Genetic variation in blood proteins within and between 19 sheep breeds from southern Africa

J. Sargent, F.H. van der Bank*, A. Kotzé
Rand Afrikaans University, Department of Zoology, P.O. Box 524, Auckland Park, 2006 South Africa

The amount of allozyme variation within, and the extent of genetic differentiation between, 19 sheep breeds from southern Africa were determined by six genetic blood systems commonly used to distinguish between animal breeds. Another eight enzyme-coding loci were analysed for five breeds. Between 55 and 66.67% of the protein-coding loci were polymorphic (95% criterion) in all the breeds, except for the Namaqua sheep which were less polymorphic (33.33%). Values of 1.67 to 2.5 were obtained for the mean number of alleles per locus and average heterozygosities per locus were calculated at 16.6 to 35.9%. The allelic constitution particularly at the transferrin (**TF**) locus varied appreciably for the different breeds. For example, the **TF*H** allele was exclusively noted in the Dormer sheep and the **TF*G** allele was found in the Afrino, Van Rooy, Border Leicester, Blackhead Persian and Skilder-Persian breeds. The only polymorphic breeds at the albumin locus were the South African Meat Merino and Van Rooy breeds. The allelic constitution at the other polymorphic loci was similar for the breeds, but the allele frequencies of the South African Merino differ from Merino breeds in other countries at the **TF** locus. Unbiased genetic distance values were the smallest between the Dorper and Dormer breeds and the largest between the Romanof and Blackhead Persian breeds, and the mean genetic distance between the 19 breeds was 0.067. The mean amount of differentiation among the breeds relative to the limiting amount under complete fixation (F_{ST}) was calculated at 0.123 for polymorphic loci, which is an indication of small genetic differentiation between the breeds studied. However, this statistic is not reflected by the allele distribution which was not identical for 25 breed pairs (15%) of the total (171) at all the genetic blood systems studied. None of the breed pairs showed identical allele distributions at all the loci studied, for at least one locus differed at each breed pair compared. The results of this study can be used in breeding programs, and present the first study of the current genetic structure of the different southern African sheep breeds.

Die hoeveelheid allosiemvariasie binne en die mate van genetiese differensiasie tussen 19 skaaprasse van suider Afrika is bepaal deur van ses algemeen genetiese bloedsisteme gebruik te maak. Daarna is nog agt ensiem-koderende lokusse vir vyf rasse ontleed. Tussen 55 en 66.67% van die proteïen-koderende lokusse was polimorfies (95% kriterium) vir al die rasse, behalwe vir die Namaqua skaapras wat slegs 33.33% polimorfies was. Die gemiddelde aantal allele per lokus wissel van 1.67 tot 2.5, en die gemiddelde heterosigositeit per lokus was 16.6 tot 35.9%. Die alleliese samestelling, veral by die transferrien (**TF**) lokus het merkbare verskille tussen die verskillende rasse getoon. Byvoorbeeld, die **TF*H** alleel was slegs by die Dormer skaapras gevind en die **TF*G** alleel in die Afrino, Van Rooy, Border-Leicester, Swartkop-Persie and Skilder-Persie rasse gevind. Die Suid-Afrikaanse Vleis Merino en Van Rooy rasse was die enigste polimorfiese rasse by die albumien lokus. Die alleliese samestelling by die res van die lokusse was soortgeyk vir al die rasse. Die Suid Afrikaanse Merino verskil by die **TF** lokus van Merino rasse in ander lande. Die genetiese afstand was die kleinste tussen die Dorper en Dormer rasse en die grootste tussen die Romanof en Swartkop-Persie rasse, met 'n

gemiddelde genetiese afstand van 0.067 tussen die 19 rasse. Die gemiddelde hoeveelheid differensiasie tussen die rasse, relatief tot die beperkte aantal onder algehele fiksasie (F_{ST}) is bereken as 0.123 vir polimorfiese lokusse, wat 'n aanduiding van min genetiese differensiasie tussen die rasse is. Hierdie statistieke word egter nie gereflekteer by al die lokusse wat bestudeer is nie, deurdat 25 raspare (15%) van die totaal (171) nie identiese alleelverspreiding het nie. Geen van die raspare het dieselfde alleelverspreiding by al die lokusse getoon nie; ten minste een lokus het by al die raspare verskillende alleelverspreidings getoon. Die resultate van hierdie studie kan in teelprogramme gebruik word en dit is die eerste studie wat die huidige genetiese struktuur van die verskillende suider Afrikaanse skaaprasse aandui.

Keywords: Sheep, allozyme variation, genetic distance, polymorphism

* To whom correspondence should be addressed

Introduction

Currently there are 33 sheep breeds in southern Africa, which include seven pure indigenous breeds. Indigenous sheep breeds generally have a high resistance to diseases, they can tolerate the warmer climate in the summer and extreme cold climate during the winter, and their production of wool and of red meat is much higher compared to other breeds. For example, the Dormer breed has a high adaptability to winter rainfall dominions. The crossbreeding of a Dorset Horn ram (with excellent meat form and rapid growth ability) and a South African Meat Merino ewe (with high fertility and the ability to produce more than one lamb) led to the origin of the Dormer. The Dormer is, therefore, one of the best crossbreeds in southern Africa. The South African Meat Merino is used for its wool and exceptional meat production; the Dorper is the best meat producer among the sheep breeds; the Ronderib Afrikaner is a fat-tailed sheep breed and the Blackhead Persian has poor meat quality with a lot of fat, but is used by Karakul farmers for upgrading and to ensure that the good characteristics (high fertility, hardness, etc.) of the breed are used to their advantage. It is, therefore, beneficial to characterize the pure sheep breeds and to use them in breeding programmes to improve breeding stocks since a combination of different indigenous sheep breeds could lead to better utilization of the natural resources, which would be economically viable (Terblanche 1978).

