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Response to Partial Selection on Clean Fleece Weight in South Australian Strong-wool Merino Sheep III.* Genetic Distance Between Flocks

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Abstract

Divergence over time in flocks selected for clean fleece weight and for fecundity is measured by gene frequency changes at the R-r-i blood group, haemoglobin, and transferrin loci. No unequivocal effect of selection is demonstrable. Problems of interpretation even in flocks of known history are discussed.

Introduction

In animal breeding, truncation selection for traits such as fleece weight in Merinos obviously involves changes at specific gene loci. However, perhaps because of the general failure to find gene loci usefully (or even consistently) associated with important production traits, very few studies have been conducted of gene frequency change at marker loci within breeds. The most comprehensive studies have been made with poultry. For example, Brown and Nordskog (1962) followed blood group gene frequencies in four lines selected on different criteria, demonstrating correlated changes in these gene frequencies.

We report here on genetical change at three loci (R-r-i blood group, haemoglobin, and transferrin) in several flocks of South Australian Merinos selected for fleece weight and for fecundity. In 1964 these loci were examined for variability in the same flocks (Cooper 1966; Cooper *et al.* 1967) and later for association with production traits (Mayo *et al.* 1970). Also, we add further segregation data to those earlier reported.

Materials and Methods

Sheep

As in earlier studies, the flocks examined were those at Roseworthy Agricultural College and their parent stud, Anama. Since 1967 one of the Roseworthy flocks (now called the Generation Interval flock) had been selected for fleece weight, the other for fecundity (now called Fecundity flock, but maintained as two sub-flocks with selection for increased or decreased fecundity). Their previous history was described by Mayo *et al.* (1969). The results of the later two experiments will be discussed elsewhere (Mann, unpublished data); here we merely note that they were designed to follow up the earlier experiments in two ways: first, to attempt to estimate the actual rate of increase over time in fleece weight that had been achieved in the former experiment, and secondly, to ascertain the practicability of increasing the low fecundity of the South Australian Merino breed. Effective

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population number (N_e) and time since the previous survey are shown in Table 1. It should be noted that N_e as calculated is based purely on the numbers of sires N_m and dams N_f in each generation [i.e. by using Wright's (1931) expression $N_e = 4N_m N_f/(N_m + N_f)$]. Thus, there is an upper limit for N_e since it does not take into account the inbreeding due to artificial selection, nor does it take into account variance in the number of offspring per parent. The diminution in N_e due to such selection has been considered theoretically by Robertson (1961), but cannot be applied precisely to all flocks in the present study since it requires a knowledge of the intensity of selection i at all stages of selection and of the heritability h^2 of relevant traits as well as the assumption that strict truncation selection has been practised, which has not always been the case in any of the flocks. Assuming upper limit values $i = 1$ (cf. Table 7 of Mayo *et al.* 1969) and $h^2 = 0{\cdot}3$ (cf. Hancock *et al.* 1979), the minimum possible value of N_e for the Index flock might be 10–30% below the value given in the table; in other flocks the decrease should be smaller.

Most of the sheep examined were at Roseworthy College, numbering approximately 1030 in the flocks. These comprised lambs born and weaned in 1975 and their sires and dams, hogget rams, hogget ewes and dry ewes. A further 286 ewes from the parent stud, Anama, were also typed. The ewes, aged 2–7 years, were a random sample of 240 from 4500 in the General Stud, and 46 from the 400 in the Nucleus. The numbers of samples typed for each polymorphism are shown in Tables 2 and 3.

Table 1. Effective sizes and generation lengths of flocks

Flocks	Effective population number	Generation length (years)	Generations 1964–1975
Anama (An)	450	3·40	3·24
Generation Interval (GI) (previously Index)	70	3·20	3·44
Fecundity (F) (previously Visual)	80	2·88	3·82
Fecundity plus (F+)	40	2·88	3·82
Fecundity minus (F−)	40	2·88	3·82

Typing for Haemoglobin, Transferrin and R-r-i Blood Groups

(i) *Haemoglobin*

Typing was carried out by electrophoresis on paper with the buffer of Evans and Blunt (1961).

(ii) *Transferrin*

The method used was horizontal electrophoresis of serum samples in starch gel (Smithies 1955) in the discontinuous buffer system described by Ferguson and Wallace (1961).

(iii) *R-r-i*

Typing was based upon the procedure described by Rendel *et al.* (1954). The haemolytic test was carried out on sheep red cells using guinea pig complement and Jersey cattle serum containing the antibody anti-J.

