VARIATION IN THE GENE FREQUENCIES OF POTASSIUM AND HAEMOGLOBIN TYPES IN ROMNEY MARSH AND SOUTHDOWN SHEEP ESTABLISHED AWAY FROM THEIR NATIVE ENVIRONMENT

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Summary

The gene frequencies for high potassium and haemoglobin type *A* have been determined in flocks of Romney Marsh and Southdown sheep in New South Wales and these frequencies have been compared with those found in Great Britain.

The high potassium gene frequency in the New South Wales flocks which were examined was significantly lower in both the Romney Marsh and Southdown breeds compared with the same breeds in Great Britain.

The haemoglobin *A* gene frequency in the Romney Marsh was significantly higher than that found in Great Britain but the Southdown flocks showed the reverse tendency. However, when Down breed or Shortwool breed gene frequencies from Great Britain were compared with the Southdown results in New South Wales, a similar and significant change to that seen in the Romney Marsh was demonstrated.

All changes were toward the normal frequencies in Merino sheep, and the possible economic significance of this is discussed.

I. INTRODUCTION

Sheep can be classified into two types—low potassium (*LK*) and high potassium (*HK*)—with respect to the concentration of potassium in their red blood cells (Evans 1954). The two types are simply inherited (Evans and King 1955), and the gene frequency for *HK* varies from breed to breed (Evans 1954; Evans, Harris, and Warren 1958*a*, 1958*b*). A bimodal distribution of potassium concentrations in the erythrocytes of different animals within a species has only been recorded in the sheep (Evans 1954; Evans, Harris, and Warren 1958*a*, 1958*b*), the goat (Evans and Phillipson 1957), the Australian possum (Barker 1958), and the ox (Evans, unpublished data).

In sheep two types of haemoglobins have been separated electrophoretically and named haemoglobin *A* and *B* respectively (Harris and Warren 1955) and the inheritance of the two types has been worked out (Evans *et al.* 1956).

The genes associated with potassium types and haemoglobin types have been shown to be at different chromosomal loci (Evans *et al.* 1956) but a correlation between these two factors between breeds is present (Evans, Harris, and Warren 1958*a*).

The Merino has a very low gene frequency for *HK* (*c.* 0·07) and a gene frequency of approximately 0·45 for haemoglobin *A*. Differences between strains of Merino exist (Evans 1960). Evans and Mounib (1957) suggested an adaptive significance for the potassium types in sheep and have shown that the British Lowland breeds have a lower gene frequency for *HK* than the Hill breeds. Evans, Harris, and Warren (1958*b*) have shown that northern European breeds are almost wholly *HK*.

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The frequencies of potassium and haemoglobin types in Romney Marsh and Southdown sheep in Great Britain have been determined (Evans, Harris, and Warren 1958*a*).

TABLE 1

NUMBER, AGE, AND SEX OF SHEEP EXAMINED, AND THE NUMBER OF EWES MATED AND RAMS USED, IN FLOCKS OF ROMNEY MARSH AND SOUTHDOWN SHEEP FROM WHICH BLOOD SAMPLES WERE OBTAINED FOR ERYTHROCYTE POTASSIUM AND HAEMOGLOBIN TYPE ESTIMATION

Flock	No. of Sheep Examined and Sex		Age (months)	No. of Ewes Mated 1958	No. of Rams Used 1958
	Potassium Estimation	Haemoglobin Estimation			
Romnay Marsh					
A	48 ♀	48 ♀	9–12	714	19
B	50 ♀	49 ♀	Mixed	110	3
C	62 ♂	61 ♂	9–12	2340	54
D	52 ♀	52 ♀	9–12	260	6
E	56 ♀	53 ♀	9–12	202	8
F	53 ♀	40 ♀	9–12	273	6
G	57 ♀	57 ♀	9–12	1400	19
H	55 ♀	54 ♀	9–12	332	10
I	42 ♀	42 ♀	Mixed	—	—
J	47 ♀	—	Mixed	—	—
Totals	522	456			
Southdown					
K	54 ♀	52 ♀	9–12	212	4
L	54 ♂, ♀	54 ♂, ♀	Mixed	304	4
M	49 ♀	49 ♀	9–12	1451	29
N	32 ♀	26 ♀	9–12	59	2
O	50 ♀	50 ♀	7–10	200	5
P	65 ♀	65 ♀	9–12	454	14
Q	100 ♂, ♀	43 ♂, ♀	9–12	—	—
Totals	404	339			

It was considered possible that the very different environmental conditions between Great Britain and Australia might cause a change in the gene frequencies for *HK* and haemoglobin *A* in British breeds of sheep that had been established in Australia towards the frequencies of these genes as found in the Merino, since this latter breed seems eminently suited to the Australian environment. If a change of this nature could be demonstrated it was considered that an adaptive significance of economic importance must be postulated for these genes.

