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Evidence of a unique developmental mechanism specifying both wool follicle density and fibre size in sheep selected for single skin and fleece characters

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Summary

Skin and fleece traits have been characterized in four lines of Merino sheep selected for high- and low-fibre diameter ($D^{\pm}$) and staple length ($L^{\pm}$) from a medium-woolled flock. Over a period of 20 years, each line responded in the desired direction, producing fleeces composed of thick or thin fibres and long or short wool staples. However, variations in the amounts of wool grown that might be expected from these procedures were compensated by changes in unselected characters. Thus a predicted difference in fleece weights between high and low staple length lines was reduced by an increase in fibre crimp frequency in L^- sheep. Similarly, changes induced in fibre diameter in the D lines resulted in small effects on fleece weight in comparison to the large (and inverse) effects on follicle numbers. Towards the end of the selection regime, mean follicle density in D^- sheep was twice that of D^+ sheep. This intriguing response within the follicle population was examined further: an analysis of the relationship between follicle density and fibre diameter amongst the four lines revealed a highly significant, negative linear correlation. The implication of this statistical association is that the numbers of follicles initiated in skin during foetal life had a direct bearing on the sizes of wool fibres eventually produced. It was concluded that both features must be under the control of a single developmental mechanism. Since the expression of each of the characters is separated in time, the mechanism must be activated during the earlier event, i.e. at or before the phase of follicle initiation.

1. Introduction

Wool growth in the Merino sheep is a continuous process with little seasonal variation. Production is a function of the activities of individual follicles (each of which elaborates one fibre) and the numbers of follicles present in the skin. Although the characteristics of each fibre are a consequence of the genetic specifications of the follicle, there are a multiplicity of local, systemic and environmental factors which affect its size, shape and composition (e.g. Reis, 1979). Notwithstanding these influences, certain fibre characters have been found to be correlated with particular functional and morphological features of follicles (Rudall, 1956; Schinckel, 1961; Henderson, 1965; Wilson & Short, 1979).

The size of the follicle population is defined in the skin before birth. The total number of follicles that develop is highly variable in different lines of sheep, density being greater in fine-woolled than in strong-woolled animals. Three major types of follicles have been described in the Merino and are established during successive waves of initiation in the foetus. The primary (P)* follicles constitute 5–10% of the population and are formed first, each being distinguished by the presence of a sweat gland and an erector muscle. The remaining, secondary (S) follicles lack these associated structures and include secondary original (SO) and secondary derived (SD) follicles. The latter do not occupy areas of uninvolved skin but develop as branches from the distal regions of the piliary canals of SO follicles (Hardy & Lyne, 1956).

As part of a series of investigations to elucidate the developmental processes that specify follicular pattern and fibre type, we have examined skin and fleece traits in four Merino sheep selection lines, each differing in single fibre characters. These included high- and low-fibre diameter ($D^{\pm}$) and high- and low-staple-length ($L^{\pm}$) lines. Here we confirm that selection for particular fleece traits is accompanied by large changes in unselected characters. The nature of these changes is such as to reduce expected effects of selection on

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* *Abbreviations*: $D^{\pm}$, high/low wool fibre diameter; $L^{\pm}$, high/low wool staple length; P, primary (follicle or fibre); S, secondary (follicle or fibre); SD, secondary derived (follicle or fibre); SO, secondary original (follicle or fibre).

wool production and suggest the existence of a single developmental mechanism controlling both fibre type and follicle density in the skin. A report dealing with some of the concepts detailed here has previously appeared in abstract form (Moore & Jackson, 1984).

2. Materials and methods

(i) *Animals*

Sheep used to initiate the programme were medium-woolled Merinos originating from a single flock. The animals were grazed at Gilruth Plains Field Station, Cunnamulla, Queensland, between 1950–65 and subsequently at Longford Field Station at Armidale, NSW. A selection regime was maintained for 20 years to establish a number of lines which differed in single skin or wool characters (Turner *et al.*, 1970). Subsequently, the sheep were maintained as distinct lines without further selection. A control flock was kept by a random breeding regime. The present study examines data collected from rams and ewes selected for high- (L^+) or low- (L^-) wool staple length and for high- (D^+) or low- (D^-) fibre diameter. Further comparative data on the animals have been provided by Jackson & Downes (1979).

(ii) *Skin and fleece characters*

Data collated in Table 1 and Figs 1–5 were obtained from an average of 40 rams and ewes in each selection line and the control flock, born between 1950 and 1974. Fleece weights and wool samples were obtained at hogget shearing at 13–15 months of age.

