DNA PEDIGREEING OF COMMERCIAL SHEEP FLOCKS

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SUMMARY
Breeding programs for livestock require accurate pedigree information. DNA based parentage is being researched as a reliable and cost effective method of pedigreeing for the Australian sheep farming market. Modern molecular genetic techniques make parentage testing in the laboratory an attractive alternative to labour intensive on-farm recording.

Keywords: Parentage, DNA profiling.

INTRODUCTION
Sheep breeding programs call for accurate parentage records to be maintained. It is possible for this to be done on the sheep station itself, but this process is labour intensive and subject to error, especially if lambing is not closely monitored. Modern molecular genetic techniques makes DNA pedigreeing of sheep flocks a viable option. Polymerase chain reaction (PCR) based techniques and sheep gene mapping research have generated a large number of DNA markers that are highly polymorphic in sheep, and suitable for DNA profiling (Crawford et al. 1993). A system whereby pedigree relationships within domestic species can be determined using molecular technology is now possible (Glowatzki-Mullis et al. 1995; Marklund et al. 1994).

MATERIALS AND METHODS
Flock The study animals were bred on a commercial sheep farm as part of a research project into recessive black pigmentation in Australian Merino sheep (Parsons et al. 1997). Six half-sib rams were mated to a flock of 92 ewes, for which pedigree relationships were unknown. Mating was random and lambing was not recorded.

Microsatellite analysis All animals from the study flock were typed for 12 ovine microsatellites previously mapped to ovine chromosomes 1, 3, 6, 13 and 29 (Crawford et al. 1995). (Table 1)

Pedigree analysis Typing data on all animals was entered into a pedigree analysis computer program for parentage assignation (I. Franklin, personal communication).
Table 1. Ovine microsatellites typed for pedigree analysis

<table>
<thead>
<tr>
<th>Locus</th>
<th>Chromosome</th>
<th>PIC*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM6438</td>
<td>1</td>
<td>0.78</td>
<td>Crawford et al. (1995)</td>
</tr>
<tr>
<td>OarCP34</td>
<td>3</td>
<td>0.80</td>
<td>Crawford et al. (1995)</td>
</tr>
<tr>
<td>BM415</td>
<td>6</td>
<td>**</td>
<td>Crawford et al. (1995)</td>
</tr>
<tr>
<td>BM4621</td>
<td>6</td>
<td>0.87</td>
<td>Crawford et al. (1995)</td>
</tr>
<tr>
<td>OarJMP8</td>
<td>6</td>
<td>0.73</td>
<td>Crawford et al. (1995)</td>
</tr>
<tr>
<td>HUJ616</td>
<td>13</td>
<td>0.68</td>
<td>Crawford et al. (1995)</td>
</tr>
<tr>
<td>McM152</td>
<td>13</td>
<td>0.77</td>
<td>Smith et al. (1995)</td>
</tr>
<tr>
<td>McM253</td>
<td>13</td>
<td>0.77</td>
<td>Smith et al. (1995)</td>
</tr>
<tr>
<td>IL2RA</td>
<td>13</td>
<td>0.68</td>
<td>Crawford et al. (1995)</td>
</tr>
<tr>
<td>TGLA23</td>
<td>13</td>
<td>0.50</td>
<td>Crawford et al. (1995)</td>
</tr>
<tr>
<td>MAF18</td>
<td>13</td>
<td>0.49</td>
<td>Crawford et al. (1995)</td>
</tr>
<tr>
<td>BM6526</td>
<td>26</td>
<td>0.67</td>
<td>Crawford et al. (1995)</td>
</tr>
</tbody>
</table>

* PIC: Polymorphic Information Content - an indication of the informativeness of the marker.
** This marker was excluded from analysis due to deviation from Hardy-Weinberg expectations.

RESULTS

Of the 13 microsatellites typed one (BM415) resulted in a significant reduction in expected heterozygote frequency. Due to this deviation from Hardy-Weinberg expectations the typing data for this microsatellite was excluded from parentage analysis.

Computational analysis of typing data on the remaining 12 microsatellites resulted in definite sire allocation for 64 out of 75 progeny in the study flock. Dams were identified for 31 of these cases. Of the remaining 11 progeny, 9 showed compatibility with more than one sire and 2 were incompatible with all sires.

DISCUSSION

With the utilisation of microsatellite markers DNA pedigreeing can become a valuable tool in sheep breeding programs. Pedigree analysis is dependent on informative meioses and markers with a high polymorphic information content (PIC) will generally be the most useful. Although the PIC values for the loci used in this study were moderate to high (0.49 - 0.87), sires were not informative for all markers. This can partially be explained by the sires being half-sibs and having many alleles in common by descent. In commercial breeding flocks, however, this would be unlikely as sires tend to be unrelated.
In this study a computer program for pedigree analysis was utilised to assign parentage to a commercially bred flock designed for a research project. Microsatellite typing data for 12 loci resulted in sire identification for 85% of the progeny and dam identification for 40% of this group in the study flock. The most likely sire/dam/offspring combination was determined by a process of exclusion. Individual sires and/or dams were excluded on the basis of incompatible genotype. Matching genotypes indicate possible family relationships, and likelihood estimates, calculated using allelic frequencies, assist in identification of the best possible combination. Where more than one possible sire/dam combination results then further genetic typing is required.

Those cases for which a compatible combination was not found were most likely to due to typing error or the existence of a nonamplifying allele (Pemberton *et al.* 1995). Nonamplifying alleles, due to mutations in the primer sites, are not uncommon and can be detected through typing mismatches of known parent-offspring pairs or significant deviation from Hardy-Weinberg expectations (Pemberton *et al.* 1995). In this study typing data on one of the microsatellites, BM415, gave significant deviation from expected Hardy-Weinberg frequencies and it is possible that a non-amplifying allele is present. This result highlights the necessity of determining Hardy-Weinberg expectations for all microsatellite typing data.

**ACKNOWLEDGMENT**

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**REFERENCES**