The object of this survey was to determine the gene frequencies for *HK* and haemoglobin *A* in the Romney Marsh and Southdown breeds as found in New South Wales and to compare them with those found in Great Britain.

Table 2

Frequency of potassium types in Romney and Southdown flocks examined, with estimated frequency for the *HK* gene

Flock	No. of Sheep examined	No. High Potassium Types	No. Low Potassium Types	High Potassium Types (%)	*HK* Gene Frequency	Imported Rams Used in 1958 (%)	Imported and Sons of Imported Rams used in 1958 (%)
Romney Marsh							
A	48	2	46	4·17	0·20	0·0	0·0
B	50	2	48	4·00	0·20	—	—
C	62	4	58	6·50	0·25	1·9	3·7
D	52	15	37	28·80	0·54	66·7	66·7
E	56	0	56	0·00	0·00	0·0	0·0
F	53	6	47	11·30	0·34	0·0	50·0
G	57	6	51	10·50	0·32	10·5	94·7
H	55	0	55	0·00	0·00	0·0	0·0
I	42	2	40	4·76	0·22	—	—
J	47	0	47	0·00	0·00	—	—
Totals	522	37	485	7·09	0·27*		
Southdown							
K	54	0	54	0·00	0·00	0·0	0·0
L	54	8	46	14·8	0·38	100·0	100·0
M	49	2	47	4·1	0·20	2·9	11·4
N	32	12	20	37·5	0·61	0·0	0·0
O	50	5	45	10·0	0·32	0·0	40·0
P	65	4	61	6·2	0·25	7·1	28·6
Q	100	6	94	6·0	0·24	—	—
Totals	404	37	367	9·16	0·30†		

* *HK* gene frequency for Romney Marsh in Great Britain (Evans, Harris, and Warren 1958*a*) = 0·53. Difference significant at 0·1% level.
† *HK* gene frequency for Southdown in Great Britain (Evans, Harris, and Warren 1958*a*) = 0·44. Difference significant at 0·1% level.

II. Material and Methods

(a) *Material*

The sheep examined were from 10 Romney Marsh flocks and seven Southdown flocks. Eight of the Romney and six of the Southdown flocks were stud flocks on the Central and Southern Tablelands of New South Wales. The remaining two Romney flocks were mixed-age ewes from the C.S.I.R.O. Field Station, Chiswick, N.S.W., and one Southdown flock was a stud flock from the Northern Tablelands. Approximately 50 sheep were sampled from each flock.

The age of the sheep varied but they were mostly 9–12-month old ewe lambs. Mixed-age ewes were bled in three flocks and rams, aged 9–12 months, were sampled on three occasions.

The number, age, and sex of sheep bled in each flock are shown in Table 1. The 9–12 month old lambs which were sampled were the progeny of the 1958 mating; the number of ewes mated and the number of rams used in this season for each flock were obtained from the British Breeds Stud Book and are also shown in Table 1. The flocks in the districts from which most of the samples were taken are not very large.

(b) *Methods*

The sheep were collected into yards the night before bleeding. The blood was withdrawn by jugular vein puncture with the sheep in the standing position, and about 10–15 ml were collected into sterile 30-ml McCartney bottles containing 1·5 μg heparin. The samples were dispatched to the laboratory as soon as possible after collection.

The concentrations of potassium in whole blood were estimated using an "EEL" flame photometer (King and Wootton 1956).

The haemoglobin preparations were made by centrifuging whole blood at 3000 r.p.m. for 30 min and discarding the plasma. The cells were then washed in normal saline three times and finally haemolysed in an equal quantity of distilled water. The haemolysate was subjected to electrophoresis on Whatman No. 3 MM paper in a horizontal-type bath using a Tris–borate buffer at a pH 9·1 (Tris 60·5 g, boric acid 4·6 g, final volume 1 litre). The bath was run at 225 V for 16 hr.

III. Results

The number of sheep of each of the two potassium phenotypes in 10 Romney Marsh and seven Southdown flocks are shown in Table 2 together with the estimated frequency of the *HK* gene.

