

Detection of quantitative trait loci for internal parasite resistance in sheep. I. Linkage analysis in a Romney × Merino sheep backcross population

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SUMMARY

This study aimed to identify putative quantitative trait loci (QTL) that significantly affect internal parasite resistance in a backcross sheep population. A Romney × Merino backcross (to Merino) flock was challenged in 3 separate infections with *Trichostrongylus colubriformis* (primary and secondary) and *Haemonchus contortus* (tertiary). Haematological parameters were measured and faecal worm egg counts (FWEC) were established to estimate parasite burden. QTL mapping was conducted for FWEC and for the changes in haematocrit following *H. contortus* challenge and in eosinophil numbers following *T. colubriformis* challenge. Animals were genotyped for 55 microsatellite markers on selected chromosomes 2, 3, 6, 11, 13, 15, 21, and 22. Four putative quantitative trait loci were found; these being for eosinophil change in the primary infection (OAR 21), for FWEC in the first infection and eosinophil change in the secondary infection (OAR 3) and for FWEC in the secondary infection (OAR 22). No significant quantitative trait loci were detected for FWEC or haematocrit change during the *Haemonchus contortus* infection. The position of the putative quantitative trait loci for eosinophil change on OAR 3 is consistent with other reports of parasite resistance quantitative trait loci, implying some commonality between studies.

Key words: sheep, quantitative trait loci, eosinophils, erythrocytes.

INTRODUCTION

Genetic improvement programmes for internal parasite resistance in sheep were initiated in the early 1970s, after the first cases of helminth resistance to commercially available anthelmintics were reported (Colglazier *et al.* 1974). Since then, considerable research has shown that indirect infection traits, in particular faecal worm egg count (FWEC), have been used successfully to select sheep resistant to *Haemonchus contortus* and *Trichostrongylus colubriformis* (Winton *et al.* 1987; Woolaston *et al.* 1990; Karlsson *et al.* 1991). FWEC has been used most commonly, but other potential selection criteria include associated traits linked to immune responses such as change in blood eosinophils, antibody responses or predictive traits such as haemoglobin type or, for *Haemonchus contortus* pathology, the change in erythrocyte numbers measured as haematocrit or packed cell volume (Eady, 1995). Haematocrit change is a function of total blood loss due to worm burden and the sheep's ability to replace erythrocytes through erythropoiesis (Albers *et al.* 1987).

Commercial implementation of genetic improvement programmes would benefit from alternatives to faecal worm egg counts (FWEC) and blood

parameters, which are both time consuming and expensive to measure. There is a range of other problems with selection based on indirect measures of parasite burden such as FWEC (Hunt *et al.* 2008). For example, natural exposure can vary from year to year or, in a dry climate, can be completely absent, making collection of data in those years impossible. An artificial infection can standardize infections, but this is difficult to achieve in a commercial setting. Therefore, the application of gene marker tests or the detection of quantitative trait loci (QTL) that provide insight into the underlying pathways of internal parasite resistance would be highly beneficial for the sheep wool and meat industries.

There has been an international focus on QTL mapping for parasite resistance in sheep and a range of nematode species, infection protocols and breeds of sheep have been used (Dominik, 2005). Therefore, it is not surprising that few common findings have been discovered across the different studies. Most studies investigated either single parasite challenges or mixed species field infections. This study used a unique approach of multiple successive challenges with 2 nematode species that have differing pathological effects.

The aim of this study was to investigate the existence of putative QTL for internal parasite resistance in sheep. The mapping experiment was designed to allow comparison between nematode

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species (*H. contortus* and *T. colubriformis*), different parasite resistance indicator traits and primary and secondary challenges in *T. colubriformis*.

MATERIALS AND METHODS

The CSIRO Gene mapping flock

In 1992, the CSIRO Gene mapping flock was established with the primary aim of mapping QTL for wool quality and production. For this purpose the Merino and Romney breeds that are divergent for wool characteristics were used. The 2 breeds also differ in their responses to parasites, and Romney parasite burdens are typically much less than those of Merinos kept under the same field conditions (Winton *et al.* 1993) and potentially genes that influence resistance to internal parasites segregate in this population.

Romney sires were crossed with Merino ewes to generate the F1 generation. Two F1 sires were used between 1992 and 1997 to produce groups of half- and full-siblings. No records on parasite challenges exist for these animals. In the years 2003 and 2004 one of the F1 sires, for which sufficient cryopreserved semen was available, was backcrossed to oestrus-synchronized Merino ewes by artificial insemination. In the two years, 399 progeny were born within 10 days. Phenotypes for parasite resistance indicator traits have been collected on these animals and they were used for analysis in this study.

