Wool follicle traits of $^1/_2$ Merino $^1/_2$ Romney F_1, and backcross $^3/_4$ Merino $^1/_4$ Romney gene mapping flocks

R.N. ANDREWS, A.E. BEATTIE, K.G. DODDS, T. WULIJI AND G.W. MONTGOMERY[1]

AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand.

ABSTRACT

Wool follicle traits and mean fibre diameter (MFD) of $^1/_2$ Merino $^1/_2$ Romney F[1] (MR), and $^3/_4$ Merino $^1/_4$ Romney backcross (MRM) were compared. Skin and midside samples were collected from 25 ewes and rams of MR and 420 MRM progeny. MRM was lower than MR for MFD by 3.0 µm, follicle fibre area (FFA) by 162.4 µm^2, and diameter (FFD) by 4.1 µm, but greater for total follicle density (DEN) by 21.7 mm^{-2}, primary density (PD) by 0.8 mm^{-2}, and secondary to primary ratio (SPR) by 4.8 units. Most traits had significant differences ($P<0.05$) for birth year, breed and sire group, but only PD and SPR were significant for sex and birth/rearing rank. FFD was positively correlated ($P<0.001$) with MFD ($r = 0.22$), and the relationship had a common slope of 0.7, but intercepts were significantly different ($P<0.01$) across birth years (i.e. 13.5, 12.0, and 10.9 µm for born 1991, 1992, and 1993).

Keywords: sheep skin; wool follicle traits; fibre diameter; gene; loci.

INTRODUCTION

The number of primary and secondary wool follicles in sheep is determined in early gestation, and follicle maturation is influenced by the pre- and post-natal environment. Total follicle (i.e. primary and secondary) density (DEN), primary follicle density (PD) and ratio of secondary to primary follicles (SPR) are important traits in wool quality and quantity. Merino sheep, with low mean fibre diameter (MFD), tend to have high DEN and SPR, with follicles of even depth, clustered in large groups (Hynd *et al*., 1996). Coarse wool breeds, such as Romney, have lower DEN and SPR. Amongst the follicle traits, SPR has a distinct advantage in follicle studies, because it is independent of size and age of animal (Schinckel, 1955a, b; Jackson *et al*., 1975). Maternal effects have an important influence, with SPR and DEN of twin born and reared progeny 5 to 10% lower than singles (Schinckel, 1955b; Jackson *et al*., 1975). SPR, DEN, and PD, have moderate to high heritabilities of 0.4 to 0.6 (Jackson *et al*., 1975; Purvis and Swan, 1997; Skerritt *et al*., 1997). Meikle *et al*. (1990) suggested the genes controlling SPR involved at least 2 loci, and there was evidence of a major gene for PD. In a study of backcross wool traits and live weights, Wuliji *et al*. (1995) reported there was no convincing evidence of segregation of major genes. The objective of this study was to investigate the relationships of wool and follicle characteristics in crosses between Merino and Romney breeds generated to search for quantitative trait loci.

MATERIALS AND METHODS

The $^1/_2$ Merino $^1/_2$ Romney F_1 (MR) flock of n = 25 was generated in 1991 by joining 5 ultrafine Merino sires with 25 high fleece weight-selected Romney ewes (Wuliji *et al*., 1995). The $^3/_4$ Merino $^1/_4$ Romney (MRM) backcross (n = 420) were produced by joining a MR son from each of 4 sires with 45 Merino ewes in both 1992 and 1993. Midside fleece and skin biopsy samples of MR and MRM were taken at hogget shearing in September 1992, 1993 and 1994, with the exception, that biopsies of MR were taken from adult ewes and rams in November 1997. Midside samples were tested for MFD and coefficient of variation (MFDCV) by an Optical Fibre Diameter Analyser (OFDA) instrument. Skin histology measurements were performed on 15 MR and 333 MRM animals. Biopsy specimens were excised from anaesthetised, unstretched skin at the midside with a 10 mm diameter trephine, fixed in 10% buffered formalin, and then 8 µm paraffin embedded transverse serial sections were taken at sebaceous gland level (Maddocks and Jackson, 1988). Fibres in tissue sections were differentiated with Long Ziehl-Neelsen carbol fuchsin stain. Microscope images of 4 fields per specimen were captured for computer image and data analyses of follicle fibre area (FFA), follicle fibre diameter (FFD), coefficient of variation (FFDCV), SPR, DEN and PD (the latter two were adjusted for shrinkage). The data were analysed by least squares analysis of variance, with breed, year of birth within breed, sex, birth and rearing rank, age of dam and sire (within breed) as fixed effects. Interactions between these fixed effects were considered, but excluded from the models because they were not significant. Correlations were calculated after adjustment for these fixed effects.