Histological preparations were made from skin of six adult rams and six adult ewes born in 1974. Samples were taken from the lateral region of the trunk with a trephine 1 cm in diameter and fixed in 60% ethanol, 30% formalin and 10% glacial acetic acid for 4 h. The fixative was replaced with 70% alcohol and the tissues were processed using conventional histological procedures. Sections (8 μm thick) were cut parallel to the skin surface at the level of the sebaceous glands, and after removal of the paraffin wax, were stained with haematoxylin, eosin and picric acid.

With a projection microscope, the numbers of P and S follicles in sections were counted in eight fields equivalent to a total area of 8 mm^2. P follicles were identified by the presence of a sweat gland duct and the erector muscle (Auber, 1950). The numbers of P and P+S follicles per mm^2 were calculated after correction for skin shrinkage (Carter & Clarke, 1957).

The diameters of fibres recorded in Table 2 were also obtained from these sections; 50 P fibres and 100 S fibres were measured at a magnification of $\times 500$. Those of Figs 1 and 4 were obtained from samples of wool by the method of Turner *et al.* (1953).

(iii) *Statistical analysis of data*

For each pair of lines ($D^{\pm}$ or $L^{\pm}$), an analysis of variance was used to separate effects of line, sex, drop and handicap. Handicap divided animals into singles progeny of adult ewes, singles progeny of maidens, twins progeny of adult ewes, and twins progeny of maidens. Preliminary analyses were conducted to investigate possible interactions between the main effects but these were all non-significant sources of variation and were ignored in the present results. The means and standard errors for lines in Tables 1 and 2 were pooled over both sexes and all drops and were adjusted for disproportionate handicap subclass numbers.

Table 1. *Fleece characters of sheep from lines selected for high- and low-fibre diameter or staple length (1968–74 drops)*

Character	Diameter + line mean ± S.E.M.	Diameter − line mean ± S.E.M.	*F* value
No. of animals	115	71	
Bodyweight (kg)	29·13 ± 0·70	29·86 ± 0·91	0·8 NS
Clean wool wt (kg)	1·75 ± 0·05	1·61 ± 0·07	4·7*
Crimp frequency (per 2·5 cm)	9·19 ± 0·26	10·42 ± 0·34	16·1**
	Length + line mean ± S.E.M.	Length − line mean ± S.E.M.	
No. of animals	120	106	
Bodyweight (kg)	32·52 ± 0·52	32·82 ± 0·49	0·2 NS
Clean wool wt (kg)	2·20 ± 0·04	1·82 ± 0·04	48·5**
Crimp frequency (per 2·5 cm)	9·09 ± 0·24	13·15 ± 0·23	174·8**

NS; not significant.
* $P < 0·05$; ** $P < 0·01$.

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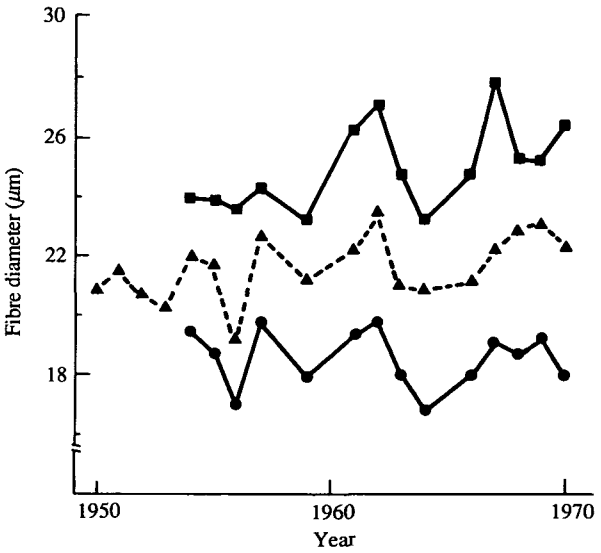

Fig. 1. Direct responses to selection for high (■) and low values (●) of fibre diameter between 1950 and 1970. ▲, Control sheep.