Table 3 shows the number of each of the three haemoglobin types found in each flock, together with the gene frequency for haemoglobin *A*, and the observed numbers are compared with the theoretically expected numbers.

Most flocks were polymorphic with respect to the potassium and haemoglobin characters and there was considerable variation between flocks. The gene frequency for *HK* in the Romneys was 0·27, compared with 0·53 in Great Britain. The range was between 0·00 and 0·54. In the Southdowns the frequency was 0·30, as against 0·44 in Great Britain, with a range of 0·00–0·61.

TABLE 3

FREQUENCY OF HAEMOGLOBIN TYPES SHOWING OBSERVED AND EXPECTED NUMBERS OF *A*, *AB*, AND *B* TYPES

Flock	No. of Sheep Examined	No. Observed			Type *A* Gene Frequency	No. Expected		
		Type *A*	Type *AB*	Type *B*		Type *A*	Type *AB*	Type *B*
Romney Marsh								
A	48	5	22	21	0·33	5·23	21·22	21·55
B	49	14	28	7	0·57	15·92	24·02	9·06
C	61	8	28	25	0·36	7·91	28·11	23·99
D	52	14	24	14	0·50	13·00	26·00	13·00
E	53	19	23	11	0·57	17·23	25·98	9·80
F	40	14	25	1	0·66	17·42	17·93	4·62
G	57	7	27	23	0·36	7·39	26·27	23·25
H	54	1	17	36	0·18	1·75	15·94	36·31
I	42	10	23	9	0·51	10·92	21·00	10·08
Totals	456	92	217	147	0·44*	96·77	206·47	151·66
Southdown								
K	52	1	24	27	0·25	3·25	19·50	29·25
L	54	2	21	31	0·23	2·86	19·13	32·02
M	49	6	23	20	0·36	6·35	22·58	20·17
N	26	0	9	17	0·17	0·75	7·34	17·91
O	50	3	35	12	0·41	8·41	24·19	17·41
P	65	4	36	25	0·34	7·51	29·17	28·31
Q	43	0	9	34	0·10	0·00	7·74	34·83
Totals	339	16	157	166	0·28†	29·13	129·65‡	179·90

* Haemoglobin type *A* gene frequency for Romney Marsh in Great Britain (Evans, Harris, and Warren 1958*a*) = 0·11. Difference significant at 0·1% level.

† Haemoglobin type *A* gene frequency for Southdown and Down breeds in Great Britain (Evans, Harris, and Warren 1958*a*) = 0·48 and 0·09 respectively. Differences significant at 1% and 0·1% levels respectively.

‡ Observed value significantly higher than expected ($P < 1 \cdot 0\%$).

The frequency for haemoglobin *A* in the Romneys was 0·44 and in the Southdowns 0·28, compared with 0·11 and 0·48, respectively, in Great Britain. The variation between flocks in the Romney was 0·18–0·66, and in the Southdowns 0·17–0·41. The expected numbers of the haemoglobin types, assuming a Hardy–Weinberg equilibrium, did not agree with those observed. In each case there was an excess of heterozygotes.

IV. Discussion

The frequency for the *HK* gene in the two breeds examined was considerably lower than that reported by Evans, Harris, and Warren (1958*a*) for the same breeds in Great Britain. These authors also showed that, in general, differences between breeds in Great Britain were greater than differences between flocks of the same breed, and that the frequencies in three Romney flocks was 0·47, 0·56, and 0·57. The range for the frequency of the *HK* gene in the various flocks examined in this survey was much wider than that observed in Great Britain.

The Merino has a gene frequency of approximately 0·07 for *HK* (Evans 1960) and appears to be able to exist under much hotter and drier conditions than British breeds (Wright 1954). *LK* animals have been shown in one breed (Scottish Blackface) to drink less water and pass less urine than *HK* animals (Evans 1957). It seems reasonable to associate the change towards a lower gene frequency for *HK* which was found in both the British breeds, which had been established in Australia and were examined in this survey, to a significant effect of these genes on the adaptation of these sheep to the Australian environment. It also seems reasonable to suggest that differences in water metabolism or lambing percentages may be involved (Evans 1960).

The argument is strengthened by the observation that most of the higher frequencies for *HK* seemed to be associated with flocks which used high percentages of imported rams. A positive correlation between these two factors is on the borderline of significance at the 5% level. The exception is flock N, which, however, used only two rams as the sires of the sheep examined. The imported rams were either from New Zealand or Tasmania.

