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Quantitative trait loci (QTL) mapping of resistance to strongyles and coccidia in the free-living Soay sheep (*Ovis aries*)

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Abstract

A genome-wide scan was performed to detect quantitative trait loci (QTL) for resistance to gastrointestinal parasites and ectoparasitic keds segregating in the free-living Soay sheep population on St. Kilda (UK). The mapping panel consisted of a single pedigree of 882 individuals of which 588 were genotyped. The Soay linkage map used for the scans comprised 251 markers covering the whole genome at average spacing of 15 cM. The traits here investigated were the strongyle faecal egg count (FEC), the coccidia faecal oocyst count (FOC) and a count of keds (*Melophagus ovinus*). QTL mapping was performed by means of variance component analysis so that the genetic parameters of the study traits were also estimated and compared with previous studies in Soay and domestic sheep. Strongyle FEC and coccidia FOC showed moderate heritability ($h^2 = 0.26$ and 0.22, respectively) in lambs but low heritability in adults ($h^2 < 0.10$). Ked count appeared to have very low h^2 in both lambs and adults. Genome scans were performed for the traits with moderate heritability and two genomic regions reached the level of suggestive linkage for coccidia FOC in lambs (logarithm of the odds = 2.68 and 2.21 on chromosomes 3 and X, respectively). We believe this is the first study to report a QTL search for parasite resistance in a free-living animal population and therefore may represent a useful reference for similar studies aimed at understanding the genetics of host-parasite co-evolution in the wild.

Keywords: QTL mapping; Soay sheep; Parasitic nematodes; Variance components; Natural population

1. Introduction

The antagonism between host and parasite is thought to be a major force in ecology and evolution due to its potential to generate and maintain genetic variation. Parasites are often characterized by high potential for diversification due to their high speed of speciation (Dykhuizen, 1998), whereas the hosts they colonize constitute a rapidly changing environment (Huyse et al., 2005). As a consequence, the host-parasite relationship generates continuously evolving host and parasite lineages (Nadler, 1995). In principle, this continuous battle can maintain genetic diversity in the antagonistic populations provided that a specificity between host and parasite genotypes is present (Haldane, 1949). Parasite resistance is likely to be controlled by several loci and therefore it may receive a strong mutational input which generates genetic variation (Houle et al., 1996). Host-parasite co-evolution may maintain genetic variation if the additive genetic value of a host genotype changes when parasites evolve as a response to the selection induced by the host (Haldane, 1949). Antagonistic pleiotropy may result in maintenance of genetic variation if the same genotype is positively selected for one fitness-related trait but negatively selected for another fitness-related trait (Roff and Mousseau, 1987). In the case of parasite resistance, this last hypothesis is suggested by the finding that

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sheep that are genetically resistant to intercellular infections may be more susceptible to infection from intracellular pathogens (Gill et al., 2000).

In sheep and other domestic ruminants, gastrointestinal nematodes are one of the most important classes of parasite. Intensive effort, therefore, has been invested in understanding, and exploiting through breeding programs, the genetic basis of parasite resistance and host-parasite co-evolution (Kaplan, 2004). Parasite resistance is complex in nature, having polygenic and environmental components (Stear et al., 1997; Bishop and Stear, 2003). Resistance to infection by gastrointestinal nematodes has moderate heritability in domestic sheep ranging from 0.13 (McEwan et al., 1992) to 0.53 (Baker et al., 1991) and resistant or susceptible lines have been selected in various countries (Dominik, 2005).

Quantitative trait loci (QTL) mapping can help to dissect the complexity of parasite resistance by identifying candidate genomic regions affecting the trait variation. To this end, different linkage mapping projects have been undertaken to find QTL for parasite resistance (Dominik, 2005). A genome scan was performed by Beh et al. (2002) using lines of sheep diverging for parasite resistance. Different regions were detected as likely to carry genes for resistance although no region was statistically significant after correcting for multiple tests. Davies et al. (2006) genotyped naturally infected lambs to scan regions previously identified as candidates for either genes for resistance or genes for other economical traits to determine whether these candidate regions could be confirmed in an independent dataset. Evidence of linkage was found on chromosomes 2, 13, 14 and 20. Recently, a genome scan performed by Crawford et al. (2006) using divergent lines and naturally infected animals detected a significant QTL on chromosome 8. In general, such selected populations have the advantage of high quality pedigrees and phenotypic data in terms of sample size and accuracy. In order to increase the power of analysis, the populations used for mapping purposes are usually grown under controlled and uniform conditions designed to maximize the genetic contribution to the phenotype (Lymbery, 1996; Lynch and Walsh, 1998). Such experimental designs, however, may not accurately reflect the interactions between genes and environment that occur in natural populations (Erickson et al., 2004; Slate, 2005) and that could contribute to host-parasite co-evolution and host population dynamics (Gulland and Fox, 1992; Gulland et al., 1993; Hudson et al., 1998).