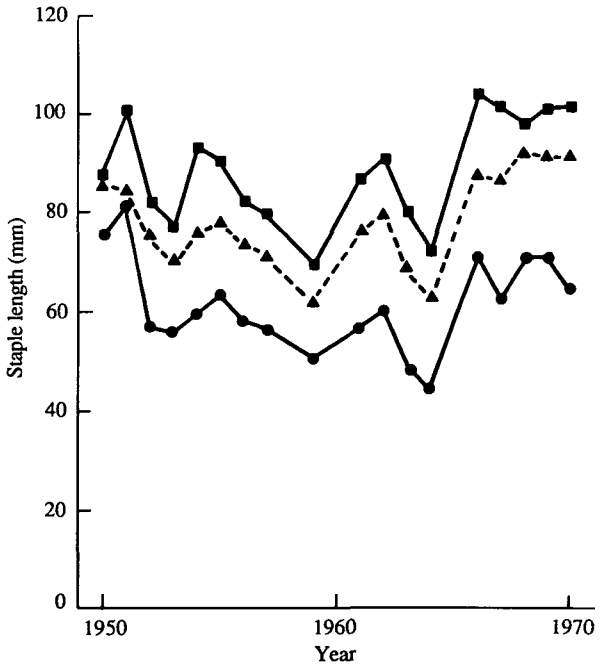

Fig. 2. Direct responses to selection of high (■) and low values (●) of staple length between 1950 and 1970. ▲, Control sheep.

3. Results

Body weights and fleece characters of sheep from four selection lines of the 1968–74 drops are collated in Table 1. Clean wool weight varied between the high and low lines: D^- sheep produced 8% less than D^+ sheep and L^- sheep 17% less than L^+ sheep.

The characters of fibre diameter and staple length responded directly to selection in each pair of the D and L lines respectively, the greater part of each effect being obtained during the initial period of the regime (Figs 1 and 2). Wool staples of L^+ sheep were

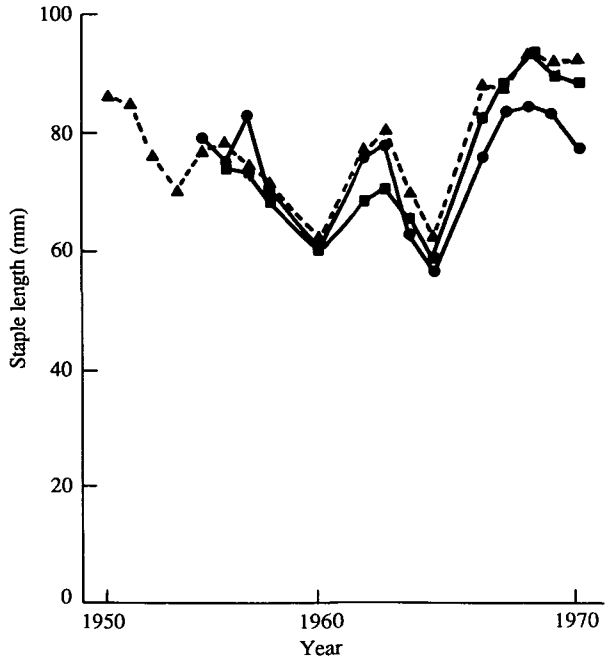

Fig. 3. Selection for high- (■) and low-fibre diameter (●); correlated responses in staple length for D lines. ▲, Control sheep.

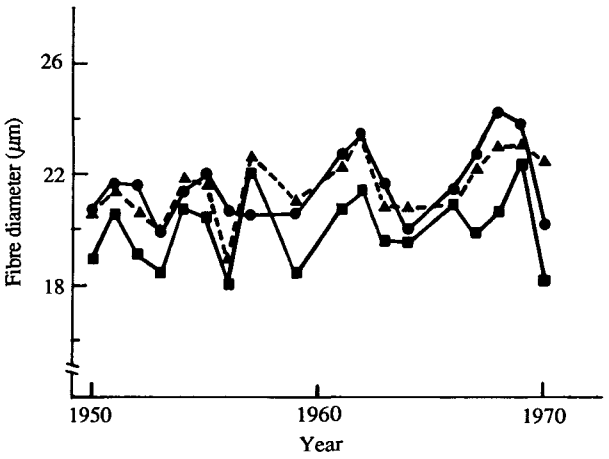

Fig. 4. selection for high (■) and low staple length (●); correlated responses in fibre diameter for L lines. ▲, Control sheep.

approximately 50% longer than those of the L^- line. However, it may be concluded from comparisons of wool weights (Table 1) fibre diameters and follicle densities (Table 2) between these lines that differences in staple length did not reflect large changes in fibre length, the observed response being due, predominantly, to an increase in crimp frequency in the L^- group (Table 1). Unselected characters of staple length in the D lines and fibre diameter in the L lines were also affected but the differences were not large (Figs 3 and 4; see also Table 2).