Phenotype data collection

Gene mapping flock backcross animals were born in late spring of 2003 and 2004. After weaning at approximately 5 months of age, all animals were faecal sampled to assess parasite burden. Around 50% of lambs had low WFEC (whole flock median WFEC = 300 eggs/g) and all lambs were treated with anthelmintic drugs (Fenbendazole, Levamisole and Naphthalaphos at the manufacturer's recommended dose). Lambs were then challenged with 3 orally administered parasite infections: a primary and secondary challenge with 20 000 McMaster susceptible strain *T. colubriformis* larvae (*Tc*) and a third challenge with 5000 Kirby1981 isolate *H. contortus* larvae (*Hc*). Each infection proceeded for 5 weeks before an anthelmintic treatment was used to clear the infection, followed by a 1-week break before the next challenge. At the end of the infection regime, animals were again treated with anthelmintics (Fenbendazole, Levamisole and Moxidectin according to the manufacturer's recommended dose).

Faecal samples were taken from the rectum on days 21, 28 and 35 of each infection and FWEC assessed using the McMaster egg flotation technique for each sample. For each infection, the mean of the FWEC at the 3 sampling times was analysed (mean

FWEC 1, 2 and 3). Peripheral blood was collected from the jugular vein into 10 ml vol. K₃EDTA Vacutainer[®] tubes. Five ml of blood was sampled for the primary infection on days 0, 14 and 30 (2003 born animals) and days 0, 12 and 26 (2004 born animals), for the secondary infection on days 0, 4, 13 and 28 (2003) and days 0, 4, 14 and 28 (2004) and for the tertiary infection days 0, 4, 15 and 28 (2003) and days 0, 4, 14 and 29 (2004). The blood was analysed using a Cell-Dyn 350R Autoanalyzer (Abbott) calibrated for ovine samples. The abundance of platelets and leukocytes and erythrocytes was recorded and the haematocrit (% blood volume comprised of erythrocytes) and eosinophil counts were analysed in this study.

The infection and sampling protocol was designed to resemble that used by Beh *et al.* (2002). Each parasite challenge was assessed using larvae cultured from pooled faeces and was found to consist predominantly of the intended parasite.

Marker selection

In total, 160 microsatellite markers were chosen for molecular characterization of the animals born in 2003. These markers were selected to be approximately 20 cM apart and spanned the whole genome. A whole genome scan on 200 animals born in 2003, analysing parasite resistance and wool phenotypes, detected significant QTL on chromosomes 2, 3, 6, 11, 13, 15, 21 and 22. Animals born in 2004 were selectively genotyped for 55 markers spread across these 8 chromosomes. Marker amplicons from the sire and grand-sire animals were assessed, and only those markers for which the sire was heterozygous were used for genotyping of their offspring. The genotypes of the Romney paternal grand-sire and in some cases the Merino paternal grand-dam were also assessed so that each paternally inherited allele in the offspring could be assigned as Romney or Merino. A third of the alleles could not be assigned to be paternal due to allelic similarities in the Romney and Merino breed. Details of all markers used in this study are available on the Australian Sheep Gene Mapping website (<http://rubens.its.unimelb.edu.au/~jillm/jill.htm>).

Data preparation

From the 399 animals of the CSIRO Gene mapping flock born in 2003 and 2004, 361 animals had phenotypic records and data on fixed effect and covariates. Mean faecal worm egg counts of the 3 sampling times (days 21, 28 and 35) for each of the 3 internal parasite infections (mean FWEC1, FWEC2 and FWEC3) were analysed. The change in erythrocyte numbers, expressed as the difference in haematocrit between day 0 and day 35 of the *H. contortus* infection (HctDiff), and the change in eosinophil

numbers as the difference between day 0 and day 28 of each *T. colubriformis* infection (EosDiff1 and EosDiff2) were also analysed. All blood cell parameters were correlated with mean FWEC1, mean FWEC2 and mean FWEC3 to prioritize the parasite resistance traits for analysis. Moderately strong negative correlations exist between erythrocyte parameters (red blood cell count, haemoglobin and haematocrit and the FWEC of the third infection with *H. contortus*) but no other significant correlations were found between blood cell parameters and FWEC (Supplementary Table 2, Online version only). From the work of others, however, eosinophil responses have been implicated in resistance to *T. colubriformis* (Douch *et al.* 1996; Windon, 1996) and in support of our findings changes in haematocrit have been correlated to *H. contortus* worm burden (Le Jambre, 1995), so we analysed these traits. Descriptive statistics for FWEC, EosDiff and HctDiff are provided in Tables 1 and 2.