Measures of Genetic Distance

(i) *Cavalli-Sforza's* f_θ

The statistic f_θ is a distance measure based on the angular transformation of allelic frequencies. As defined by Cavalli-Sforza (1969), it is

$$f_\theta = 4(1 - \cos\theta)/(n - 1)$$

where n is the number of alleles at a given locus, and $\cos\theta = \Sigma_i^n (p_{i1}\, p_{i2})^{\frac{1}{2}}$, and p_{i1} and p_{i2} are the frequencies of the ith allele in the two populations being compared. We combined the results over three loci, where appropriate, in the manner described by Cavalli-Sforza (1969).

(ii) *Latter's* ϕ

The statistic ϕ is defined by Latter (1972*a*) as

$$\phi = [\sum_i \tfrac{1}{2}(p_{i1} - p_{i2})^2]/(1 - \sum_i p_{i1}\, p_{i2})$$

where p_{i1} and p_{i2} are as before. ϕ has the useful property that

$$\phi = 1 - H/H_B,$$

where $H = 1 - \frac{1}{2}\sum_i (p_{i1}^2 + p_{i2}^2)$ is the mean level of heterozygosity, and $H_B = 1 - \sum_i (p_{i1}\, p_{i2})$ is that predicted in the F_1 population formed by crossing the two populations of interest. Information from the three loci was combined as described by Latter (1972*a*).

Table 2. Comparison of gene frequencies for transferrin alleles in the Roseworthy and Anama flocks, 1964 and 1975

Sample	Frequency of:					Total animals typed
	Tf^A	Tf^B	Tf^C	Tf^D	Tf^E	
Anama 1964	0·162	0·214	0·023	0·599	0·002	278
Anama 1975	0·114	0·190	0·088	0·602	0·004	284
Index 1964	0·265	0·202	—	0·515	0·019	206
GI 1975	0·222	0·167	—	0·608	0·003	177
Visual 1964	0·190	0·208	0·005	0·569	0·028	195
F+ 1975	0·269	0·140	—	0·574	0·017	119
F− 1975	0·211	0·328	—	0·461	—	117
Fecundity total 1975	0·241	0·232	—	0·519	0·008	236

Table 3. Comparison of gene frequencies for haemoglobin and *R-r-i* blood group alleles in the Roseworthy and Anama flocks, 1964 and 1975

Sample	Frequency of				Total number	
	Hb^A	Hb^B	R	r [A]	*Hb*	*R-r-i*
Anama 1964	Unknown	Unknown	Unknown	Unknown		
Anama 1975	0·404	0·596	0·687	0·313	286	285
Index 1964	0·359	0·641	0·402	0·598	206	207
GI 1975	0·548	0·452	0·554	0·446	177	181
Visual 1964	0·237	0·763	0·453	0·547	205	204
F+ 1975	0·219	0·781	0·430	0·570	119	120
F− 1975	0·274	0·726	0·551	0·449	117	119
Fecundity total 1975	0·246	0·754	0·486	0·514	236	239

[A] Frequency of *r* was estimated as the square root of the phenotype frequency.

(iii) *Nei's D*

Nei (1973) has defined his distance measure as

$$D = -\log_e I,$$

where $I = J_{xy}/(J_x\, J_y)^{\frac{1}{2}}$; and J_x, J_y and J_{xy} are the arithmetic means of j_x, j_y and j_{xy} respectively over the three loci. The probability of identity of two randomly chosen genes is $j_x = \Sigma p_i^2$ in population X, where p_i and q_i are the frequencies of the ith alleles in X and Y respectively.

D is strictly only a valid measure for a random sample of gene loci which we certainly have not obtained, but as it has already been used on a much larger sample of loci in the Roseworthy Merinos by Manwell and Baker (1977), it should be included for completeness.

Results for Blood Polymorphism

A comparison of gene frequencies for the alleles examined in the Roseworthy and Anama flocks for 1964 and 1975 is shown in Tables 2 and 3. The gene frequency data for 1964 were taken from Cooper *et al.* (1967) and Mayo *et al.* (1970). Since there has been some interchange of rams, during the course of the 10 years, between the Fecundity plus (F+) and Fecundity minus (F−) flocks, data are presented for these flocks as well as for the Fecundity flock as a whole.