The diameters of individual P and S fibres were measured in sections of skin taken from rams and ewes of the 1974 drop and are presented as means in Table 2. In all lines, S fibres were finer than P fibres. There were no significant differences in diameters of P

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Table 2. *Fibre diameters and follicle numbers (mean ± S.E.M.) of 6 rams and 6 ewes from each of the lines selected for high- and low-fibre diameter or staple length (1974 drop)*

Character	Fibre diameter lines			Staple length lines		
	D^+	D^-	*t* value	L^+	L^-	*t* value
Fibres						
P diameter (μm)	29·1 ± 1·0	18·4 ± 1·0	7·9**	25·3 ± 1·0	25·3 ± 1·0	0·1 NS
S diameter (μm)	25·2 ± 0·5	15·6 ± 0·5	15·0**	19·1 ± 0·5	19·8 ± 0·5	1·1 NS
Follicles						
No. P/mm²	3·6 ± 0·2	3·1 ± 0·2	1·7 NS	3·4 ± 0·2	3·1 ± 0·2	1·1 NS
No. P+S/mm²	38·2 ± 3·5	80·3 ± 3·5	8·5**	49·9 ± 3·5	49·3 ± 3·5	0·1 NS

NS, no significant difference between lines.
* $P < 0·05$; ** $P < 0·01$

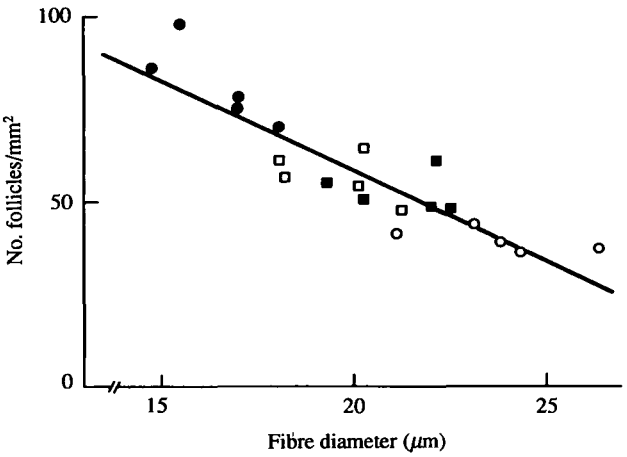

Fig. 5. Regression of mean follicle density on mean fibre diameter in the 1970–4 drops of all lines, D^+ sheep (○), D^- sheep (●), L^+ sheep (□), L^- sheep (■).

fibres or S fibres between the high and low L lines. In the D sheep, as expected, the fibres of P and S follicles of the low line were finer than those of P and S follicles respectively, of the high line. The latter observations indicated that there would be more fibres, and therefore a larger follicle population in D^- sheep since fleece weight and staple length were much less affected than the selected character (Table 1, Fig. 2). Estimates of the numbers of P+S follicles confirmed that D^- sheep had approximately twice the mean follicle density of the D^+ animals (Table 2). Secondary follicles were predominantly responsible for this difference since P density did not differ greatly amongst any of the lines (Table 2). Selection for changes in staple length had no effect on follicle numbers in the L lines.

The apparent inverse relationship between fibre diameter and follicle density revealed in the D lines was examined further. In drops of all lines at the end of the selection regime (from 1970 to 1974) a highly significant negative and linear correlation ($r = -0·89$) was found to exist between these parameters (Fig. 5).

4. Discussion

Studies of the effects of selection on single skin and wool characters of Merino sheep were begun with the objective of identifying individual traits that could be used as determinants of fleece weight (Turner *et al.*, 1970). The methods used to alter fibre diameter and staple length were effective in the present study and confirm that characters show direct and correlated responses to selection. Differences in clean wool weights which might have been expected between high and low lines were observed, but were smaller than effects induced in other, unselected characters. Thus in L sheep, the difference in staple length between high and low lines was caused predominantly by an increase in crimp frequency in the L^- line rather than a reduction in fibre length. Similarly, whilst fibre diameter of both P and S follicles of D sheep altered in the desired directions, S follicle densities showed reverse responses. These data are consistent with those of Rendel & Nay (1978) who concluded that selection aimed at altering a single character within the fleece induced compensatory changes in fibre structure or follicle numbers rather than affecting the total mass of wool. The productive capacity of the skin may thus be seen as being independent of these characters (see also Galpin, 1948).