Unadjusted data were checked for normality using a Shapiro-Wilk test. Mean FWEC1, 2 and 3 were cube root transformed (Cube_FWEC1, Cube_FWEC2, Cube_FWEC3) to adjust the normality properties of the data distribution. Phenotypic data were then adjusted for significant fixed effects and covariates. Fixed effects and covariates included: birth year (2003 and 2004), sex (male and female), birth weight, birth type (single and multiple), rearing type (single and multiple) and weaning weight.

From the 55 markers genotyped across the 2003 and 2004 animals of the CSIRO Gene mapping flock, 5 markers failed in the 2004 animals. Genotype data were systematically checked for genotype errors using a function in R/QTL (Broman *et al.* 2003) which is based on a method developed by Lincoln and Lander (1992). The error LOD provides the significance criterion and genotypes above the threshold Error LOD >3 were removed. Map positions of markers were taken from the Australian Gene Mapping website (<http://rubens.its.unimelb.edu.au/~JILLM/jill.htm>).

Linkage analysis

QTL analyses were performed using R/QTL (Broman *et al.* 2003). The data were analysed using a maximum likelihood approach (Dempster *et al.* 1977). To explore whether results were affected by the analysis method used, additional analyses were undertaken using Haley-Knott regression (Haley and Knott, 1992) and a non-parametric approach, an extension of the Kruskal-Wallis test as described by Kruglyak and Lander (1995). All analysis approaches gave very similar results and only the maximum likelihood results are reported. Genotype probabilities were calculated at 1 cM steps. Experiment-wide significance thresholds of $P < 0.05$ were evaluated by permutation testing

Table 1. Descriptive statistics of faecal worm egg counts (in eggs/gram) at 3 sampling times and their means after infection of the sheep from the CSIRO Gene Mapping flock born in 2003 and 2004 ($n = 399$)

Faecal worm egg count	Mean	Min	Max	Stddev
Infection 1 (<i>T. colubriformis</i>)				
FWEC Day 21	2302	0	6300	1206
FWEC Day 28	2761	0	6700	1205
FWEC Day 35	3391	100	8200	1349
Mean FWEC1	2818	33	5600	981
Infection 2 (<i>T. colubriformis</i>)				
FWEC Day 21	2502	0	7800	1286
FWEC Day 28	3061	0	12 800	1578
FWEC Day 35	3777	0	10 800	1673
Mean FWEC2	3113	0	7400	1174
Infection 3 (<i>H. contortus</i>)				
FWEC Day 21	6654	0	32 400	4662
FWEC Day 28	14 477	0	52 200	9215
FWEC Day 35	15 823	0	56 200	8710
Mean FWEC3	12 307	0	40 400	6710

(1000 permutations). Confidence intervals were calculated as 95% Bayesian credible interval in R/QTL (Broman *et al.* 2003). The false discovery rate (FDR) was established by calculating the ratio of expected to observed QTL resulting from the analysis. The expected number of QTL found by chance is the product of the number of chromosomes, the number of traits and the significance threshold.

RESULTS

Descriptive statistics

Table 1 shows the descriptive statistics of FWEC on the 3 sampling days following each of the 3 infections and their means. The data demonstrate that the challenge protocol applied in this experiment resulted in high levels of individual variation within each infection.

Table 2 summarizes the mean, minimum, maximum and standard deviations of the eosinophil counts for the primary and secondary infections and haematocrit level for the tertiary infection. Eosinophil counts increased and haematocrit decreased with increasing length of parasite infection.

Linkage analysis

Table 3 summarizes the significant results from the linkage analysis and provides detailed information on the position of the putative QTL, adjacent markers, permutation LOD score and the 95% confidence interval. The LOD profiles are shown in Figs 1–3.

Chromosome 22 showed the most significant QTL for Cube_FWEC2. The 95% confidence interval was 23 cM and is the smallest for the putative QTL found in this study. Chromosome 21 yielded a putative

Table 2. Descriptive statistics of eosinophil counts ($10^6/\text{ml}$) (Eos) and haematocrit (%) (Hct) at different sampling times, the mean difference of eosinophil counts between day 0 and day 28 (EosDiff), and the mean difference in haematocrit between day 0 and day 35 (HctDiff) of the sheep from the CSIRO Gene Mapping flock born in 2003 and 2004 ($n=399$)

Haematology measure	Mean	Min	Max	Stddev
Infection 1 (<i>T. colubriformis</i>)				
Eos Day 0	0.05	0.00	0.60	0.06
Eos Day 14	0.07	0.00	0.34	0.05
Eos Day 28	0.09	0.00	0.71	0.08
EosDiff1	0.04	-0.54	0.69	0.09
Infection 2 (<i>T. colubriformis</i>)				
Eos Day 0	0.08	0.00	0.57	0.07
Eos Day 4	0.09	0.00	0.61	0.08
Eos Day 14	0.10	0.00	0.44	0.07
Eos Day 28	0.09	0.00	0.48	0.07
EosDiff2	0.02	-0.36	0.38	0.08
Infection 3 (<i>H. contortus</i>)				
Hct Day 0	30.00	19.00	36.00	2.00
Hct Day 4	29.00	22.00	37.00	2.00
Hct Day 14	29.00	23.00	35.00	2.00
Hct Day 35	22.00	11.00	33.00	3.00
HctDiff	-7.00	-18.00	2.00	3.00

QTL for EosDiff1 at 66 cM at the end of the chromosome. Only 3 markers were genotyped on chromosome 21.