In the Roseworthy flocks the transferrin gene Tf^C appears to have been lost and Tf^E is at a lower frequency in 1975 as compared with 1964. In the Anama flock, however, Tf^E gene frequency has remained almost the same and Tf^C has increased. Tf^D is still the most frequent allele while Tf^A and Tf^B alleles, at intermediate frequencies,

Table 4. Tranferrin family data

Mating (sire × dam)		Phenotype				χ^2	P^A
A/B × D/D		AD	BD				
	Weaners	9	2			4·46*	0·21
	Hoggets	7	7			0	
	Total	16	9			1·96	
A/D × D/D		AD	DD				
	Weaners	18	21			0·23	0·14
	Hoggets	7	2			—[B]	
A/B × B/D		AB	AD	BD	BB		
	Weaners	0	1	3	2	—	0·75
	Hoggets	1	1	7	1	—	
A/B × A/D		AB	AA	BD	AD		
	Weaners	2	2	3	4	—	0·67
	Hoggets	0	1	4	3	—	
B/D × B/D		BB	BD	DD			
	Weaners	4	3	2		—	1·00
	Hoggets	4	3	2		—	
	Total	8	6	4		3·83	
A/D × A/D		AA	AD	DD			
	Weaners	6	8	4		1·33	0·72
	Hoggets	0	1	1		—	
	Total	6	9	5		0·05	

[A] Probability given by Fisher's exact contingency table test for homogeneity in phenotypes of weaners and hoggets.
[B] Number insufficient for computation of χ^2.
* $P < 0·05$.

have shown both increases and decreases in the various flocks compared. The marked increase in frequency of Hb^A for the Generation Interval (GI) flock contrasts with its virtually constant frequency in the Fecundity (F) flock. Manwell and Baker (1977) sampled a proportion of the animals typed in the current survey, with conflicting results, possibly resulting from some misclassification as their pooled results are similar to pooled results for the F and GI flocks.

In the R-r-i blood group system the frequency of *R* is noticeably higher in the parent flock, Anama, than in the other flocks. The frequency of this allele has increased in the GI, F and F− flocks, and decreased in the F+ flock.

The effective population numbers and generation times for the flocks (shown in Table 1) were used to calculate changes in gene frequency per generation and the variances of gene frequency changes. Using the square root of the expected variance of gene frequency change as an estimate of the standard error of gene frequency change, the only significant change in gene frequency per generation was found for Tf^C in the Anama flock. The observed phenotypes at the *Tf* and *Hb* loci agreed in all cases with Hardy–Weinberg expectations, in contrast to Cooper *et al.* (1967) who found an excess of heterozygotes at the transferrin locus in the Roseworthy Visual flock. There was no apparent trend in the flocks towards an excess of either homozygotes or heterozygotes.

Table 5. Transferrin type of progeny from reciprocal matings of BD × AD and BD × DD

Sire × dam	Progeny				χ^2	P^A
	(*a*) Weaners (born 1975)				χ^2_3	
	A/B	A/D	B/D	D/D		
B/D × A/D	0	9	4	8	9·67*	<0·009
A/D × B/D[B]	4	3	8	0	—[C]	
Total	4	12	12	8	4·89 n.s.	
	(*b*) Hoggets (born 1974)					
	A/B	A/D	B/D	D/D		
B/D × A/D	3	3	2	3	—	0·35
A/D × B/D[B]	0	5	1	1	—	
Total	3	8	3	4	—	
	(*c*) Weaners				χ^2_1	
	B/D	D/D				
B/D × D/D[B]	10	19			2·79 n.s.	>0·8
D/D × B/D	6	13			2·56 n.s.	
Total	16	32			5·33*	
	(*d*) Hoggets					
	B/D	D/D				
B/D × D/D[B]	13	5			4·25*	0·35
D/D × B/D	1	0			—	
Total	14	5			4·26*	
	(*e*) Cooper's (1966) data for hoggets					
	B/D	D/D				
B/D × D/D	4	8			1·33 n.s.	
D/D × B/D	10	17			1·82 n.s.	
Total	14	25			3·10 n.s.	

[A] Probability given by χ^2 or Fisher's exact contingency test for homogeneity in reciprocal crosses. (Cooper's data and data for weaners were homogeneous; $\chi^2_1 = 0{\cdot}06$, $P > 0{\cdot}95$.)

[B] A/D × B/D and B/D × D/D weaner and hogget data were heterogeneous ($P < 0{\cdot}05$). All other weaner–hogget comparisons were homogeneous.

