Establishing a flock for gene mapping in wool traits

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ABSTRACT

A gene mapping flock for wool traits was established by backcrossing of Merino cross Romney (F_1) to both parental breeds. The objectives for this flock were to investigate the segregation of wool and skin characteristics in crosses between the Merino and Romney and to investigate methods for the analysis of genetic markers and quantitative trait loci. Analysis of Merino backcross animals showed no evidence of major genes segregating for any of the wool traits studied. The analysis estimated a finite number of genes affecting fibre diameter and bulk. A genetic marker study of these family groups may provide more powerful analysis for the presence of genes affecting wool traits that may be segregating in these crosses.

Keywords: gene; quantitative trait loci; backcross; Merino; segregation.

INTRODUCTION

Sheep breeds in New Zealand produce a range of wools with different fleece characteristics, such as fibre diameter, staple length, fibre crimp and bulk. Each of these traits can be characterised by objective measurement of wool or skin follicles. Single genes such as the Drysdale gene (Dry, 1955) and the Lustre gene (Blair, 1990) are known to affect wool traits in sheep. However, most wool traits show polygenic variation. The inheritance of these traits is controlled by a number of alleles at several loci together with environmental factors. Little is known about the likely numbers of loci influencing different wool traits.

Recently, methods have been developed to study the inheritance of these polygenic traits (Paterson *et al.*, 1989). The segregation of individual chromosome segments can be followed in appropriate pedigrees using genetic linkage maps based on DNA markers. The chromosomal location of loci influencing quantitative traits can be determined in out-bred populations using this approach together with appropriate analytical methods. A large part of the difference between pig breeds for growth and fatness was accounted for by a single region on chromosome 4 (Anderson *et al.*, 1994).

A primary genetic linkage map for sheep has been developed (Crawford *et al.*, 1995) and systematic searches for genes affecting production in sheep are now possible. Experimental methods for locating markers linked to polygenic traits have been developed for species with inbred lines. We have established a backcross flock using Merino and Romney as parental breeds to investigate appropriate methods for the analysis of genetic markers and quantitative trait loci (QTL) in an outbred species. The parental breeds differ by 7-12 phenotypic standard deviations for a number of traits including fibre diameter, follicle density, secondary to primary follicle ratio and crimp frequency. These large breed differences for wool and follicle characteristics provide a good model system for the analysis of QTL in traits of importance to the New Zealand sheep industry.

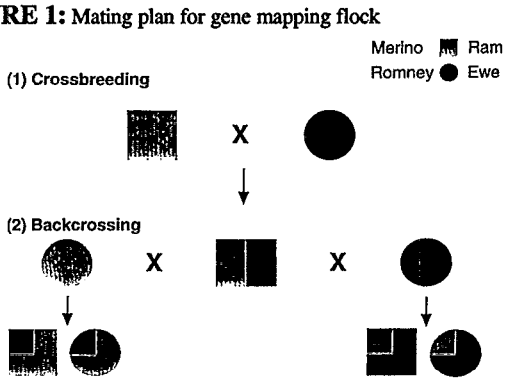
The flock was established by backcrossing F1 Merino x Romney rams to Merino ewes. Wool measurements were taken from the Merino x Romney flock and the (Merino x Romney) x Merino backcross flock. This paper outlines the establishment of these flocks, presents the analysis of their wool characteristics and investigates the sources of environmental variation in the Merino backcross flock.

MATERIALS AND METHODS

Crossbreeding and backcrossing

Five ultrafine Merino rams from the Tara Hills research flock were single sire mated to 25 high fleece weight-selected Romney ewes from the Woodlands Research Station in April 1991 to generate Merino x Romney F_1 (MR). One ram lamb from each of four sires was single sire mated to 45 Merino ewes in April 1992 to generate backcross (3/4 M 1/4 R: MRM) gene mapping groups, and this mating was repeated in 1993 aiming to expand the groups to 100 - 120 progeny per sire family. Backcrossing to Romney ewes (producing 1/4 M 3/4 R: MRR) was carried out in April 1994 at Woodlands Research Station single sire mating the four rams, that had previously been mated to Merino ewes, to 110 Romney ewes each (Fig 1).

