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ANALYSIS OF A SOUTH AFRICAN MERINO FLOCK DIVERGENTLY SELECTED FOR REPRODUCTIVE POTENTIAL

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SUMMARY

A South African Merino sheep flock has been divergently selected for more than 8 generations for the ability of ewes to rear multiple offspring. Selection has resulted in a High line and a Low line that differ markedly in their reproductive output. The causative mutations and/or quantitative trait loci responsible for the difference in reproductive traits between these 2 lines have not yet been determined. Genomic regions under selection would be expected to demonstrate the highest level of genetic differentiation between these lines and would also exhibit a higher than expected degree of homozygosity within lines. Selected individuals were genotyped using the OvineSNP50 BeadChip and the genotype data were analysed to identify differences between the 2 lines. The High line and Low line ewes were shown to be phenotypically and genetically discrete; confirming the presence of 2 distinct lines. Several markers subjected to selection could be identified between the 2 lines. It can be assumed that most of these markers differ as a result of the differential selection pressure applied on reproduction. Further investigation into these loci could provide valuable information on the genes and/or quantitative trait loci involved in an improved phenotype with greater reproductive success.

INTRODUCTION

Reproduction traits are of importance in the improvement of the economic output of industry sheep flocks (Olivier 1999; Safari *et al*. 2005). Net reproduction, defined as the number of lambs (or weight of lamb) weaned per ewe mated, is a lowly heritable, gender-limited, composite trait, recorded later in life. The recording of some of the net reproduction components, such as ovulation rate, conception rate and embryo survival, is notoriously complicated, costly and labour-intensive. Conventional breeding efforts depend on recorded data linked to pedigree information to implement an efficient improvement strategy and therefore rely heavily on the recording of relevant traits (Notter 2012). This necessitates accurate performance testing of all potential breeding animals, thereby increasing costs and slowing down the rate of any potential genetic gains. Molecular markers associated with reproductive traits could accelerate genetic improvement by facilitating a more accurate estimation of reproductive potential at a much earlier age (Dodds *et al*. 2007; Hayes *et al*. 2009).

Several studies have reported mutations in a single ovine gene or closely linked group of genes that result in highly proliferative lines. Although lamb rearing ability is assumed to be a complex trait (Notter 2012), the chromosomal regions of previously identified mutations could serve as candidate regions for the current study. Mutations in 3 major genes, the bone morphogenetic protein receptor 1B, bone morphogenetic protein 15 and growth and differentiation factor 9, result in increased ovulation rate in sheep. These genes are located on chromosome 6, the X chromosome and chromosome 5, respectively (Davis 2005; McNatty *et al*. 2007).

A South African Merino sheep flock has been divergently selected for their ability to rear multiple offspring since 1986. Selection has been applied for more than 8 generations and has resulted in a High line and a Low line that differ markedly in their reproductive output (Cloete *et*

al. 2004). The causative mutations and/or quantitative trait loci responsible for the difference in reproductive traits between these 2 lines have not been determined. These lines could potentially serve as a model for identifying the genomic regions underlying reproductive traits and to determine whether mutations identified in other highly proliferative lines are also segregating in this flock.

MATERIALS AND METHODS

The divergently selected lines are maintained at the Elsenburg research farm in the Western Cape province of South Africa. The location, animal resource and the selection protocol followed is detailed in the literature (Cloete *et al*. 2004; 2009). Blood samples were obtained from 112 individuals from the lines and were genotyped using the OvineSNP50 BeadChip (Illumina) at GeneSeek Inc (Lincoln, USA). Sampled individuals were representative of the recent genetic composition of the lines (born between 2002 and 2008); with accurate estimated breeding values for number of lambs weaned per parity and total weight weaned per parity; and represent the extremes of the phenotypic distribution. Pedigree information was considered to minimise the inbreeding and relatedness in the sampling cohort to reduce potential within-line population substructure. A t-test was performed to confirm that significant differences exist between the phenotypes of the 2 lines.

