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The search for quantitative trait loci affecting wool colour

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ABSTRACT

Farmers have been attempting to breed whiter wool for some time using quantitative and subjective genetic selection based on phenotypic traits. The improvement of wool colour using this form of selection has achieved limited success.

The advent of DNA marker maps has introduced the possibility of more directly relating phenotypic and genetic variation. Work presented here has identified a total of ten potential quantitative trait loci (QTL) that are significant at the suggestive level, and one additional QTL that approached this significance threshold that influence wool colour. There have been five putative QTL identified for base colour or challenge colour brightness. Two QTL affecting challenge colour yellowness was identified, while three QTL identified were linked to predictive colour variation. An additional QTL that affected predictive colour has also been mapped to a non-specific location on chromosome 24. Several further key areas of research need to be addressed before the DNA markers can be developed for use in the improvement of wool colour.

Keywords: wool colour; QTL; phenotypic variation.

INTRODUCTION

The local and export earnings of the New Zealand wool industry rely heavily on the production of high quality wool. One feature that affects the use of wool throughout the world is its colour, in particular its yellowness and brightness. There is considerable phenotypic variation in the yellowness of wool within any given flock of sheep exposed to the same local environmental conditions. Benavides *et al.* (1998) and Benavides and Maher (2000) calculated the heritability of yellow predictive colour (predictive colour) and base colour to be low, suggesting that the expression of wool colour is a result of both genetic and environmental effects. Evidence for the influence of environmental and genotype by environment interactions on wool colour has been reported (Sumner, 2000). However, the level of the complexity of these effects is not well understood.

Quantitative Trait Loci (QTL) mapping is one approach to finding genes of importance, such as growth and fatness in pigs (Anderssen *et al.*, 1994), milk yield (van Tassell *et al.*, 2000) and the Booroola fecundity (FecB) gene (Montgomery *et al.*, 1993). Various methods have been developed to study inheritance of chromosomal segments

through pedigrees and to identify QTL responsible for phenotypic variation (Paterson *et al.*, 1988; Haley, 1995). The long-term goal of this research is the implementation of marker assisted selection programmes that have the potential to increase selection efficiency (Staub *et al.*, 1996).

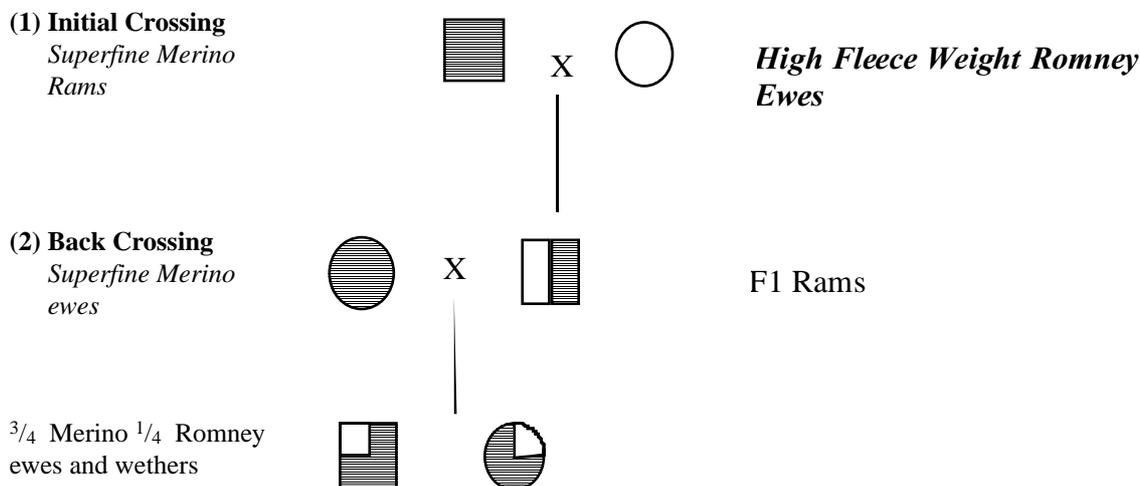
In this experiment, a backcross population of $3/4$ Merino X $1/4$ Romney animals was used to search for QTL affecting brightness and yellowness of wool in the ovine genome.