From an evolutionary perspective, it is of interest to know whether major genes for parasite resistance explain observed variation in natural populations in situ. Perhaps such genes can only be detected under highly controlled environmental conditions when genetic variation and statistical power are maximised. In free-living populations, environmental noise and interactions between genetic variation and environmental variation may mask the effects of individual genes (Lynch and Walsh, 1998). In this paper we present a QTL analysis of parasite resistance in a free-living sheep population.

The free-living Soay sheep population on Hirta, St. Kilda, UK, is the subject of a long term project aimed at addressing a wide range of ecological and evolutionary issues (extensively documented in Clutton-Brock and Pemberton, 2004) including the genetics and evolution of parasite resistance in the wild. The Soay sheep is naturally parasitized by several gastrointestinal nematode species (Wilson et al., 2004; Wimmer et al., 2004; Craig et al., 2006), the most prevalent and abundant being strongyles (of which the predominant species are *Teladorsagia circumcincta*, *Trichostrongylus axei*, *Trichostrongylus vitrinus*; see Craig et al., 2006). Different species of protozoans also infect the intestinal tract of Soay sheep; these belong mainly to the genus *Eimeria* but *Cryptosporidium parvum* and *Giardia duodenalis* also occur (Wilson et al., 2004; Craig et al., in press). Keds (*Melophagus ovinus*) also parasitize the Soay sheep, living in the wool and feeding on blood, causing anaemia and irritation (Wilson et al., 2004).

The evolution of parasite resistance in Soay sheep has previously been addressed using two different population genetics strategies, the first being a quantitative genetics approach. Parasite resistance, measured as strongyle faecal egg count (FEC), is under directional selection (Coltman et al., 1999). In addition, there is a positive genetic correlation between body traits and resistance to strongyles (Coltman et al., 2001a) so that resistant sheep also experience better growth. Because body size and parasite resistance are under directional selection, it is expected that in this population the allelic variants associated with small body size and/or low parasite resistance will be eliminated by selection and additive genetic (heritable) variation will be reduced to near zero (Fisher, 1958; Endler, 1986). However, parasite resistance in Soay sheep has low but not null heritable variation – previous population-wide estimates based on an animal model found a heritability for FEC in summer of 0.11 ± 0.02 in males and 0.13 ± 0.01 in females (Coltman et al., 2001a).

In a second approach, previous studies in Soay sheep have examined a number of candidate loci for parasite resistance in simple association studies. Three loci appear to be associated with parasite resistance: the interferon gamma gene (IFNG) on chromosome 3 (Coltman et al., 2001b); the major histocompatibility complex (MHC) on chromosome 20 (Paterson, 1998; Paterson et al., 1998); and the adenosine deaminase gene (ADA) on chromosome 13 (Gulland et al., 1993). It is of great interested to know whether these association studies can be supported in a more rigorous QTL search.

Here, we make use of a previously established mapping pedigree and linkage map (Beraldi et al., 2006; Beraldi et al., unpublished data) and phenotypic data for three classes of parasites (gastrointestinal nematodes, coccidia and keds), to ask: (i) whether we can detect heritable variation for resistance; (ii) whether we can detect QTL for resistance; and (iii) whether any QTL found coincide with previous domestic sheep or Soay sheep studies.

