, this loss must be balanced against the benefits of using the indicator trait which may be apparent in terms of cost of measurement or impact on production.

#### 2.2.5.2 Phenotypic variation

The indicator trait should be highly variable and the method used to measure it should reflect this variation. Parasite infection, in terms of worm numbers, provides a continuous scale for measurement of disease resistance with the only proviso being a certain challenge may be necessary for genetic differences to be expressed. Selection on a continuous trait will allow greater discrimination between animals for resistance. For example, selection for OLA type or Hb type will result in a flock homozygous for these traits as they are under the control of a limited number of alleles at identifiable loci. Using this type of an indirect trait then precludes the utilisation of genetic variation that exists within OLA or Hb type. This loss of discrimination occurs with disease traits such as fleece rot and fly strike where disease incidence varies depending on environmental conditions and animals not exhibiting the disease cannot be differentiated between for resistance.

#### 2.2.5.3 Expression in both sexes and age of measurement

To optimise genetic gain an indicator trait should be expressed in both sexes and be able to be measured at an early age. Selecting on both sexes increases the overall selection intensity for the flock. However, the measurement would need to be simple and inexpensive to warrant measuring replacement females. The ability to gain a

measure of resistance at an early age also allows generation interval to be shortened. The measurement of weaners for FEC or even lambs for an indicator trait would allow 2-2.5 year old sires to be assessed on the performance of their progeny. For selection on individual performance early measurement is not as critical, as the limiting factor will be the age of measurement for other production traits such as liveweight gain, clean fleece weight and fibre diameter.

Early culling for low resistance has non-genetic benefits in terms of pasture contamination and subsequent exposure of susceptible stock to infection. Studies of the frequency distribution of trichostrongylid nematodes has demonstrated that these parasites have an over-dispersed distribution with most animals carrying a less than average worm burden, while a few heavily infected animals harbour a large proportion of the total parasite population (Barger 1985). In this study the four species of parasites monitored were sufficiently aggregated that a culling of 21% of the flock would achieve a 50% reduction in worm burden. Studies of the distribution of worm egg counts (Cummins *et al.* 1991) have also demonstrated that a large proportion of the pasture contamination (50%) is caused by a relatively low proportion of the flock (15%), the removal of these sheep having a potentially important impact on parasite epidemiology and subsequent strategies needed in a resistant flock to control parasites.

### 2.2.5.4 Genetic co-variation with other traits of importance

Genetic associations between the resistance trait and other traits of importance in the breeding objective need to be considered. When assessing the merit of any indirect trait, its effect must be evaluated in terms of the aggregate genotype which comprises all traits contributing to profit (Smith and Smith 1993). Neutral or favourable associations allow the incorporation of the indicator trait without unduly compromising the overall breeding objective. Unfavourable associations do not preclude the use of an indicator trait, but do make its inclusion in the breeding objective in any other form except selection index difficult. For example, fibre diameter has an antagonistic genetic relationship with clean fleece weight in many Merino flocks. Selection for reduced fibre diameter and increased fleece weight can be

achieved by the combination of these traits with their relative economic weightings in a selection index. Should disease resistance be unfavourably correlated with production it can be treated in the same manner.

### 2.2.5.5 Measured independent of disease

An ideal indicator trait for disease resistance is one that can be measured independently of the presence of the disease. This attribute allows a resistance measurement to be made without the animals experiencing the harmful effects associated with infection and the subsequent loss in production that may result. If the indicator trait is an infection trait the breeder must wait until the disease actually occurs before the measurements can be made. With nematode infection this involves monitoring the FEC of the animals until the incidence of the disease and its level of infection is great enough to allow genetic differences to be detected. With seasonal variation in the availability of larvae on pasture the animals may not become infected during the period that is most suitable for measurement from a management point of view. To allow greater control over time of measurement and the period over which an infection must run, artificial infection has been used. Artificial infection has the advantages of ensuring all animals are challenged at the same time and have an established egg producing worm population within 4 weeks of dosing with infective larvae. However, there are potential disadvantages associated with artificial infection, namely that the animal is not given the opportunity to experience the trickle infection that occurs when sheep graze infective pasture, and the challenge is single-species whereas natural challenge usually comprises a mixture of worm species.

### 2.2.5.6 Cost effective and simple to measure

Cost and ease of measurement need to be considered for each candidate trait. An expensive test will restrict the number of animals that can be measured with a subsequent effect on selection intensity and response. A measurement that involves complicated sampling procedures and laboratory techniques will obviously increase costs but may also preclude breeders from making the measurements because of their

accessibility to facilities. For instance, if blood samples need to be collected by a qualified technician and processed within a short time after sampling, breeders at a distance from laboratories may not be able to participate, regardless of the cost of measurement. However, cost and ease of measurement should not preclude traits from being evaluated as potential indicators of disease resistance as technology has a habit of catching up with successful and profitable innovation.

## 2.2.6 Assessment of resistance

FEC is the only measurement to date that has been used extensively to measure the parasite resistance of sheep. It meets many of the above requirements and is a reasonably practical indirect trait. Its major disadvantage is that it is a disease trait and the animals must become parasitised to obtain a resistance measurement.

Repeatability analysis allows the separation of variation in FEC due to temporary effects, which are entirely environmental in origin, and permanent effects both genetic and environmental (Falconer 1989). The repeatability of a trait sets an upper limit to its possible heritability, although this is rarely achieved in practice due to the occurrence of undefined permanent environmental effects. Repeatability is a useful parameter to consider when assessing the potential value of an indicator trait as it gives a relatively simple and fast estimate of the potential heritability.

### 2.2.6.1 Repeatability within infection cycle

Within an infection cycle the repeatability of two FECs made a week apart, 4-5 weeks into the infection, is high. Repeatability estimates were 0.6-0.7 for a single artificial infection of *H. contortus* (Woolaston *et al.* 1991; Barger, unpublished data) and 0.69 for an artificial infection of *T. colubriformis* after vaccination with irradiated *T. colubriformis* (Windon, unpublished data). Over an extended infection repeatability of fortnightly FECs was also found to be high, 0.6 for an artificial trickle infection with *H. contortus* (Barger and Dash 1987) persisting for 13 weeks.