Biochemical blood polymorphism is increasingly being used to study the variability within populations and to estimate racial divergence between breeds because differences in allele frequencies indicate each breed's particular identity (Van Zeveren 1995). The aim of the present study is to characterize 19 sheep breeds from southern Africa by comparing them in respect of gene and phenotype frequencies at the following polymorphic serum markers which are routinely used in animal genetic studies: albumin (**ALB**), carbonic anhydrase (**CA**), diaphorase (**Dia**), hemoglobin (**HB**), transferrin (**TF**) and X-protein (**X**). Another eight enzyme-coding loci were analysed for the Dorper, Van Rooy, Romanof, Dormer and South African Meat Merino breeds. The additional loci were analysed to test the accuracy of the initial phenetic relationships obtained from only the six polymorphic loci used in routine analyses.

Materials and Methods

Blood samples of each breed were collected from at least three different breeders. The breeds that were analysed, their status and sample sizes are presented in Table 1. Sample size was subject to availability. All breeds studied were bred in the Northern and Western Cape Provinces, and the characteristics for which the different breeds were selected include growth rate, percentage meat, reproductivity and improved adaptability to the environment.

Table 1 Population number, sheep breed, sample size (*n*) and status of each population studied

No	Breed	*n*	Status
1	Dorper	151	Adaptive synthetic breed
2	Pedi	100	Indigenous
3	South African Meat Merino	97	Adaptive synthetic breed
4	Landrace	99	Exotic
5	Merino	99	Adaptive synthetic breed
6	Afrino	100	Adaptive synthetic breed
7	Namaqua	169	Indigenous
8	Van Rooy	180	Adaptive synthetic breed
9	Russian Red Wooled Persian	105	Exotic
10	Border Leicester	71	Exotic
11	Blackhead Persian	217	Indigenous
12	Ronderib Afrikaner	138	Indigenous
13	Damara	98	Indigenous
14	Skilder Persian	52	Indigenous
15	Dormer	286	Adaptive synthetic breed
16	Vandor	31	Adaptive synthetic breed
17	Karakul	25	Indigenous
18	Perinoff	12	Adaptive synthetic breed
19	Romanof	28	Exotic

Hydrolysed horizontal starch and two-dimensional polyacrylamide gels were used to separate allele products, using the buffer systems and gel concentrations described by Shaw & Prasad (1970). For **TF**, tris-boric acid was used as buffer (pH 8.7); in the two-dimensional electrophoresis, tris-citric acid was used as buffer (pH 6.3) for separating **ALB**; for **CA** and **X**, boric-NaOH was used as buffer (pH 8.7) and a tris-boric acid buffer was used for **HB** and **Dia** but with pH values of 8.9 and 8.6 respectively. Staining recipes adapted from Harris & Hopkinson (1976) were used. The enzymes stained for were: glucose dehydrogenase (**GLD**; E.C 1.1.1.47), glycerol-3-phosphate dehydrogenase (**GPD**; E.C 1.1.1.8), 6-phosphogluconate (**PGD**; E.C 1.1.1.44), esterase (**EST-1**, **-2**; E.C 3.1.1.-), glucose-6-phosphate isomerase (**GPI**; E.C 3.5.1.9), lactose dehydrogenase (**LDH**; E.C 1.1.1.27) and peroxidase (**PER**; E.C 1.11.1.7). The following buffer systems were used to analyse an additional eight enzymes: (a) **MF** – a continuous Tris, boric acid and EDTA buffer system (pH 8.6; Markert & Faulhaber, 1965; for **GLD**, **GPD** and **PGD**) and (b) **RW** – a discontinuous, citric acid (gel pH 8.7), lithium hydroxide and boric acid buffer system (electrode pH 8.0; Ridgway *et al.* 1970; for **EST-1, -2**, **GPI**, **LDH** and **PER**). Twelve per cent starch gels were used to resolve these eight enzymes.

Six loci for each population, and thereafter an additional eight loci for five populations were studied with a maximum of nine alleles per locus. The **BIOSYS-1** computer program (Swofford & Selander 1981) was used to calculate average heterozygosity values (**H**) using the formula of Nei (1978). We have calculated Wright's (1978) fixation index (**F**) values for polymorphic loci and Chi-squared (χ^2) values for deviations of allele combinations from expected Hardy-Weinberg equilib-

rium was tested at each locus. The coefficients of heterozygosity deficiency or excess (**d**) for each polymorphic locus, mean number of alleles per locus (**A**), and percentage of polymorphic loci (**P**) using the 0.95 criterion were calculated. The genetic distances between breeds were determined with Nei's (1972, 1978) formuli, and **DISPAN** (Ota 1993) was used to construct a dendrogram from Nei's (1978) genetic distance values using neighbour-joining and bootstrap tests (1 000 replications). **GENEPOP** (Version 3.1b, Raymond & Rousset 1995) was used to determine if significant ($p<0.05$) differences in allele distribution were present between breed pairs.