MATERIALS AND METHODS

Pedigree Structure

The $3/4$ Merino X $1/4$ Romney (MRM) pedigree structure have been previously discussed in detail in Wuliji *et al.* (1995). Figure 1 summarises the pedigree structure of the MRM backcross progeny. One F1 ram lamb from each of the four original Merino sires was single-sire mated to Merino ewes to generate approximately 100 backcross progeny per sire, born in each of two consecutive years, 1992 and 1993. The progeny were initially grazed at Tara Hills Research Station, Omarama for two years. The 1992-born progeny were then transported to Lincoln University and mid-side wool sampled in 1996, while the 1993-born

FIGURE 1: Mating plan for the Merino X Romney backcross population.



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cohort was grazed prior to sampling at AgResearch Woodlands, Invercargill. The cross between the superfine Merinos and the High Fleece Weight Romneys was selected because of the extensive differences in a number of traits including fibre diameter and wool colour of the parent breeds (Henry *et al.*, 1998), creating an F1 population from which segregating QTL could be traced in the backcrosses.

Phenotype Measurement

Pedigree, birth date and birth/rearing rank were recorded for all progeny (Henry *et al.*, 1998). Yellowness, assessed as predictive colour (Wilkinson, 1981), and yellowness and brightness, by each of the base colour (NZS 8707:1984) and challenge colour (Gutierrez, 1996) methods were measured on all backcross progeny.

Genome Scan

Two hundred and forty eight markers were used to perform a genome wide scan of the ovine genome for QTL for each trait measured within the four MRM families. The majority of the genetic markers used in this study were microsatellites with positions on the ovine map given in de Gortari *et al.* (1998). Restriction fragment length polymorphism and gene markers sourced elsewhere were also used. The genotypes of the 248 markers, spaced at 20-30 cM intervals throughout the genome, were generated by AgResearch Molecular Biology Unit using the touch-down polymerase chain reaction conditions reported by Crawford *et al.* (1995). To determine which markers were informative, the original four sires were genotyped across all 248 markers. Subsequently, the genotyping of the MRM progeny was restricted to the informative loci only. The number of informative markers per family ranged from 160 to 177. Following the genotyping of the progeny, two independent persons scored the genotypes to limit discrepancies. The data were screened for the following anomalies: abnormal segregation of markers within families; the presence of double recombinants within short distances; abnormal rates of between family recombination; within-family map distances disparate from previously

published reports. Potential errors were retested or corrected.

Statistical Analysis

The statistical model included the fixed effects of year, sex, birth/rearing rank, dam's age, dam's selection flock and sire, and the covariate of birth date. Significant ($P < 0.01$) two-way interactions were also included, these being sire by year for predictive colour and sire by dam's selection flock for challenge colour yellowness. The phenotypic data for wool colour and brightness was analysed by the Haley-Knott interval mapping method using a single-QTL model (Haley and Knott, 1992). This method calculated the probability of the presence of QTL at 2 cM intervals between each pair of flanking markers. The presence of QTL was tested within each of the four MRM families. The results reported used suggestive Lander-Botstein probability (LBProb) significance thresholds calculated using the Kruglyak method (Lander and Kruglyak, 1995). The position of QTL on each chromosome were measured in cM from the starting position of the analysis, which was located 2 cM before the first marker.