2. Materials and methods

2.1. Study population

The Soay sheep on the islands of Soay and Hirta (St. Kilda archipelago, North West Scotland, UK, 57°49′N, 08°34′W) are feral populations of a breed regarded as the most primitive in Europe (Campbell, 1974; Doney et al., 1974); nowadays, the sheep population of Hirta varies between 600 and 2000 individuals. Since 1985 regular expeditions have been sent to St. Kilda to monitor the population dynamics and to record the life histories of individuals living in Village Bay, Hirta (Clutton-Brock et al., 2004). No predators are present on St. Kilda. All animal handling was undertaken under the appropriate UK Home Office licences.

2.2. Mapping pedigree and linkage map

The whole Soay sheep pedigree file numbers more than 3900 animals. Within this pedigree maternal links were assigned through observation of the animals in the field, whereas paternal links were inferred through molecular analysis (Overall et al., 2005). From the total pedigree, a panel of 588 animals was genotyped at 247 microsatellite and four isoenzyme markers. This subset comprised all the half-sibships with 10 or more individuals and their common parents. The ancestors of the genotyped individuals and the animals linking different sibships ($n = 294$) were not genotyped, but they were included in the mapping pedigree to improve the estimates of kinship and the identity by descent (IBD) coefficients in the variance component analysis. A more thorough description of the mapping pedigree and selection criteria is included in Beraldi et al. (2006). The Soay sheep map covers approximately 90% of the genome with an average inter-marker spacing of 15 cM. Further details of the map characteristics and the technical procedures can be found in Beraldi et al. (2006).

2.3. Phenotypic dataset and measures of parasitism

Phenotypic records of the animals in the mapping pedigree were retrieved from the Soay sheep database. The data analyzed in this study were collected between 1988 and 2005 from animals born between 1979 and 2002.

In the present study, the quantification of sheep resistance to gastrointestinal parasites was based on the indirect measures of strongyle FEC and coccidia faecal oocyst count (FOC). The direct count of parasites would involve the sacrifice of animals and post-mortem examination: this alternative is not feasible because the Soay sheep are protected and the sacrifice of animals would be in conflict with the study of the Soay sheep as a free-living population. However, previous work has shown a correlation between FEC and burden in island Soay populations (Wilson et al., 2004). Strongyle FEC and coccidia FOC were determined as the number of parasite eggs (FEC) or oocysts (FOC) per gram (wet weight) of faeces using a modification of the McMaster technique (MAFF, 1986). Other distinctive helminth species (*Nematodirus* spp., *Moniezia expansa*, *Capillaria longipes* and *Trichuris ovis*) are routinely classified and quantified in Soays but were not abundant enough for analysis. A few hosts that had previously been treated with either anthelminthics or hormones for experimental purposes were excluded from analysis. The count of keds was the total number of keds observed during a 1 min search of the wool on a sheep's belly. The raw data (strongyle FEC, coccidia FOC and ked count) were transformed into the natural logarithm to achieve a distribution closer to normality (all the measurements were increased by one unit before transformation, i.e. Ln(trait + 1), so that zero values remained unchanged after transformation). The genetic and environmental sources of variation of parasite resistance are expected to change with the age of the animals and time of year (Bishop et al., 1996; Coltman et al., 2001b). Therefore, only the samples collected in the August catch up, when most of the data are collected, were included in the analyses. In addition, each parasitic group was analyzed separately in lambs (4-month-old animals) and adults (animals older than 4 months). Sample sizes and summary statistics for each trait are reported in Table 1.

2.4. Definition of fixed effects

Fixed effects influencing the study traits were fitted in the variance component models. In order to facilitate comparisons with previous studies in Soay sheep, the fixed effects fitted for strongyle FEC were the same as those fitted by Coltman et al. (2001b). For consistency, coccidia FOC was also analyzed with the same model. A general linear model analysis implemented in Minitab 14.1 (Minitab Inc.) was applied to determine the amount of variation explained by each fixed effect (Table 2).

2.5. Estimation of variance components

Under the null hypothesis of no segregating QTL, the additive genetic variation of a trait is supposed to be composed by many genes of small effect scattered across the genome. The trait can be modelled as a combination of fixed and random effects (Lynch and Walsh, 1998; Williams and Blangero, 1999):

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{a} + \mathbf{e}$$

where $\mathbf{y}$ is a vector of records on individuals; $\boldsymbol{\beta}$ is a vector of fixed effects; $\mathbf{a}$ is a vector of additive genetic effects (or breeding values) estimated on the basis of the coefficient of co-ancestry between any pair of individuals in the pedigree; $\mathbf{e}$ is a vector of residual effects. $\mathbf{X}$ and $\mathbf{Z}$ are design matrices relating records to the appropriate fixed or random effects.