RESULTS

Many of the traits had significant differences ($P<0.05$) for breed, year of birth and sire (Table 1). Diameter measurements of MR were greater than MRM, with SPR, DEN and PD lower. For respective MR and MRM genotypes, FFD measurements were greater than MFD by 4.9 µm and 3.8 µm, with FFDCV lower than MFDCV by 8.2 and 1.8%

[1] AgResearch Molecular Biology Unit & Centre for Gene Research, Department of Biochemistry, University of Otago, Dunedin, New Zealand.

TABLE 1: Midside fibre diameter and follicle characteristics by breed, year of birth, sex, birth and rearing rank, and sire, for 1/2 Merino 1/2 Romney (MR) and 3/4 Merino 1/4 Romney (MRM) backcross flocks.

	n	MFD (µm)	MFD CV(%)	FFA (μm^2)	FFD (µm)	FFD CV(%)	DEN (mm^{-2})	PD (mm^{-2})	SPR (units)
Breed									
MR	24	23.6	27.2	652.4	28.5	19.0	20.2	2.0	10.7
MRM	417	20.6	24.5	490.0	24.4	22.7	41.9	2.8	15.5
SED	[441]	0.46***	0.85**	95.43ns	2.04*	2.35ns	3.36***	0.16***	1.76**
Year of Birth									
1991 (MR)	24	23.6^{a}	27.2^{a}	652.4^{a}	28.5^{a}	19.0^{a}	20.2^{a}	2.0^{a}	10.7^{a}
1992 (MRM)	185	21.0^{b}	24.4^{b}	519.6^{a}	25.2^{a}	19.6^{a}	41.0^{b}	2.7^{b}	16.0^{b}
1993 (MRM)	232	20.2^{c}	24.6^{b}	460.5^{b}	23.7^{b}	25.8^{b}	42.7^{b}	2.9^{c}	15.0^{c}
SED	[441]	0.36	0.67	72.64	1.56	1.79	2.56	0.12	1.34
Sex									
Ewe	231	22.3	25.8^{a}	594.4	27.0	20.8	29.1	1.8^{a}	14.8^{a}
Ram	9	21.9	26.6^{ab}	543.0	25.7	21.1	34.0	3.6^{b}	9.2^{b}
Wether	201	22.1	25.1^{b}	576.3	26.6	20.8	29.8	1.8^{a}	15.3^{a}
SED	[441]	0.49	0.89	108.71	2.33	2.67	3.83	0.18	2.00
Birth & Rearing Rank									
1,1	214	22.1	25.7	576.5	26.7	20.7	31.4	2.4^{a}	13.8^{a}
2,1	19	22.0	26.0	530.5	25.4	20.6	31.4	2.4^{ab}	13.3^{ab}
2,2	208	22.2	25.7	606.7	27.3	21.3	30.2	2.5^{b}	12.3^{b}
SED	[441]	0.30	0.54	49.2	1.05	1.21	1.74	0.08	0.91
Sire (MR)									
88017	7	23.1^{a}	26.9	734.9	30.3	18.7	19.0	1.9^{a}	10.5
89068	6	24.9^{b}	27.0	621.4	28.2	15.8	21.9	1.5^{a}	12.9
89074	4	24.4^{ab}	29.2	607.2	27.2	22.5	19.5	2.2^{ab}	10.7
89130	7	23.0^{a}	28.8	646.1	28.3	19.2	20.2	2.4^{b}	8.9
SED	[24]	0.89	1.63	188.66	4.04	4.64	6.65	0.32	3.47
Sire (MRM)									
91003	111	20.5	23.8^{a}	510.6^{a}	24.8^{a}	23.4	40.9	2.8^{ab}	15.3^{a}
91011	93	20.7	24.9^{b}	507.9^{ab}	24.9^{a}	22.6	41.6	2.7^{a}	16.7^{b}
91015	110	20.5	24.8^{b}	498.2^{ab}	24.7^{ab}	23.1	42.5	2.9^{bc}	15.3^{a}
91018	103	20.7	24.6^{b}	443.4^{b}	23.4^{b}	21.8	42.4	2.9^{c}	14.7^{a}
SED	[417]	0.21	0.38	34.00	0.73	0.84	1.20	0.06	0.63

* P<0.05; ** P<0.01; *** P<0.001; ns not significant.

