

MEDIUM DENSITY BEADCHIP GENOTYPE DATA REVEALS GENOMIC STRUCTURE OF SOUTH AFRICAN MERINO-BASED BREEDS

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SUMMARY

In South Africa, the Merino sheep was used to develop a number of Merino-derived breeds with the goal of improving productivity and robustness to challenging production systems. The current study evaluated population genetic structure and breed relationships of Merino and Merino-based sheep breeds of South Africa using the Ovine50K beadchip. Five breeds evaluated consisted of the Dohne Merino (n=50), South African Merino (n=10), South African Mutton Merino (n=10), Meatmaster (n= 48) and the Afrino (51). The Dohne Merino and Afrino were observed to have the highest genetic diversity of $H_E=0.3699$ and $H_E=0.3642$, respectively. PCA clustered Meatmaster divergently from other breeds forming an outgroup with no indications of admixture or gene flow into other breeds. Highest differentiation was observed between Meatmaster and South African Mutton Merino ($F_{ST}=0.1699$). Lowest F_{ST} values were found between Afrino and Dohne Merino $F_{ST}=0.01746$. Meatmaster differed from Dohne Merino and South African Merino on chromosomes 19; 11 and 9. The SNP *s10035.1* on chromosome 11 had an F_{ST} of 0.6361 and is associated with *SEPT9*, which encodes septin-9 protein while that on chromosome 9 (*s71002.1*; $F_{ST}=0.6198$) is within 1MB region of *SOX17* gene. A SNP that differentiated the subpopulation of Afrino vs Dohne Merino and South African Merino on chromosome 12 (SNP *s62606.1*, $F_{ST}=0.5690$) was observed to be associated with *THRSP* gene that plays a role in lipogenesis regulation, more especially in lactating mammary gland. Results obtained in this study indicated breed relations but also genetic structuring in South African Merino based sheep breeds.

INTRODUCTION

The Merino sheep breed is regarded as one of the oldest and most economically influential breeds in the world (Al-Atiyat *et al.* 2016). These sheep are known for their fine and soft wool (DAFF 2015). In South Africa, the breed was introduced in the 1780's from Spain (Buduram 2004; DAD-IS 2009) and has become adapted to South African climatic conditions (Snyman 2014). It is suggested that South African Merino carry a variety of genes that confer adaptation to a range of production environments (Peters *et al.* 2010). The South African Merino is believed to be a composite breed between Spanish, Saxony, Rambouillet, American and Australian sheep breeds (Mason 1996). From the South African Merino, several Merino based breeds have been developed (Hlophe 2011) for either wool, mutton or as dual-purpose breeds such as the Afrino, Meatmaster and Dohne Merino breeds in South Africa (Snyman 2014).

The Dohne Merino is regarded as a synthetic breed (Buduram 2004), developed from crossing German Mutton Merino rams, commonly known as South African Mutton Merino with the South African Merino ewes (Kotze 1951; Jordan 2013). This breed was developed to improve productivity and robustness to survive in a parasite infested region of the Eastern Cape province of South Africa. Afrino sheep originated from a cross between Ronderib Afrikaner, Merino and South African Merino (Snyman 2014) and are reared predominantly for meat production. Meatmaster was developed from multiple breeds of South African Mutton Merino, Van Roy and Ile deFrance, Damara, Wiltshire Horn,

Dorner and other breeds with the objective of improving meat qualities of the fat-tailed sheep breeds (Peters *et al.* 2010; Snyman 2014).

This study investigated population genetic structure and breed relationships between Merino and Merino-based sheep breeds of South Africa. Based on the crossbreeding history and the divergent breeding goals amongst breeds, it was hypothesised that there is genetic diversity and population substructure among these Merino-type breeds.

MATERIALS AND METHODS

The study used SNP genotype data from a total of 169 animals obtained from five different Merino and Merino-based sheep populations consisting of South African Mutton Merino (n=10), Dohne Merino (n=50), South African Merino (n=10), Afrino (n=51) and Meatmaster (n=48). The samples were obtained from different farms in the Eastern Cape region close to Middelburg.

The Illumina OvineSNP 50K Bead Chip array was used to genotype a total of 54,241 SNPs on 169 individuals. The genotype data was subjected to quality control using PLINK V1.07 (Purcell 2009) to remove individuals with more than 5% missing genotypes, SNPs with a call rate less than 95% and a minor allele frequency (MAF) less than 5% as well as individual animals that deviated from Hardy Weinberg equilibrium ($P < 0.0001$) from further downstream analysis.