Heritability (h^2), permanent environment effect (c^2) and residual effect (e^2) were calculated as the ratio of the rele-

Table 1
Characteristics and estimated variance components of the study traits

Trait	Dataset	No. records	No. animals (genotyped)	Mean (SE)[a]	V_A[b] (SE)[a]	CV_A[c] (%)	h^{2d} (SE)[a]	V_C[e] (SE)[a]	CV_C[f] (%)	c^{2g} (SE)[a]	V_E[h] (SE)[a]	CV_E[i] (%)	e^{2j} (SE)[a]
Strongyles	Lambs	383	381 (307)	5.376 (2.150)	0.944 (0.472)	18.08	0.26 (0.12)	NS	—	—	2.626 (0.441)	30.14	0.74 (0.12)
Coccidia	Lambs	230	228 (204)	7.979 (1.228)	0.254 (0.254)	6.32	0.22 (0.21)	NS	—	—	0.915 (0.243)	11.99	0.78 (0.21)
Keds	Lambs	376	374 (310)	1.221 (0.804)	0.022 (0.054)	12.06	0.04 (0.09)	NS	—	—	0.563 (0.067)	61.45	0.96 (0.09)
Strongyles	Adults	962	345 (192)	3.073 (2.749)	0	—	—	0.789 (0.236)	28.90	0.13 (0.04)	5.479 (0.311)	76.17	0.87 (0.04)
Coccidia	Adults	694	240 (155)	5.224 (2.517)	0.310 (0.183)	10.66	0.06 (0.03)	NS	—	—	4.890 (0.305)	42.33	0.94 (0.03)
Keds	Adults	1303	396 (229)	0.123 (0.312)	0.002 (0.002)	40.97	0.03 (0.02)	NS	—	—	0.089 (0.004)	242.50	0.97 (0.02)

[a] Standard error.
[b] Additive genetic variance.
[c] Coefficient of additive genetic variation.
[d] Heritability.
[e] Permanent environmental variance.
[f] Coefficient of permanent environmental variation.
[g] Permanent environmental effect.
[h] Residual variance.
[i] Coefficient of residual variation.
[j] Residual effect.

vant variance component (V_A, additive genetic variance; V_C, permanent environmental variance; V_E, residual variance) to total phenotypic variance (V_P), i.e. $h^2 = V_A/V_P$; $c^2 = V_C/V_P$; $e^2 = V_E/V_P$.

The coefficients of variation were calculated as:

$$CV_i = 100V_i^{1/2}/\bar{x},$$

where the subscript i stands for the additive genetic (A), permanent environment (C) and residual components (E) and $\bar{x}$ is the trait mean.

Variance components were estimated by the restricted maximum likelihood procedure (Lynch and Walsh, 1998) implemented in the software package ASReml (Gilmour et al., 2002).

2.6. QTL mapping

To map putative segregating QTL, an IBD (identity by descent) matrix estimated at any given map position was fitted in the model described above as an additional random effect (George et al., 2000):

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Za} + \mathbf{Zq} + \mathbf{e}$$

where $\mathbf{q}$ is a vector of additive QTL effect. IBD sharing statistics were estimated using pedigree relationships, marker data and map distances described above and in Beraldi et al. (2006). For an initial scan, IBD matrices and variance components were estimated every 10 cM. Putative QTL regions, i.e. those reaching a logarithm of the odds (LOD) score of at least 1, were then scanned every 1 cM. The calculation of the IBD matrices was performed by a Markov chain Monte Carlo (MCMC) which allows the handling of very large and complex pedigrees. This process was implemented in the program Loki (Heath, 1997). LOD scores were calculated as the difference in log-likelihood between QTL and polygenic model according to the equation:

$$\mathrm{LOD} = (L_1 - L_0)/\ln(10)$$

where L_1 is the natural log-likelihood of the QTL model and L_0 the natural log-likelihood of the polygenic model.