These estimates are high and have been calculated from FEC measured on animals artificially infected with nematode parasites. However, there may be situations on farm where the repeatability of FEC could be considerably lower. In a ram breeding flock fed a grain supplement due to drought the repeatability of two FEC's 7 days apart after an artificial challenge with *T. colubriformis* was 0.35 (Eady, unpublished data). This result indicates that management practices such as drought feeding may influence the reliability of FEC for predicting worm numbers and these issues should be carefully considered when making assessments of parasite resistance.

### 2.2.6.2 Repeatability between infection cycles

The repeatability between artificial infections with *H. contortus* was found to be 0.3 (Barger and Dash 1987). Selection line flocks in New Zealand and in Victoria, Australia have used FEC in two consecutive natural infection cycles as their criteria for measuring resistance. In the New Zealand flocks the repeatability of FEC across these two infection cycles has ranged from 0.4 to 0.5 (Baker *et al.* 1991) and for the Victorian flock ranged from 0.11 to 0.48 with a mean of 0.27 (Cummins *et al.* 1991). The time for the completion of the two infection cycles in each country was approximately 3 months.

There have been instances where the repeatability between infection cycles separated by a number of months has been low. In a sire evaluation program the repeatability of FEC measured at 9 months of age and again at 17 months of age after natural infections was 0.18±0.05 (Eady, unpublished data). The two infection cycles were separated by 3 drenches. FEC of rams in a group breeding scheme were measured at 10 months and again at 15 months of age giving a repeatability of 0.08±0.06. The first FEC was 28 days after an artificial infection with *T. colubriformis* and the second and subsequent FEC was after a natural infection (*T. colubriformis* 68% and *Ostertagia* spp 32%) which took 12 weeks to build up to a level of greater than 1500 epg. There is evidence that changes in supplementary feeding and paddocks can influence the repeatability of FEC (Kelly and Gray 1995). Changes to management, feeding or any

exposure to stress should be considered when deciding on the timing of FEC measurements.

There is still a lot to be understood about what influences the repeatability of FEC especially under commercial conditions where there is little control over the rate of infection and management of animals between measurements. When sheep are exposed to parasite infection from birth with no drenching it appears that the repeatability of FEC from month to month is low and variable (0.03-0.34, Karlsson *et al.* 1991). This may be due to animals being at different stages of resistance development, unadjusted by anthelmintic treatment which can itself cause a loss of natural resistance (Barger 1988).

The results to date suggest that multiple measurements of FEC in consecutive cycles, at fairly close intervals, may be the best way to utilise two independent measurements. Assuming a repeatability of 0.6 for FEC and a heritability of 0.3, two measurements within the same infection cycle would increase the heritability estimate to 0.37 (Falconer 1989), resulting in only a small increase in response for the extra investment. If the repeatability between infection cycles is 0.3 and the heritability of a single measurement is 0.3 then the use of two measures in different infection cycles will increase the heritability of FEC to 0.46. This theoretical increase is consistent with heritability estimates obtained in flocks where two FEC have been made (Table 2.2). To gain the most return from the investment of resources into a breeding program for parasite resistance it would be of greater benefit to measure FEC over two consecutive infection cycles, rather than within the one cycle or over a longer interval.

### 2.2.6.3 Repeatability across ages

Measurements to aid in the selection of breeding stock are usually made at an early age and are assumed to be indicative of lifetime performance. Atkins and Mortimer (1987) have demonstrated that this is not the case for clean fleece weight in Merino sheep and, as lifetime clean wool production is obviously an important determinant of income, a genetic correlation of less than 1 between hogget fleece weight and lifetime

production must be accounted for. Although weaners are the most susceptible class of livestock to parasite infection, a lifetime improvement in resistance is desirable as peri-parturient ewes also suffer from parasite challenge as their immune response weakens during this period. The rise in egg output in lambing ewes then provides a reservoir of infective larvae for their offspring. There are no published genetic correlations between weaner FEC and FEC at a later age but studies of selection line flocks have indicated that resistant ewe weaners maintain their superiority as adult ewes both as dry sheep and more importantly during the peri-parturient peak in FEC (Watson *et al.* 1992; Woolaston 1992). As a consequence ewes selected for lower FEC as weaners should assist to reduce seasonal pasture contamination during lambing by developing a lower peri-parturient peak as well as resisting reinfection.

#### 2.2.6.4 Artificial versus natural infection

The desired trait to be changed by selection is FEC after natural infection and this may not be the same trait as FEC after artificial infection because the artificial challenge will remove some of the variation in FEC due to level or pattern of larval ingestion which may have a some genetic basis. For instance, a characteristic such as grazing behaviour may have some impact on the number of worms that an individual sheep picks up from pasture and it is possible that grazing behaviour has some genetic component. However, selection flocks have demonstrated that using either artificial or natural selection will result in offspring with lower FEC after natural infection. In each instance the trait FEC may not be controlled by exactly the same set of genes but the end result is comparable. Heritability estimates for FEC after artificial or natural infection have been similar (See Table 2.2).

Although there have not been any published estimates of the genetic correlation between artificial and natural infection, evidence from selection line flocks suggest that selecting for FEC after artificial challenge results in similar differences in resistance to natural infection (Watson *et al.* 1992; Woolaston 1992).

### 2.2.7 Conclusion

Phenotypic traits used to measure resistance to internal parasites can be divided into two classes. The first class is infection traits such as FEC, blood eosinophil count and resilience which are only possible to measure during infection with internal parasites. The second class is predictor traits such as whole blood lymphocyte culture, ovine lymphocyte antigen type, haemoglobin type and enzyme linked immunosorbent assay which can be measured at any time. For any of these traits to be usefully included in a selection program they must meet a number of criteria from a genetic and practical point of view. The desirable genetic criteria are that the trait be heritable, well correlated with the disease at a range of ages, highly variable, expressed in both sexes and has a neutral or favourable correlation with other traits in the breeding objective. Practical requirements are that the trait can be measured independent of the presence of the disease, be cost effective and simple to undertake by breeders. FEC is the only measurement to date that has been used extensively to measure the parasite resistance of sheep. It meets many of the above requirements and is a reasonably practical indirect trait. Its major disadvantage is that it is a disease trait and the animals must become parasitised to obtain a resistance measurement.