FIGURE 1: Mating plan for gene mapping flock



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Measurements

Lambs were recorded for pedigree, birth date and birth/rearing rank, and MRM male lambs were castrated. Weaning weight (WWT), autumn liveweight (ALW) and spring liveweight (SLW) were measured in MR and MRM progeny. A midside fleece sample was collected at each shearing as lambs (4 month) and hoggets (12 month old) and yield (%), greasy fleece weights, clean fleece weights, fibre diameter (FD) and fibre diameter variation (FDCV) were measured. Mean fibre diameter was determined by the air flow method (IWTO) for MR lamb fleece samples and by Optical fibre diameter analyser (SGS Ltd) for hoggets. Medullation score (MS) was assessed on two scoured staples in an aniseed oil/benzyl alcohol solution on a 5 point scale with zero being no medullation and 1 to 4 being low to heavy medullation. Staple strength (SS), staple length (SL) and fibre crimp (FC) were measured on the same five staples per fleece sample. SS was measured using a staple breaker (Agritest Ltd, Australia). Tristimulus scoured wool colour, specifically brightness (Y) and yellowness (Y-Z) (Bigham *et al.*, 1984), and loose wool bulk and resilience (WRONZ Bulkometer) were also measured on conditioned wool (65 ± 2% r.h. and 20 ± 2°C).

Segregation Analyses

The data were analysed by least squares (analysis of variance), with year of birth, sex, birth/rearing rank, age of dam (2 year vs older or unknown) and sire as fixed effects and birth date as a covariate. Two-factor interactions were fitted and dropped by backwards elimination of non-significant effects leaving year of birth by sex for live weight, year of birth by birth date for FD and MS, birth/rearing rank by birth date for yield and sex by age of dam for SL. Segregation analyses were performed on 'residual data' (i.e. the residual of a particular variate from a least square model as above, but without sire fitted) within sire groups, using the methods described in Hawker *et al.*, (1988) for progeny of heterozygous sires and homozygous ewes. To perform this test, an estimate of the within major gene standard deviation is required. This was taken as the mean of that from the group of sires with the lowest standard deviations, as those with higher standard deviations were hypothetical major gene carriers. Significance tests are performed using the results of Goffinet *et al.*, (1992) and the significance level taken as 0.05 divided by the number of tests (60) performed. Progeny distributions were plotted as an additional aid for detecting bimodality.

Estimation of the minimum number of genes involved in a trait were calculated using methods analogous to those of Lande (1981) using the formula $n_E = d^2/8\sigma^2_s$ where d is an estimate of the difference in parental means, and σ^2_s is the extra genetic variance segregating in an F_2 population compared with an F_1 population. To enable these calculations to be performed, it was necessary to define parental population parameters, as well as the backcross mean (μ_B) and variance (σ^2_B). For Romneys (R), the mean (μ_R) and variance(σ^2_R) were estimated using the hoggets, born during 1985-93, from the high fleece weight line of the trial described by Hawker *et al.*, (1988) which is the flock which provided the parental Romneys. The Merino (M) mean (μ_M) and variance(σ^2_M) were estimated using the hoggets, born during 1988-93, from the superfine and control lines of the trial described by Wuliji *et al.*, (1990). To reduce the effects of the R flock being farmed in a different environment, *d* was estimated by $4(\mu_M - \mu_B)$, while σ^2_s was estimated as the maximum of zero or $(4\sigma^2_B - 3\sigma^2_M - \sigma^2_r)/2$, where $\sigma^2_r = (4\mu_B - 3\mu_M)^2\sigma^2_R/\mu_R^2$, and is a CV-invariant scaled version of σ^2_R. These calculations assume that heterosis effects (in MRM) are minimal.

RESULTS

The mean weaning weight, autumn and spring live weights, and greasy and clean fleece weights of MR progeny are shown in Table 1. Rams were significantly (P<0.05) heavier than ewes at weaning and in the autumn, but by spring there was no difference. The mean ALW and SLW in MR were higher than they were in MRM while WWT was lower though these means were unadjusted for factors considered in MRM progeny data. Liveweights generally differed significantly between years, and levels of maternal effects (not shown).

TABLE 1: Mean live weights (kg) and fleece weights (kg) of Merino x Romney and (Merino x Romney) x Merino progeny

	Merino x Romney			(Merino x Romney) x Merino		
Traits	Ram	Ewe	S.E.D	Wether	Ewe	S.E.D
Animal number	9	16		201	216	
Live weights						
Weaning	20.7	17.4	0.9*	26.6	25.4	0.33***
Autumn	42.4	34.3	1.1***	33.5	32.6	0.32**
Spring	41.2	40.4	1.6^{ns}	36.3	33.8	0.33***
Fleece weights						
Lamb greasy fleece	1.58	1.47	0.09^{ns}	-	-	-
Lamb clean fleece	1.24	1.18	0.06^{ns}	-	-	-
Hogget greasy fleece	3.08	2.79	0.18^{ns}	2.82	2.79	0.04^{ns}
Hogget clean fleece	2.38	2.33	0.12^{ns}	2.07	2.06	0.03^{ns}

– lamb shearing was not practiced for (Merino x Romney) x Merino back crosses; *: P<0.05; ** :P<0.01; ***: P<0.001; ^{ns}: non-significant.