Results

Analysis of six polymorphic loci

Relative allele frequencies for 19 sheep breeds from southern Africa for each polymorphic locus are given in Table 2. Loci where significant ($p<0.05$) deviations of allele combinations from expected Hardy-Weinberg proportions occurred were estimated (Table 2). The **D**-allele is the most common allele at the **TF** system for most of the breeds, except for the South African Meat Merino and Perinoff (**A**-allele), Russian Red Wooled Persian and Border Leicester (**B**-allele) and Karakul (**C**-allele) breeds; the **H**-allele was exclusively found in the Dormer breed, although at a low frequency, and the **G**-allele was found in the Afrino, Van Rooy, Border Leicester, Blackhead Persian and Skilder Persian breeds only. Only seven of the nineteen breeds did not comply with Hardy-Weinberg expectations at this locus (Table 2).

All of the breeds, except for the South African Meat Merino and the Van Rooy breeds, were monomorphic at the **ALB** system and only the South African Meat Merino had allele frequencies which complied with Hardy-Weinberg expectations at this locus. Thirteen of the breeds were monomorphic for the **B**-allele at the **CA** system, **C**-alleles were observed at the Russian Red Wooled Persian and Border Leicester breeds, whereas **A**-alleles were present in the remaining four populations (Landrace, Afrino, Namaqua and Vandor). All of the polymorphic breeds at the **CA** system had allele combinations which approximated Hardy-Weinberg proportions. The Namaqua and Romanof breeds were the only monomorphic breeds observed for the **X** system, and all of the breeds studied had the **B**-allele as the most common allele at these three loci (**ALB**, **CA** and **X**). All of the breeds where polymorphism occurred had allele combinations which did not comply with Hardy-Weinberg expectations at the **X** system (Table 2).

The **HB** system was polymorphic in all but the Pedi and Skilder Persian breeds, and the Ronderib Afrikaner was the only breed with allele classes which did not approximate Hardy-Weinberg expectations at this locus. The **Dia** system was polymorphic, with most breeds showing the **A**-allele at the largest frequency, except for the South African Meat Merino, Perinoff and Romanof breeds (**B**-allele). Eight breeds did not have allele classes which approximated Hardy-Weinberg expectations at this locus (Table 2).

The **A**, **P**, **H** and **D** values are given in Table 4. Values of 1.67 (Pedi and Romanof) to 2.5 (Afrino, Van Rooy and Border Leicester) were obtained for **A**, and **H** values ranged from 16.6 (Namaqua) to 35.9% (Russian Red Wooled Persian) with an average of 27% for all the breeds. Between 50.00 to 66.67% of the protein coding loci were polymorphic in all the breeds, except for the Namaqua sheep (33.33%). The unbiased **D** (Nei, 1978) values were the smallest between the Dormer and Dorper breeds (0.004) and also between the Perinoff and Romanof breeds (0.004), and the largest between the Romanof and Blackhead Persian breeds (0.140). The mean **D** value between the breeds was 0.067.

Table 2 Relative allele frequencies for each polymorphic locus in 19 sheep breeds from South Africa, see Table 1 for breed designation. * Loci where significant ($p<0.05$ deviations of allele classes from expected Hardy-Weinberg proportions occurred

	Breed																		
Locus (Allele)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
TF																			
(A)	0.186*	0.030	0.469*	0.162	0.025	0.125	0.142*	0.347*	0.157	0.239	0.205*		0.031	0.375*	0.076	0.097	0.120	0.400	0.208
(B)	0.236	0.012	0.025		0.025	0.124	0.069	0.338	0.373	0.078	0.112*	0.077	0.163	0.334	0.032	0.100	0.100		
(C)	0.050	0.125	0.173	0.146	0.475	0.275	0.036	0.047	0.129	0.204	0.021	0.163	0.117	0.010	0.059	0.210	0.400	0.350	0.375
(D)	0.529	0.845	0.148	0.419	0.500	0.485	0.698	0.463	0.186	0.162	0.694	0.652	0.765	0.442	0.479	0.661	0.380	0.150	0.417
(E)			0.198	0.247		0.070		0.009	0.190	0.014		0.072	0.010		0.051				
(G)						0.020		0.066		0.007	0.002			0.010					
(H)															0.002				
ALB																			
(A)			0.019					0.006*											
(B)	1.000	1.000	0.981	1.000	1.000	1.000	1.000	0.994	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CA																			
(A)				0.015		0.042	0.003									0.017			
(B)	1.000	1.000	1.000	0.985	1.000	0.958	0.997	1.000	0.971	0.991	1.000	1.000	1.000	1.000	1.000	0.983	1.000	1.000	1.000
(C)									0.029	0.009									
X																			
(A)	0.170*	0.188*	0.140*	0.182*	0.155*	0.084*		0.215*	0.324*	0.053*	0.458*	0.067*	0.171*	0.375*	0.261*	0.400*	0.080*	0.050*	
(B)	0.830	0.813	0.860	0.818	0.845	0.916	1.000	0.785	0.676	0.947	0.542	0.933	0.829	0.625	0.739	0.600	0.920	0.950	1.000
HB																			
(A)	0.103		0.031	0.230	0.482	0.297	0.031	0.045	0.267	0.079	0.015	0.213*	0.112		0.160	0.033	0.100	0.325*	0.375
(B)	0.897	1.000	0.969	0.770	0.518	0 703	0.969	0.955	0.733	0.921	0.985	0.787	0.888	1.000	0.840	0.967	0.900	0.675	0.625
Dia																			
(A)	0.776*	0.563	0.418	0.670	0.696*	0.717	0.651*	0.781*	0.529	0.842	0.803*	0.907*	0.729	0.694	0.787*	0.667	0.620*	0.474	0.375
(B*)	0.224	0.438	0.582	0.330	0.304	0.283	0.349	0.219	0.471	0.158	0.197	0.093	0.271	0.306	0.213	0.333	0.380	0.526	0.625

Additional loci

Seven of the eight additional loci were monomorphic for the five breeds studied. Only the **PER** locus was polymorphic in all the sheep breeds, with **A** being the most common allele. None of the allele classes approximated Hardy-Weinberg expectations at the **PER** locus.

