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An ovine quantitative trait locus affecting fibre opacity in wool

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ABSTRACT

This experiment was originally structured as a genome-wide screen for quantitative trait loci (QTL) affecting resistance or susceptibility to facial eczema disease (FE), a liver mycotoxicosis. Two Finnish Landrace (F) rams were crossed to three Texel (T) ewes to produce three cross-breed (designated F × T) rams. The F × T rams were then outcrossed to 210–230 Coopworth ewes each to generate three half-sib families, and 200 progeny per sire family were used in the FE QTL experiment. In a FE-phenotyping trial, the 600 five-month old lambs were orally challenged with a fixed dose rate of sporidesmin (FE mycotoxin) to determine their disease status. About 217 informative microsatellite markers, which were evenly spaced throughout the 26 ovine autosomes, were analysed in the FE study. Genotyping for the FE experiment was conducted in two stages: a primary and a secondary screen. In the initial primary screen, 46 most FE-resistant and 46 most FE-susceptible progeny of each sire family were selectively genotyped with the markers and analysed. Resulting chromosomes carrying suggestive and significant FE QTL were then followed up in a secondary screen when all progeny were genotyped for final analysis; there were seven chromosomes that underwent secondary screening, viz. OAR1, OAR2, OAR6, OAR13, OAR18, OAR19 and OAR20. During the FE-phenotyping trial period, mid-side wool samples were collected from the unshorn lambs for fibre measurement; the wool traits measured included yield, staple length (StapLen), crimp frequency, fibre curvature (FCurv), fibre opacity (FOpac), fibre diameter mean (FDMean) and fibre diameter standard deviation (FDsd). In a linkage analysis of wool traits, using the Haley–Knott regression method with the available "FE-generated" genotypes, a highly significant QTL for fibre opacity was detected on OAR20.

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1. Introduction

A series of steps are required when conducting a genome-wide screen experiment to detect quantitative trait loci (QTL) for a complex trait. The steps include the generation of animals with a structured pedigree, phenotyping the resource animals, genotyping animals with informative DNA markers, and finally the linkage analysis for co-segregation of phenotypes with genotypes. The time and financial resources involved for such experiments are often prohibitive. An ideal approach is to generate an animal resource and use it for as many trait studies as possible. This is not always possible for many disease traits; for facial eczema (FE) as an example, the liver damage would inadvertently affect other production traits of interest such as growth rate and meat yield or quality (Kirton et al., 1979; Kirton et al., 1976). Here we incorporated a wool QTL study into a FE genome-screen experiment on an assumption that the wool parameters measured were largely expressed before the sheep were challenged with sporidesmin so would be relatively unaffected.

The dimensions and composition of wool fibres are commercially important through their effects on performance during wool processing and their attributes on the end products. For instance, the optimum length of wool fibres lies between 40 mm and 150 mm and depends very much on the choice of end product. Fibre diameter affects stiffness: fine fibres which are soft are suitable for comfortable apparel, while coarse, strong fibres make hard-wearing carpets. Fibres with frequent crimp make textiles with high insulation and resistance to compression, while straight fibres make lustrous or felted products. Medullated fibres have a core of cells not filled with keratin proteins which take up dye differently from solid fibres; medullated wool gives an uneven dyed colour that can be marketed as an attribute (e.g. tweed fleck), or avoided in products for which evenness of colour is important. When these traits are of sufficient commercial importance, they become the

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subject of manufacturing specifications and the target of animal breeding programmes. Accumulated historical demand for different fibre types has given rise to the wide divergence in fleece types seen between sheep breeds. Implicitly this means that the different fibre characteristics are governed by genetic factors.

For wool traits, the heritability and inter-trait correlations are generally high relative to other sheep production traits (Fogarty, 1995). Traits such as fibre loss, halo hair, and lustrous or silky wool, were listed by Dolling et al. (1996) as affected by major genes, but the identities of the causative genes are not known. In a review by Purvis and Franklin (2005), putative genes or DNA markers associated with variation in fibre diameter and staple length were reported. Bray et al. (2002) had reported some segregation of wool characteristics in the progeny of Finnish Landrace sires selected for long and short wool length.

The animal resource used in the present study was originally designed for a FE QTL experiment (unpublished data). It consisted of three half-sib sire families, generated from Finnish Landrace/Texel (F x T) rams outcrossed to Coopworth ewes. Assuming that the FE challenge trial had limited effect on wool characteristics under our experimental conditions, we attempted to maximize the use of the FE resource animals by also measuring their wool fibre traits as an add-on QTL study: the traits included yield, staple length, crimp frequency, fibre curvature, fibre opacity and fibre diameter. The genotypes of the animals generated from the original FE experiment were used for linkage analyses with the wool traits, and the results are reported here.