Within breed diversity estimates of observed heterozygosity (H_o); expected heterozygosity (H_e) and inbreeding co-efficient (F_{is}) were estimated using Plink v1.07 (Purcel, 2009).

To evaluate population structure, Principle Component Analysis (PCA) was carried out using Golden Helix SNP and Variation Suite (SVS) v8.1 (Golden Helix Inc 2012). The eigen values for the principle components (PC) 1 and 2 were used in plotting the clustering of individuals.

An analysis of molecular variation (AMOVA) was done to understand the partitioning of diversity in the five sheep breeds using ARLEQUIN V3.1 (Excoffier and Lischer 2010). AMOVA was analysed for (i) all five breeds and (ii) within and between the developed (Afrino; Meatmaster and Dohne Merino) versus pure breeds (SA Merino and SA Mutton Merino).

The per population and per marker F_{ST} based outlier loci method was used to determine genetic differentiation between pairs of populations of the five sheep breeds and the affected SNPs using Golden Helix SNP and Variation Suite (SVS) V8.1 (Golden Helix Inc. 2012). Associated genes were mapped within 1MB region of significant SNPs ($F_{ST} > 0.50$) using ENSEMBLE genome browser and NCBI (NCBI; www.ncbi.nlm.nih.gov) ENSEMBL Ovine (*Ovis Aries*) genome build AOR4.

RESULTS AND DISCUSSION

Highest genetic diversity values were observed in Dohne Merino and Afrino with the expected heterozygosity (H_e) of 0.3699±0.1286 and 0.3642±0.1294, respectively (Table 1). Across all populations, the observed heterozygosity values (H_o) were higher than the expected heterozygosity with an average inbreeding coefficient (F_{is}) of 0.0567. Low inbreeding values were obtained ranging from -0.0897 in South African Mutton Merino to -0.0191 observed for Dohne Merino.

Table 1. Expected and observed heterozygosity in the five Merino sheep breeds of South Africa

Breed	No. of animals	$H_o \pm SD$	$H_e \pm SD$	F_{is}
Afrino	51	0.3746±0.1439	0.3642±0.1294	-0.0287
Meatmaster	48	0.3664±0.1520	0.3537±0.1356	-0.0359
SA Mutton Merino	10	0.3846±0.1960	0.3529±0.1327	-0.0897
SA Merino	10	0.3861±0.1857	0.3363±0.1290	-0.0634
Dohne Merino	50	0.3769±0.1425	0.3699±0.1286	-0.0191
All breeds	166	0.3672±0.1077	0.3893±0.1094	0.0567

Principle component analysis resulted in four main clusters of (i) Dohne Merino and SA Merino; (ii) SA Mutton Merino (iii) Afrino and (iv) Meatmaster (Figure 1). The first principle component (PC1) explained 50.48% of the variation and clustered the Meatmaster divergently from other breeds forming an outgroup. Clustering of Meatmaster separately from other breeds can be explained by the history of the breed. The breed was developed from fat-tailed hair sheep and its clustering is possibly reflecting the composite blood lines of South African Mutton Merino and other breeds with 50% of its blood lines coming from Damara (Snyman 2014). The Afrino, which is also a composite breed with Merino and other none Merino sheep of the Afrikaner breed formed a unique cluster albeit closer to the SA mutton Merino. The Dohne Merino on the other hand is a result of selection and crossing of Merino breeds, which explains its clustering close to the SA Merino sheep.

