QTL MAPPING FOR PRODUCTION TRAITS IN A MULTI-STAGE AWASSI X MERINO BACK-INTER-CROSS DESIGN

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SUMMARY

Identification of genes that contribute to economically important traits will increase our understanding of the biology leading to superior or inferior production, and may allow for additional selection strategies. The first step in this process is the chromosomal localisation of such genes. We have initiated a sheep-genome research programme aimed at identifying quantitative trait loci for wool, meat and milk production within the one resource population based on an extreme breed back-cross and inter-cross design between Awassi fat-tail sheep and Merino superfine and medium wool sheep.

Keywords: QTL, Merino, Awassi, milk, meat, wool

INTRODUCTION

Wool, meat and milk represent the most important products in sheep production. Genetic improvement in each of these traits has led to the development of specialist breeds for each of these products. In addition, numerous genetic investigations have revealed an extensive source of genetic variation within breeds for each of the production traits and their components. Heritability estimates for wool production and quality range from 0.3 to 0.5. Similarly heritability estimates for lamb and meat production range from 0.15 to 0.6. Heritability estimates for milk production are relatively scarce by comparison with the other traits, but for the major milk-breeds estimates range from 0.25 to 0.6 for milk yield and composition. See Piper and Ruvinsky (1997) for a comprehensive overview.

All these historical developments and detailed investigations demonstrate that genes make a significant contribution to the differences we observe in desired production charactersistics of sheep. To date, selection has largely been through exploitation of estimated breeding values derived from phenotypic expression of selection traits. With the rapid expansion of genome information for most livestock species, including sheep, it is now considered feasible that future selection decisions can be made through additional information provided by screening individuals at the level of the gene- ie. DNA-based selection. Such selection strategies can target direct selection for favourable alleles of known genes (genotypic selection), or DNA markers closely linked to anonymous production genes (marker-assisted selection). Such information can be incorporated in traditional within-flock selection programmes through marker assisted selection (MAS), or rapid introgression of desirable genes with the aid of DNA markers (marker assisted introgression, MAI).

In order to develop the necessary information for such selection tools, considerable genomic research is required to locate the genes or chromosomal regions which contain genes that affect production traits. The genes we wish to target are generally referred to as quantitative trait loci (QTL). The cornerstones of such research programmes require at least the following two components: a) a comprehensive linkage or physical map with both class I loci (expressed genes) and class II loci (anonymous polymorphic markers), together with comparative map information across other species in which candidate genes with known function and economic significance have been identified, and b) resource populations with appropriate pedigree structure and detailed performance records.

The development of a comprehensive linkage map in sheep (Gortari *et al.* 1998) and comparative genome data bases, now allows researchers to target all regions in the genome with an average resolution of 1 marker per 3 million base-pairs (3 centimorgan resolution), in addition 344 functional genes have now been mapped in sheep. Specialist resource families have been established to map QTL for production traits, disease resistance, and traits with a simple Mendelian inheritance.

Most resource populations that have been established allow detailed examination of a limited range of production traits, since their utility is largely determined by the founder populations used to generate the families in which genes of interest are segregating. In a large study at the Centre for Advanced Technologies in Animal Genetics and Reproduction (*ReproGen*), we have exploited a unique breed combination to map important QTL for wool, meat, and milk production in a single resource population.

REPROGEN RESOURCE POPULATION

Our resource population utilises extreme breed differences between the specialist wool producing Merino breed, and the recently imported specialist milk strains of Awassi sheep developed in Israel (Gootwine and Goot 1997). With the low milk yield in the Merino, and the poor carpet wool quality of the Awassi, these two breeds can considered to be extremes for these two production traits. Further contrasts between the two breeds show a small frame size in the Merino and major differences in fat distribution between Merino and the fat-tail Awassi. The matings and development of the resource population has been staged in three phases.

Phase 1. Heterozygous F1 males and females were created by crossing 4 Awassi sires (each representing a major founder (F0) family developed in Israel and imported to Australia), with 30 superfine and medium wool Merino ewes. A total of 25 progeny were born and raised till sexual maturity. An F1 ram lamb from each of the 4 F0 Awassi sire families (representing both medium and superfine wool Merino bloodlines on the maternal side) were selected and back-crossed to medium and superfine Merino ewes. In total, 400, 150, 150, and 150 progeny were born for each of the 4 F1 sires. The mating structures and progeny details are shown in Figure 1. We utilised a one-way back-cross design for simplicity and cost (Merino ewes were readily available, whereas purebred Awassi ewes were unavailable, and creation of an F2 resource population would have require large numbers of F1 females or multiple ovulation and embryo transfer).

Phase 2. To exploit the necessity to mate and lamb all back-cross females in order to measure milk production, all back-cross ewes are being mated to F1 sires. Allocation is such that the same F0

family is not represented again. This is shown in Figure 1. These progeny will be used for strategic verification of putative QTL obtained from phase 1. In addition, unrelated families from elite industry sires will be used to verify putative QTL in industry relevant flocks.

Phase 3. Primary genome screens of back-cross progeny (combined with subsequent verification) is suitable to detect QTL with large effect, but is relatively insensitive to obtain a precise location of the QTL. For this latter aim a fine mapping strategy is required. Davarsi (1998) outlines several fine mapping strategies. One of these is an advanced inter-cross between two parental lines. We have made provisional resources available to inter-cross F1 ewes and sires to produce F2 females with the aid of sexed semen and MOET. This may be followed by JIVET and use of sexed semen to reduce generation interval and maximise the number of recombination events in a closed population to obtain more precise location of QTL. The possible strategy is shown in Figure 1.