Deficiencies of heterozygotes occurred in all of the breeds that did not have allele classes which approximated Hardy-Weinberg expectations at the **ALB, CA, X, HB, Dia** and **PER** loci; with no heterozygotes observed at the **ALB** locus in the Van Rooy breed. Four of the breeds (Dorper, South African Meat Merino, Blackhead Persian and Skilder Persian) had allele combinations which did not comply to Hardy-Weinberg expectations at the **TF** locus owing to an excess of heterozygotes. However a deficiency of heterozygotes occurred in the other breeds at this locus. Individual heterozygosity values for polymorphic loci ranged from 0.016–0.774 at **TF**, 0.012– 0.036 at **ALB**, 0.007–0.081 at **CA**, 0.095–0.497 at **X**-protein, 0.030–0.499 at **HB** and 0.168–0.499 at the **Dia** locus. Details can be obtained from the senior author (e-mail: genetika@na.rau.ac.za). Allele frequencies of South African Merino breeds at the **TF**-locus were also compared with allele frequencies in Merino breeds in other countries (Table 3). The South African Merinos differed from Merino breeds in other countries at this locus.The mean **F** value of individuals relative to the subpopulation (F_{IS}) is 0.260, 0.351 for the total population (F_{IT}) and F_{ST} = 0.123 for the amount of differentiation among populations relative to the limiting amount under complete fixation (Table 5). The loci that contributed least to the inter- and intraspecific differences are **ALB** (F_{ST} = 0.014) and **CA** (F_{ST} = 0.023) because all of the breeds studied shared the most common allele at these loci (Table 5).

Table 3 Comparison of the frequency of transferrin alleles in seven populations of Merino sheep

Population	No. of samples	Alleles					
		TF-A	TF-B	TF-C	TF-D	TF-E	TF-G
South African Meat Merino	81	0.469	0.012	0.173	0.148	0.198	
South African Merino ♣	99	0.025		0.475	0.500		
Mailliard Merino ♦	39	0.080	0.240	0.350	0.330		
Peppin Merino from Central Western Queensland ♠	248	0.180	0.150	0.200	0.390	0.020	0.060
Peppin Merino from Badgery Creek ♠	71	0.110	0.080	0.190	0.460	0.080	0.060
Tasmanian Fine Wooled Merino ♠	210	0.010	0.310	0.280	0.370	0.020	
Spanish Merino ♥	200	0.190	0.150	0.120	0.437	0.102	

♣ Current study, ♦ Stormont *et al.* (1968), ♠ Ashton & Ferguson (1962), ♥ Nguyen *et al* (1992)

Figure 1 presents a dendrogram that reflects the unbiased genetic distances between the breeds and their phylogenetic relationships. The closest genetically related breeds are the Dorper and Dormer breeds, Perinoff and Romanof breeds, Van Rooy and Skilder Persian breeds, Blackhead Persian and Vandor breeds, Pedi and Damara breeds and Landrace and Afrino breeds, respectively (Table 4, Figure 1). The results compared well with results obtained after the additional enzymes were analysed (Figure 2). However, the bootstrap values at the nodes increased with an average of 35%. No distinct genetic differentiation (Figure 1) was obtained between the indigenous and exotic sheep breeds (e.g. the Landrace and Afrino breeds which are adaptive synthetic indigenous breeds).

Table 4 Mean number of alleles per locus (**A**), percentage of loci polymorphic (**P**), average heterozygosity per locus (**H**), Nei (1978) unbiased genetic distance above diagonal) and Nei (1972) genetic distance (below diagonal). See Table 1 for breed designation