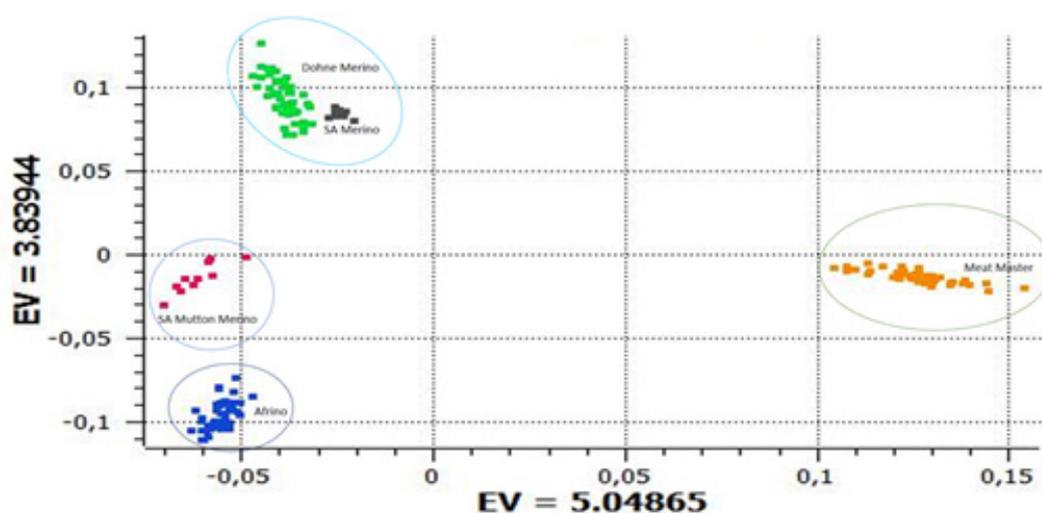


Figure 1. Principal component analysis for the 5 South African Merino-type breeds

AMOVA results (Table 2) suggested some level of population sub-structuring that exist in the South African merino based sheep breeds under study. Slightly over 10% of the variation was between breeds whilst variation amongst the individuals within breed was over 80%. A small percentage (1.25%) was observed between pure breeds and composite breeds developed from Merino and other sheep breeds. Overall, $\pm 10\%$ of the variation was observed between breeds, demonstrating existence of some divergence between breeds albeit small.

Table 2. Analysis of Molecular Variance (AMOVA) for the South African Merino-type breeds

Dataset	Percentage of variation (%)		
	Between population	Between populations within group	Within populations
All 5 breeds	10.62	-	91.38
Developed and pure breeds	1.25	10.74	88.01

Pairwise population F_{ST} ranged from 0.0175 – 0.1699. The Meatmaster diverged significantly from other sheep breeds with F_{ST} ranging from $F_{ST} = 0.1100$ between Meatmaster and Dohne Merino

to $F_{ST}=0.1699$ between Meatmaster and SA Mutton Merino. Low F_{ST} values was observed between Dohne Merino and Afrino ($F_{ST}=0.01746$). This suggest that the breeds have not been kept in isolation from each other for a long period with a possibility for gene flow between breeds. This is regarded as outcrossing and appears to shape the genetic structure of the breeds. Genetic differentiations between Meatmaster from Dohne Merino and South African Merino were observed on chromosome 19 (SNP *OAR19_45719557.1*; $F_{ST}=0.6475$); 11 (SNP *s10035.1*, $F_{ST}=0.6361$ and SNP *s56939.1*, $F_{ST}=0.6011$ and chromosome 9 SNP *s71002.1*, $F_{ST}=0.6198$) as illustrated in Table 3. SNP *s10035.1* on chromosome 11 is found to be within 1MB region of *SEPT9* (which encodes septin-9 protein) and while that on chromosome 9 (*s71002.1*) is within 1MB region of *SOX17* gene. A SNP that differentiated the subpopulation of Afrino vs Dohne Merino and South African Merino from chromosome 12 (SNP *s62606.1*, $F_{ST}=0.5690$) is associated with *THRSP* gene (a gene that plays a role in lipogenesis regulation, more especially in lactating mammary gland).

Table 3. Breed differentiating SNPs ($F_{ST} > 0.5$) and the associated ENSEMBLE genes

Subpopulation	F_{ST}	SNP ID	CHR	Related gene
MMA VS DME & SAM	0,6475	<i>OAR19_45719557_X,1</i>	19	No gene
	0.6361	<i>s10035.1</i>	11	<i>SEPT9</i>
	0.6011	<i>s56939.1</i>	11	<i>EXOC7,FOXJ1</i>
	0.6198	<i>s71002.1</i>	09	<i>SOX17</i>
AFR VS DME & SAM	0.6197	<i>OAR5_51499408.1</i>	5	<i>ARHGAP26</i>
	0.5690	<i>s62606.1</i>	12	<i>THRSP</i>

CONCLUSIONS

In conclusion, results showed that the variation within the breeds was greater compared to between breed diversity. Significant genetic variation is still maintained in South African Merino-type sheep breeds. These results are in agreement with the origins, breeding practices as well as the geographical regions from which these breeds are found. The low but present genetic variation between Merino and merino derived breeds could be beneficial in the exchange of genes through outbreeding. Unfavourable traits observed in a certain group can be improved by outcrossing to increase genetic diversity against a population structure.

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