Only samples with a call rate of >85% and single nucleotide polymorphism (SNP) loci with a call rate of >85%, GenCall score of >0.25, GenTrain score of >0.50 and minor allele frequency of >0.01 were included in downstream analyses. In an attempt to investigate the possibility that chromosomes cited in literate play a significantly more important role in reproduction in the current study, the genotypic data were partitioned by chromosome. A factorial component analysis was conducted in Genetix (Belkhir *et al*. 2004) to assess the multi-factorial variance between the lines and evaluate the degree of clustering on a three-dimensional scale. Markers subjected to selection were identified by an Fst outlier approach using a Bayesian method in BAYESCAN (Foll and Gaggiotti 2008) and using the FDIST2 method (Beaumont and Nichols 1996) in Lositan (Antao *et al*. 2008). Markers found to be subjected to selection based on both methods of analyses were further investigated using the *Ovis aries* genome –Annotation release 100 (www.ncbi.nlm.nih.gov/projects/mapview).

RESULTS AND DISCUSSION

Ninety-one individuals and 23 781 SNP loci met the quality control measures and were included for downstream analyses (Table 1). The relatively low SNP loci yield after quality control is most probably due to a loss of DNA quality during processing rather than a lack of polymorphic markers in the study cohort. Genotyping of other South African Merino sheep have yielded a higher number of markers after quality control (data not shown).

Table 1. A total of 91 of the original 112 samples were included in further analyses

Total number of sampled individuals		Samples included in analyses	
27 Rams	19 High line	23 Rams	16 High line
	8 Low line		7 Low line
85 Ewes	45 High line	68 Ewes	38 High line
	40 Low line		30 Low line

The t-test confirmed significant differences in the phenotypic values of the 2 lines for number and weight born per joining, consistent with the study by Cloete *et al*. (2004). The factorial component plot for each chromosome indicated 2 distinct clusters representing the divergently selected lines (Figure 1). The lines can therefore be considered to be phenotypically and genetically distinct as a result of several generations of selection.

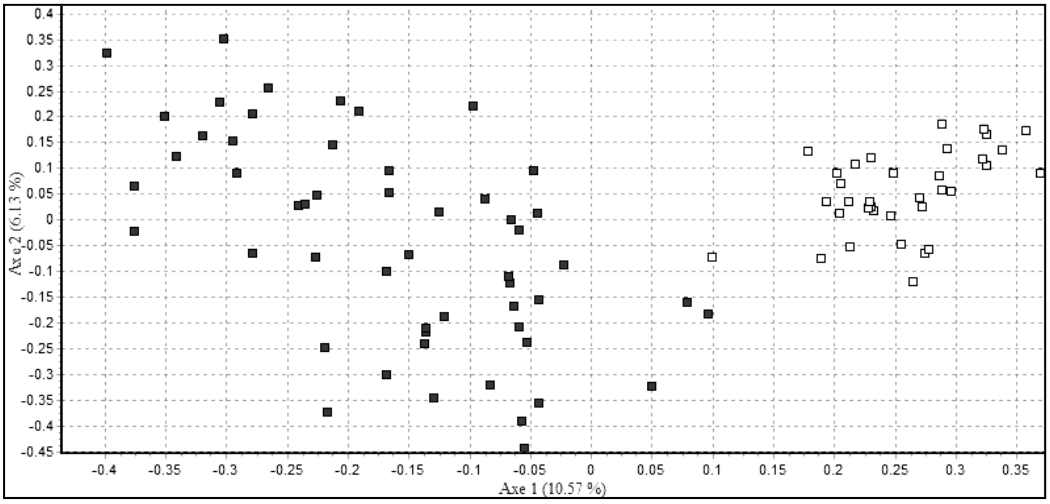

Figure 1. A factorial component plot of chromosome 5, indicating 2 distinct clusters. Dark squares represent individuals from the High line and white squares that of Low line.