Genome-wide suggestive and significant thresholds were obtained by solving Equation 1 of Lander and Kruglyak (1995) assuming a map length of 33.5 Morgans spanning 27 chromosomes. Solutions were 1.9 and 3.3, respectively. The genome-wide significance (LOD = 3.3) corresponds to the probability of finding a false positive every 20 genome scans; the suggestive significance (LOD = 1.9) corresponds to the probability of finding a false positive once per genome scan (Lander and Kruglyak, 1995). Here, all the LOD scores exceeding the arbitrary threshold of 1 are reported. For LOD scores above the suggestive threshold, support intervals for the presence of a putative QTL were defined by the map range within a one-LOD score drop from the peak value; which is equivalent to approximately 95% confidence (Lander and Botstein, 1989).

Table 2
Fixed effects for the study traits fitted in the polygenic and quantitative trail loci models

Trait	Dataset	Sex	Litter size	Birth year	Collection year	Weight	Collection age	Total deviance explained (df)
Strongyles	Lambs	4.2 (1)	0.3 (2)[a]	—	17.4 (14)	3.5 (1)	NF	25.5 (18)
Coccidia	Lambs	0.7 (1)[a]	~0 (2)[a]	—	20.8 (9)	5.6 (1)	NF	27.4 (13)
Ked count	Lambs	5.8 (1)	3.1 (2)	—	4.1 (14)[a]	NF	NF	13.0 (17)
Strongyles	Adults	13.2 (1)	NF	NF	4.0 (17)	0.4 (1)	NF	21.2 (22)
Coccidia	Adults	1.4 (1)	NF	NF	13.4 (12)	1.2 (1)	2.1 (1)	18.2 (15)
Ked count	Adults	NF	NF	4.1 (22)	5.8 (16)	NF	NF	9.9 (38)

The deviance explained (in percentage) and the degrees of freedom used by each term (in brackets) are reported.
NF, Not fitted.
[a] Effect non-significant ($P > 0.05$) but fitted for consistency with previous analyses.

3. Results and discussion

Six (non-independent) traits reflecting the resistance of Soay sheep to gastrointestinal strongyles, coccidia and keds at ages 4 months and 16 months or older were modelled.

3.1. Variance component analysis

Results of the variance component analysis under the polygenic model are presented in Table 1. In lambs, the additive genetic component of strongyle FEC and coccidia FOC accounted for a moderately high proportion of the phenotypic variation although the estimates were not very precise due to the large standard deviations. The heritabilities of strongyle FEC and coccidia FOC in lambs were similar, being 0.26 ± 0.12 and 0.22 ± 0.21, respectively. In adults, no genetic variation was detected for strongyle FEC and very low heritability was detected for coccidia FOC ($h^2 = 0.06 \pm 0.03$). The estimates of heritability reported by Coltman et al. (2001a) for strongyles FEC in Soay sheep were between 0.11 and 0.14. The inconsistency between results from Coltman et al. and the present study could be explained by differences in the pedigree and data selection. In particular, the estimates of Coltman et al. (2001a) are based on animals of any age whereas in this study we differentiated between lambs (4-months-old animals) and adults (animals older than 4 months). Also, higher estimates of heritability in this study may be explained by more reliable inference of parentage. The genotyping of more than 200 markers in the genome scan allowed the detection of pedigree errors that can downwardly bias the estimate of genetic parameters (Charmantier and Reale, 2005). With respect to domestic sheep, the estimates of FEC heritability in Soay sheep reported here do not particularly differ from farmed or experimental populations. However, as heritability is a property of the population and not the species, care should be taken in comparing Soay and domestic sheep because of the differences in life history and environment between a free-living population and managed, selected flocks.

With respect to the coefficients of variation, the CV_A of strongyle FEC in lambs was about three times the CV_A of coccidia FOC in lambs (18.08 and 6.32, respectively) although the heritability of the two traits was similar (0.26 and 0.22, respectively). This suggests that there is greater genetic variation responsible for strongyle FEC than coccidia FOC, but also that the phenotypic variation of strongyle FEC is higher than that of coccidia FOC.