The mean wool characteristics of MR lambs and hoggets, and MRM hoggets are shown in Table 2. There was a noticeable age difference in MR for SS and Y-Z value, both being lower in hogget fleeces than in lamb fleeces, but little change in the other traits, though these are unadjusted averages. Mean wool characteristics in MRM hoggets apparently differed from those in MR for yield, FD, FDCV, FC and yellowness.

Mean year, sex and sire effects for clean fleece weight and wool characteristics of MRM are presented in Table 3. There was a significant (P<0.01) year of birth difference in all characteristics except MS, FDCV and yield. Wethers had a lesser degree of medullation (P<0.05), lesser variability in FD (P<0.01) and shorter SL (P<0.05) compared with ewes. There were small, but significant differences in the traits of interest between sire groups, with sire 91003 and 91011 higher (P<0.05) for bulk and MS, while 91003 was lower (P<0.05) for FDCV.

One of the segregation tests (Sire 91015 for SLW) achieved significance (uncorrected P = 0.0002) in the segregation analysis.

TABLE 2: Mean wool characteristics of Merino x Romney (MR) and (Merino x Romney) x Merino (MRM) progeny

Traits	MR Lamb	S.E	MR Hogget	S.E	MRM Hogget	S.E
Yield (%)	79.2	3.5	80.4	2.4	74.6	0.2
Bulk (cm^3/g)	30.5	2.4	26.9	1.6	27.0	0.1
Fibre diameter (μm)	23.7	1.3	23.8	1.4	20.9	0.1
Fibre diameter C.V. (%)	–	–	28.0	3.0	24.2	0.1
Staple Length (mm)	82.6	9.3	86.3	8.5	85.7	0.5
Staple Strength (N/ktex)	25.0	7.1	21.5	7.1	32.0	0.5
Brightness (Y)	70.0	2.0	66.8	0.8	68.5	0.0
Yellowness (Y-Z)	5.5	0.7	1.4	1.5	0.4	0.0
Staple crimp (No/cm)	3.4	0.5	3.4	0.5	4.9	0.1

Table 4 shows the results of estimating the minimum number of genes. Only two traits (FD and Bulk) gave finite estimates.

DISCUSSION

The FD of MR hoggets was very similar to a previous crossbreeding trial of Merino and Romney sheep (Meikle *et al.*, 1988). The maternal (not shown) and sex effects were similar to those found for the pure breeds (Baker *et al.*, 1979; Hawker *et al.*, 1988) and crossbreds (Sidwell and Miller, 1971).

Schinckel and Hayman (1960) found that follicle population density and fibre weight of F_1 and F_2 Border Leicester x Merino breeds did not differ in their means but the F_2 animals had a highly significant increase in variance. The hybrid vigour in MR or MRM was not estimated since there were confounding environmental effects, however these would be expected to be very low for wool characteristics. McGuirk *et al.*, (1978) showed that crossbreds produced more wool than the average of the pure parental breeds, however hybrid vigour was negligible in the components of wool production including SL, FD or follicle density. In their study, crossbreds

TABLE 3: Mean clean fleece weight and wool characteristics by year of birth, sex and sires in (Merino x Romney) x Merino backcross progeny