# Chapter 3 Transformation and Standardisation of Faecal Egg Count Data

## 3.1 Introduction

Internal nematode populations in sheep have generally been found to be over-dispersed (Barger 1985) where most hosts carry few parasites, while a few heavily infected hosts carry a large proportion of the total parasite population. The negative binomial distribution provides a good description of observed distributions of worm counts from a range of worm genera, with all genera equally over-dispersed (Barger 1985). This is significant, as worm infections in the field will usually be a mixture of worm species over which the sheep breeder will have little control. FEC closely follows the same pattern of dispersion as it is a good indicator of worm burden (McKenna 1981) and FECs measured on a large group of sheep are generally not normally distributed.

Non-normal distributions and heterogeneity of variance in data can be addressed by scale transformation, although transformations should not be made without good reason. There are, broadly speaking, three main reasons for making a scale transformation: to make the distribution of residuals normal; to make the variance independent of the mean; and to remove or reduce non-additive interactions such as dominance and epistatic interaction (Falconer 1989).

An assumption in analysis of variance is that errors of prediction are normally distributed around each and every predicted dependent variable score. For conforming data a scatter plot of residuals should show a random pattern about a mean of zero. An additional test of normality is a normal probability plot of residuals where expected normal values are plotted against their actual normal values. Expected normal values are estimates of the $z$ score that a residual should have, given its rank in the original distribution if the original distribution is normal (Tabachnick and Fidell 1989).

A common effect that can also be attributed to the scale of measurement is a change in variance with changing mean. Heterogeneity of variance may affect the accuracy of selection unless proper transformation and perhaps even standardisation to a common variance is applied (Hill 1984). The implications of lack of homogeneity of variance in animal breeding are such that there will be a bias towards the selection of animals from the group with the highest variance, the extent of bias amplified with increasing selection intensity.

One method of testing for homogeneity of variance is to examine the relationship between mean and variance of the groups to be compared. A technique to do this is Levene's test which performs an analysis of variance of the statistic $z_{ij} = |x_{ij} - \bar{x}_i|$ where $x_{ij}$ is the $i$th measurement in the $j$th group, $\bar{x}_i$ is the mean of the random variable $x_i$. The variable $z_{ij}$ is the absolute value of the residual for the $i$th measurement of the $j$th group from a least-squares analysis (Levene 1960). If there is a significant difference in $z_{ij}$ between groups this indicates the assumption of independence of mean and variance is being violated.

The aims of this chapter are to examine the distribution of FECs, to assess the impact of different transformations on the correlation between estimated breeding values (EBVs) for FEC and the ranking of animals on these EBVs, to investigate the appropriateness of standardising data across sites and years, and to assess the effect of different transformations on heritability estimates.

## 3.2 Materials and methods

### 3.2.1 Distribution of FEC

The distribution of FEC and transformed FEC was examined in the Katanning, Turretfield and CSIRO resource flocks. A full description of each flock and the collection of FEC data is given in Chapter 4. These data sets were selected for this analysis because of the large numbers in each flock. Data were transformed using the

most appropriate transformation based on Levene's test for homogeneity of variance (Levene 1960) and the correlation between residuals and normal scores (Tabachnick and Fidell 1989). Levene's test was used in preference to Bartlett's test, as the latter is sensitive to departures from the assumption of an underlying normal distribution (Bailey 1981) while Levene's test is more robust in dealing with non-normal distributions.

### 3.2.2 Effect of data transformation on sheep selection

The effect of using different transformations on FEC EBVs was assessed using data from the New England Sire Evaluation Scheme (NESES). This analysis was done using the NESES data, rather than the data from the resource flocks, because its structure in terms of design and numbers of progeny per sire is typical of an industry situation in which FEC EBVs are calculated. Each year the scheme included approximately 16 predominantly finewool Merino sires. In 1993 two sires were entered from CSIRO research flocks at Armidale. In 1990 and 1991 ewes were inseminated at "Mirani", Walcha and in 1992 and 1993 at "Gostwyck", Uralla. The comparison of FEC EBVs with a range of transformations used the following FEC data. Progeny from each sire group born in 1990 were tested for worm resistance in July 1991 (number of animals, n=35 ) at 9 months of age. The 1991 progeny (n=439) were tested in March 1992 at 5 months of age, the 1992 progeny (n=462) in July 1993 at 11 months of age and the 1993 progeny (n=553) in March 1994 at 7 months of age. The 1990 and 1993 animals were sampled again as hoggets and these data were added to the analysis for the presentation of FEC EBVs for individual sires but was not used in the comparison of transformations or evaluation of data standardisation.

Following the period of natural worm challenge, FEC was determined for each animal using a modified McMaster technique (Whitlock 1948) with a lower limit of detection of 100 epg. The following transformations were made to FEC: $FEC^{0.5}$, $FEC^{0.33}$ and $\log_e((FEC/100)+1)$. These transformations were assessed, based on a normal probability plot (Tabachnick and Fidell 1989) and Levene's test for homogeneity of variance (Levene 1960). Least squares analysis of variance (Harvey 1987) was used to

estimate the effects of sire as well as the fixed effects of sex and year. First order interactions of main effects were tested for significance and sequentially omitted from the model if non-significant ($P<0.05$) or accounted for less than 2% of the variation. The following linear model was used to estimate sire and error components of variance for transformed FEC:

$$Y_{ijk} = \mu + S_i + s_j + y_k + \text{interactions} + e_{ijk}$$

where Y is transformed FEC; $\mu$ is the common mean; $S_i$ is the effect of the $i$th sire; $s_j$ is the effect of the $j$th sex (ewe or wether); $y_k$ is the effect of $k$th site-year group and $e_{ijk}$ is the random error.

The sire evaluation scheme data was also used to examine the effect, on the distribution of EBVs, of standardising FEC data across sites and years. Standardised FEC ($SDFEC^{0.33}$) was calculated as ($FEC^{0.33}_i$ - $FEC^{0.33}_{mean}$) x 1/SD. EBVs for the transformed FECs were calculated using PEST (Groeneveld 1990). Genetic links between years were used to calculate EBVs.