The adult datasets of strongyle FEC, coccidia FOC and ked count in both lambs and adults showed little or zero heritable variation (Table 1). However, the CV_A in adult coccidea FOC (10.66) is higher than in lambs (6.32). This suggests the genetic variation in adults is overwhelmed by environmental variation so that the genetic component makes little contribution to phenotype. The same speculation could be applied to strongyle FEC and ked count but in this case the fact that the means and variances are similar and close to zero make the coefficient of variation difficult to interpret due to its mathematical properties.

3.2. Variance component QTL mapping

Genome scans were performed for strongyle FEC and coccidia FOC in lambs, the two traits with moderate heritability. The LOD score profiles for these traits are shown in Fig. 1 and characteristics of LOD scores higher than 1 are listed in Table 3.

Three LOD scores above 1 but below the suggestive threshold were detected for strongyle FEC (Table 3). These were located on chromosomes 6 (LOD = 1.58), 12 (LOD = 1.49) and 1 (LOD = 1.43). Beh et al. (2002) detected a suggestive QTL for resistance to *Trichostrongylus colubriformis* in 20-week-old sheep on chromosome 6 and a pointwise significant peak (significant at $P < 0.05$ but unadjusted for multiple tests) for 27-week old animals. This group also identified one region on each of chromosomes 1 and 12 reaching the pointwise significance in 27-week-old animals. The scan published in that study does not report the position of the LOD peaks so that it is not possible to determine whether their peaks correspond to those presented here. To the best of our knowledge no study, other then that mentioned above, detected QTL for parasite resistance in the regions reported here.

The scan for coccidia FOC in lambs produced two LOD scores exceeding the suggestive threshold (chromosome 3 with LOD = 2.68 and support interval of approximately 30 cM, and chromosome X with LOD = 2.21 and support interval of approximately 17 cM; Table 3 and Fig. 2), and

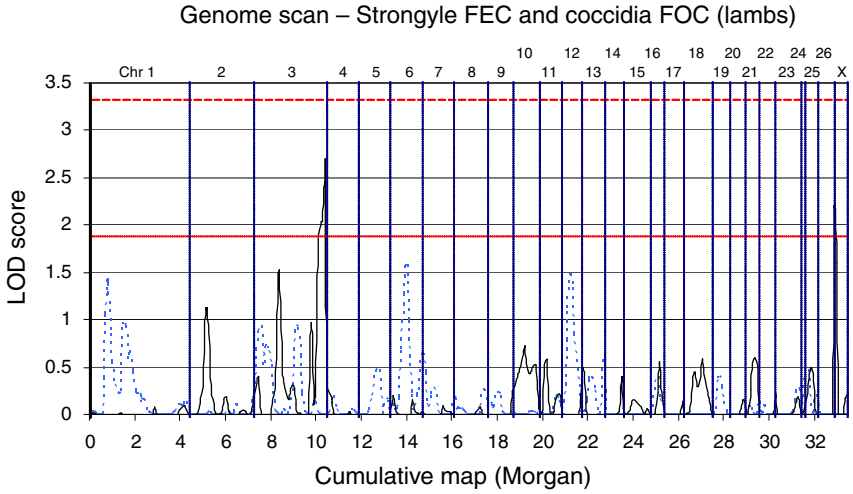

Fig. 1. Whole genome scans of strongyle faecal egg count (FEC; dashed line) and coccidia faecal oocyst count (FOC; continuous line) in lambs. Logarithm of the odds (LOD) score values (ordinate) were plotted against genetic position (abscissa, Morgan scale). Dotted horizontal lines show the genome-wide significance threshold (3.3); dashed lines are the suggestive significance threshold (1.9). Vertical lines mark the chromosome boundaries and chromosome names are displayed at the top.

Table 3
Logarithm of the odds (LOD) scores higher than 1 detected for strongyle faecal egg count and coccidia faecal oocyst count in lambs

Trait	Dataset	LOD	Chr.	Position (cM)	Flanking markers (cM)[a]	
Strongyles	Lambs	1.58	6	74	BMS360 (4)	McM140 (4)
		1.49	12	44	CSSM3 (1)	MCMA52 (19)
		1.43	1	79	BM6465 (7)	CSAP36E (1)
Coccidia	Lambs	2.68[b]	3	328	CSAP39E (17)	CSSME76 (0)
		2.21[b]	X	3	McM158 (3)	MAF45 (32)
		1.52	3	127	RM96 (6)	BM2818 (14)
		1.13	2	89	CSSM37 (7)	FCB128 (3)