[C] Numbers insufficient for computation of χ^2.

* $P < 0{\cdot}05$. n.s., not significant.

Family Data

Family data for the transferrin phenotypes were written down separately for weaners and hoggets where matings provided a reasonable number of progeny (Table 4). Sex of the parents was not taken into consideration except for the two matings shown in Table 5. Three matings show significant departures from expec-

tation for the number of progeny produced (Tables 4 and 5); in the mating (B/D × A/D) only the weaners show significant departure; weaners and hoggets, being heterogeneous, could not be pooled. Cooper (1966) found, by contrast, no departures from expectation other than the mating B/D × B/D which gave 5 B/B, 7 B/D and 12 D/D [χ^2(2 d.f.) = 8·25, 0·02 < P < 0·05]. Here there was no evidence to confirm this but in the B/D × D/D mating there was found to be a similar excess of D/D progeny in the weaners. (Again hoggets and weaners were heterogeneous.) A similar trend was observed when Cooper's data for this mating were examined. However, the phenotypes of the hoggets for the B/D male × D/D female mating showed an excess of B/D progeny. In our own data there was a tendency towards an excess of B/D type progeny in the A/B × B/D mating, although overall numbers for this mating were small.

The minor deficiency of A/B phenotype in the B/D × A/D mating and D/D phenotype in the A/D × B/D mating (Table 5) was not found by Cooper (1966).

Matings were then grouped according to presence versus absence of a particular allele. There were two significant departures from expectation, namely the matings of B/+ male × +/+ female (weaners) with 41 +/+ : 18 B/+, and of +/+ male × D/+ female (hoggets) with 4 +/+ : 12 D/+. Most of the matings of the former type would involve the B/D × D/D mating already mentioned. The latter were largely A/B × B/D and A/B × A/D matings. Overall, there appears to be no consistent effect which might be attributed to a particular allele, or to a specific sire or dam effect, though the D allele, where present in matings, tended to give rise to either an excess of D/+ or D/D progeny. When matings were grouped according to whether the progeny are like or unlike dam there were no significant deviations; this finding, with the results already discussed, provides no evidence to support the possibility of maternal–foetal incompatibility.

Tests for the expected 1:1 ratio of heterozygotes to corresponding homozygotes in the progeny of heterozygous sires showed no evidence for superiority of heterozygotes. This was in contrast to the results of Rasmusen and Tucker (1973) who found, by using the method of incomplete family data (Cooper 1966), that there was a highly significant excess of heterozygotes which was due, according to them, to a general advantage of heterozygotes. Cooper had also found an excess of heterozygotes in the offspring of two sires, one of *Tf* type A/C and one of type A/D, and he suggested that this might be due to inbreeding.

Since Mayo *et al.* (1970) had found significant heterogeneity at the 5% level between transferrin phenotypes for numbers of ewes lambing and not lambing this was re-examined in the present data but found not to be significant. Also, there was no evidence to support an association of transferrin type or haemoglobin type of ewe and fertility.

Flock Divergence

To assess flock divergence three measures of population differentiation were used and the results are given in Tables 6 and 7. It can be seen that for each comparison made all three measures D, ϕ and f_θ are similar in magnitude. As expected, the isolated flocks tend to show a greater divergence than descendant and ancestral flocks. The measures show that divergence between flocks tended to increase with time and where comparisons were made between flocks of small effective population sizes (the F+ and F− flocks being the smallest) divergence tended to increase.

Some indication, for example, of the effect of flock size on divergence of isolated flocks can be gained by examining from Table 6 the comparisons of (1) Index–Visual and F+–F−, and (2) Anama 1975–F+ and F− and Anama 1975–GI. Because of the limited number of flocks examined and the difficulty of separating the effect of time from the effect of flock size (e.g. GI and F flocks) it is not possible to gain an accurate assessment of either of these effects.