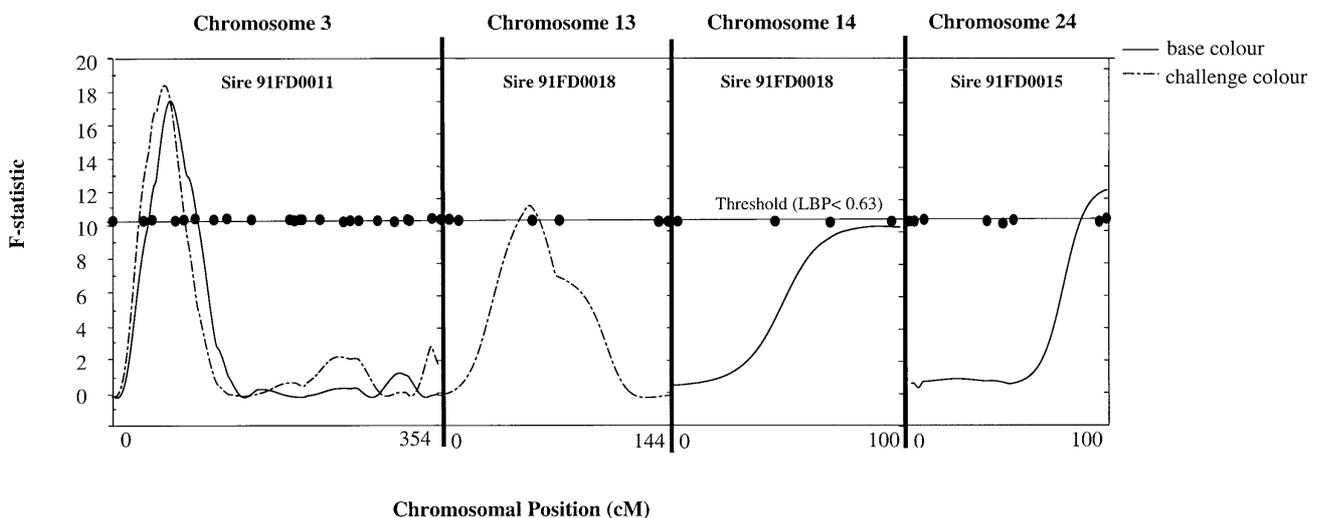
RESULTS

There were several significant marker-trait associations found within sire groups for colour traits, and in most instances there was evidence for more than one putative QTL per trait.

QTL affecting the Brightness of Wool

Four putative QTL affecting the brightness of wool have been detected at the suggestive significance (LBProb < 0.63) level within individual sire groups. One QTL on each of chromosomes 3 and 24 for the brightness of wool, as determined using the base colour method, were detected (Figure 2). Two further QTL were mapped to chromosomes 3 and 13 that affected brightness by challenge colour. The two QTL mapped to chromosome 3 for challenge colour and base colour were segregating in the same sire group and were located at 56 cM and 60 cM, respectively. The

FIGURE 2: Statistical profiles calculated at 2 cM intervals for QTL found for the brightness of wool. The dots along the threshold line represent the chromosomal position of markers used in the analysis.



microsatellite marker TGLA77 was the closest marker to both QTL. A comparison between the maximum F scores for the brightness by both base colour and challenge colour suggests little evidence that the QTL were in different positions. The QTL identified on chromosome 24 was situated at 76 cM but the overall marker coverage on this chromosome was poor. Figure 2 also shows an additional QTL mapped to chromosome 14 that approached the suggestive threshold for base colour brightness.

QTL affecting the Yellowness of Wool

Linkage analysis revealed that the variation in the yellowness of the wool, as measured by the challenge colour method, was significantly associated with one marker mapped to each of chromosomes 12 and 16 (Figure 3). The F-score peak of the QTL on chromosome 12 was located at the distal end at 88 cM, and was flanked by the markers BM3509 and HUIJ625. The QTL on chromosome 16 was located on chromosome 16 at 116 cM, between the markers BM1225 and RM106. Both putative QTL segregated in progeny of the same sire. There was no evidence for any QTL that affected the variation observed for base colour yellowness.

QTL affecting the Yellow Predictive Colour of Wool

Interval mapping detected suggestive QTL on chromosomes 13, 17 and 24 which affected predictive colour variation in wool (Figure 4). The QTL mapped at 144 cM on chromosome 13, the QTL mapped to 92 cM on chromosome 17 and the QTL mapped to chromosome 24 segregated in the same sire group. An additional area of interest was also identified on chromosome 17. This QTL was mapped to 8 cM on this chromosome and segregated in a different family to the other QTL mapped on this

chromosome.