Standardised FEC EBVs were converted back to the units of eggs per gram (epg) of faeces as a means of explaining to ram breeders how standardised EBVs can be interpreted. This required the calculation of the average coefficient of variation for $FEC^{0.33}$ using FEC data collected from 24 flocks, including the resource flocks described in Chapter 4, as well as a number of commercial ram breeding flocks. The average coefficient of variation was then used to convert FEC EBVs to estimated progeny differences in FEC (epg) for worm infections averaging 500 epg and 1000 epg.

### 3.2.3 Effect of data transformation on heritability estimates

The effect on heritability estimates of using different transformations was assessed using data from the Katanning, Turretfield and CSIRO resource flocks. Least squares analysis of variance (Harvey 1987) was used to estimate the effects of strain (where

applicable), bloodline-within-strain, bloodline and sire-within-bloodline as well as the fixed effects and their interactions. Day of birth was fitted as a covariate. The statistical models for all flocks are given in Chapter 4.

## 3.3 Results

### 3.3.1 Distribution of FEC

In the Katanning, Turretfield and CSIRO flocks the coefficient of variation for FEC was high, ranging from 55-187% (Table 3.1). The transformation of FEC did not always result in a near-normal distribution of residuals, but the general outcome was that the assumption of normality was not overtly violated once data had been transformed. The correlation between residuals and normal scores for the data, after the most appropriate transformation, ranged from 0.954 to 0.997 (Table 3.1). Examination of means and variances for FEC in the Katanning, Turretfield and CSIRO flocks (Table 3.1) showed that a transformation was also required to make the variance of FEC independent of mean FEC. Before any transformation the correlation between mean and variance for the six measurements was 0.69; after a cube root transformation was applied to all data sets the correlation between mean and variance was -0.04.

### 3.3.2 Transformation and sheep selection

The distribution of FEC in each of the NESES groups was significantly skewed to the right as indicated by estimates of the parameter k of the negative-binomial ranging from 1.8 to 4.1. Regardless of transformation, significant ($P<0.05$) sire effects for FEC were present in the 1990, 1992 and 1993 drops, but not for the 1991 drop which was excluded from further analyses. Site-year group and sex both had a significant effect on $FEC^{0.33}$ ($P<0.05$). Using the criteria of normally distributed residuals and homogeneity of variance, the square root transformation was the most appropriate for the 1993 data and the log transformation for the 1990 and 1992 data. When all data were combined the best transformation was the cube root. The correlation between

FEC EBVs of all progeny, calculated using the three transformations, ranged from 0.98 to 1.00 (Table 3.2). The same correlations, calculated from EBVs for the 45 sires used across years, ranged from 0.91 to 0.98.

Table 3.1 Mean, variance and coefficient of variation for FEC measured in the Katanning, Turretfield and CSIRO flocks and correlation of residuals and normal scores after transformation

| Flock | Mean FEC (epg) | Variance FEC $(epg)^2$ | Coeff. of Var. (%) | Correlation of residuals and normal scores after specified transformation | No of Sheep | Worm spp. |
|---|---|---|---|---|---|---|
| Katanning 1990 | 4452 | 19241996 | 99 | 0.976 Log ((FEC/100)+1) | 954 | Hc |
| Katanning 1991 | 2637 | 2109951 | 55 | 0.997 Cube root FEC | 980 | Tc |
| Turretfield 1992 | 918 | 2242495 | 163 | 0.996 Log ((FEC/100)+1) | 1614 | Hc |
| Turretfield 1993 | 2790 | 2944350 | 62 | 0.954 Cube root FEC | 881 | Tc |
| CSIRO 1991 | 4244 | 56458239 | 177 | 0.992 Log ((FEC/100)+1) | 1076 | Hc |
| CSIRO 1992 | 311 | 337561 | 187 | 0.971 Cube root FEC | 1072 | Tc |

Table 3.2 Correlation between FEC EBVs using different transformations within sites

| Site and Year | Correlation (±standard error) between FEC EBVs using different transformations | | |
|---|---|---|---|
| | $FEC^{0.5}$ $FEC^{0.33}$ | $FEC^{0.5}$ Log((FEC/100)+1) | $FEC^{0.33}$ Log((FEC/100)+1) |
| Mirani 1990 | 1.00±0.0007 | 0.97±0.0003 | 0.99±0.001 |
| Gostwyck 1992 | 0.99±0.001 | 0.97±0.0006 | 0.99±0.002 |
| Gostwyck 1993 | 0.98±0.002 | 0.98±0.0006 | 0.99±0.002 |
| Sires - all sites | 0.98±0.01 | 0.91±0.002 | 0.97±0.01 |

These correlation coefficients show that there is strong agreement in FEC EBVs between the different transformations. This relationship was also examined for sheep at the extreme lower end of the distribution (most resistant 10% of sheep). Sheep were ranked on FEC EBV using each transformation and the possible loss of selection efficiency from using an inappropriate transformation was examined (Table 3.3). The

average EBV for the most resistant 10% of animals using the best transformation (that with the most nearly normal distribution and homogeneity of variance) is given in Table 3.3. EBVs using alternate transformations were then used to rank the animals. The change in average EBV (in the original units) was taken as an indicator of loss of efficiency.

Table 3.3 Relative efficiency of different transformations for ranking animals in the lowest 10% of FEC EBVs within sites

| Site and year | Efficiency of different transformations for selecting the most resistant 10% of animals, relative to the most normal transformation*, and evaluated on the latter scale | | |
|---|---|---|---|
| | FEC$^{0.5}$ | FEC$^{0.33}$ | Log ((FEC/100)+1) |
| Mirani 1990 | 100 | 100 | 100* |
| Gostwyck 1992 | 97.6 | 100 | 100* |
| Gostwyck 1993 | 100* | 99.7 | 98.1 |

For example, in the Gostwyck 1993 group, the average EBV of the lowest 10% of animals, using the best transformation (FEC$^{0.5}$), was -3.14 FEC$^{0.5}$ units. When the animals were ranked on FEC$^{0.33}$ the average EBV was -3.13 FEC$^{0.5}$ units and when ranked on log FEC was -3.08 FEC$^{0.5}$ units. These figures were used to calculate the relative efficiency of 99.7% for the cube root transformation and 98.1% for the log transformation as shown in Table 3.3. The results of this comparison indicate that there is little or no loss in selection accuracy if a sub-optimal transformation is used.