Table 6. Measures of population differentiation

ϕ is Latter's (1972*a*) measure, D is Nei's (1973) measure, f_θ is Cavalli-Sforza's (1969) measure, and the standard error of f_θ is given by

$$(\{[16\,\Sigma(1-\cos\theta)^2/(n-1)]/\,\Sigma(n-1)\}-f^2)^{1/2}/l^2\,,$$

where n is the number of alleles and l is the number of loci (Cannings and Cavalli-Sforza 1973)

Flocks compared	Time of isolation (years)	Measure of differentiation D	ϕ	f_θ	s.e. of f_θ
Index and Visual	11	0·014	0·014	0·015	0·004
GI and Fecundity	22	0·071	0·063	0·054	0·027
GI and F−	22	0·070	0·061	0·060	0·020
GI and F+	22	0·086	0·079	0·069	0·032
Anama 1964 and Index[A]	16	0·021	0·015	0·021	
Anama 1975 and GI	27	0·034	0·031	0·036	0·001
Anama 1964 and Visual[A]	16	0·003	0·003	0·003	
Anama 1975 and Fecundity	27	0·055	0·052	0·048	0·008
Anama 1975 and F−	27	0·045	0·042	0·033	0·002
Anama 1975 and F+	27	0·079	0·075	0·084	0·010
F− and F+	11	0·028	0·028	0·036	0·005

[A] Based on *Tf* frequency only.

Table 7. Flock differentiation with time

ϕ is Latter's (1972*a*) measure; D is Nei's (1973) measure; f_θ is Cavalli-Sforza's (1969) measure; ϕ_E is the expected value of population divergence involving genetic drift, and is given by $\phi_E = 1 - \exp(-t/2N_e)$, where t is time in generations; and $\Delta F_T = \Delta F \times t$, where $\Delta F = 1/2N_e$ i.e. the inbreeding increment per generation, and t is time in generations

Flocks compared[A]	Time (years)	Gener-ations	D	f_θ	ϕ	ϕ_E	ΔF_T
Anama 1964 and Anama 1975[B]	11	3·24	0·008	0·009	0·007	0·004	0·004
Index and GI	11	3·44	0·046	0·033	0·040	0·024	0·025
Visual and Fecundity	11	3·82	0·003	0·003	0·004	0·024	0·024
Visual and F−	11	3·82	0·016	0·018	0·016	0·047	0·048
Visual and F+	11	3·82	0·004	0·006	0·004	0·047	0·048

[A] 1964 and 1975 descendant flocks.
[B] Based on *Tf* frequency only.

The Index flock shows a greater differentiation from its descendant flock, GI, than does the Visual flock from its F flocks. The measures used also show non-additivity. [For example, with Nei's measure, D (GI and F) is greater than D (GI and Index) plus D (F and Visual).]

Measures of population differentiation involving the 1964 Anama flock are limited by the fact that information on only transferrin frequencies was available. The value obtained for Anama 1964 and 1975, however, is consistent with the lower differentiation expected with a flock of larger effective population size (Table 7).

A relatively large divergence was shown with comparisons involving the F flock with either the GI flock or Anama 1975 flock. Since the F and GI flocks are both subject to different forms of artificial selection, the possibility exists that this is partly reflected in the degree of differentiation.

Table 8. Heterozygosity (H) within flocks

The estimate of heterozygosity at a locus is given by $h = 1 - \Sigma x_i^2$, where x_i is the frequency of the ith allele. Average heterozygosity for r loci is given by $H = \sum_{j=1}^{r} h_j/r$, and its sampling variance can be estimated by

$$V(H) = \sum_{j=1}^{r} (h_j - H)^2/[r(r-1)] \text{ (Nei 1975),}$$

where subscript j refers to the jth locus

Flock	Heterozygosity at locus			Average	$[V(H)]^{1/2}$
	Tf	*Hb*	*R-r-i*	*H*	
Anama 1964	0·569	—	—		
Anama 1975	0·593	0·482	0·430	0·502	0·048
Index	0·623	0·460	0·481	0·521	0·051
GI	0·553	0·495	0·494	0·514	0·019
Visual	0·596	0·362	0·496	0·484	0·068
F+	0·578	0·342	0·490	0·470	0·069
F−	0·635	0·398	0·495	0·509	0·069
Fecundity	0·619	0·371	0·500	0·496	0·071

Table 9. Heterozygosity and inbreeding

H is heterozygosity; $F = (\bar{H} - \bar{H}_{obs})/\bar{H}$

Flocks	Mean level of heterozygosity ($\bar{H}$)	$\bar{H}$ observed, based on phenotypes present ($\bar{H}_{obs}$)	F (estimate of inbreeding)	ΔF ($F_{1975} - F_{1964}$)
Index and Visual	0·503	0·515	0[A]	0·004
GI and Fecundity	0·505	0·503	0·004	

[A] Actual numerical value = −0·024.