Chromosome 3 showed 2 significant QTL for Cube_FWEC1 and EosDiff2 in 2 different positions on the chromosome. The 95% confidence intervals for both QTL were very large. No QTL were found for any indicator traits for the tertiary infection with *H. contortus*.

The significant putative QTL found in this study only explain between 1.36% and 3.26% of the total phenotypic variance and their effect sizes are small (Table 4). The QTL effects are expressed as the phenotypic average of the progeny carrying the Romney allele minus the phenotypic average of those carrying the Merino allele. The QTL effect for Cube_FEC2 was negative, indicating that the Romney allele was associated with lower egg counts.

Six parasite resistance traits were analysed on 8 chromosomes at a significance level of $P < 0.05$ and 2.4 QTL were expected to be found by chance. Four putative QTL were found. This results in a false discovery rate (FDR) of FDR = 80%.

DISCUSSION

This study investigated putative QTL for measures of parasite resistance using *H. contortus* and *T. colubriformis* infections in sheep. Four putative QTL for measures of parasite resistance to *T. colubriformis* in sheep were found; two on chromosome 3, and one each on chromosomes 21 and 22. Previously

published studies have not reported QTL for internal parasite resistance on chromosomes 22, but Moreno *et al.* (2006) found a putative QTL for FWEC in response to a field infection on chromosome 21 and several studies have found evidence for QTL on chromosome 3. In a fine mapping study on a Romney population, a QTL was mapped close to a region that contains the interferon gamma gene on chromosome 3 (Paterson *et al.* 2001). Similarly, Coltman *et al.* (2001) confirmed an association between alleles at the interferon gamma locus and resistance indicator traits in response to a natural *Teledorsagia circumcincta* infection in Soay sheep. A more recent study by Davies *et al.* (2006) also found strong evidence for parasite resistance QTL on chromosome 3. Beh *et al.* (2001) reported a putative QTL on chromosome 3 for cube root transformed mean FWEC of a secondary infection with *T. colubriformis*. Although, Beh *et al.* (2001) pointed out that the marker distances were large in their study for this particular region. The conjunction of findings between our study and others on chromosome 3 suggests strongly that future work on this chromosome is warranted. Crawford *et al.* (2006) detected 6 QTL for parasite burden and immune response to infection in a large outcross design on chromosomes 2, 8, 11 and 23. None of these results overlap for the 3 chromosomes that were genotyped across both studies, but the phenotypes recorded were different.

The QTL described herein have large confidence intervals and account for a small percentage of the total phenotypic variation of the traits. The expression of a particular level of resistance to internal parasites is the consequence of a cascade of events that underlie the control of a large number of genes, as shown in a number of recent gene expression studies (Diez-Tascon *et al.* 2005; Andronicos *et al.* 2009). This study found QTL with much smaller effect sizes probably because a large number of genes control a complex trait like parasite resistance; a hypothesis that is shared by Crawford *et al.* (2006). It is also supported by findings of Marshall *et al.* (2009), who found a large number of QTL with moderate size effects in a mapping experiment for resistance to *H. contortus*. A simulation study by Hayes and Goddard (2001) has shown that the majority of putative QTL for any trait are expected to have a small to moderate size effect. If internal parasite resistance is truly controlled by a large number of genes with small effect, then this study might not have been powerful enough to detect the small effect sizes, which could explain the lack of significant QTL for internal parasite resistance in this study.