DISCUSSION

This study is the first to report the use of interval mapping to identify markers that affect wool yellowness and brightness. This study incorporated approximately 250 markers in the analysis and identified ten genomic regions that affected wool colour traits at the suggestive level and an additional area of interest that approached the suggestive level. Overall, the numbers of markers used in the genome scan provided a wide coverage of the ovine genome (Henry *et al.*, 1998). One factor that may have limited the successful identification of genomic regions linked to quantitative variation in the wool colour traits studied was the pedigree of the flock. The initial mating design for the flock was chosen to maximise the variation in fibre diameter and was not necessarily focussed on wool colour. There is anecdotal evidence of genetic differences between the colour of Romney and Merino wool. Merino animals tend to produce whiter wool while the wool of Romney animals tends to yellow to a greater degree. The lack of differences across combined data suggests less genetic difference between the breeds than first thought. The results of the analysis across the four sire groups may have been different if a greater emphasis had been placed on wool colour prior to selection of parent animals.

The identification of potential QTL on chromosomes 3, 12, 13, 14, 17 and 24 indicated a complex system of genetic determination, where in some cases multiple QTL on multiple chromosomes were found to be associated with wool colour traits. The interpretation of the results from the genome scan is difficult because of the uncertainty surrounding the relationships between the traits under

FIGURE 3: Statistical profiles calculated at 2 cM intervals for QTL found for Challenge Colour yellowness of wool. The dots along the threshold line represent the chromosomal position of markers used in the analysis.

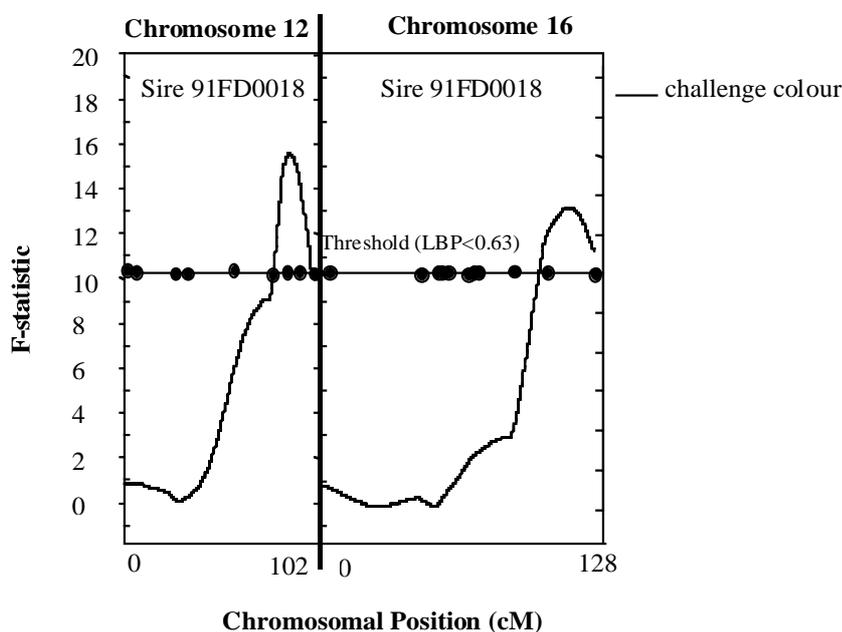
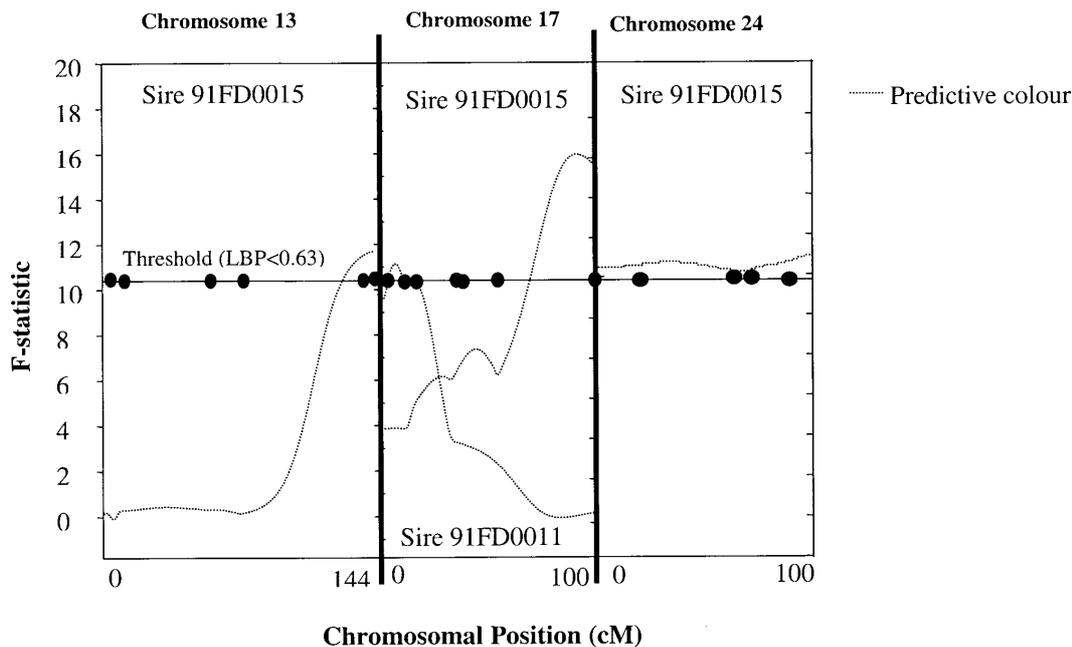