Breed	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A	2.00	1.67	2.33	2.33	1.83	2.50	2.00	2.50	2.33	2.50	2.17	2.00	2.17	2.00	2.33	2.17	2.00	2.00	1.67
P	66.67	50.0	50.0	66.67	66.67	66.67	33.33	50.0	66.67	66.67	50.0	66.67	66.67	50.0	66.67	50.0	66.67	66.67	50.0
H	0.240	0.178	0.252	0.306	0.285	0.288	0.166	0.239	0.359	0.211	0.219	0.193	0.212	0.255	0.273	0.255	0.245	0.286	0.263
Breed																			
1	****	0.030	0.066	0.018	0.063	0.021	0.013	0.007	0.043	0.022	0.025	0.016	0.011	0.016	0.004	0.022	0.022	0.065	0.073
2	0.031	****	0.082	0.038	0.080	0.042	0.014	0.037	0.084	0.091	0.034	0.042	0.008	0.042	0.043	0.013	0.034	0.100	0.068
3	0.068	0.084	****	0.042	0.120	0.067	0.065	0.049	0.045	0.064	0.105	0.118	0.091	0.048	0.085	0.077	0.037	0.025	0.049
4	0.020	0.040	0.044	****	0.037	0.008	0.028	0.021	0.027	0.041	0.048	0.025	0.022	0.035	0.022	0.031	0.017	0.038	0.038
5	0.065	0.082	0.123	0.039	****	0.014	0.076	0.080	0.072	0.085	0.106	0.040	0.052	0.109	0.063	0.070	0.036	0.049	0.029
6	0.023	0.044	0.069	0.010	0.016	****	0.027	0.030	0.049	0.039	0.064	0.014	0.020	0.056	0.029	0.041	0.011	0.033	0.025
7	0.014	0.015	0.067	0.030	0.078	0.028	****	0.023	0.073	0.048	0.049	0.025	0.010	0.040	0.030	0.034	0.023	0.071	0.056
8	0.008	0.038	0.051	0.023	0.082	0.023	0.023	****	0.053	0.029	0.021	0.032	0.022	0.007	0.019	0.022	0.028	0.064	0.082
9	0.045	0.086	0.048	0.030	0.074	0.051	0.074	0.055	****	0.047	0.078	0.080	0.069	0.043	0.031	0.058	0.041	0.038	0.057
10	0.024	0.093	0.067	0.043	0.087	0.042	0.049	0.031	0.050	****	0.081	0.043	0.059	0.046	0.026	0.074	0.026	0.051	0.088
11	0.026	0.035	0.107	0.049	0.108	0.066	0.050	0.022	0.080	0.082	****	0.050	0.025	0.012	0.027	0.007	0.066	0.135	0.140
12	0.017	0.043	0.119	0.026	0.042	0.015	0.026	0.033	0.082	0.044	0.051	****	0.012	0.062	0.021	0.043	0.033	0.091	0.080
13	0.012	0.009	0.093	0.024	0.054	0.022	0.011	0.024	0.071	0.061	0.026	0.013	****	0.036	0.018	0.012	0.026	0.088	0.067
14	0.019	0.045	0.051	0.038	0.112	0.060	0.043	0.009	0.046	0.049	0.015	0.064	0.039	*****	0.022	0.016	0.043	0.079	0.103
15	0.005	0.044	0.087	0.024	0.064	0.030	0.031	0.020	0.033	0.028	0.028	0.021	0.020	0.025	****	0.025	0.032	0.078	0.088
16	0.026	0.016	0.082	0.035	0.074	0.045	0.037	0.026	0.063	0.078	0.010	0.046	0.016	0.021	0.029	****	0.032	0.096	0.087
17	0.026	0.038	0.042	0.022	0.041	0.015	0.026	0.032	0.045	0.030	0.069	0.037	0.030	0.049	0.036	0.038	****	0.023	0.023
18	0.071	0.106	0.032	0.044	0.055	0.039	0.076	0.069	0.045	0.058	0.141	0.096	0.094	0.086	0.084	0.104	0.032	****	0.004
19	0.081	0.076	0.058	0.047	0.038	0.034	0.064	0.090	0.066	0.097	0.148	0.088	0.076	0.114	0.096	0.097	0.034	0.017	****

Table 5 Summary of F-statistics (Wright, 1978) at all loci for 19 sheep breeds from South Africa

Locus	F_{IS}	F_{IT}	F_{ST}
TF	0.019	0.165	0.146
ALB	0.241	0.251	0.014
CA	-0.029	-0.005	0.023
X	1.000	1.000	0.112
HB	0.014	0.157	0.146
DIA	0.217	0.332	0.084
PER	-0.066	0.047	0.113
Mean	0.260	0.351	0.123

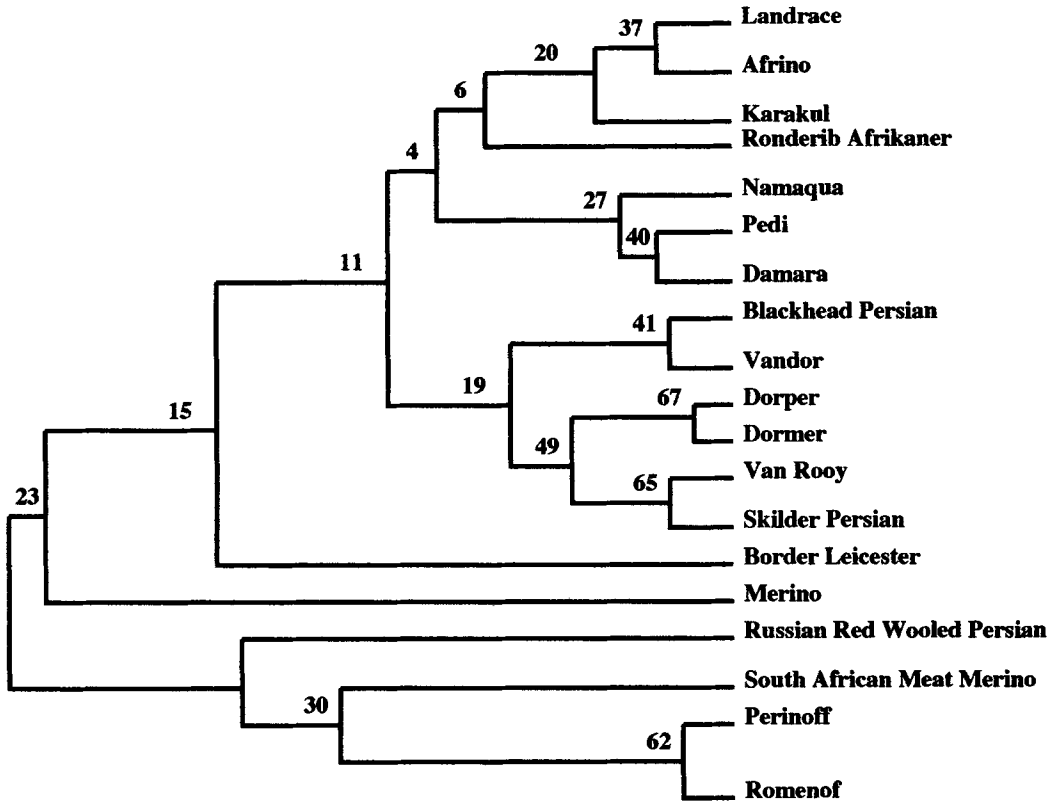

Figure 1 Dendrogram showing relationships between 19 sheep breeds using the unbiased genetic distance coefficient of Nei (1978). Numbers at nodes are bootstrap values.