The same comparison was done for the 45 progeny tested sires (Table 3.4). When the 10% most resistant sires were identified, across all sites, there was a greater loss in selection accuracy, with efficiency of selection dropping to 87.2% when the log transformation was used to rank the sires, compared to using the "correct" cube root transformation (Table 3.4). The individual sires selected using each transformation are given in Table 3.4. The apparently greater loss in selection efficiency for the sires could be a function of both the correlation between EBVs for the sires (Table 3.3) and the small number of animals selected in the 10% most resistant.

Table 3.4 Relative efficiency of different transformations for ranking progeny tested sires in the lowest 10% of FEC EBVs across all sites and the sires selected to be the most resistant

| | Efficiency of different transformations for selecting the most resistant 10% of sires, relative to the most normal transformation*, and evaluated on the latter scale | | |
|---|---|---|---|
| | $FEC^{0.5}$ | $FEC^{0.33}$ | Log ((FEC/100)+1) |
| Sires - all sites | 96.2 | 100* | 87.2 |
| Selected 10% of sires | Woolaroo B204 | Woolaroo B203 | Nerstane 473 |
| | Yalgoo 942 | Nerstane 473 | Woolaroo B203 |
| | Roseville Park 69 | Yalgoo 942 | Europambela B980 |
| | Nerstane 473 | Roseville Park 69 | Winton 6339 |
| | Sierra Park Urq64 | Europambela B980 | CSIRO 91A 0619 |

Because the impact of using a sub-optimal transformation appeared to be small, the cube root transformation was selected for further data analysis. Mean and variance for $FEC^{0.33}$ from the three sites in the sire evaluation scheme are given in Table 3.5. A strong relationship remained between mean $FEC^{0.33}$ and its variance. The effect of this heterogeneity of variance, on selection of animals across sites, was investigated. From an across site-year analysis, identifying the most resistant 5% of animals using EBVs based on $FEC^{0.33}$ gave the proportions selected from each site-year group shown in Table 3.5. There was a predominance of animals selected from the Mirani 1990 drop, the most variable group, and no animals were selected from the Gostwyck 1993 drop, which had the lowest mean and standard deviation. Once the EBVs were based on standardised $FEC^{0.33}$ ($SDFEC^{0.33} = (FEC^{0.33}_{i} - FEC^{0.33}_{mean}) \times 1/SD$), there was more uniform selection across groups. This result is show graphically in Figure 3.1.

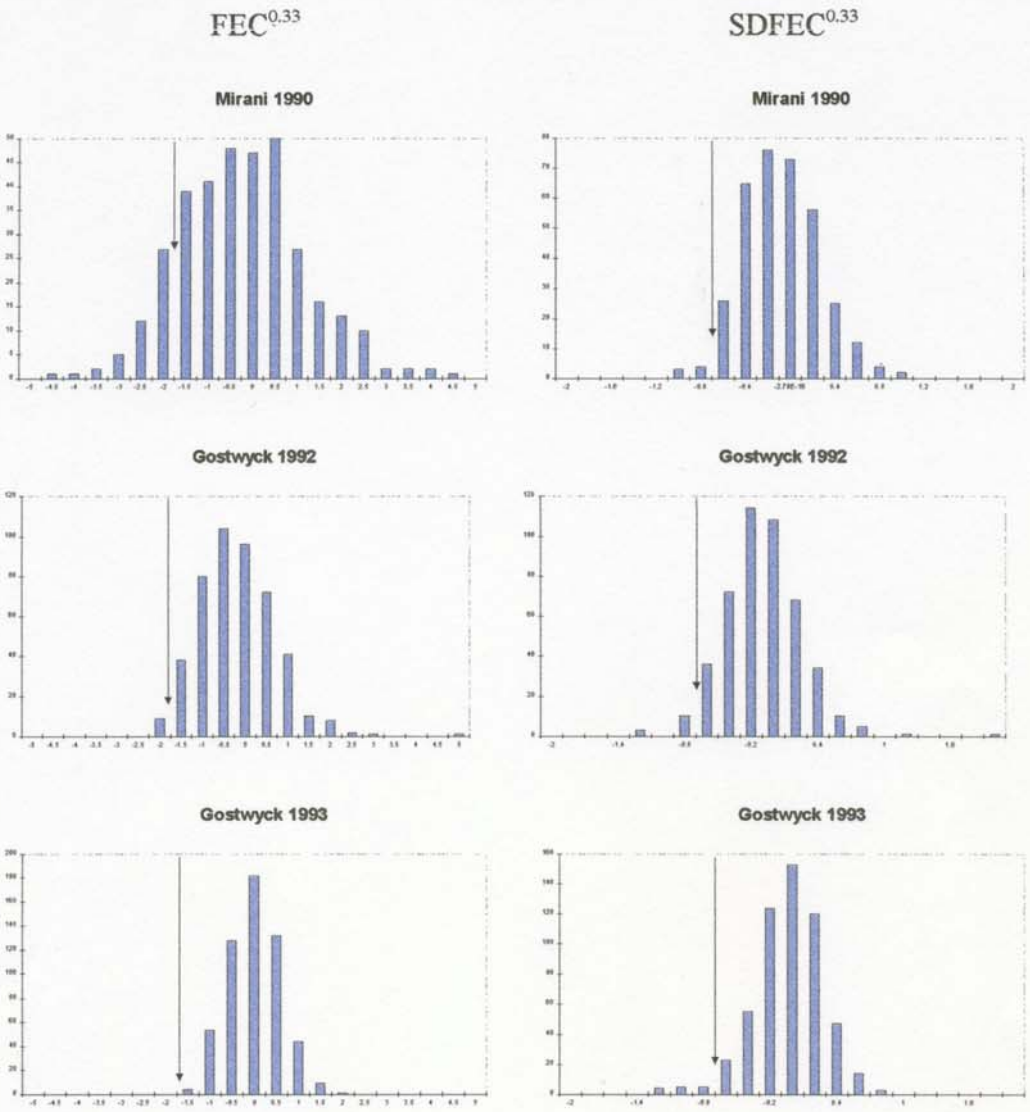


Figure 3.1 Distribution of transformed FEC EBVs at each site with approximate cut-off point (marked by arrow) for selection of the most resistant 5% of animals.