The parameter ϕ of Latter (1972*a*) has an expected value (ϕ_E) which is identified with the coefficient of kinship for a model of population divergence involving genetic drift alone (Latter 1972*b*), i.e. $\phi_E = 1 - \exp(-t/2N_e)$, where t is time in generations. The Visual and F flocks show the greatest departure from expectation (Table 7). The values for ϕ_E agree with inbreeding increment levels calculated from the formula $\Delta F = 1/2N_e$, which would be expected from the small number of generations that have elapsed.

According to Nei (1975) average heterozygosity per locus is a measure of genetic variation in a population. For estimating average heterozygosity of gene diversity a large number of loci should be examined. Nei recommends that to minimize the sampling variance for average heterozygosity (an essentially imprecise measure), it

is better to examine a large number of loci rather than a large number of individuals per locus. Nevertheless, average heterozygosity was examined in these flocks to gain information about possible changes in genetic variability. In Table 8 there is shown to be considerable inter-locus variation in heterozygosity, the sampling variances of which are high. The average heterozygosities for the different flocks, however, show little variation overall and no obvious trend between former and current flocks.

An attempt was made to provide an estimate of change in inbreeding (1964–1975). Table 9 shows the values for heterozygosity based on (1) calculation from gene frequencies, and (2) phenotypes present. A low estimate for change in inbreeding was obtained and this supports the estimates obtained previously. Matings between close relatives in the Roseworthy flocks are avoided, and according to Robertson (1964) this would result in a slower decline in heterozygosity in the initial generations.

Discussion

The loss of the Tf^C gene from the F flock and the lower frequencies of Tf^E in the F and GI flocks were not altogether unexpected, since these genes were at a low frequency in the flocks examined in 1964, and these flocks have always been of small effective population size. Some idea of the rate of loss of genes at low frequency in small populations such as these can be gained from the comparison of gene frequencies of Tf^C and Tf^E in the Index and GI flocks, and the Visual and F flocks. As well as small population size and low gene frequency, selective differences between genotypes may lead to rapid gene loss, or delay it. However, this would have been unlikely to have prevented the ultimate loss of Tf^C or Tf^E from such low initial gene frequencies. At present little is known of any selective differences between genotypes in these flocks (or any other). The loss of these alleles could be predicted from changes of gene frequency with genetic drift, after testing the hypothesis that the alleles sampled are selectively neutral. Ewens (1972) has derived a gene frequency distribution for neutral alleles and a test based on this, but its use here would be inappropriate.

At Anama gene frequencies have changed significantly between 1964 and 1975, despite the larger effective population size (Table 2). However, there were introduced to the Nucleus stud of Anama some 90 ewes from Bungaree stud (1973 and 1974), though it is not clear that this could have had much effect since the 46 ewes of the Nucleus sampled from 450 were found to be homogeneous with the 238 ewes from the General stud (sampled from 4500).

Rasmusen and Tucker (1973) concluded from their study of four flocks that transferrin types of sires, dams and offspring somehow influence reproductive performance but that their effects were not easily measured. Matings involving BD appeared to give the largest effects. [The matings, however, of B/D × B/D gave 4 B/B:7 B/D:2 D/D, which did not agree with Cooper's (1966) 5 B/B:7 B/D:12 D/D.] The present findings also suggest that BD appears to be involved significantly. There is a tendency for some matings (Tables 4 and 5) to give an excess of B/D type progeny [A/B × B/D, not significant; A/B × A/D, not significant; B/D × D/D (hoggets) significant].

In the B/D × D/D reciprocal matings (weaners) there is no consistent unlike-dam or unlike-sire effect, both matings producing an excess of D/D. Cooper's data also support these findings. However, there is a significant shortage of D/D in the present hogget data (B/D male × D/D female mating). The poor repeatability in this mating

and for the B/D × B/D mating may reflect the importance of between-year environmental variation affecting the fitness of genotypes. With particular regard to the excess number of homozygotes being produced, it is not necessary with multiple alleles that each heterozygote be fitter than any homozygote; there can be stable equilibrium without this (Mandel 1959).

Tf^D is the most frequent allele in the Merino flocks sampled in Australia (Cooper 1966). The possibility that it has a significant effect on numbers of different types of phenotypes produced is indicated from Cooper's and the present findings.