Interpretation of the changes in the frequencies of the haemoglobin phenotypes is more difficult. In the Romney Marsh sheep there is a greater frequency for haemoglobin *A* compared with the same breed examined in Great Britain ($P<0\cdot1$), whereas the Southdowns have a lower frequency for haemoglobin *A* compared with the same breed in Great Britain ($P<1\cdot0$). However, Evans, Harris, and Warren (1958*a*) who only examined one Southdown flock in Britain state "The most anomalous result is that obtained with respect to the haemoglobin gene in the Southdown . . . Its position in the overall geographic pattern is anomalous". The mean gene frequency for the five Down flocks examined by them was 0·09 and for the nine Lowland Shortwool flocks 0·07, but the frequency for the Southdown was 0·48 despite the fact that the Southdown had been used in the establishment of all the other Down breeds. Evans, Harris, and Warren suggest that the Southdown haemoglobin *A* frequency obtained by them "may be due to chance fluctuations". The Southdown *HK* gene frequency was in no way anomalous, however, with respect to other Down or Shortwool sheep. The significant increase in the gene frequency for haemoglobin A in the Romney Marsh in Australia compared with Great Britain and the fact that a similar significant tendency ($P<0\cdot 1$) would be shown in the Southdown if mean Down or Lowland breed haemoglobin A frequencies rather than Southdown frequencies obtained in Britain were taken suggests that the Southdown flock examined by Evans, Harris, and Warren may not have been representative of the breed and that in Australia the change to a higher frequency for haemoglobin A in the Romney Marsh breed may be paralleled by a similar change in the Southdown.

TABLE 4

COMPARISON BETWEEN THE GENE FREQUENCIES FOR HK AND HAEMOGLOBIN A IN SHEEP IN GREAT BRITAIN AND AUSTRALIA

Breed	HK Gene Frequency		Haemoglobin A Gene Frequency	
	Great Britain	Australia	Great Britain	Australia
Romney Marsh	0·53	0·27	0·11	0·44
Southdown	0·44	0·30	0·09* 0·48†	0·28
Merino		0·07		0·45

* Down breeds excluding Southdown (Evans, Harris, and Warren 1958*a*).

† Southdown breeds (Evans, Harris, and Warren 1958*a*).

The excess of heterozygotes (haemoglobin AB) was significant at the 1% level in the Southdowns but not significant in the Romneys.

If the distribution of haemoglobin types expected in a Hardy–Weinberg equilibrium is compared with the observed numbers within each breed it will be seen that the greatest deviations occur in those flocks using a small number of rams. As a general rule those flocks in which 10 or more rams were used showed the highest correlation between observed and expected numbers; the only exception was flock P, which used 14 rams during the season but the 65 sheep examined in this survey were, however, only sired by 6 of these. The significant difference between expected and observed numbers in the Southdown should therefore be interpreted with caution.

It has been shown that in British breeds in Great Britain there is a correlation between those breeds having a high gene frequency for HK and those with a high gene frequency for haemoglobin A in spite of an absence of association within breeds (Evans, Harris, and Warren 1958*a*) and these authors suggest that HK and haemoglobin A have some relative advantage over LK and haemoglobin B in those areas where this type of flock is indigenous. An investigation of these types in Scottish Blackface sheep in a single environment, however, did not reveal any major differences (King *et al.* 1958).

If *LK* and haemoglobin *A* are of adaptive significance in Australia—and this is suggested by the frequencies of these genes in Merino sheep (gene frequency for $LK = 0 \cdot 93$ approx., gene frequency for haemoglobin $A = 0 \cdot 45$ approx.)—one might expect the frequencies of these genes in British breeds in Australia to tend towards the Merino frequencies. The results reported here suggest that this does occur (Table 4).

The interpretation of these data is not clear cut but it would appear likely that *LK* sheep are better adapted to the environment from which these samples were taken and that haemoglobin *A* or haemoglobin *AB* may also have advantages over the homozygous haemoglobin *B* type animal in the same environment. If these postulated advantages can be substantiated and shown to be associated with adaptation to the Australian environment (e.g. drought, parasitism, heat) the genes controlling them become of considerable economic importance in this environment. As both potassium type and haemoglobin type are simply inherited, breeding for the superior animal would be both easy and quick.

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