A preliminary study of molecular divergence of the Elsendburg merino flock based on RAPD markers

P. Naidoo1,2, S. W. P. Cloete1,3 & A. Fossey2 – pavarnij@elsenburg.com
1Directorate: Animal Sciences, Western Cape Department of Agriculture; 2School of Life Sciences, University of KwaZulu-Natal; 3Department of Animal Sciences, Stellenbosch University.

Take home message

The study shows that the H and L lines of South African Merino sheep divergently selected for and against their ability to rear multiple offspring are different at a molecular level. Ten RAPD markers were used to study divergence. Phenotypic data on the lifetime reproduction of ewes born in 1999 and 2000 indicated that reproduction in the high line ewes was markedly higher than that of low line contemporaries (P < 0.01). The preliminary RAPD assay, conducted on 15 ewes from each line, used eight primers and produced 87% polymorphic loci. The mean coefficient of genetic differentiation between lines (GST) was estimated to be 0.25.

Introduction

Over several years, marked fluctuations were observed in the wool: meat price ratio, with the relative monetary value of wool generally declining and meat becoming more expensive. These trends have resulted in the selection strategy of South African Merinos being adapted accordingly (Olivier, 1999). The genetic improvement of reproduction, expressed as total weight of lamb weaned per lambing opportunity, is thus seen as one of the cornerstones of increased productivity.

This situation prompted a divergent selection experiment for and against the ability of ewes to rear multiple offspring. The composite trait is affected by the expression of several genetically influenced traits. Variation in the component traits contributes to the phenotypic variation in the composite trait. Lamb survival, and in particular the survival of multiples, was improved in the High (H) line. The selection strategy based primarily on the maternal phenotype has proven successful in the establishment of the two phenotypically distinct Merino lines (Cloete et al., 2004).

Furthermore, results from this selection experiment have shown a marked, divergent response in total weight of lamb weaned per parity amounting to +1.8% per annum in the H line and -1.3% per annum in the low (L) line (Cloete et al., 2004). These distinct differences between lines possibly suggest that one or more putative loci with a marked effect on overall reproduction may perhaps be present. Random Amplified Polymorphic DNA (RAPD) markers could be used to estimate the molecular genetic divergence between the H and L lines in an initial attempt to estimate the genetic distance between lines. The minimal infrastructure and equipment required for RAPD studies makes this technique useful as a preliminary method for screening for DNA based diversity on limited funds.

The objective of this study is to show phenotypic data that establishes the divergence between the two lines, as well as the first investigation of DNA based diversity using RAPD markers to find evidence that the H and L lines are significantly divergent at a molecular level.

Materials and Methods

Experimental animals were obtained from two lines of Merino sheep divergently selected from the same base population since 1986. Details of the procedure for the selection of replacements can be found in the literature (Cloete et al., 2004). In short, ewe and ram progeny of ewes rearing more than one lamb per joining (ie reared twins at least once) were preferred as replacements in the H line. Replacements in the L line were preferably descended from ewes rearing fewer than one lamb per joining (ie barren, or lost all lambs born at least at one lambing opportunity). Replacements were preferably descended from ewes with more than one reproduction record, particularly in rams. The general management and husbandry of the flocks as well as the experimental sites were also described by Cloete et al. (2004).

To elucidate the phenotypic divergence between the lines, records of the two most recent ewe groups present for three lambing opportunities (regarded as lifetime reproduction for this study) were analysed. Thus only 68 ewes, born in 1999 and 2000, and present in the flock at lambing at four years of age were considered. Number of lambs born, number of lambs weaned as well as total weight of lamb weaned were expressed per ewe joined.

Preliminary RAPD assays were conducted on 15 randomly chosen ewes per line from the H and L lines. Eight individuals born during 1999 and seven individuals born in the period from 1996-1998 were used. Total genomic DNA was extracted from whole blood samples collected by jugular venipuncture, and isolated using the Gentra Puregene DNA Purification Kit (Acodek Ingram). DNA concentrations were determined by comparison with the molecular weight marker III (Roche) on 1% agarose gel was performed. DNA samples were diluted in 10mM Tris (pH 8.0) to 20 DM working stock solutions.