FIGURE 4: Statistical profiles calculated at 2 cM intervals for QTL found for the Yellow Predictive Colour of wool. The dots along the threshold line represent the chromosomal position of markers used in the analysis.



investigation. Research is yet to fully determine the relationships between the colour measurement traits employed in this study but some association is highly likely (Reid, 1992; Benavides and Maher, 2000).

The F-score peaks on chromosome 3 for each brightness trait were located at essentially the same position, suggesting a single gene or cluster of genes affecting both traits. However, it must be noted that apparently independent QTL for the two brightness traits were also found, and that no common or proximal QTL for yellowness by base colour and challenge colour were found.

The wide span of the F-score peaks on chromosome 13 and 24 are indicative of regions of the genome where the marker density was low, therefore making the position of the QTL hard to accurately locate. The location of the QTL could change considerably with additional marker information. Additional markers that provide greater coverage across the entire genome would have resulted in a more effective and accurate estimation of QTL location.

The results presented in this report suggest a number of potential QTL that partially explain the variation observed in wool colour. Although the evidence for marker-QTL association was not conclusive, the results warrant further investigation in two key areas. First, additional research to increase the number of genes mapped onto the ovine genome could provide a useful tool for identifying potential candidate genes in the regions identified. There were no obvious candidate genes found on the Roslin Institute website SheepBase (<http://www.ri.bbsrc.ac.uk>) in the vicinity of putative QTL at the time of publication. A search of the genomes of other closely related species, cattle and goats, did not reveal any likely candidate genes. Second, the range of genotypes examined for QTL for wool colour needs to be extended both within and outside the parental breeds used in this study.

Eleven separate suggestive or near suggestive QTL positions were identified for wool colour in total. Other factors, including epistasis, genetic by environment interactions and the magnitude of background effects of other genes, could have limited the findings in the population (Gerbens *et al.*, 2000). These data have not been analysed for the presence of epistatic or interactive effects but it is the intention of the authors to do so for the colour traits reported.

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REFERENCES

- Anderssen, L.; Haley, C.S.; Ellegren, H.; Knott, S.A.; Johansson, M.; Andersson, K.; Andersson-Eklund, L.; Edfors-Lilja, I.; Fredholm, M.; Hansson, I.; Håkansson, J.; Lundström, K. 1994: Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science* 263: 1771-1774.
- Benavides, M.V. and Maher, A.P. 2000: Quantitative genetic studies on wool yellowing in Corriedale sheep -II- Clean wool colour and wool production traits: Genetic parameter estimates and economic returns. *Australian journal of agricultural research* 51: 191-196.
- Benavides, M.V.; Maher, A.P.; Young, M.J. and Reid, T.C. 1998: Quantitative genetic studies on wool yellowing in Corriedale sheep -I- Wool yellowing susceptibility and wool production traits - Genetic parameter estimates. *Australian journal of agricultural research* 49: 1195-1200.
- Crawford, A.M.; Dodds, K.G.; Ede, A.J.; Pierson, C.A.; Montgomery, G.W.; Garmonsway, H.G.; Beattie, A.E.; Davis, K.; Maddox, J.F.; Kappes, S.W.; Stone, R.T.; Nguyen, T.C.; Penty, J.M.; Lord, E.A.; Broom, J.E.; Buitkamp, J.; Schwaiger, W.; Epplen, J.T.; Matthew, P.; Matthews, M.E.; Hulme, D.J.; Beh, K.J.; McGraw, R.A.; Beattie, C.W. 1995: An autosomal genetic linkage map of the sheep genome. *Genetics* 140: 703-724.