Table 3.5 Mean FEC and mean and standard deviation for $FEC^{0.33}$ within site-year, and proportion of animals from each site-year group selected among the most resistant 5% of animals

| Site and Year | n | FEC (epg) | $FEC^{0.33}$ | | Proportion (%) of each flock selected in most resistant 5% . | |
|---|---|---|---|---|---|---|
| | | | Mean ($epg^{0.33}$) | SD ($epg^{0.33}$) | EBV $FEC^{0.33}$ | EBV $SDFEC^{0.33}$ |
| NESES Mirani 1990 | 351 | 7010 | 18.12 | 4.27 | 14.8 (52/351) | 5.1 (18/351) |
| NESES Gostwyck 1992 | 462 | 1071 | 9.40 | 2.77 | 3.5 (16/462) | 6.9 (32/462) |
| NESES Gostwyck 1993 | 553 | 612 | 8.01 | 2.11 | 0.0 ( 0/553) | 3.3 (18/553) |

FEC EBVs for the sires evaluated in the NESES in 1990, 1992 and 1993 are presented in Figure 3.2. FEC breeding values have been estimated from standardised $FEC^{0.33}$ with a mean of zero and phenotypic variance of 1.

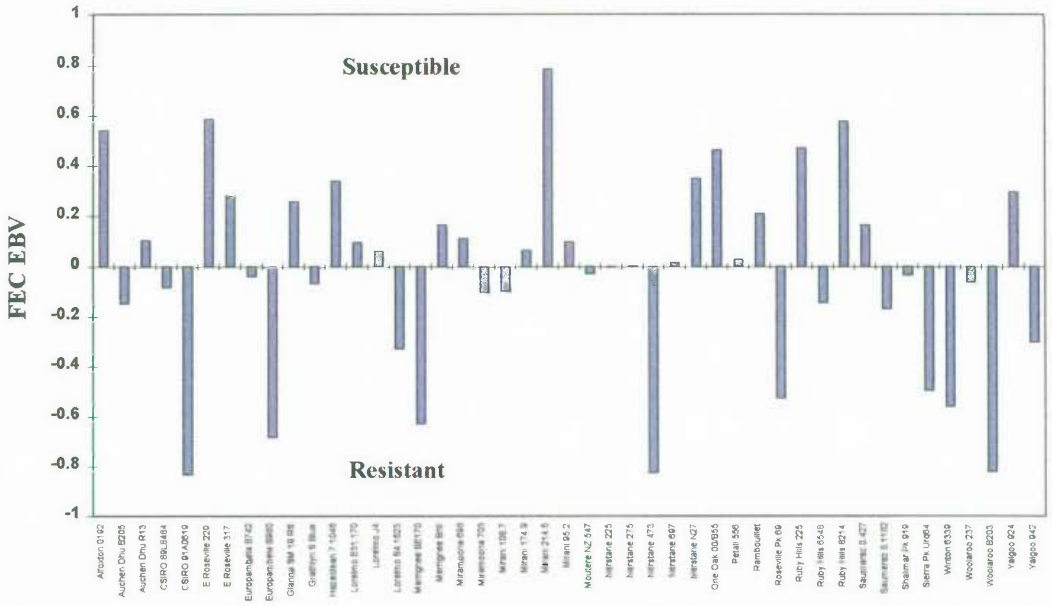


Figure 3.2 FEC EBVs for sires evaluated in the NESES in 1990, 1992 and 1993.

The average coefficient of variation for $FEC^{0.33}$ was 40%, with a standard deviation of 24.2%. This is based on 24 data sets which ranged from 16.5% to 115.7% (Table 3.6).

Table 3.6 Mean $FEC^{0.33}$ and standard deviation and coefficient of variation for $FEC^{0.33}$ from a range of Merino resource flocks, sire evaluation flocks and commercial stud flocks

| Data Source | Mean $FEC^{0.33}$ | SD $FEC^{0.33}$ | Coefficient of variation for $FEC^{0.33}$ (%) |
|---|---|---|---|
| Camden 1991 | 17.08 | 5.06 | 29.6 |
| Camden 1990a | 10.05 | 4.86 | 48.3 |
| Camden 1990b | 7.10 | 5.45 | 76.8 |
| Deniliquin 1992 | 9.67 | 3.34 | 34.6 |
| CSIRO 1991 | 10.84 | 8.85 | 81.6 |
| CSIRO 1992 | 3.72 | 4.30 | 115.7 |
| Gostwyck 1992 | 9.40 | 2.77 | 29.5 |
| Gostwyck 1993 | 8.01 | 2.11 | 26.4 |
| Katanning 1991 | 14.91 | 4.98 | 33.4 |
| Katanning 1992 | 13.38 | 2.47 | 18.4 |
| Mirani 1990 | 18.12 | 4.27 | 23.6 |
| Mirani 1991 | 8.40 | 2.39 | 28.4 |
| Turretfield 1992 | 8.28 | 3.52 | 42.5 |
| Turretfield 1993 | 13.66 | 2.42 | 17.7 |
| Trangie 1990 | 20.52 | 6.06 | 29.5 |
| Trangie 1991 | 15.35 | 2.53 | 16.5 |
| Southrose 1993 | 10.14 | 3.13 | 30.9 |
| deMestre 1994 | 6.34 | 3.49 | 55.1 |
| England 1994 | 6.56 | 3.57 | 54.4 |
| Nareen 1994 | 6.30 | 3.68 | 58.5 |
| Hazeldean 1993 | 11.62 | 2.57 | 22.2 |
| Nerstane 1993 | 11.09 | 1.96 | 17.7 |
| Pocock 1993 | 10.06 | 2.30 | 22.9 |
| Richardson 1994 | 7.61 | 3.58 | 47.1 |
| Mean (±sd) | | | 40.1±24.2 |

## 3.3.3 Transformation and heritability estimates

Data from three resource flocks were used to examine the effect of different FEC transformations on the estimation of heritability of FEC. The optimal transformation for each data set, based on Levene's test and normality of residuals, is indicated in Table 3.7. Heritability estimates using other transformations varied little from the estimate using the optimal transformation.