^{abc} data bearing a different superscript were significant at P<0.05.

MFD: mean fibre diameter; MFDCV: MFD coefficient of variation; FFA: follicle fibre area; FFD: follicle fibre diameter; FFDCV: FFD coefficient of variation; DEN: total follicle density; PD: primary follicle density; SPR: ratio of secondary to primary follicles.

(actual), whilst SED's of the 2 fibre traits were 20 to 40% of their follicle counterparts. Year of birth included the breed component, and hence the largest significant differences (P<0.05) were those that contrasted breed (i.e. between born 1991 and born 1992 or 1993). SPR and PD were significantly different (P<0.05) for birth and rearing rank and sex, and MFDCV for sex only. MFDCV, PD and SPR of rams were about 1% and 1.8 mm^{-2} greater, and 6 units lower than ewes and wethers. Twins with an SPR of 12.3 units and PD of 2.5 mm^{-2} were 1.5 units lower, and 0.1 mm^{-2} greater than singles. More traits had significant differences (P<0.05) in MRM than MR sire groups, but for most traits the differences were relatively small.

Table 2 shows the phenotypic correlations between

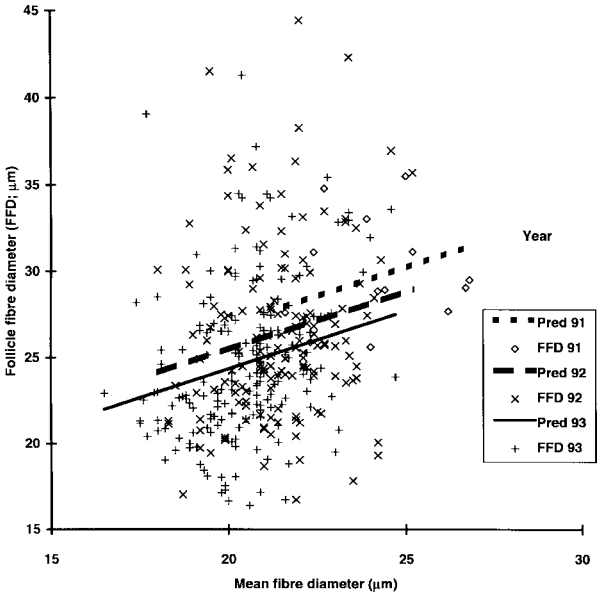
TABLE 2: Phenotypic correlations of midside fibre diameter, coefficient of variation, and follicle traits for pooled data.

Trait	MFDCV	FFA	FFD	FFDCV	DEN	PD	SPR
MFD (µm)	0.14**	0.20***	0.22***	0.08ns	-0.42***	-0.25***	-0.17***
MFDCV (%)		0.12*	0.11*	0.21***	-0.04ns	0.04ns	-0.07ns
FFA (μm^2)			0.98***	0.57***	0.04ns	-0.26***	0.24***
FFD (µm)				0.50***	-0.01ns	-0.27***	0.22***
FFDCV (%)					0.19***	-0.14**	0.29***
DEN (mm^{-2})						0.33***	0.62***
PD (mm^{-2})							-0.49***

*P<0.05; **P<0.01; ***P<0.001; ns not significant.

MFD: mean fibre diameter; MFDCV: MFD coefficient of variation; FFA: follicle fibre area; FFD: follicle fibre diameter; FFDCV: FFD coefficient of variation; DEN: total follicle density; PD: primary follicle density; SPR: ratio of secondary to primary follicles.

wool and follicle traits. The regression of FFD with MFD was affected by year of birth only, with significantly different ($P<0.01$) intercepts for born 1991 (i.e. MR) 13.45 µm (SE 4.11), born 1992 (i.e. MRM) 12.04 µm (3.53) and born 1993 (i.e. MRM) 10.90 µm (3.38), but the slopes were not significantly different, and the common slope estimate was 0.67 (SE 0.16) (Fig. 1).



