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BUILDING AN INTEGRATED QTL MAP FOR ALL MAJOR PRODUCTION TRAITS IN SHEEP BASED ON MULTIPLE QTL MAPPING POPULATIONS

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SUMMARY

Two experimental sheep populations comprising 3579 progeny, have been established to map QTL for over 125 phenotypes covering wool, growth/carcass, parasite traits, lactation traits, feed intake, feed efficiency and energy partitioning, morphology, behaviour, and reproductive performance. Genome wide scans using microsatellite markers have been completed, and preliminary results of a whole genome scan are described. The results of this will provide a valuable resource for further fine-mapping, functional studies and gene identification. An integrated QTL map and framework linkage map is being constructed.

INTRODUCTION

By comparison with cattle and pig, relatively few QTL studies have been conducted in sheep. A notable feature of ovine QTL studies is that they have been conducted in a wide diversity of commercial breeds, and in many cases for a limited range of target traits such as meat, wool, milk or disease resistance. In addition many QTL studies have been based on partial genome scans in regions which were thought to contain putative QTL. We have taken an approach to maximise efficiency of QTL discovery by utilising extreme breed crosses where the founder breeds have specialist attributes such as: wool, carcass composition, lactation performance or adaptation to extreme environmental conditions, and maximise the range of phenotypes measured. Not only does this strategy maximise efficiency against genotyping investment, but this also allows examination of pleiotropic effects.

RESOURCE FLOCKS

The Centre, in partnership with collaborators, has generated two major QTL mapping resource populations for QTL identification, gene discovery and functional studies on genes for all major wool, growth, carcass, lactation, and disease resistance traits in sheep. The first is a (Merino x Awassi) x Merino backcross and intercross population. This population is described as the AMM population. The second population is also an extreme breed cross (Merino x Indonesian Thin Tail (ITT)) x Merino backcross and intercross population for study of parasite resistance- (*Haemonchus contortus* and *Fasciola gigantica* repeated challenge), growth and fleece traits and an extensive range of immunological and morphological traits. This population is described as the ITTMM population.

AMM population. As summarised by Raadsma *et al*. (1999), four Awassi sires were crossed with medium and superfine Merino ewes to produce 16 F1 families. Four F1 ram lambs were selected to represent each of the founder families, and these were backcrossed to superfine and medium Merino

ewes, producing families with approximately 611, 202, 141, and 186 progeny respectively. Additionally, 1391 double-backcross and intercross progeny were generated from all F1 sires by mating them to backcross females, thus providing informative markers from both sire and dams, with a representation of all 3 QTL genotypes (QQ, Qq, qq). Final family sizes were 987, 703, 274 and 567 for the four families.

ITTMM population. This population was established in Bogor Indonesia from reciprocal matings for both Sumatra and Garut Thin Tail parents to medium and fine wool Merino parents. Approximately 86% of the subsequent matings were backcrosses of F1 sires to Merino ewes, the remainder (14%) were F2 and backcross to ITT ewes. A total of 1048 progeny were generated across 10 families with four families specifically amplified to over 120 progeny per family for QTL mapping. All progeny were raised indoors free from parasites during the entire experiment and raised on controlled diets until slaughter at 16 months of age. Parasite challenge was conducted by experimental means for both *Haemonchus contortus* (two separate challenges 1500 L3 larvae via trickle infection) and *Fasciola gigantica* (challenge with 250 or 400 metacercarea).

Genotyping. Initially, 838 microsatellite markers were identified on the basis of map location (Maddox 2001) and PIC scores from the Australian Sheep Gene Mapping Resource (http:// rubens.its.unimelb.edu.au/~jillm/jill.htm) and bovine resources. A panel of 558 of pre-selected polymorphic micro-satellite markers was screened for a genome wide scan, of which a total of 204 markers were informative in the AMM flock. A framework genetic linkage map was constructed using Carthagene software (http://www.inra.fr/bia/T/CartaGene/index.html) for each chromosome at a minimum significance of LOD > 2 for next best map order (average LOD: 6.92). The total number of markers in the final framework map was 191 and varied between 4 and 16 loci per chromosome. The average adjacent marker density across the genome was 22.2 cM and predicted genome coverage was approximately 95% for each chromosome. This framework map provides maximum power for first pass QTL discovery.A low density marker screen with an average inter-marker distance of 30cM using 136 markers was conducted in the ITTMM flock. An average of 97 informative markers per family was used in the primary screen with 7.13 informative families per locus and 376 phase known informative individuals per locus. Mapping was carried out using preexisting knowledge of linkage group assignments. Mean map distance between markers was 33.1cM (range: 1.9 – 190cM, median: 25.2cM). Incorporation of the additional information from the two maps into the Australian Sheep Gene map will strengthen a framework map position, confirming marker order and distances.