Allele distributions were not identical between the following 25 breed pairs at all the loci studied: Dorper and Russian Red Wooled Persian, Ronderib Afrikaner and Romanof; Pedi and Afrino,

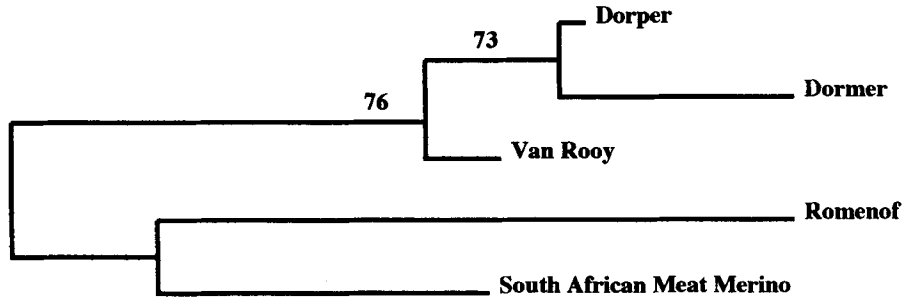

Figure 2 Dendrogram showing the relationships between 5 sheep breeds using the unbiased genetic distance coefficient of Nei (1978). Numbers at nodes are bootstrap values.

Blackhead Persian, Ronderib Afrikaner and Dormer; South African Meat Merino and Ronderib Afrikaner; Landrace and Blackhead Persian and Dormer; Merino and Afrino, Russian Red Wooled Persian, Blackhead Persian and Ronderib Afrikaner; Afrino and Blackhead Persian; Namaqua and Russian Red Wooled Persian; Blackhead Persian and Russian Red Wooled Persian, Karakul, Pnoff and Romanof; Ronderib Afrikaner and Dormer and Skilder Persian; Dormer and Pnoff; Damara and Romanof; and Skilder Persian and Romanof and Pnoff.

Discussion

Genetic variation

Cryptic polymorphism of blood groups or proteins, that have not deliberately been selected by man, show simple patterns of evolutionary change. For this reason the use of allozyme polymorphism can give accurate estimates of relationships between populations (Ordas & San Primitivo 1986). Maintaining genetic variance within a pure breeding population is a complex problem. A possible solution would be to determine the degree of distribution of the current genetic diversity because no management or conservation action can be taken without a complete understanding of the current genetic structure of a species. This is an important consideration since the existence of some of the indigenous sheep gene pools are threatened by crossbreeding. The theoretical basis of the study of polymorphic proteins is that breeds can be defined as populations which differ from each other in the relative distribution and frequencies of genes (Hasselholt 1969).

This study genetically characterizes and describes 19 sheep breeds from South Africa. Some differences in allelic constitution occurred between the sheep breeds studied, especially at the **TF** locus (Table 2); this indicates clear genetic differentiation between the breeds studied. For example, the **TF*H** allele is a rare allele exclusively found in the Dormer breed (at a very low frequency) and the **G**-allele was only found in five of the 19 sheep breeds studied (Afrino, Van Rooy, Border Leicester, Blackhead Persian and Skilder Persian breeds). Manwell & Baker (1977) suggested that electrophoretic variants with low frequencies may represent, in many cases, relative recent mutations occurring after divergence of the breeds; this could be the case with the Dormer breed. The **D**-allele is the most common allele at the **TF**-locus for most of the breeds with a few exceptions already mentioned in the Results section (Table 2). The genetic differences between the breeds are to be expected for breeds found in separate locations throughout the Cape Province, where little or no gene flow occurs. The gene frequencies at the **TF**-locus were compared with those reported by other researchers to obtain information on the degree of divergence between breeds (Table 3). Ash-

ton & Ferguson (1962) reported the frequencies of alleles **TF *A, -B*, -C*, -D*, -E*** and **-G*** in three different populations of Australian Merino; Stormont *et al.* (1968) reported the frequencies of these alleles in Mailliard Merino and Nguyen *et al.* (1992) published results on these alleles in Spanish Merino. Expressive differences can be noted between the Australian, Spanish, Mailliard and South African Merino's. For example the **TF*D** allele appears to be the most common allele in all of the Merino breeds, except for the South African Meat Merino (showing a low frequency of 0.148 at this allele). The other Merino breeds had **TF*B** and **TF*E** alleles, but not the South African Merino in the current study. Mailliard Merino also did not have **TF*E** and **TF*G** alleles (Stormont *et al*, 1968). These differences could be due to small sample size (39 Mailliard Merino), directed selection and different populations studied.

