A SCREEN OF CHROMOSOME 1 FOR QTL AFFECTING NEMATODE RESISTANCE IN AN OVINE OUTCROSS POPULATION

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INTRODUCTION

Gastrointestinal nematodes infect sheep grazing in contaminated pastures leading to reduced growth and productivity. Parasite control using anthelmintics is being questioned due to the development of nematode strains resistant to one or multiple classes of drugs (Sangster, 1999). Another aspect to consider is the increasing demand of consumers for products free of residues that might enter the food chain from the environment (Herd *et al*., 1993). Selecting animals naturally resistant to these parasites constitutes an alternative to complete reliance on drugs.

Resistance to nematode parasites is associated with the development of an early immune response. The control of the type, rapidity and size of this response is very complex and involves many genes. However, it is possible that only a few play a significant role in the resistance process. An efficient first step towards the identification of these genes is the detection of the chromosomal regions where they are located, using a quantitative trait loci (QTL) finding approach. A previous study (Beh *et al*., 1998) showed evidence of QTL segregation for nematode resistance on sheep chromosome 1. As a part of a project that aims to identify the loci responsible for parasite resistance in sheep, we have undertaken the study of this chromosome as a candidate for QTL segregation using an outcross population.

MATERIALS AND METHODS

Outcross design. The Wallaceville divergent faecal egg count (FEC) selection lines of Romneys commenced in 1979 (Baker *et al.,* 1991). Reciprocal crosses between the "high FEC" (susceptible) and the "low FEC' (resistant) lines were made to generate rams putatively heterozygous at QTL for parasite resistance. These sires were crossed with between 100 and 300 unselected Coopworth ewes to generate five half-sib pedigrees. The number of offspring in each family ranged from 100 to 348 and totalled 953.

Marker data. The 18 microsatellite markers used in this study have been selected from the ovine chromosome 1 linkage map (Maddox *et al*., 2001). Whenever a sire was heterozygous at a particular marker the offspring were genotyped. A summary of the markers analysed in each family is shown in Table 1. Microsatellite genotyping was performed by polymerase chain reaction (PCR) and denaturing polyacrylamide gel electrophoresis.

Phenotypic data. Two natural field challenges of infective nematode larvae were given to all outcross lambs. Mean faecal egg counts were recorded after each challenge for strongyle (FEC1 and FEC2) and *Nematodirus* (NEM1 and NEM2) (Baker *et al.*, 1991). Adult *T. colubriformis* parasites from the abomasum were collected and counted at slaughter (ATRICH), which took place approximately one week after the end of the second challenge.

QTL analysis. Prior to analysis, traits were log(x+c) transformed (LFEC1, …, LATRICH), where c was the lowest possible non-zero count for the trait. Markers were assumed to be in the positions estimated by the male linkage map of Maddox *et al.,* (2001). The information content (IC; Spelman *et al.,* 1996) along the map was calculated for each family and averaged. The presence of segregating QTLs was tested by interval mapping using a multimarker regression method (Knott *et al*., 1996) using a model that included sex, site by year, day of birth, and sire. Chromosome-wise statistic thresholds were calculated from 1,000 phenotype permutations following the method proposed by Churchill and Doerge (1994).

Table 1. Markers analysed per family

Markers	Sire				
	1	**2**	**3**	**4**	**5**
EPCDV010	-	-	y	y	y
BMS2145	y	y	y	-	y
ILSTS044	y	y	y	-	y
BM6465	y	y	y	y	y
ILSTS029	y	-	y	y	-
BM4129	y	y	y	y	y
BL41	-	y	y	y	y
TGLA49	y	-	-	y	y
ILSTS004	y	y	y	y	y
CSSM004	y	-	y	y	-
INRA011	y	y	-	y	y
DB6	y	y	y	y	y
BM6506	y	y	-	y	-
MCM130	y	-	-	-	y
CSSM032	-	y	-	y	-
MAF109	y	y	y	y	y
MAF4	y	y	y	y	y
BMS2263	y	y	y	y	y

Markers analysed are indicated by "y".

RESULTS AND DISCUSSION

Figure 1 illustrates the marker map of chromosome 1 and the IC extracted from the genotypic data generated in this study. The average IC exceeded 60% for most of the chromosome with values higher than 80% at *BM6465, ILSTS029, ILSTS004, INRA11, DB6* and *MAF4*. However, it didn't reach 50% in the intervals *EPCDV010-BMS2145* and *BL41-TGLA49.*

Interval mapping showed no significant results for the parameters LNEM1, LNEM2 and LFEC1. However, in the case of LFEC1, the F value observed for sire 4 in the interval *MAF4-BMS2263* was under the 10% probability level. It is interesting to note that the orthologous area in mouse contains a QTL identified as *Cypr1* (cytokine production 1) that controls *IL-4* production, a cytokine directly involved in the immune response against nematode parasites (Kosarova *et al.,* 1999). Regarding LFEC2, sire 1 showed a significant F value of 12.04 (the

5% chromosome-wise threshold is 10.89) in the area between *EPCDV010* and *ILSTS044*. No significant results were obtained in the across family analysis for this trait.