DISCUSSION

MFD and SPR traits for MR and MRM (Table 1), were similar to results of Meikle *et al.* (1988) with the same genotypes, but DEN and PD were 40 to 60% higher in their study, which may indicate these had no adjustment for specimen shrinkage. Breed differences in this study were confounded with year and age effects. However MR biopsies taken from adult sheep in lieu of hoggets would be unlikely to affect the veracity of DEN, PD or SPR measurements, since most follicle populations mature by 12 months age (Schinckel, 1955a). The other traits would be expected to be influenced by age to a similar relative magnitude as MFD, for which Atkins (1990) reported an increase of 1.8 µm from 1 to 6 years age. Differences between traits of born 1991 (i.e. MR) and born 1992 or 1993 (i.e. both MRM) were of greater magnitude than between born 1992 and 1993, and thus year of birth effects were more often different when they included breed differences. The lower SPR of rams was accompanied by a higher PD, whilst the higher DEN was influenced by PD. The maternal handicap of twins for SPR of 1.5 units, was 0.6 units less than that reported for Merinos by Schinckel (1955b), and Jackson *et al.* (1975), but the lower birth weight of Merino twins, harsher rearing conditions, and post-natal restriction of food supply could have accentuated this maternal handicap. The significant differences ($P<0.05$) between sire groups in MRM for SPR and DEN were similar to differences between mating groups for wool weight and MFD Merino selection lines reported by Jackson *et al.* (1975).

There was only a modest association of FFD with MFD (Fig. 1; Table 2). Despite reports of positive correlations between FFD and MFD, the relationship between the 2 measurements is inconsistent. In our study with MR and MRM (see Table 1), and with Merino and Border Leicester rams (Steinhagen *et al.*, 1986), FFD was 5 to 21% greater than MFD, but 22% lower with Merino rams (McCloghry *et al.*, 1997). Histological processing and measurement techniques may be factors in these differences (McCloghry *et al.*, 1997). Overall, the correlations in Table 2 were consistent with reports of other workers (Jackson *et al.*, 1975; Steinhagen *et al.*, 1986; Meikle *et al.*, 1988; Purvis and Swan, 1997; Skerritt *et al.*, 1997). Comparisons of FFD with other follicle traits were restricted by the lack of published results. Recent evidence suggests genetic relationships of follicle traits differ between Merino strains. Hynd *et al.* (1997) showed that improvement in economic gain (up to 25%) could be made by adding DEN, skin quality (appraised) and biopsy weight (i.e. weight of excised skin) to an index including MFD, MFDCV and clean fleece weight.

Schinckel and Hayman (1960) and Pattie and Smith (1964) reported wool and follicle traits of F_1 and F_2 Border Leicester x Merinos similar to our study. Schinckel and Hayman (1960) found the CV of DEN increased from 14% in F_1 to 24% in F_2, and concluded there was unequivocal evidence of segregated genetic factors in F_2, whilst Pattie and Smith (1964) found no such evidence for follicle traits. In their investigation of SPR and PD traits in F_1 to F_6 MR and MRM genotypes, Meikle *et al.* (1990) showed there was no evidence of gene segregation of SPR, but higher SPR's were independent of PD, and high within genotype variation in F_1 may indicate a major gene was involved in PD inheritance.

The MRM animals in this study form part of a flock developed to identify chromosomal regions associated with variation in wool traits (Wuliji *et al.*, 1995). Segregation with the Horns locus in this flock located this gene to chromosome 10 (Montgomery *et al.*, 1996). A genome screen with 216 markers in the progeny born in 1992 failed to identify major genes for wool traits (Henry *et al.*, 1998). There was evidence for segregation of MFD with one marker locus resulting in a difference in MFD of 1.7 µm between progeny groups (Henry *et al.*, 1998). The wool follicle traits reported here will be adjusted for significant environmental variation and analysed together with genetic markers to search for chromosomal regions influencing characteristics of wool follicle distributions.

CONCLUSION

This study has demonstrated the effectiveness of histological processing and image analyses of wool follicles of sheep of different genotypes. The information produced here will increase the understanding of relationships among wool and follicle traits. Recent knowledge of

between strain differences in follicle traits and their association with wool production, points to substantial economic gains by inclusion of traits such as DEN, skin thickness and quality, in selection indices for low MFD and high fleece weight. Other gains for the woolgrower may arise from the development of a more efficient fibre-follicle production system, through an understanding of fibre and follicle relationships, and the potential of selection to exploit sire differences within sheep breeds.

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