- de Gortari, M.J.; Freking, B.A.; Cuthbertson, R.P.; Kappes, S.M.; Keele, J.W.; Stone, R.T.; Leymaster, K.A.; Dodds, K.G.; Crawford, A.M.; Beattie, C.W. 1998: A second generation linkage map of the sheep genome. *Mammalian genome* 9: 204-209.
- Gerbens, F.; de Koning, D.J.; Hardens, F.L.; Meuwissen, T.H.E.; Janss, L.L.G.; groenen, M.A.M.; Veerkamp, J.H.; Van Arendonk, J.A.M.; te Pas, M.F.W. 2000: The effect of adipocyte and heart fatty acid-binding protein genes on intramuscular fat and backfat content in Meishan crossbred pigs. *Journal of animal science* 78: 552-559.
- Gutierrez, J.L.A. 1996: Development of the challenge test for the susceptibility of wool to yellow discolouration. Thesis, Masters of Agricultural Science, Lincoln University, New Zealand.
- Haley, C.S. 1995: Livestock QTLs-bringing home the bacon? *Trends in genetics* 1: 488-492.
- Haley, C.S.; Knott, S.A. 1992: A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* 69: 315-324.
- Henry, H.M.; Dodds, K.G.; Wuliji, T.; Jenkins, Z.A.; Beattie, A.E.; Montgomery, G.W. 1998: A genome screen for QTL for wool traits in a Merino X Romney backcross flock. *Wool technology and sheep breeding* 46: 213-217.
- Lander, E.S.; Kruglyak, L. 1995: Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature genetics* 11: 241-247.
- Montgomery, G.W.; Crawford, A.M.; Penty, J.M.; Dodds, K.G.; Ede, A.J.; Henry, H.M.; Sise, J.A.; Swarbrick, P.A.; Hanrahan, V.; Buchanan, F.C.; Hill, D.F. 1993: The ovine Booroola fecundity gene (FecB) is linked to markers from a region of human chromosome 4q. *Nature genetics* 4: 410-414.
- NZS 8707: 1984. 1984 : Method for the measurement of the colour of wool. Standards Association of New Zealand.
- Paterson, A.H.; Lander, E.S.; Hewitt, J.D.; Peterson, S.; Lincoln, S.E.; Tanksley, S. D. 1988: Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature* 335: 721-726.
- Reid, T.C. 1992: Procedures for Identifying Resistant or Susceptible Wool. Milestone 1. Report to New Zealand Wool Board (May) 9.
- Staub, J.E.; Serquen, F.C.; Gupta, M. 1996: Genetic markers, map construction, and their application in plant breeding. *HortScience* 31: 729-740.
- Sumner, R.M.W. 2000: On the sheep's back. *Wool grower Autumn*, pp23.
- van Tassell, C.P.; Ashwell, M.S.; Sonstegard, T.S. 2000: Detection of putative loci affecting milk, health, and conformation traits in a Holstein population using 105 microsatellite markers. *Journal of dairy science* 83: 1865-1872.
- Wilkinson, B.R. 1981: Studies on fleece yellowing Part 1: Prediction of susceptibility to yellow discolouration in greasy fleeces. *Wool technology and sheep breeding* 29: 169-177.
- Wuliji, T.; Montgomery, G.M.; Dodds, K.G.; Andrews, R.N.; Beattie, A.E.; Turner, P.R.; Rogers, J. 1995: Establishing a flock for gene mapping in wool traits. *Proceedings of the New Zealand Society of Animal Production* 55: 285-288.