The FDIST2 method indicated 1476 markers to be subjected to selection on the 27 chromosomes in the ovine genome. This number was reduced to 926 after a correction for multiple testing was implemented (Figure 2). The Bayesian-based analysis however, identified only 47 markers to be subjected to selection. The overall percentage of markers subjected to selection was 4.00% using the FDIST2 method and 0.20% using the Bayesian method.

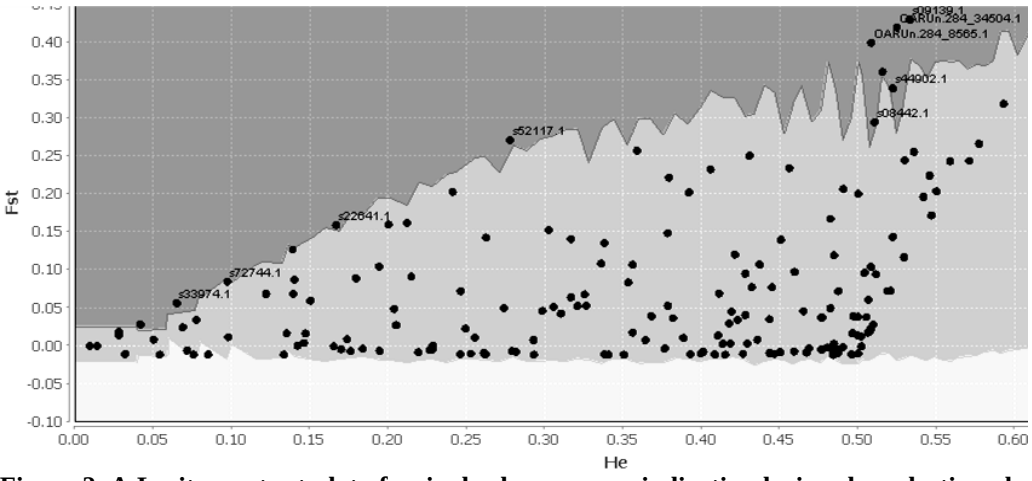

Figure 2. A Lositan output plot of a single chromosome indicating loci under selection above the 95% percentile (darker shaded area), neutral markers (lighter shaded area) and markers under balancing selection (white area).

All markers identified according to the Bayesian method were also identified by the FDIST2 method. The disparity between the numbers of markers identified by the 2 different methods has been noted in other related studies (Narum and Hess 2011). The Bayesian method implemented in BAYESCAN has been shown to be less prone to type I errors (false positives) compared to the method employed in Lositan and this could explain the larger number of markers being identified by Lositan in the current study.

Chromosome 5, 6 and the X chromosome did not exhibit a significant difference in the percentage of markers subjected to selection. No significant difference was seen between the number of markers or extent of putative markers under selection within any of the individual chromosomes.

Several markers were found to be located in or near annotated genes. One of these genes, the corticotropin releasing hormone gene on chromosome 9 is especially noteworthy as the corticoid pathway has been shown to influence reproduction in sheep (Breen *et al*. 2005).

CONCLUSIONS

The divergently selected Elsenburg flock can be considered a valuable genetic resource for studies aiming to identify genomic regions playing a role in reproductive traits. This study identified a large number of markers across the ovine genome that appear to be subjected to selection, thereby supporting the premise that reproductive traits are under the control of several loci spread throughout the genome.

Chromosome-specific partitioning of the data did not identify specific chromosomes with a greater significance pertaining to reproductive traits. However, it did facilitate the identification of loci associated with genomic areas under selection. Further investigation of these loci could provide valuable information on the genes and/or quantitative trait loci involved in an improved reproduction phenotype. A whole-genome analysis to identify signatures of selection could shed more light on the genomic regions involved in the reproduction traits of this divergently selected flock.

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