Six of the breeds were polymorphic at the **CA**-locus. The **C**-allele found in the Russian Red Wooled Persian and Border Leicester breeds were at low frequencies; the **A**-allele found in the remaining four polymorphic breeds (Landrace, Afrino, Namaqua and Vandor breeds) was also present in low frequencies at this locus. A study by Ordas & San Primitivo in 1983 on Churra sheep breeds was compared with the current investigation. They discovered a new allele (**CA*M**) at the red cell carbonic anhydrase locus in Churra sheep. They found the **CA*S** allele to be present in all of the breeds studied (Churra, Lacha and Manchega), and this allele was fixed in most of the breeds. In the current study, the **CA*B** allele was also present in all of the breeds studied, it is therefore speculative that the latter allele is the same as the **CA*S** allele reported by Ordas & San Primitivo (1983). This can only be verified if their samples are compared with the current samples on the same gel.

The allelic constitutions were similar at the last five (**ALB, CA, X, Dia** and **PER**) polymorphic loci, with three exceptions at the **Dia** locus. Three of the breeds (South African Meat Merino, Perinoff and Romanof) had the **B**-allele at the largest frequency, whereas all the other breeds had the **A**-allele as the most common allele. This difference could be due to small sample size (especially for the Perinoff and Romanof breeds).

Significant ($p<0.05$) deviations of allele frequencies may occur owing to crossing and linking, inbreeding, sample error, population bottlenecks and random genetic drift. Ideal Hardy-Weinberg populations do not actually occur in nature owing to various factors, which can shift the equilibrium and disrupt the stability of a population, giving rise to change in the genetic structure. Since the sheep breeds studied do not represent natural populations, and because of directed selection of domesticated stock, it came as no surprise that the allele classes deviated from expected Hardy-Weinberg proportions at most loci. Deficiencies of heterozygotes were found in all of the breeds that did not have allele combinations which approximated Hardy-Weinberg expectations (**ALB, CA, X, HB, Dia** and **PER**). This may be the result of strong selection against heterozygous genotypes or the Wahlund (1928) effect (the inclusion of two or more genetically distinct units into a single population sample). The directed selection criteria would be most appropriate in this case.

The values of **P** and **H** were the lowest in the Namaqua breed (**P** = 33.33%, **H** = 0.166; Table 4). Although **A** was calculated at 2.00 in this breed, it was not the lowest of the 19 breeds studied. This is due to four alleles being present at the **TF** locus in the Namaqua breed, in contrast to only three **TF**-alleles in the Pedi, Merino and Romanof breeds, with lower **A** values of 1.67, 1.83 and 1.67 respectively. Nevertheless, sufficient genetic variation exists between breeds for selection purposes. The Russian Red Wooled Persian presented the highest **H** value (0.359), and has the highest genetic variation among the breeds studied. The individual heterozygosity values (0.774) were also the highest in the Russian Red Wooled Persian. Selander & Kaufman (1973) and Clarke *et al.* (1989) reported **P** values of 10 to 20%. Our results of 50 to 66.67% are much higher owing to only 14 loci being studied, whereas Selander & Kaufman (1973) studied 32 loci and Clarke *et al.* (1989)

studied 40 loci. The latter authors also reported an average **H** value between different sheep breeds at 19% (range: 13 to 53%); this compares well with the results obtained from the current study (16.6 to 38.9%). However, Selander & Kaufman (1973) reported **H** values of natural vertebrate populations (rats, seals and humans) at approximately 6%. This difference could be due to the fact that the sheep are not natural populations and that selection occurs in favor of heterozygosity.

Genetic differentiation

We have used **D** values to indicate genetic differentiation. The mean **D** value between the breeds (0.067) indicates little genetic differentiation between the breeds. According to Nei (1976) the **D** values for local breeds is between 0.000 and 0.058. Buis & Tucker (1983) reported **D** values of 0.181 to 0.308 between different sheep breeds and an average **D** value of 0.248. The topology of the dendrogram constructed from the matrix of **D** values (Figure 1) allows us to differentiate two groups: (1) South African Meat Merino, Perinoff, Romanof and Russian Red Wooled Persian and (2) the remaining sheep breeds studied. It can be inferred from the dendrogram that the flocks that are closest genetically are the Dorper and Dormer, and Perinoff and Romanof breeds. This dendrogram was identical to the one constructed after additional enzymes were analysed (Figure 2). It indicates that the first six loci analysed gave reliable results. The Dorper and Dormer breeds are both descendants from the Dorset Horn and are therefore closely related (Terblanche 1978). This association is also reflected in the dendrograms constructed from genetic distance values, and the high bootstrap value (65) for the grouping of the Van Rooy and Skilder Persian breeds. This relationship is probably the result of Skilder Persian rams being crossed with Van Rooy ewes to improve their gene pools (Campbell 1995). Similarly, the Blackhead Persian breed was introduced to the Vandor breed, which explains the small genetic distance value (0.007) between them (*Farmers Weekly* 1 May 1998). The basal position of the South African Meat Merino breed in Figures 1 and 2, compared to that of the Dormer is also supported by historical events. The crossbreeding of a Dorset Horn ram (with excellent meat form and rapid growth ability) and a South African Meat Merino ewe (with high fertility and the ability to produce more than one lamb) led to the origin of the Dormer.

Wright's (1978) fixation index is another measure to describe the level of differentiation between populations (i.e. a test whether or not they are from the same gene pool). The mean measure of the relatedness of individuals within populations ($\mathbf{F_{ST}}$) value of 0.123 for polymorphic loci (Table 5) is an indication of relatively little genetic differentiation between the breeds studied. The extent of allelic fixation of individuals relative to the subpopulations ($\mathbf{F_{IS}}$ = 0.260) also reflects the above phenomenon since values of $\mathbf{F_{IS}}$ are less (close to zero) in most natural populations where random mating within subpopultions occurs (Nei 1986). In addition the $\mathbf{F_{IT}}$ value of 0.351 (which quantifies inbreeding owing to population subdivision) is indicative of relatively little gene flow between populations. This is not surprising, because each breeder protects his breed from outbreeding in order to maintain high production characteristics. None of the breed pairs showed identical allele distribution at all the loci studied. Identical ($p>0.05$) allele distributions were obtained for 146 of the 171 breed pairs and there is no obvious relationship between **D** values and allele distribution patterns. This is probably the influence of the small number of loci studied.