Table 3.7 Heritability estimates (±standard error) for $FEC^{0.5}$, $FEC^{0.33}$ and log ((FEC/100)+1) from three resource flocks

| Site and Flock | $FEC^{0.5}$ | $FEC^{0.33}$ | Log ((FEC/100+1) |
|---|---|---|---|
| Katanning 1991 | 0.14±0.07 | 0.16±0.07 | 0.19±.08* |
| Katanning 1992 | 0.25±0.09 | 0.26±0.09* | 0.26±0.09 |
| Turretfield 1992 | 0.34±0.09 | 0.34±0.09 | 0.35±.09* |
| Turretfield 1993 | 0.11±0.06 | 0.10±0.06* | 0.09±0.06 |
| CSIRO 1991 | 0.38±0.10 | 0.40±0.10 | 0.40±0.10* |
| CSIRO 1992 | 0.38±0.10 | 0.39±0.10* | 0.40±0.10 |

* Most appropriate transformation based on Levene's test and normality of residuals.

## 3.4 Discussion and conclusion

The need to transform and standardise FECs was clear from the data sets examined. Transformation of data does not make an appreciable difference unless the coefficient of variation is relatively high, for example in the case of transformation to logarithms above approximately 20% (Falconer 1989). The coefficient of variation observed in the three resource flocks was well in excess of 20% (Table 3.1) so that a transformation is indeed appropriate for these data sets. The marked skewness in distribution of FEC, demonstrated in the Merino resource flocks, was observed in all flocks described in this thesis. Therefore, it is highly likely that all FEC data will need transformation before analysis and, for the reasons that follow, this is recommended even for the basic task of calculating mean resistance and ranking for resistance using individual animals' phenotypes.

There is a tendency for stud breeders to interpret measurements before appropriate statistical analysis, which in the case of FEC may be misleading because of its skewed distribution. Where animals are being selected on the basis of their own FEC, the ranking of sheep will be consistent before and after transformation so the use of unadjusted egg counts instead of transformed counts will not introduce any bias into

the selection of sires for stud use. However, if resistance of the remaining flock rams is defined in terms of their egg count relative to the mean of the group, which is the current method used for fleece weight and fibre diameter, the use of unadjusted FEC will result in greater than 50% of the sheep being below average for FEC. Since the intrinsic worth of FEC cannot be defined, as it can be for traits such as wool weight or fibre diameter, the commercial ram buyer is going to focus on buying rams that are above average for parasite resistance. For this type of scoring system to be rational the classification of above and below average resistance should be made using transformed FEC values. If BLUP is introduced, to use information from related animals or to adjust for management group effects, then the transformation of FEC is mandatory for the assumptions of homogeneity of variance and normality to be met. As the majority of stud breeders have sire pedigree information for a proportion of their lambs (Casey and Hygate 1992) it is likely that those breeding for parasite resistance, a trait that tends to be lower in heritability than fleece traits, will use BLUP for estimating breeding values.

After establishing that FEC transformation is required for data analysis the question remains as to which transformation is most appropriate. Commonly used transformations are square root (Albers *et al.* 1987), cube root (Woolaston *et al.* 1991; Blattman *et al.* 1993; Woolaston and Piper 1996) or $\log_e$ (Barnes and Dobson 1990a). There appears to be no universally applicable transformation. For the resource flocks (Table 3.1), a certain transformation was appropriate in one year but this changed across years and flocks. With FEC data from a number of Romney flocks in New Zealand the square root transformation proved to be better for three of the six flocks, while a log transformation was better in the remaining flocks (Bisset *et al.* 1992).

The choice of transformation to use with the sire evaluation FEC data was also unclear, with the log transformation best for two site-year groups and square root for the other. Falconer (1989) summarises this dilemma with the statement that the scale appropriate for one population may not be appropriate for another, and the scale appropriate to the genetic and environmental components of the variation may be different. As there appears to be no easy solution to this dilemma the approach taken here was to determine the loss of selection efficiency with a sub-optimal

transformation. The correlations between EBVs calculated with three transformations, given in Table 3.2, show that there is strong agreement in EBVs calculated using different transformations. For the transformations considered here, it appears that the use of a sub-optimal transformation will not have a significant effect on the estimation of EBVs for a large group of animals from a number of sites and varying levels of infection.

Examining the correlation between EBVs for all animals at each site one finds a strong general relationship between EBVs regardless of transformation, but this does not answer the question whether individuals of merit are being omitted from selection at the extremes of the EBV distribution. The further analysis of efficiency of selection (Table 3.3) indicates that there was little or no loss in selection intensity, as evaluated on the most appropriate scale, if a sub-optimal transformation is used when ranking the most resistant 10% of animals in a relatively large group of sheep. The strong general association between EBVs appears to be maintained at the lower extreme of the FEC EBV distribution.

The impact that the type of transformation has on either the correlation between EBV's for FEC or the selection intensity achieved for the trait in a large group of animals appears to be minimal, at least when pedigree structure is relatively simple. Identifying the most resistant 10% of animals would be a similar procedure to that which a ram breeder would use to select young rams (offspring of a progeny test), current home-bred sires and outside sires for parasite resistance. The same type of comparison was repeated for progeny tested sires only with similar results. The proportion of animals chosen to examine selection emphasis was arbitrary (10% for all animals and for progeny tested sires), but it represented what is likely to happen in practice. A breeder conducting a home progeny test linked to a central test will be interested in the top 10% of all animals as some offspring of the progeny test will be potential sires. On the other hand a breeder who does not have a home progeny test is only going to be interested in the best sires from the evaluation scheme rather than any of their offspring. In both situations the effect of the type of transformation used for FEC will be minimal. In only one of the site-year groups examined (Gostwyck 1993), was there any substantial loss of selection pressure by the use of a sub-optimal

transformation. The degree of loss will be dependent on the proportion of animals to be selected and the number in the selected group. That is, changes of ranking for one or two animals will have a larger effect in a group of 5 than a group of 25.

It appears that the selection of one transformation as universal for all data in both situations will not have an adverse effect on selection outcomes if that transformation turns out to be sub-optimal for a particular data set. This will assist greatly with the integration of FEC into a measurement regime as it can be then treated in a standard manner, simplifying the procedure that will apply to data analysis.