Fraser (1951) and Fraser & Short (1952) attempted to explain these observations by proposing that wool production in adult animals was governed by competition amongst follicles for a limited amount of fibre-forming substrate. The inverse relationship between follicle density and fibre size was explained in a similar way: during foetal life, developing follicles competed for essential materials, effectively inhibiting the initiation of other follicles within the immediate vicinity. Large follicles would have inhibitory fields of greater ranges than those of smaller follicles and therefore, fewer would be initiated (Fraser & Short, 1960). New wool follicles only appeared as a consequence of foetal growth: expansion of the skin area separated initiated follicles and reduced their inhibitory influence. In this way the pattern of follicles that developed

was dependent on the distribution of inhibitory fields originating from primordia of the first initiation wave. The competition model represents an early attempt to relate components of fleece structure to follicle distribution. However, in a comprehensive review, Ryder and Stephenson (1968) queried Fraser's interpretation of some data cited in support. Recent evidence also fails to sustain the model. For example, the premise linking initiation to skin area is at variance with observations of Nagorcka & Mooney (1985) who reported that the rate of P follicle formation exceeded that at which skin area increased in the foetus. Furthermore, competition between follicles was not evident in the sheep lines of the present study. The Fraser model predicts that more P follicles would occur in D^- sheep because of their smaller size and consequently reduced range of inhibition during the first wave of initiation. However, our observations show that the P follicle numbers in all lines are approximately equivalent.

The effects of selection for fibre and skin characters on unselected traits other than on wool growth thus remain to be explained. The most intriguing changes were those resulting from selection for fibre diameter. Even if other sources of variation are ignored, regression analyses of pairs of characters within the four lines, (i.e. staple length, follicle density and fibre diameter) from the 1970–74 drops show that fibre diameter is highly negatively correlated with follicle number: $r = -0{\cdot}89$ (staple length *vs.* follicle number: $r = -0{\cdot}04$, staple length *vs* fibre diameter: $r = 0{\cdot}04$). The strong inverse relationship implies that both parameters are linked during development, i.e. the mechanism involved in generation of the whole follicle population also specifies each follicle with respect to the size of its fibre. The final number of follicles is not predetermined by the mechanism. The data show that the skin simply produces greater or lesser numbers as fibre diameter declines or increases during selection. One further conclusion may be drawn: since follicle initiation and fibre specification are temporally distinct processes, both parameters must be determined at, or prior to, the earlier developmental event, i.e. initiation.

An hypothesis to account for the compensatory changes in fibre and skin traits observed is that there was a genetically determined developmental capacity in the original, medium-woolled flock to elaborate defined amounts of fibre-producing tissue. The four lines of sheep inherited this potential relatively unaltered; the effect of single character selection was to change the distribution of fibre-producing tissue amongst initiation sites. The genetically determined, developmental capacity resides in a particular group of cells of defined population size. Evidence accumulated so far support the likelihood that these cells are progenitors of the dermal papillae, because of their roles in follicle formation (Cohen, 1965; Kollar, 1970; Oliver, 1970; Pisansarakit & Moore, 1986) and fibre specification (Rudall, 1956; Ibrahim & Wright, 1982). The pre-papilla cells are established in the dermis by a determinative event during foetal life. Such programming of particular cells within a population resident in a homogeneous environment has been described in other differentiating systems (e.g. Adler, 1987). All of the follicles that develop to maturity arise as a result of the inductive activities of these papilla cell precursors. Since fibre and papilla dimensions are correlated in mature follicles (Rudall, 1956; Ibrahim & Wright, 1982) we propose that eventual fibre size is a function of the number of cells that engage in initiation of each follicle. The developmental mechanism controlling fibre size and follicle density may thus be viewed as an expression of the requirement that most of the cells participate in follicle formation, each initiation event progressively depleting the population. The number of follicles that are formed and the sizes of their prospective fibres is a consequence of the manner in which the cell population is utilized. Thus in D^+ sheep the number of cells participating in the initiation of each follicle would be greater than for D^- sheep. The more rapid removal of cells from the parent population by this process would result in fewer follicles, but each would have the capacity to elaborate a larger fibre.

Mrs R. Joseph carried out the measurements of fibre diameters in adult sheep skin and Mr P. Pisansarakit gave valuable assistance. Mr. R. M. Farrell maintained the selected lines of sheep and kept service data records. Mrs R. L. Bartle assisted with data management and statistical analysis.

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