2. Materials and methods

2.1. Resource animals

The structured half-sib pedigrees were designed specifically for a FE genome-screen experiment. It is commonly known that as a breed the Finnish Landrace is naturally tolerant to FE and the Texel is very susceptible (Phua et al., 2013). Hence, two Finnish Landrace (F) rams were mated to three Texel (T) ewes to produce three cross-bred F × T rams, designated F × T1, F × T2 and F × T3; F × T1 and F × T2 were half sibs from the same Finnish Landrace sire but different Texel dams. Semen was collected from the three F × T rams and used to artificially inseminate 210–230 Coopworth ewes per ram (Animal Ethics Committee approval code AEC-P503), to generate three half-sib families with 270–290 progeny each. All lambs were born at about the same time and within a two-week period.

Two hundred normal healthy lambs were selected per sire, at five months of age, for the FE QTL experiment. To determine their FE phenotypes, the 600 selected lambs were orally challenged with a fixed dose rate of sporidesmin (at 0.3 mg/kg live-weight) in a FE-phenotyping trial of 5-week duration (AEC-P538); the FE traits were measured in terms of blood levels of the liver-specific enzymes, viz. gamma-glutamyl transferase (GGT) and glutamate dehydrogenase (GDH), and liver injury score of the livers at time of slaughter (at six weeks after sporidesmin dosing). In the latter scoring, the livers were collected during commercial slaughter and subjectively assessed for overall damage; they were assigned a score on a 0–5 (unaffected — severe) scale, with 0.5 increments (Smith et al., 1977). The animals were unshorn since birth; at five weeks post dosing when the animals were six months old, mid-side fleece were sampled to skin level.

2.2. Measurements of wool traits

The wool traits measured included clean wool yield (yield (%)), staple length (StapLen), crimp frequency, fibre curvature (FCurv), fibre opacity (FOpac), fibre diameter mean (FDMean) and fibre diameter standard deviation (FDsd) (Table 1). Yield (%) was determined by washing the greasy wool sample and expressing the weight of clean wool (at 16% moisture) as a percentage of the greasy wool weight. Staple length and crimp frequency were measured manually on three staples (naturally formed bundles of fibres) per animal. Fibre curvature, opacity and diameter were measured on 2-mm snippets of clean wool fibres using an optical fibre diameter analyser (OFDA 100) (Baxter et al., 1992). The mid-side wool samples were white, and in white wool, opacity is a measure of medullation (Lupton and Pfeiffer, 1998). Where there are pigmented fibres, these will also be detected as opacity on the OFDA.

2.3. Genotyping with microsatellite markers

Blood samples were collected in sodium heparin vacutainers, and genomic DNA was extracted from white blood cells using the high-salt method of Montgomery and Sise (1990). DNA genotyping with microsatellite markers was as described in Phua et al. (2008). Briefly, the reverse primers were end-labelled with [γ-^{33}P] ATP using T4-polynucleotide kinase. A touchdown programme of PCR amplification was used: that is, three cycles at 95 °C for 45 s and 60 °C for 1 min; three cycles at 95 °C for 45 s and 57 °C for 1 min; three cycles at 95 °C for 45 s and 54 °C for 1 min; three cycles at 95 °C for 45 s and 51 °C for 1 min; 20 cycles at 92 °C for 45 s and 48 °C for 1 min. The PCR products were resolved in 6% denaturing polyacrylamide gels, which were then dried and visualised using autoradiography. The progeny were scored for their inheritance of the heterozygous sire's alleles.

2.4. Markers selection and genotyping of animals

About 240 microsatellite markers, spaced throughout the 26 ovine autosomes, were tested. They were sourced from the linkage maps of Crawford et al. (1995), de Gortari et al. (1998) and Maddox et al. (2001). Eventually, 217 informative markers, that showed heterozygosity in at least one of the three F × T sires, were used to genotype the progeny. Genotyping of animals in the FE experiment was done in two stages, namely a primary and a secondary screen. In the primary screen, the 217 markers were used to selectively genotype the 46 most FE-resistant and the 46 most FE-susceptible progeny of each F × T sire that showed respective marker heterozygosity. Significant and suggestive FE QTL detected in the primary screen were then followed up in a secondary screen: markers on the corresponding chromosomes carrying the QTL were genotyped across all the progeny of heterozygous F × T sires. Seven chromosomes underwent secondary screening, viz. OAR1, OAR2, OAR6, OAR13, OAR18, OAR19 and OAR20. All the genotype data generated above were used directly for the wool QTL analyses.

2.5. Genome-wide linkage analysis of markers to wool traits

The method of analysis followed the least-squares interval mapping of Knott et al. (1996), using *F*-statistic profiles for the regression of phenotype on the conditional probability of inheriting the sires' alleles calculated at 2-cm intervals (Phua et al., 2008). Besides genotype probability within sire, the models included birth/rearing rank, sex and sire as fixed effects, and birth day of year as a covariate; all genotyped and un-genotyped animals were included in each analysis. Our initial assumption of FE having minimal impact on wool traits may be incorrect: liver injury score was found to have a significant effect on staple length, crimp frequency and yield, and was hence fitted in the analysis. The theoretical *F*-statistic critical values (Lander and Kruglyak, 1995) were used: the point-wise probabilities of $P = 0.000054$ and $P = 0.0016$ correspond to significant and suggestive levels, respectively, and their theoretical *F*-values were 7.7 and 5.2 (Table 2). The actual significant and

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