	No	Clean Fleece kg	Yield %	Bulk cm^3/g	MS 0-4	Crimp No/cm	FD μm	FDCV %	SL mm	SS N/ktex	Brightness Y	Yellowness Y-Z
Year of birth												
1992	185	2.09	73.7	27.7	0.28	4.5	21.1	24.0	77.8	24.3	68.7	0.5
1993	232	2.03	74.0	26.6	0.39	5.0	20.3	24.2	84.2	36.2	68.2	0.3
S.E.		0.03^{ns}	0.4^{ns}	0.2***	0.07^{ns}	0.2***	0.1***	0.3^{ns}	1.3***	1.5***	0.1***	0.1**
Sex												
Ewe	216	2.06	74.2	27.1	0.40	4.6	20.9	24.5	82.5	29.9	68.5	0.4
Wether	201	2.07	73.6	27.2	0.27	4.8	20.6	23.8	79.5	30.6	68.4	0.3
S E D		0.03^{ns}	0.3^{ns}	0.2^{ns}	0.06*	0.1^{ns}	0.2^{ns}	0.3**	1.5*	0.9^{ns}	0.1^{ns}	0.1^{ns}
Sire												
91 003	108	2.06	73.6^{a}	27.7^{c}	0.34^{ab}	4.9^{a}	20.6	23.3^{a}	84.1^{c}	32.5^{b}	68.6^{b}	0.5^{c}
91 011	94	2.06	74.6^{b}	27.6^{bc}	0.45^{b}	4.9^{ab}	20.8	24.4^{b}	77.1^{a}	29.7^{a}	68.4^{ab}	0.3^{ab}
91 015	111	2.07	73.5^{a}	27.1^{b}	0.29^{ab}	4.5^{b}	20.7	24.5^{b}	80.6^{b}	28.8^{a}	68.6^{b}	0.2^{a}
91 018	104	2.06	73.8^{ab}	26.1^{a}	0.26^{a}	4.7^{ab}	20.9	24.2^{b}	82.2^{b}	30.1^{ab}	68.2^{a}	0.5^{c}
S.E.D.		0.04	0.5	0.3	0.9	0.2	0.2	0.4	1.6	1.3	0.1	0.1**

MS: medullation score; FD: fibre diameter; FDCV: FD variation; SL: staple length; SS: staple strength; *: P<0.05; **: P<0.01; ***:P<0.001; ^{ns}: non-significant; ^{abc}: sires bearing a different superscript differ at P<0.05.

TABLE 4: Estimation of the minimum number of genes (n_E) affecting productive traits and hogget fleece traits.

Trait	μR	μM	μB	σ^2_R	σ^2_M	σ^2_B	n_E[1]	SE
WWT	23.19	23.05	26.70	9.42	9.82	12.29		
SLW	39.85	38.07	36.65	21.06	23.25	12.44		
CFW	2.13	2.02	2.15	0.11	0.08	0.08		
Yield	72.54	66.28	74.12	9.31	13.05	11.42		
FD	35.66	17.38	20.82	3.10	1.14	1.98	22.3	6.2
SL	114.77	61.67	86.26	195.70	45.21	76.47		
Bulk	22.47	31.03	26.97	5.41	2.42	4.28	8.7	1.5
SS	26.49	23.712	8.61	74.66	29.72	60.78		
Brightness	59.69	70.89	68.48	3.64	3.58	0.99		
Yellowness	2.28	1.89	0.44	0.66	0.41	0.33		

[1] Infinite estimates not shown.

were close to the pure breed average in fibre length and FD, but lower than their pure breed average for density and secondary to primary follicle ratios. Meikle *et al.*, (1988) found MR crosses had a positive heterosis in liveweight and greasy fleece weight but negative heterosis in follicle traits. The crimp frequency in MR of this trial was higher than Merino x Border Leicester crosses in the Australian trial (McGuirk *et al.*, 1978), while FD was similar to that of Merino x Border Leicester cross, and MR of a previous trial in NZ (Meikle *et al.*, 1988). Sidwell *et al.*, (1971) found there was no great variation in wool traits such as FD and FDCV in down-breed crosses.

Segregation analyses of the backcross sire family progeny have not produced compelling evidence that any differences in the wool traits between the breeds are controlled predominantly by single genes. Though one sire/trait combination gave a significant segregation test, his progeny distribution did not appear bimodal, and so the result is difficult to interpret. The progeny of this sire were heavier ($P<0.001$) than the progeny of the other sires, so the higher variation contributing to the significant segregation result may be due, in part, to this higher mean.

The variance in the backcross population was often low, giving rise to zero estimates of σ^2_s, and consequently infinite estimates of n_E. The two traits that gave finite estimates of n_E. The two traits that gave finite estimates of n_E (FD and Bulk) are among those that differ markedly between the parental breeds, and therefore are likely to have QTLs detected if they exist. The infinite estimates in part reflects bias resulting from management and environmental differences, the assumption of heterosis effects, and the different breed groupings from the true parental populations. Using the same methodology to try and adjust for differences across environments, the parental breeds appear to differ by 7 and 8 phenotypic standard deviations for bulk and FD respectively.

Although the segregation tests and minimum number of genes analysis have not provided compelling evidence that the breed differences in any of the traits investigated are controlled by predominantly a single gene, they do illustrate the difficulties with searches for major genes without the aid of genetic markers. A QTL study using genetic markers is a more powerful technique for the investigation of segregating major genes in this cross. The distribution of wool traits in these and the (Merino x Romney) x Romney families could provide a leading clue as to which are likely to have QTL detected by marker typing and linkage analysis.

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