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Fine mapping of trypanosomiasis resistance loci in murine advanced intercross lines

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Abstract. We have previously reported the results of genome-wide searches in two murine F_2 populations for QTLs that influence survival following *Trypanosoma congolense* infection. Three loci, *Tir1, Tir2,* and *Tir3,* were identified and mapped to mouse Chromosomes (Chrs) 17, 5, and 1 respectively, with confidence intervals (CIs) in the range 10–40 cM. The size of these CIs is to a large degree the consequence of limited numbers of recombination events in small chromosomal regions in F_2 populations. A number of population designs have been proposed to increase recombination levels in crosses, one of which is the advanced intercross line (AIL). Here we report fine mapping of *Tir1, Tir2,* and *Tir3* in G6 populations of two independent murine AILs created by crossing the C57BL/6J strain with the A/J and BALB/cJ strains, respectively. Data were analyzed by two methods that gave equally informative and similar results. The three QTLs were confirmed in the A/J × C57BL/6J AIL and in the combined data set, but *Tir2* was apparently lost from the BALB/cJ × C57BL/6J AIL. The reduction in CIs for the *Tir* loci ranged from 2.5 to more than ten-fold in G6 populations by comparison with CIs obtained previously in the equivalent F_2 generations. Mapping in the AILs also resolved the *Tir3* locus into three trypanosomiasis resistance QTLs, revealing a degree of complexity not evident in extensive studies at the F_2 level.

Introduction

Most quantitative trait loci (QTL) detection and mapping studies in mice have been carried out in F_2 intercross and backcross designs. As a consequence of limited numbers of recombination events in small chromosomal regions, this has generally restricted mapping to relatively large confidence intervals (CIs) (Darvasi and Soller 1995). However, precise localization of quantitative trait loci (QTL) is required for positional cloning, and positional candidate gene identification is facilitated by high-resolution mapping. In order to meet this need, a number of population designs have been proposed that increase recombination levels in QTL-segregating populations. Among these is the advanced intercross design (Darvasi and Soller 1995). An advantage of this approach over congenic approaches is its suitability for simultaneous refinement of map positions of multiple loci. In addition, the advanced intercross design lends itself to situations where information on the number of QTLs in a particular region is equivocal, as data obtained with advanced intercross lines potentially allow linked QTLs to be dissected into the constituent loci (Darvasi 1998).

An advanced intercross line (AIL) is produced from an F_2 population by random intercrossing (avoiding sibling pairing) in each generation from F_2 onwards, until the desired advanced intercross (G3, G4, G5 etc.) is attained. For QTL mapping purposes, only individuals in the final generation are phenotyped and genotyped (Darvasi and Soller 1995). In this way, the many recombinations required for high-resolution mapping of QTL are accumulated in a relatively small population over many generations, which avoids producing and examining many progeny in a large F_2 or backcross population. With the same population size and QTL effect, and when marker spacing is not limiting, the 95% CI of a QTL map location can be reduced, in principle, by a factor of $t/2$, where t is the number of the advanced generations. This reduction can be obtained, in practice, up to the sixth or eighth generation, if each generation is derived from a minimum of approximately 50 breeding pairs of the previous generation (Darvasi and Soller 1995).

Trypanosoma congolense causes a form of tsetse fly-transmitted African trypanosomiasis (Murray and Gray 1984), which is of economic importance in livestock (Winrock International 1992). Previously, we reported the results of genome-wide searches in two murine F_2 populations for QTLs that influence survival following *T. congolense* infection (Kemp et al. 1996, 1997). Three loci, *Tir1, Tir2,* and *Tir3,* were identified and mapped to mouse Chromosomes (Chrs) 17, 5, and 1 respectively, with CIs in the region of 10–40 cM.

In this report we describe the application of the advanced intercross design in higher resolution mapping and further characterization of *Tir1, 2,* and *3* using two analysis methods in two G6 populations.

Materials and methods

Advanced intercross lines. AILs were developed by crossing mice of the trypanosomiasis-resistant C57BL/6J strain (B6) with a) mice of the susceptible A/J strain (A) and b) mice of the susceptible BALB/cJ strain (B). The new lines were designated Ilri:A,B6 and Liv:B,B6 respectively. Phenotyping, genotyping, and linkage analysis were performed in G6 populations designated (AxB6)G6 and (BxB6)G6. C57BL/6J OlaHsd (B6), A/J OlaHsdnd (A), and BALB/cJ OlaHsdce (B) were purchased from Harlan UK Ltd. (Bicester, Oxon, UK). F_1 mice were produced by using both males and females of each parental strain. F_2 mice of each cross were generated by intercrossing F_1s, and then 50 G3 litters were produced from 50 F_2 pairs. Sibling pairing was avoided, but otherwise pairing was at random. Similarly, G4 and G5 generations were each produced from 50 successful pairings. In the final stage, multiple mating of 161 separate pairs of (AxB6) G5 mice produced 1986 G6 offspring; 95 (BxB6) G5 pairs produced 912 G6 offspring.