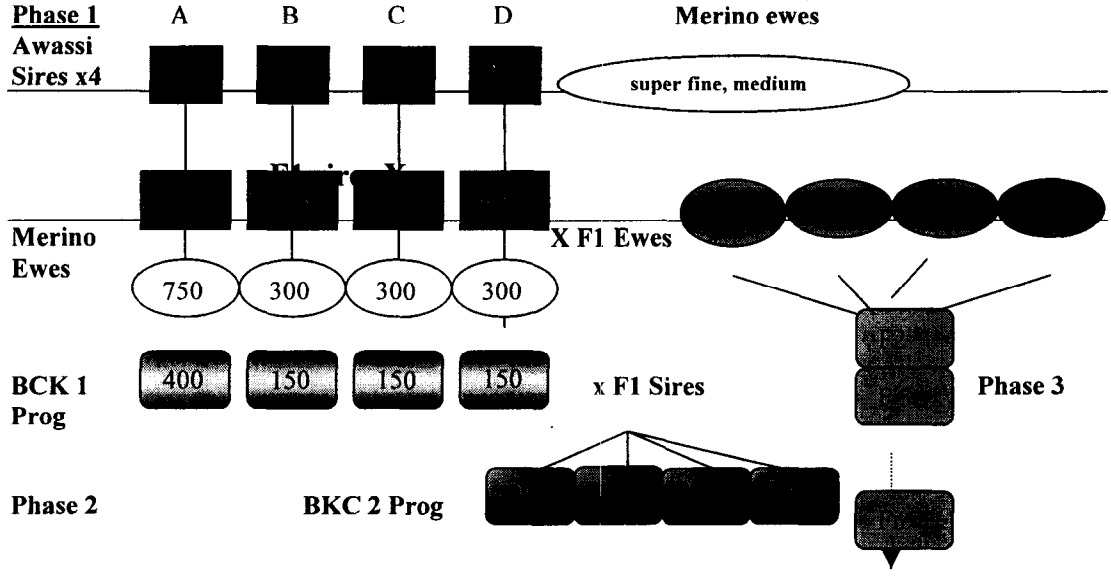

Figure 1. Mating strategy for generation of progeny for QTL mapping of major genes for all major production traits in sheep (see text for details on matings in Phase 1,2,3)

GENOTYPING STRATEGY

Class II anonymous micro-satellites are the main markers used in the genotyping strategy. Almost complete genome coverage can be achieved at pre-determined intervals with highly polymorphic markers. Furthermore, these markers are amenable to large-scale genotyping throughput with semi-automatic platforms as described by Wise *et al* (1998). For phase 1, we have selected 125 informative markers following a pre-screening of 245 markers. The selected markers represent 90 % coverage of the genome at 25cM intervals in the 4 families with a total of 125,000 genotypes for phase 1 progeny. Simulation suggests that putative QTL with 0.25 SD effect could be detected at 95 % confidence, using this strategy. Genotyping in Phase 2 will target only those regions with putative QTL, using a marker density with 10cM resolution. Genotyping strategy for phase 3 progeny has not

yet been decided, since high-capacity genotyping technologies utilising high-density single nucleotide polymorphic markers (SNP) and micro-arrays may become available.

PHENOTYPING

The importance of high quality phenotypic data is sometimes underestimated in major genome projects. Multi-trait QTL detection analytical programmes are now available and can exploit the use of repeated records or the option to sample related traits to increase power of QTL detection. The nature of traits measured in our resource population is detailed in Table 1. At present we are recording 75 biological traits over 1,000 progeny generated in phase 1. This represents approximately 275,000 primary record entries. Data management is being developed in collaboration with the Australian National Genomic Information Service (ANGIS) to develop generic relational data bases for QTL mapping experiments.

Table 1. Nature of traits measured in Awassi-Merino QTL mapping-resource flock.

Meat production	Wool production	Milk production	Miscellaneous
Growth (pasture/feedlot)	Fleece weight-4x	Lactation yield 2x	Conformation
Feed conversion	Fleece quality 4x *	Milk quality 2x	Disease resistance
Body composition-CAT scan*	Production efficiency (mid side patch)	Milk composition 2x Feed conversion 2x	Behaviour Reproductive
Meat/skin quality*	Skin histology 4x	Milking behaviour	capacity females

* Denotes traits evaluated in collaboration with South Australian Research & Development Institute (SARDI) meat and wool section. Repeat sampling denoted by x.

RELEVANCE TO OTHER GENOMES

The utility of putative QTL found in sheep is likely to be higher than the target species alone, and will lead to equivalent/candidate genomic regions which can be targeted in cattle. The main advantage of using sheep instead of cattle is the cost of maintenance and infrastructure required for the resource populations. The equivalent extreme breed cross in cattle is likely to invoke a Holstein Friesian or high lactation dual-purpose dairy breed, crossed with *Bos indicus* or possibly Scottish highland cattle. We estimate that we can maintain 7-10 experimental sheep for the cost of one beast. Furthermore, the ability to monitor body composition changes *in vivo* in sheep provides experimenters with greater research options. The added advantage of using sheep is the power to map wool-production QTL in the same population. The disadvantage is that putative QTL found in sheep will certainly need to be validated in cattle before they can be used in that species.

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