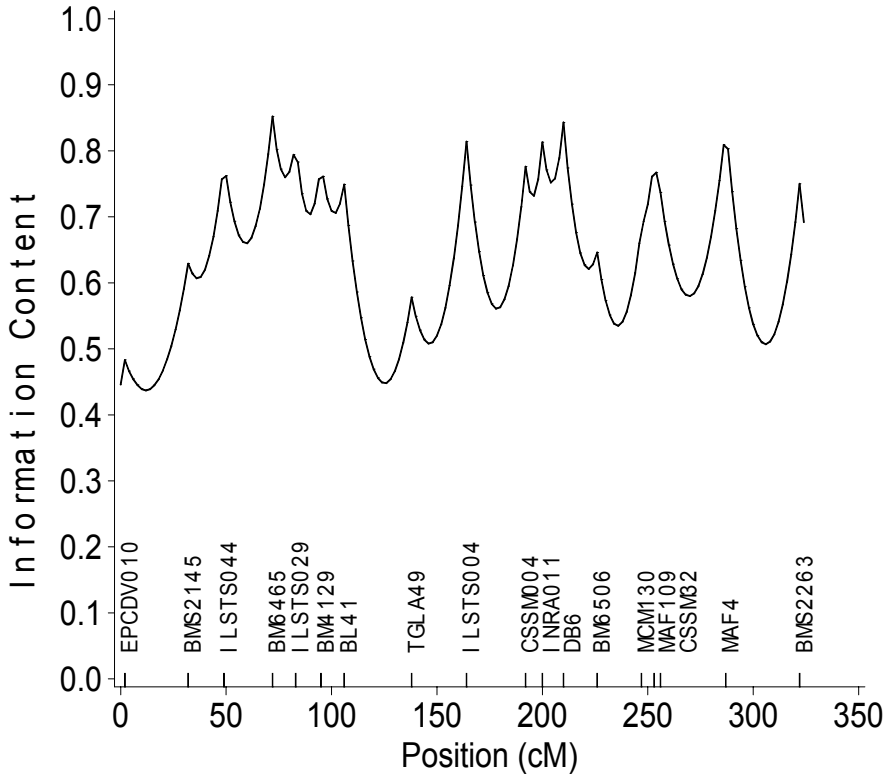

Figure 1. Information content along chromosome 1

The results for interval mapping for LATRICH are illustrated in Figure 2. The across family analysis was significant in the interval *EPCDV010-ILSTS044*. The individual family analysis showed that sire 1 was significant in this area. As commented above, a significant value was obtained in the same family and region for LFEC2, suggesting the segregation of a putative QTL affecting both, strongyle faecal egg count and the number of adult *T. colubriformis* parasites in the abomasum. However, this result has been found in a low IC interval, and sire 1 in particular was homozygous for the most proximal marker (*EPCDV010*). In order to verify these results, new markers need to be analysed in this area. Beh *et al.* (1998) found evidence of the segregation of a putative QTL affecting nematode resistance in the proximal part of ovine chromosome 1. Although their results were not conclusive, the chromosomal area involved is fairly coincident to the one reported here. Further studies will help to validate these results and to determine if the same QTL is segregating in different populations.

The individual analysis also shows a significant value for sire 2 in the proximal part of chromosome 1. Nevertheless, the area involved is associated with the marker *BM6465* rather than in the already mentioned interval.

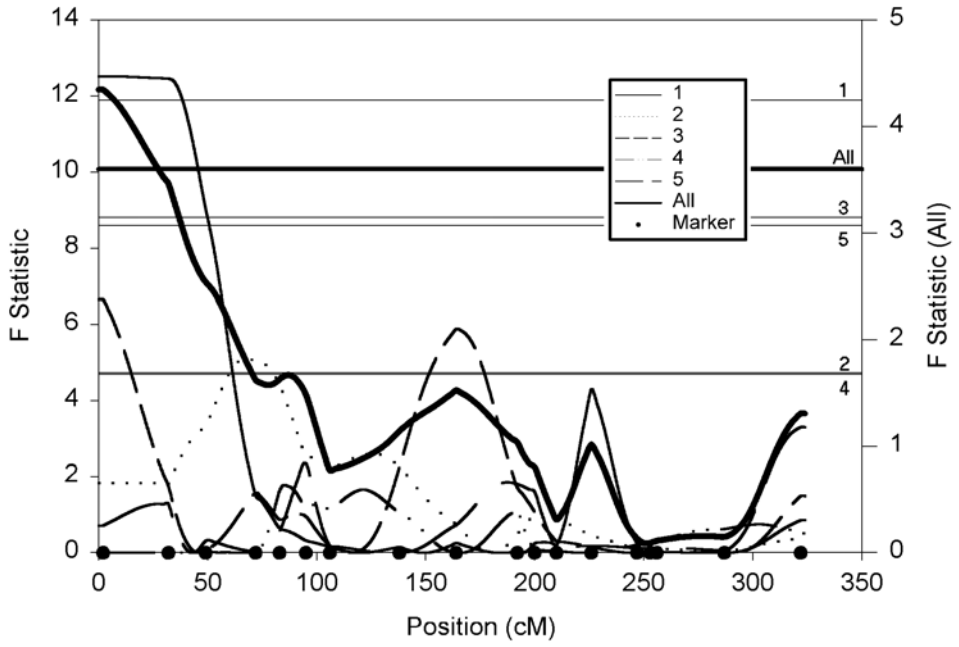

Figure 2. F-value of interval mapping for LATRICH. The scale on the left represents the individual family analysis and on the right the across family analysis. Thresholds obtained by permutation test are indicated in every case. Abscissa shows the marker position ordered along the chromosome

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