Our study shows the non-alignment of QTL for FWEC following *T. colubriformis* infection and the change in eosinophil numbers as a result of these infections for this particular experiment. Also there was no evidence of a phenotypic relationship between the two traits (Supplementary Table 3, Online

Table 3. QTL position, marker bracket and 95% confidence interval (95% CI) of significant QTL as assessed by the 5% permutation LOD score (PermLOD) for the parasite resistance traits

Trait	Chr	LOD score	Perm LOD	Position (cM)	Marker bracket		95% CI (cM)
					Marker 1	Marker 2	
Cube_FWEC1	3	1.93	1.83	62	OARCP34	CSAP31e	278.4
EosDiff2	3	2.20	1.77	213	CSAP019	MAF23	182.0
EosDiff1	21	1.80	1.22	66	CSR272	BMS1948	33.4
Cube_FWEC2	22	3.03	1.24	52	HEL11	BM6041	23.0

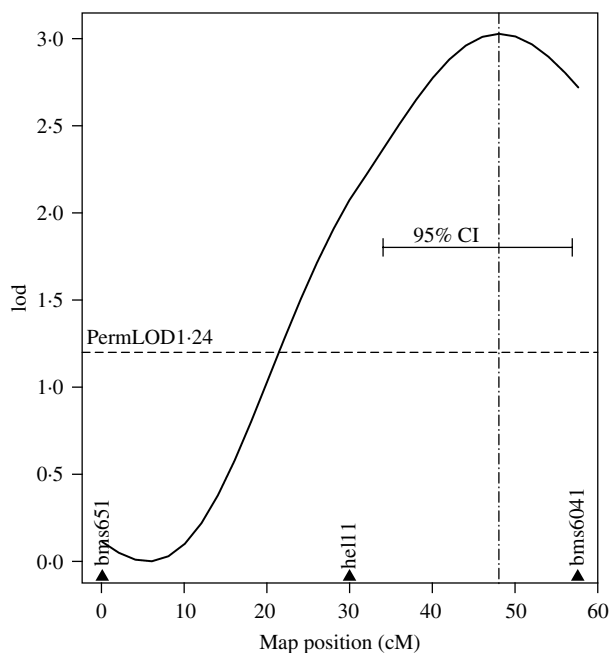


Fig. 1. LOD profile of chromosome 22 for Cube_FWEC2, indicating the significant QTL by the vertical broken line, the permutation threshold (PermLOD) by the horizontal broken line and the confidence interval (95% CI).

version only) even though Davies *et al.* (2005) found strong negative genetic and moderately negative phenotypic correlations between FWEC and eosinophil count in Scottish Black face sheep. Earlier research on the relationship between eosinophil numbers and FWEC has also resulted in conflicting results. Windon *et al.* (1987) observed an increase in eosinophil responsiveness as characteristic of parasite resistance, whereas Woolaston *et al.* (1996) observed no relationship between FWEC and eosinophil count.

A novel aspect of the experimental protocol used in this study was that the sheep were artificially challenged with 2 parasites in separate infections. This is a different approach from that used in other studies in which resistance of sheep to a single parasite (Beh *et al.* 2001; Marshall *et al.* 2005) or the responses to mixed field infections (Paterson *et al.* 2001; Davies *et al.* 2006) were investigated. In the most recent major mapping study on parasite resistance

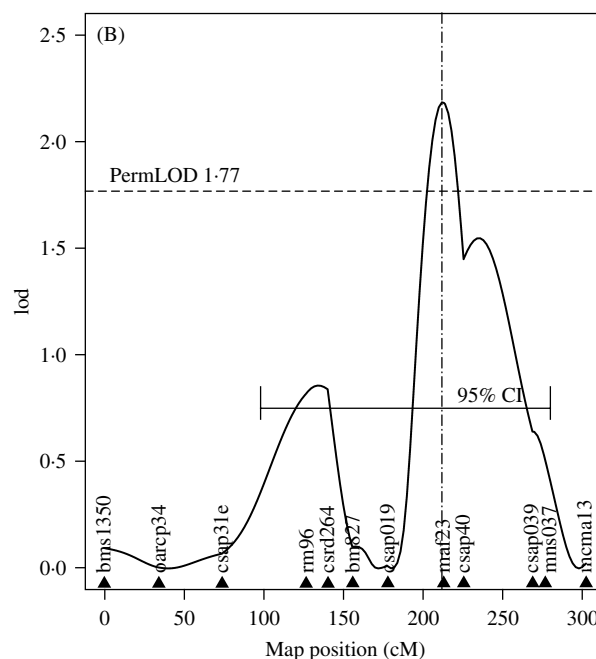
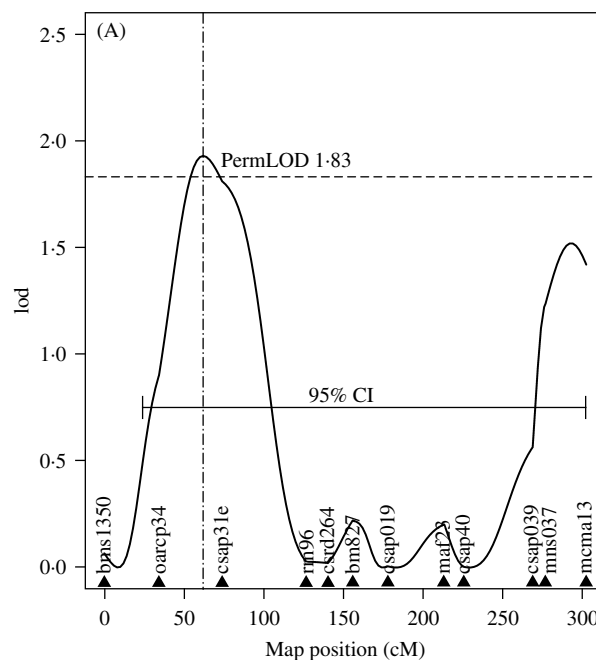