All RAPD reactions used Taq DNA polymerase in Buffer A (Promega) and PCR Nucleotide Mix (Promega). The RAPD assays followed the protocols of Cushwa et al. (1996), but altered the reaction volume to 25 E. Amplification was effected on the Geneamp PCR System 2700. RAPD assays were screened on 15% agarose gels at 150 V for three hours, using 1 X TBE buffer and ethidium bromide UV fluorescent stain. Bands were scored by visual analysis. Two RAPD primers were discarded: primer BO8 selected from Cushwa et al. (1996) showed no polymorphism, while primer JO9 of the randomly selected primers failed to amplify. All primers were tested for repeatability of amplification patterns under constant reaction conditions.

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence 5'-3'</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>BO8</td>
<td>GTC CAC AGC G</td>
<td>Cushwa et al. (1996)</td>
</tr>
<tr>
<td>B20</td>
<td>GGA CCC TTA C</td>
<td>Cushwa et al. (1996)</td>
</tr>
<tr>
<td>C08</td>
<td>TGG ACC GGT G</td>
<td>Cushwa et al. (1996)</td>
</tr>
<tr>
<td>C19</td>
<td>GGT GCC AGC C</td>
<td>Cushwa et al. (1996)</td>
</tr>
<tr>
<td>D20</td>
<td>ACC CGG TCA A</td>
<td>Cushwa et al. (1996)</td>
</tr>
<tr>
<td>F03</td>
<td>CCT GAT CAC C</td>
<td>-</td>
</tr>
<tr>
<td>X07</td>
<td>GAG CGA GGC T</td>
<td>-</td>
</tr>
<tr>
<td>J09</td>
<td>TGA GCC TCA C</td>
<td>-</td>
</tr>
<tr>
<td>P16</td>
<td>CCA AGC TGC C</td>
<td>-</td>
</tr>
<tr>
<td>K03</td>
<td>CCA GCT TAG G</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1: RAPD markers tested for polymorphic alleles in the preliminary genome scan.

Average reproduction records were subjected to analysis of variance, involving the effects of line (H or L) and birth year (1999 or 2000). Least squares procedures were used, to account for uneven subclasses. In the absence of significant selection line X birth year interactions, only line effect means were subsequently computed and tabulated.
Results and Discussion

Reproduction of the H line ewes was markedly higher than that of L line contemporaries (P < 0.01; Table 2). Average weight of lamb weaned per joining in the H line was thus more than double that in the L line. This clearly supports marked divergence between the two lines (Cloete et al. 2004), presumably resulting from divergent genetic selection since 1986. Means for total weight of lamb weaned accorded with earlier figures of 25.1 kg in the H line and 16.1 kg in the L line for the period from 1997 to 2002 (Cloete et al. 2003).

The eight RAPD primers used for the analysis produced 51 polymorphic loci in the H line and 48 in the L line. The percentage of polymorphic loci was 69% and 65% for respective lines. The total percentage of all polymorphic loci for both lines was 86%. The means of the observed number of alleles and the effective number of alleles were 1.69 and 1.36 for the H line, and 1.65 and 1.34 for the L line. Cushwa et al. (1996) correspondingly reported a total percentage of 97% polymorphic loci; when using 131 primers in RAPD assays using the Agreeresearch international mapping flock Kantanen et al. (1995) generated only 47 fragments using 27 RAPD primers on Finnsheep. In comparison, the present study produced 51 fragments in the high line and 48 in the low line using only eight RAPD primers. The RAPD markers in this study on Merinos therefore appear to be highly polymorphic. The mean coefficient of genetic differentiation (GST) was estimated to be 0.25. This indicates that approximately 25% of the total genetic variation is accounted for by differences between lines, while the remaining 75% corresponds to differences among individuals within lines. Nei’s genetic distance (Nei 1978) between the two lines amounted to 0.26. Despite a relatively small sample size and a low number of markers used in the present study, some indications of genetic differentiation between lines were observed in the RAPD assays. Such an outcome would support the significant phenotypic divergence reported. Table 3 shows the significance of the line differences at polymorphic loci as Chi² statistics. While there are several non-significant differences in gene frequencies, there are also a noticeable number of significant (P < 0.05) differences in gene frequencies.

Table 2: Average (± SE) reproduction of 1999 and 2000 born ewes in the H (n = 40) and L lines (n = 18) over three lambing seasons

Table 3: Chi-squared comparisons of gene frequencies between lines at polymorphic loci, as derived from the RAPD assays

References