[a] In parentheses the distance (cM) of the flanking markers from the quantitative trait loci peak.
[b] Suggestive linkage (LOD >1.9).

two LOD scores exceeding the value of 1 (chromosome 3 with LOD = 1.52 and chromosome 2 with LOD = 1.13; Table 3). The LOD score peak for coccidia FOC in lambs on chromosome X is in the vicinity of one of the telomeres. No previous studies have investigated chromosome X for parasite resistance QTL or genes. Davies et al. (2006) identified three regions on chromosome 3 likely to be linked to different traits related to parasite resistance (IgA activity, *Nematodirus* FEC in August, and strongyle FEC in October) and one region on chromosome 2 linked to *Nematodirus* FEC in September. Although the support intervals of the present study and that of Davies et al. (2006) overlap, the statistical significance of the results and the differences in the two approaches make it difficult at this stage to understand whether the two studies have identified the same regions. Other studies in domestic sheep did not identify QTL in the regions detected here.

Candidate regions identified in previous association studies in Soay sheep did not produce any evidence of linkage in our genome scans. It is known that the IFNG region on chromosome 3 is related to strongyle parasite resistance in domestic sheep (Paterson et al., 1999) and Soay lambs (Coltman et al., 2001b). The IFNG region, located at approximately 244 cM on the Soay map, did not produce any particular evidence of linkage and it is outside the support interval of the suggestive QTL identified for coccidia FOC. The effect of IFNG on strongyle FEC in Soay sheep was estimated by Coltman et al. (2001b) in a dataset larger than the one analyzed here and using a general linear model in which the two alleles of a microsatellite in the IFNG gene were fitted as a fixed factor (Coltman et al., 2001b). The association between FEC and microsatellite alleles was significant at $P = 0.047$ but the variation explained by the microsatellite was very low (0.5% of the total deviance). Consequently, it is not surprising that no QTL was detected in this region considering the differences in datasets and methods of analysis. Similarly, the ADA locus, mapped to chromosome 13 (Beraldi et al., 2006), and the MHC region on chromosome 20 did not produce any evidence of linkage. As with IFNG, the association between the MHC region and strongyle FEC was detected using a larger dataset with a generalized linear model. Failure to detect linkage could be due to insufficient power of the current sample size or lack of informative markers in the target regions although good marker coverage was achieved in the putative regions (Beraldi et al., 2006). However, associ-