Fig. 2. LOD profiles of chromosome 3 for Cube_FWEC1 (A) and EosDiff2 (B), indicating the significant QTL by the vertical broken line, the permutation threshold (PermLOD) by the horizontal broken line and the confidence interval (95% CI).

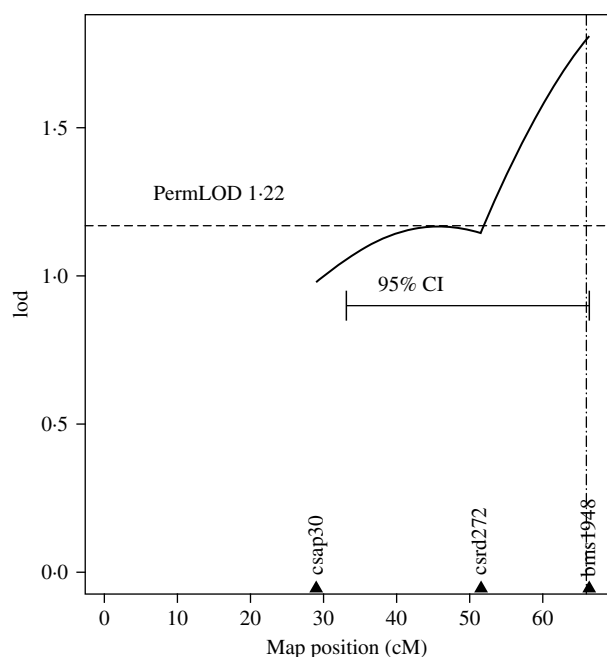


Fig. 3. LOD profiles of chromosome 21 for EosDiff1, indicating the significant QTL by the vertical broken line, the permutation threshold (PermLOD) by the horizontal broken line and the confidence interval (95% CI).

(Crawford *et al.* 2006) lambs were subjected to consecutive field challenges, in a manner relevant to commercial sheep production. However, the artificial challenges utilized in this study enabled the investigation of resistance traits to specific Strongyloid nematodes (*H. contortus* and *T. colubriformis*) separately.

No overlapping QTL were found for primary and secondary infections with *T. colubriformis*. One reason might be that around 50% of lambs in this study carried a parasite burden prior to the first infection, because it is difficult to avoid an infection of lambs under field conditions. We minimized exposure by using anthelmintic treatment of the dams and by paddock rotation, and also by beginning the experiment as soon as possible after weaning the animals, but there was still a low level of exposure to infection. Consequently, the first infection with *T. colubriformis* was not a primary infection for at least half the animals, although it was a synchronized infection given after resident parasites had been removed using anthelmintic drugs.

Our approach enabled the investigation of underlying resistance pathways to only one or both parasites in series. It has been shown that selection of resistance to one or other of these parasite species leads to cross-resistance to both parasites (Ingham *et al.* 2007). In this study, no significant results were found that would suggest overlapping putative QTL for resistance to *H. contortus* and *T. colubriformis* infection. Nevertheless, genetic relationships between FWEC from infections with one

Table 4. Percentage of phenotypic variance explained by the QTL (%Var) and the QTL effect and its standard error (S.E.) for significant QTL

Trait	%Var	QTL effect	S.E.
Cube_FWEC1	1.36	2.534	1.151
EosDiff2	2.66	0.028	0.009
EosDiff1	2.06	0.023	0.008
Cube_FWEC2	3.26	-3.636	1.031

species of nematode and FWEC from infections with other species has been demonstrated (Windon *et al.* 1987; Gruner *et al.* 2004) and might be attributable to loci that could not be detected in this study.

The extent of haematological phenotypes collected has not been reported in previous mapping studies for internal parasite resistance in sheep. The combination of measurement of FWEC and blood cell parameters enables the possibility of gaining insight into the genetic relationships between egg counts taken at different infection times and changes in the numbers of circulating blood cells. This first analysis has concentrated upon the changes in cell numbers between day 0 and day 28 of infections, and finds no common chromosomal regions associated with FWEC and haematological measures. However, this does not exclude the possibility of QTL influencing both these traits in other populations.