Phenotyping. Over 125 traits covering wool, growth, carcass composition, behavioural, morphological, lactation, feed efficiency, and disease resistance measurements have been collected in both resource flocks. Many phenotypes are recorded in both flocks as well as many traits not normally recorded in commercial populations highlighting benefits of a purpose designed QTL mapping flock. In addition skin section, wool, muscle, milk and DNA, and serum samples have been stored, where relevant, for further study.

Genetic and statistical analyses. QTL analyses were conducted using QTL Express (http://qtl.cap.ed.ac.uk). A chromosome-wide threshold for statistical significance was calculated for each chromosome, based on a permutation test of 1000 re-samples. In addition QTL-MLE was used as a maximum likelihood based approach specifically for half sib designs in non-inbred strains (Thomson *et al.* these proceedings).

INITIAL RESULTS

The results for all QTL analyses are still incomplete, but results to date are summarised in Table 1 Goddard *et al.* (2003) compared Australian QTL resource flocks in relation to False Discovery Rate (FDR), which is the proportion of reported QTL that are likely to be false positives. Of the resource flocks compared, the Merino-Awassi flock had the lowest FDR. The ITTMM flock has a higher FDR.

Table 1 Summary of QTL results to date ((Awassi x Merino)x Merino) ((ITT x Merino)xMerino) flocks.

Traits listed by grouping similar traits.	Number of traits Analyzed to date	Number of QTL with chromosome wide P<0.01	Expected number of false positives[1]	False Discovery Rate (FDR) %	Range of QTL effects SD
AMM flock					
Wool/fleece quality	25	42	6.50	15.5	0.29-1.52
Body/carcass/growth	48	50	12.48	25.0	0.38-1.02
Lactation traits	12	15	3.12	20.8	0.31-1.10
Food intake/growth	3	3	0.78	26.0	Tba
Pigmentation	6	14	1.56	11.1	Tba
TOTAL	**97**	**124**	**22.1**	**20.3**	
ITTMM flock					
Wool/fleece quality	7	6	1.82	30.3	Tba
Body/carcass/growth	2	0	0.52	Undefined	Tba
Parasite response	5	4	1.3	26.0	Tba
Immune response	34	14	10.14	72.0	Tba
Pigmentation	1	1	0.26	26.0	Tba
TOTAL	**49**	**21**	**12.74**	**60.6**	

Tba= to be analysed

RELEVANCE OF RESULTS FROM A WIDE CROSS

From time to time, concern is expressed that QTL discovered in a wide cross may provide little, if any, useful information on QTL segregating within commercial breeds. Extensive studies in pigs and poultry (Evans *et al*. 2003; Nagamine *et al*. 2003, 2004; de Koning *et al*. 2004) suggest just the opposite, namely that most QTL discovered in wide crosses are also segregating within commercial populations. In addition the likelihood of discovery of major genes (QTL with large effect) for traits such as unique wool/fleece quality traits, fat deposition traits, reproductive traits, is greater in a diverse cross than within breed studies, where major genes have been fixed within breeds. Furthermore the utility of researching genes with extreme effects can be shown by the *Callipyge* polymorphism which has no direct practical relevance to commercial sheep production in this country, and would not have been discovered, nor "validated" in Australian Industry QTL mapping resources, but yet provides an excellent frame work for mechanistic studies. With the advent of genome-wide selection (GWS) (Raadsma *et al.,* 2007 these proceedings), the main focus for QTL

[1] Expected number of false positives = 26 autosomes x number of traits x 0.01

studies should be on gene discovery and functional analyses to broaden our understanding of complex polygenic traits. In our studies preliminary results from both our populations have strong alignment with published QTL for meat, growth, milk and host resistance to parasites in a broad range of commercial breeds. The challenge for gene discovery underlying QTL is that often too many QTL are presented and enormous resources are required to analyse each QTL in turn. A positional-functional genomics approach as described by Singh *et al*. (2007), leads to positional-functional candidate genes for high resolution analysis. This requires well characterised QTL in a live animal resource population. These two resource populations and associated data sets represent a major resource on which full genome scans have been completed and this has identified segregating QTL with large effects in wool, carcass, and parasite resistance traits. Integration of information from multiple sources will allow for a strategic reference map of QTL for all major production traits in sheep.

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