The measures of population differentiation show the order of divergence one might expect with time and with flocks of differing size. Unfortunately, however, the flocks are not large enough to give enough confidence in the results, compared, for example, with those of Heuch (1975) for the divergence of Nordic cattle breeds. The Index and Visual flocks after 11 years (1953–1964) of selecting replacement rams by two different methods showed homogeneity with respect to *Tf* gene frequency, and limited divergence as indicated by the measures of population differentiation. After a further 11 years (1964–1975), however, the descendant flocks, GI and F respectively, show relatively marked divergence. Interpretation of these differences is limited by the fact that only a small number of loci were examined. Estimates for divergence due to genetic drift alone provide little evidence for effects of selection. Supporting this is the observation that gene frequency changes, which result in differentiation, manifest no consistent pattern among the various lines (Anama 1964–Anama 1975; Index–GI; Visual–F+; Visual–F−). The Visual and F flocks remain close to each other with respect to the three loci examined, but it is unlikely that stabilizing selection affected these lineages alone, especially in view of relative population sizes.

Differences in artificial selection between the F and GI flocks cannot be excluded as a cause for the differences in divergence. However, this is an unlikely contingency since selection will usually require many generations to make large changes in gene frequency, except where it is both intense and directed at a highly heritable trait determined by a very few genes. This is supported by the results of Mayo *et al.* (1970) and many other workers (especially Robertson 1973) who have found little evidence for the effect of polymorphisms on fertility and production traits. The more likely dominant cause for the differences in population measures is, therefore, sampling variation. As noted on page 668, artificial selection would in any case have some of its effect through the decline in N_e as a result of concentration of particular genotypes in the selected individuals.

Manwell and Baker (1977), using 30 polymorphisms, obtained a value for the distance between the South Australian Merino and Poll Dorset breeds at Roseworthy Agricultural College of $D = 0{\cdot}014$. This value of D, which is more precise (assuming no misclassification other than that mentioned on page 670) than ours for the various Merino flocks, further illustrates the problem of interpretation of genetic distance. The Poll Dorset breed is a very recent breed (about 40 years old) and was established by crossing Corriedale (a fixed halfbred Lincoln × Merino) and Ryeland sheep with Dorset Horns (Pattie 1973). Despite this knowledge of the Poll Dorset ancestry, it is impossible to know whether the ancestral genotypes led to the distance observed through the founder effect, or whether there has been selection at some of the loci involved, or merely genetic drift, or whether some of the biochemically assayed loci might have been linked to polling loci.

Another linkage problem in the estimation of genetic distance concerns linkage disequilibrium, i.e. non-independence of the gametic combinations. The three loci were examined for non-random association between genotypes (i.e. they were taken pairwise and values for χ^2 heterogeneity were obtained). There was no evidence to support the possibility that there is any linkage, in agreement with the work of Cooper (1966) and Rasmusen *et al.* (1974).

There are a number of factors apart from selection which might influence the inbreeding levels in these flocks. Theoretically one expects an approximate loss of $\frac{1}{8}N_m + \frac{1}{8}N_f$ per generation of the remaining heterozygosis. If, however, N_m and N_f represent the effective numbers these would be less than the actual numbers and the amount of inbreeding would be greater, though reduced by the systematic avoidance of close matings. (It can be shown that the maximum effect of avoiding all inbreeding as far as possible within a closed population tends towards halving the expected rate, but this optimum was not attained in the flocks considered here.) Also, in small closed flocks, as already discussed, one might find an apparent excess of heterozygotes above the number calculated from the gene frequency. This apparent excess is expected to be on average $\frac{1}{8}N_m + \frac{1}{8}N_f$. Thus the observed degree of inbreeding in these flocks is the resultant of various partly conflicting factors.

Selection or inbreeding would decrease genetic variability but there were only small changes in genetic variation as measured by average heterozygosity, although the number of loci examined here is far too small for accurate prediction. Heterozygosity is higher at the transferrin locus than at the other two loci and it is important to understand the significance of this where a larger number of loci with two or more alleles are examined. Rendel (1967) reported that the degree of homozygosity and the number of alleles within the B system of various cattle breeds seemed to give a relatively good indication of the amount of inbreeding which has taken place in the past. It might be possible to gain similar information from sheep by selecting suitable multi-allelic systems. However, increasing evidence of wide heterogeneity for levels of heterozygosity at different loci (cf. Singh *et al.* 1976), which as yet remains unexplained, implies that polymorphism will be more use in demonstrating divergence than in describing the level of inbreeding.

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