This study detected a low number of putative QTL. Due to constraints, data were only available for 1 single sire half-sib family with 399 progeny. Potentially this could have been another reason for the small number of significant QTL found, if this particular sire did not segregate for QTL of detectable size even though the design provides sufficient power for the detection of moderate size QTL. The validity of the results of this study is limited to this particular sire family and can not be generalized. Furthermore, it could be possible that the Romney and Merino breeds were genetically not as divergent as expected from phenotypic observations (Windon *et al.* 1993). Further work needs to investigate and verify the results in multiple families with higher marker density.

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REFERENCES

- Albers, G. A. A., Gray, G. D., Piper, L. R., Barker, J. S. F., LeJambre, L. F. and Barger, I. A.** (1987). The genetics of resistance and resilience to *Haemonchus contortus* infection in young Merino sheep. *International Journal for Parasitology* **17**, 1355–1363.
- Andronicos, N., Hunt, P. W. and Windon, R. G.** (2010). Expression of genes in gastrointestinal and lymphatic tissues during parasite infection in sheep genetically resistant or susceptible to *Trichostrongylus colubriformis* and *Haemonchus contortus*. *International Journal for Parasitology* **40**, 417–429.
- Beh, K. J., Callaghan, M. J., Leish, Z., Hulme, D. J., Lenane, I. and Maddox, J. F.** (2001). A genome scan for QTL affecting fleece and wool traits in Merino sheep. *Wool Technology and Sheep Breeding* **49**, 88–97.
- Beh, K. J., Hulme, D. J., Callaghan, M. J., Leish, Z., Lenane, I., Windon, R. G. and Maddox, J. F.** (2002). A genome scan for quantitative trait loci affecting resistance to *Trichostrongylus colubriformis* in sheep. *Animal Genetics* **33**, 97–106.
- Broman, K. W., Wu, H., Sen, S. and Churchill, G. A.** (2003). R/QTL: QTL mapping in experimental crosses. *Bioinformatics* **29**, 889–890.
- Colglazier, M. L., Kates, K. C. and Enzie, F. D.** (1974). Cambendazole-resistant *Haemonchus contortus* strain in sheep: further experimental development. *Journal of Parasitology* **60**, 269–292.
- Coltman, D. W., Wilson, K., Pilkington, J. G., Stear, M. J. and Pemberton, J. M.** (2001). A microsatellite polymorphism in the gamma interferon gene is associated with resistance to gastrointestinal nematodes in a naturally-parasitized population of Soay sheep. *Parasitology* **122**, 571–582.
- Crawford, A. M., Paterson, K. A., Dodds, K. G., Diez Tascon, C., Williamson, W. A., Thomson, M. R., Bisset, S. A., Beattie, A. E., Greer, G. J., Green, R. S., Wheeler, R., Shaw, R. J., Knowler, K. and McEwan, J. C.** (2006). Discovery of quantitative trait loci for resistance to parasitic nematode infection in sheep: I. Analysis of outcross pedigrees. *BMC Genomics* **7**, Article number 178.
- Davies, G., Stear, M. J., Benothman, M., Abuagob, O., Kerr, A., Mitchell, S. and Bishop, S. C.** (2006). Quantitative trait loci associated with parasitic infection in Scottish blackface sheep. *Heredity* **96**, 252–258.
- Davies, G., Stear, M. J. and Bishop, S. C.** (2005). Genetic relationships between indicator traits and nematode parasite infection levels in 6-months-old lambs. *Animals Science* **80**, 143–150.
- Dempster, A. P., Laird, N. M. and Rubin, D. B.** (1977). Maximum likelihood for incomplete data via the EM algorithm. *Journal of the Royal Statistical Society B* **39**, 1–38.
- Diez-Tascon, C., Keane, O. M., Wilson, T., Zadissa, A., Hyndman, D. L., Baird, D. B., McEwan, J. C. and Crawford, A. M.** (2005). Microarray analysis of selection lines from outbred populations to identify genes involved with nematode parasite resistance in sheep. *Physiological Genomics* **21**, 59–69.
- Dominik, S.** (2005). Quantitative trait loci for internal nematode resistance in sheep: a review. *Genetics Selection Evolution* **37** (Suppl. 1) S83–S96.
- Douch, P. G. C., Green, R. S., Morris, C. A., McEwan, J. C. and Windon, R. G.** (1996). Phenotypic markers for selection of nematode resistant sheep. *International Journal for Parasitology* **26**, 899–911.
- Eady, S. J.** (1995). Phenotypic traits associated with resistance to internal parasites. In *Breeding for Resistance to Infectious Disease in Small Ruminants* (ed. Gray, G. D., Woolaston, R. R. and Eaton, B. T.), pp. 219–233. ACIAR, Canberra, Australia.
- Gruner, L., Bouix, J. and Brunel, J. C.** (2004). High genetic correlations between resistance to *Haemonchus contortus* and to *Trichostrongylus colubriformis* in INRA 401 sheep. *Veterinary Parasitology* **119**, 51–58.
- Hayes, B. J. and Goddard, M. E.** (2001). The distribution of the effects of genes affecting quantitative traits in livestock. *Genetics Selection Evolution* **33**, 209–229.
- Haley, C. S. and Knott, S. A.** (1992). A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* **69**, 315–324.
- Hunt, P., McEwan, J. and Miller, J.** (2008). Future perspectives for the implementation of genetic markers for parasite resistance in sheep. *Tropical Biomedicine* **25**(1s), 18–33.
- Ingham, A., Reverter, A., Windon, R., Hunt, P. and Menzies, M.** (2008). Gene expression changes in genetically resistant and susceptible sheep following gastrointestinal nematode challenge. *International Journal for Parasitology* **38**, 431–442.
- Karlsson, L. J. E., McLoed, I. M., Leelwardana, D. H., Sissoev, K. and Simmons, J.** (1991). Selection for nematode resistance in sheep in the Australian Mediterranean climate zone. In *Breeding for Disease Resistance in Sheep* (ed. Gray, G. D. and Woolaston, R. R.), pp. 131–138. Australian Wool Cooperation, Melbourne, Australia.
- Kruglyak, L. and Lander, E. S.** (1995). A non-parametric approach for mapping quantitative trait loci. *Genetics* **139**, 1421–1428.
- Le Jambre, L. F.** (1995). Relationship of blood loss to worm numbers, biomass and egg production in *Haemonchus* infected sheep. *International Journal for Parasitology* **25**, 269–273.
- Lincoln, S. E. and Lander, E. S.** (1992). Systematic detection of errors in genetic linkage data. *Genomics* **14**, 604–610.
- Marshall, K., Maddox, J. F., Lee, S. H., Zhang, Y., Kahn, L., Graser, H. U., Gondro, C., Walkden-Brown, S. W. and van der Werf, J. H. J.** (2009). Genetic mapping of quantitative trait loci for resistance to *Haemonchus contortus* in sheep. *Animal Genetics* **40**, 262–272.
- Marshall, K., van der Werf, J. H. J., Maddox, J. F., Graser, H. U., Zhang, Y., Walkden-Brown, S. W.**