Transforming FEC data alone may not always result in homogeneous variances across data cells. Woolaston and Piper (1996) showed that a cube root transformation did not equalise variances between data collected in different years but it did reduce the range in variances of year-line cells from 118-fold to 10-fold. After a cube root transformation there is still obviously a strong relationship between mean and variance of $FEC^{0.33}$ for the site-year groups from the sire evaluation flocks (Table 3.4). This will tend to bias selection of animals in favour of those measured in site-year groups that were highly variable, especially for rams, due to a low proportion being selected. For instance ranking the most resistant 5% of animals on FEC EBV from the combined data from all sites, genetically linked by common sires, gave unequal proportions from each site-year group (Table 3.5, Figure 3.1) with sheep from the highly variable group favoured for selection. Pre-adjusting the transformed data to a common variance markedly reduced this effect. It is difficult to determine the expected proportion from each site-year to be selected, because the distribution of resistance for each group of sires is unknown. If this is assumed to be the same in each year, then the expectation is that an equal proportion of animals will be chosen from each site-year.

To standardise data in such a manner assumes the differences in variance are related only to the mean and scale of measurement and that the pattern of distribution of environmental deviations is the same as that for breeding values, in the untransformed units. The danger in using such a technique arises when data are standardised within site-year-sex cells and then used to test hypotheses concerning these fixed effects, as

standardising the variance could change the ratio of mean squares. Changing the mean or adjusting for a constant fixed effect across data will not affect the estimation of breeding values, but these fixed effects should remain in the model to ensure the appropriate degrees of freedom are used.

Artificially removing differences in variance so that EBVs will not be biased by greater variation in some groups overcomes the challenges of problematical transformations. It is also an important consideration when planning the national reporting of breeding values for parasite resistance, as these will have been estimated in sire evaluation schemes and on-property evaluations located in diverse environments. The performance of individual sires in the NESES (Figure 3.2) indicates there is a large amount of genetic variation for parasite resistance. This variation is comprised of both between-sire and between-bloodline components which can only be separated by examining FEC variation in a population with the appropriate structure. The standardised format for reporting FEC EBVs allows EBVs to be interpreted independent of the mean egg count of the particular infection when the animals were measured. Reporting EBVs in terms of eggs per gram without also reporting the mean would not be informative.

However, it is useful for breeders to know how the standardised EBVs can be interpreted in terms of egg counts. Given the coefficient of variation for $FEC^{0.33}$ (Table 3.6), a range of conversions can be made to express the predicted progeny means in terms of egg count. This is done for worm infections with means of 500 epg and 1000 epg and the conversions are given in Table 3.8. There are a couple of points to make about the conversion table. The conversions are dependent on the mean egg count, so at different levels of infection the predicted progeny FECs will vary for each FEC EBV. The FEC EBVs are halved to give expected progeny differences, because the ram only contributes half the genes to each offspring, the other half coming from the ewes, which are assumed to be allocated to rams at random. The spread in predicted egg counts is not symmetrical about the mean. Given a mean of 1000 epg, it is predicted that a ram with a FEC EBV of -1 will produce progeny with an egg count that is closer to the mean than a ram with a FEC EBV of +1. It is predicted that a ram with a FEC EBV of -1 will produce offspring with an egg count of 512 epg, while a

ram with a FEC EBV of +1 produces offspring with an egg count of 1728 epg. This unbalance is caused by the skewed distribution of the original FECs and is another reason why standardising FECs simplifies the interpretation of EBVs.

Table 3.8 Predicted FEC for progeny of rams with varying standardised FEC EBVs

| Standardised FEC EBV | Standardised FEC EPV | Predicted FEC of progeny Mean count 500 epg | Predicted FEC of progeny Mean count 1000 epg |
|---|---|---|---|
| -1.0 | -0.5 | 257 | 512 |
| -0.8 | -0.4 | 297 | 593 |
| -0.6 | -0.3 | 341 | 681 |
| -0.4 | -0.2 | 390 | 779 |
| -0.2 | -0.1 | 443 | 885 |
| 0.0 | 0 | 500 | 1000 |
| 0.2 | 0.1 | 563 | 1125 |
| 0.4 | 0.2 | 630 | 1260 |
| 0.6 | 0.3 | 703 | 1405 |
| 0.8 | 0.4 | 781 | 1561 |
| 1.0 | 0.5 | 864 | 1728 |

However, the conversion is useful for explaining to a ram buyer what the difference is between two rams with different FEC EBVs. For example, it is predicted that a ram with a FEC EBV of -0.6 will produce progeny that will average 681 epg (Table 3.8), when the progeny of a ram with a FEC EBV of +0.4 will average 1260 epg, given the mean infection is 1000 epg. Some breeders may not regard an egg count of this magnitude as relevant to their flock and so the same comparison can be made using a lower mean. The same example now shows that a ram with a FEC EBV of -0.6 will produce progeny that will average 341 epg, when the progeny of a ram with a FEC EBV of +0.4 will average 630 epg.

The heritability of parasite resistance influences the potential rate of genetic change for this trait. The effect of different transformations on heritability estimates was examined in the three largest resource flocks where the number of sires and offspring per sire were maximised to give the most precise estimates. Heritability estimates for transformed FEC using alternate transformations varied little from the estimate using the optimal transformation (Table 3.6). From these results it appears that the adoption of a uniform transformation for all data will have a negligible effect on heritability estimates. This conclusion is consistent with results from New Zealand Romneys

(Bisset *et al.* 1992) and Merino *Trichostrongylus* selection lines (Piper 1987; Woolaston *et al.* 1991) where heritability estimates, based on different functions of FEC, were not substantially different.

If there is no major compromise in accuracy of selection and estimation of heritability by using one uniform transformation the question still remains which transformation should it be? In one of the sire evaluation site-year groups $FEC^{0.5}$ was the optimal transformation, whereas log((FEC/100)+1) was best for the other two. When all three groups were combined $FEC^{0.33}$ was the most appropriate transformation. Woolaston and Piper (1996) concluded that $FEC^{0.33}$ was the most appropriate transformation for data from Merinos selected for divergent levels of resistance to *Haemonchus contortus*. Given this evidence the decision was made to adopt a cube root transformation for all FECs collected throughout the following studies reported in this thesis.