Conclusion

Allozyme studies can mesh genetic and ecological information to strengthen inferences about specific aspects of population structure, especially breeding structure and effective gene flow. The results of this study can, therefore, be used in breeding programs, and present the first study of the

current genetic structure of the different sheep breeds found in southern Africa. Furthermore, the results for the sheep breeds where large sample sizes were involved are important for the future monitoring of gene flow in populations, to determine levels of inbreeding and crossbreeding in each breed, and to enhance the global information on domestic animal diversity.

Acknowledgments

Sasol has provided financial funding and the Animal Improvement Institute (Irene) assisted with the practical assessment of the sheep breeds.

References

ASHTON, G.C. & FERGUSON, K.A., 1962. Serum transferrins in Merino sheep. *Genetical Research, Cambridge* 4, 240–247.

BUIS, R.C. & TUCKER, E.M., 1983. Relationships between rare breeds of sheep in the Netherlands as based on blood-typing. *Animal Blood Groups Biochemical Genetics* 14, 17–26.

CAMPBELL, Q., 1995. The indigenous sheep and goat breeds of South Africa. Dreyer Printers and Publishers.

CLARKE, S.W., TUCKER, E.M. & OSTERHOFF, D.R., 1989. Blood groups and biochemical polymorphism in the Namaqua sheep breed. *Animal Genetics* 20, 279–286.

HARRIS, H. & HOPKINSON, D.A., 1976. Handbook of enzyme electrophoresis in human genetics. North Holland Publising Company, Amsterdam. American Elsevier Publishing Company, Inc. New York.

HASSELHOLT, M., 1969. Serum protein polymorphisms in swine electrophoretic identification. *Genetics and Application*. Munksgaard, Copenhagen pp. 48–52.

MANWELL C. & BAKER, C.M.A., 1977. Genetic distances between the Australian Merino and the Poll Dorset sheep. *Genetical Research* 29, 239–244.

MARKERT, C.L. & FAULHABER, I., 1965. Lactate dehydrogenase isozyme patterns of fish. *J. Exp. Biol.* 159, 319–332.

NEI, M., 1972. Genetic distance between populations. *The American Naturalist* 106 (949), 283–292.

NEI, M., 1976. Mathematical models of speciation and genetic distance. In: *Population Genetics and Ecology* (ed. S. Karlin and E. Nevo) pp. 723–766. New York: Academic Press.

NEI, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89, 583–590.

NEI, M., 1986. Definition and estimation of fixation indices. *Evolution* 40, 643–645.

NGUYEN, T.C., MORERA, L., LLANES, D., & LEGER, P., 1992. Sheep blood polymorphism and genetic divergence between French Rambouillet and Spanish Merino: role of genetic drift. *Animal Genetics* 23, 325–332.

ORDAS, J.G. & SAN PRIMITIVO, F., 1983. A new allele at the red cell carbonic anhydrase locus in sheep. *Animal Blood Groups and Biochemical Genetics* 14, 233–234.

ORDAS, J.G. & SAN PRIMITIVO, F., 1986. Genetic variation in blood proteins within and between Spanish dairy sheep breeds. *Animal Genetics* 17, 255–266.

OTA, T., 1993. **DISPAN.** Pennsylvania State University, Philadelphia.

RAYMOND, M. & ROUSSET, F., 1995. **GENEPOP** (version 3.1b): Population genetics software for exact tests and ecumenicism. *J. Heredity*, 86, 248–249.

RIDGEWAY, G., SHERBOURNE, S.W. & LEWIS, R.D., 1970. Polymorphism in the esterases of Atlantic herring. *Trans. Am. Fish. Soc.* 99, 147–151.

SELANDER, R.K. & KAUFMAN, D.W., 1973. Genetic variability and strategies of adaptation in animals. *Proceedings of the National Academy of Science USA* 70-76, 1875–1877.

SHAW, C.R. & PRASAD, R., 1970. Starch Gel Electrophoresis of Enzymes — a Compilation of Recipes. *Biochemical Genetics* 4, 297–320.

STORMONT, C., SUZUKI, Y., BRADFORD, B.E. & KING, P., 1968. A survey of hemoglobins, transferrins and certain red cell antigens in nine breeds of sheep. *Genetics* 60, 363–371.

SWOFFORD, D.L. & SELANDER, R.B., 1981. **BIOSYS 1:** a **FORTRAN** program for the comprehensive

analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* 72, 281–283.

TERBLANCHE, E., 1978. *Ken ons kleinvee rasse.* Human en Roussou Uitgewers (Edms.) Bpk. Nasionale Boekdrukkery, Goodwood.

VAN ZEVEREN, A., 1995. A genetic study of four Belgian pig populations by means of seven microsatillite loci. Pig workshop abstracts. *International Society for Animal Genetics* pp.14.

WAHLUND, S., 1928. The combination of populations and the correlation examined from the standpoint of the study of heredity. *Hereditas* 11, 65–106.

WRIGHT, S., 1978. Evolution and Genetics of Populations, Vol 4. Variability within and among Natural Populations. University of Chicago, Chicago.