- and Kahn, L.** (2005). A genome scan for quantitative trait loci for resistance to gastrointestinal parasite *Haemonchus contortus* in sheep. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **16**, 115–118.
- Moreno, C. R., Gruner, L., Scala, A., Mura, L., Shibli, L., Amigues, Y., Sechi, T., Jacquet, P., Francois, D., Bouix, J., Carta, A. and Rupp, R.** (2006). QTL for resistance to internal parasites in two designs based on natural and experimental conditions of infection. *Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, August 13–18, Belo Horizonte, MG, Brazil, Paper number 15-05*.
- Paterson, K. A., McEwan, J. C., Dodds, K. G., Morris, C. A. and Crawford, A. M.** (2001). Fine Mapping a locus affecting host resistance to internal parasites in sheep. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **13**, 91–94.
- Windon, R. G.** (1996). Genetic control of resistance to helminths in sheep. *Veterinary Immunology and Immunopathology* **54**, 245–254.
- Windon, R. G., Dineen, J. K. and Wagland, B. M.** (1987). Genetic control of immunological responsiveness against the intestinal nematode *Trichostrongylus colubriformis* in lambs. In *Merino Improvement Programs in Australia* (ed. McGuirk, B. J.), pp. 371–375. Australian Wool Corporation, Melbourne, Australia.
- Windon, R. G., Gray, G. D. and Woolaston, R. R.** (1993). Genetic control of resistance to gastro-intestinal nematodes. *Proceedings of the 3rd International Sheep Veterinary Society Conference* **17**, 37–51.
- Woolaston, R. R., Barger, I. A. and Piper, L. R.** (1990). Response to helminth infection of sheep selected for resistance to *Haemonchus contortus*. *International Journal for Parasitology* **20**, 1015–1018.
- Woolaston, R. R., Manuelli, P., Eady, S. J., Barger, I. A., Le Jambre, L. F., Banks, D. J. D. and Windon, R. G.** (1996). The value of circulating eosinophil count as a selection criterion for resistance of sheep to trichostrongyle parasites. *International Journal for Parasitology* **26**, 123–126.