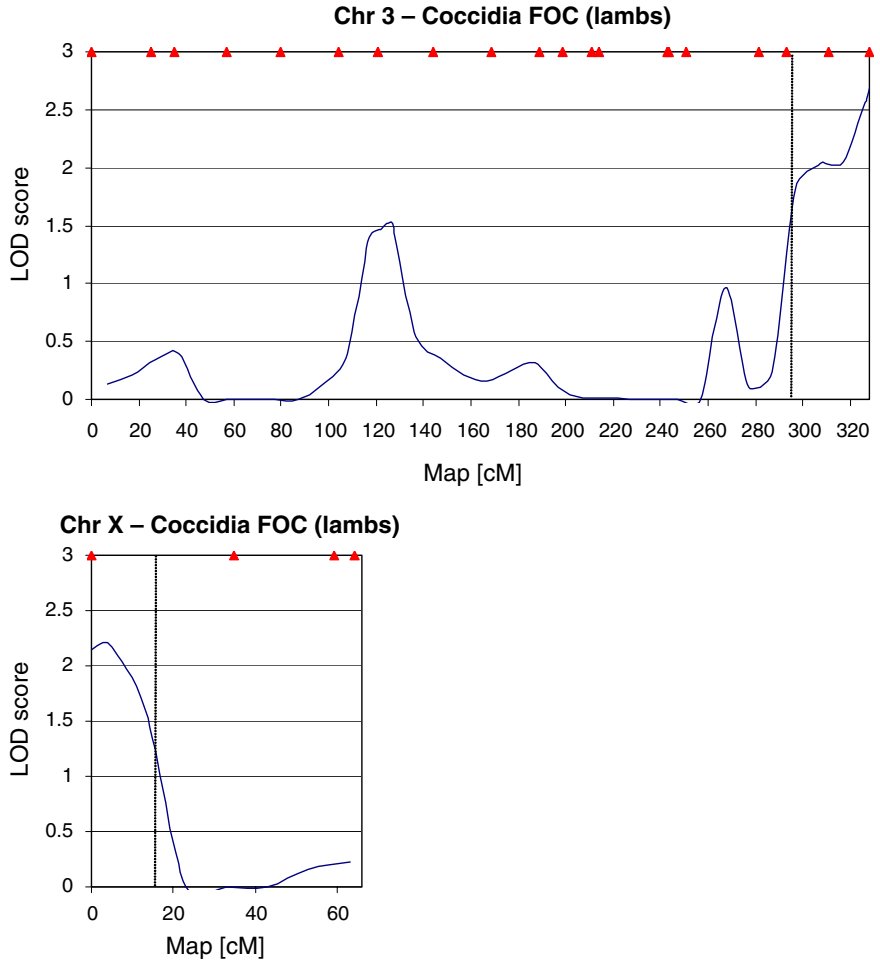

Fig. 2. Detailed map positions of the suggestive quantitative trait loci (QTL) peaks identified by the genome scan for coccidia faecal oocyst count (FOC) in lambs. The abscissas represent the chromosome maps (centimorgan scale). Vertical lines define the 1-LOD (logarithm of the odds) drop support intervals. Triangles on top of the graphs show the marker positions.

ation studies are prone to produce false positive results due to, for example, population structure (Cardon and Bell, 2001). Population stratification occurs if the sample consists of a number of divergent populations which differ in both candidate-locus frequencies and phenotypic frequency. In this case, an association can be detected in the absence of linkage. It should be noted in this respect that, despite its small size, the Soay sheep population is structured into at least three sub-units which are genetically and spatially differentiated (Coltman et al., 2003; Charbonnel and Pemberton, 2005).

3.3. Conclusions and future developments

This paper describes a genome scan in the free-living Soay sheep to detect QTL associated with resistance to different kinds of parasites, here measured as strongyle FEC and coccidia FOC and ked count, through variance component analysis. This is one of the first genome-wide scans for parasite resistance performed in sheep and the first which makes use of a free-living population. Future projects aimed at undertaking the same approach may find the methods and findings of this study to be a useful reference; for this reason, results that are liable to be false positives have been presented. Overall, the methods and data described here show that FEC of strongyle and FOC of coccidia have moderate heritability in lambs and lower or undetectable heritability in adults. Two genomic regions reached the suggestive linkage threshold which could be confirmed or rejected by the genotyping of additional markers mapping in the target region or by analysing more families.

At this stage, the regions identified in this study do not reach high enough significance to allow in depth speculations about the genetic architecture of the study traits and the possibility that these regions are false positives cannot be rejected. However, this is not unusual in QTL mapping projects which start, as in this case, without a priori information and without targeting a specific genomic region. As a comparison, the study of Beh et al. (2002) only detected one region above the suggestive threshold and five regions with pointwise significance. Davies et al. (2006) identified four regions above genome-wide significance but only after having targeted regions previously reported in the literature as responsible for parasite resistance.

The power of analysis of the present study is probably not high enough to detect genes with small effect. Parasite

resistance measured as FEC or FOC is the result of a large number of physiological pathways that from the original infection lead to the egg or oocyst count. The target phenotype is therefore a composite of different traits, many with a strong environmental component. The use of more specific measures of parasite resistance, for example the egg count of individual parasitic species, should make the analysis more powerful as it would focus on a better-defined phenotype. The molecular identification of eggs of single species of nematodes has been initiated by Wimmer et al. (2004). In order to reduce the statistical noise due to the environment it would be advisable to collect as many measurements as possible in different years on the same host individuals in order to detect the component of phenotypic variation due to the permanent environmental conditions.

In the long term, knowledge of the map position of a gene affecting parasite resistance can be used to examine selection at, and molecular evolution of, resistance genes. The future statistical and biological models aimed at explaining the maintenance of genetic variation in Soay sheep will be improved by accounting for variability at specific genomic regions known to affect the level of parasitic infection.

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