All authors made equal contributions to this study.

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Table 1. Murine *T. congolense* trypanosomiasis resistance QTLs. Localization and significance in advanced intercross lines at the 6th generation.[a]

QTL	Chrom-osome	Population	LOD score	Location (cM)[b]	Nearest marker(s)	95% C.I.[c] (cM)
Tir1	17	BxB6	20.08	18.2	*D17Mit16*	2.8 (17.4–20.2)
Tir1	17	AxB6	19.35	17.9	*D17Mit175*	1.3 (17.3–18.6)
Tir1	17	Combined	37.56	18	*D17Mit16*	0.9 (17.4–18.3)
Tir2	5	AxB6	4.63	42	*D5Mit113*	16 (38–54)
Tir2	5	Combined	5.46	44	*D5Mit114*	12 (39–51)
Tir3a	1	BxB6	4.15	60	*D1Mit87*	9 (56–65)
Tir3a	1	AxB6	8.07	62.5	*D1Nds2*	0.7 (62.3–63)
Tir3a	1	Combined	9.94	59.2	*D1Nds2*	1.8 (58.8–60.6)
Tir3b	1	BxB6	5.88	74	*D1Mit286–D1Mit268*	11 (69–80)
Tir3b	1	AxB6	6.8	70	*D1Mit286–D1Mit102*	16 (63–79)
Tir3b	1	Combined	11.3	71	*D1Mit286–D1Mit102*	10 (68–78)
Tir3c	1	BxB6	3.78	92	*D1Mit113*	9 (86–95)
Tir3c	1	AxB6	5.49	96	*D1Mit16*	18 (83–101)
Tir3c	1	Combined	7.62	93	*D1Mit113*	8 (90–98)

[a] Data are derived from analyses of three populations: (BxB6)G6, (AxB6)G6, and the two G6 populations combined. Analyses were according to Haley and Knott (1992).
[b] Distance from centromere.
[c] The 95% CI is taken as the map distance between the points on either side of the LOD score peak at which the LOD score falls by 2. This column shows size (in cM) of CI, with position (cM from the centromere) in parentheses.

Trypanosomiasis challenge. 1986 (AxB6)G6 and 892 (BxB6)G6 mice, together with control parental strain mice, were challenged at 12 weeks of age by intraperitoneal inoculation of 4×10^4 bloodstream forms of *T. congolense* clone IL1180 (Masake et al. 1983; Nantulya et al. 1984). In the following 14 days, blood samples were collected daily from the tail tip of all challenged mice and examined for evidence of infection. Additional unchallenged control mice were caged alongside challenged mice and were sampled in the same way.

Response to challenge and phenotyping. a) (AxB6)G6. Infections were confirmed in 1685 (AxB6)G6 mice, of which 74 were excluded from the experiment for technical reasons. All of the remaining 1611 mice succumbed to infection, with a mean survival time of 81 days. b) (BxB6)G6. Of 892 (BxB6)G6 mice inoculated, 37 did not become detectably parasitemic and were excluded from further analysis. The challenge was terminated after 167 days, when 39 of 43 surviving mice had become aparasitemic and had thus apparently self-cured. The survival times of these mice ranged from 11 to 167 days, with a mean of 75 days. When survivors were excluded, the mean survival time was 71 days.

The phenotype studied was survival time measured in days following the day of challenge (day 0).

Genotyping and linkage analysis. In total, 1611 (AxB6)G6 and 855 (BxB6)G6 mice were infected and provided survival data for analysis. Following the challenge and monitoring period, genomic DNA of G6 and parental strain mice was prepared from tails by conventional methods. Sixty-eight and 88 microsatellites in (AxB6)G6 and (BxB6)G6 mice, respectively, located at intervals of 0.5–3 cM across Chrs 17, 5, and 1, were genotyped as described previously (Kemp et al. 1997). Genotyping was initially restricted to mice representing the phenotypic extremes. Thus, in the (AxB6)G6 population, the first 200 mice to succumb to infection and the last 200 mice that died were genotyped. In the (BxB6)G6 population, the first 75 mice to succumb, the last 37 that died, as well as 39 self-curing survivors, were genotyped. Subsequently, 100 (BxB6)G6 with mid-range survival times were genotyped at microsatellite loci around LOD score peak points.

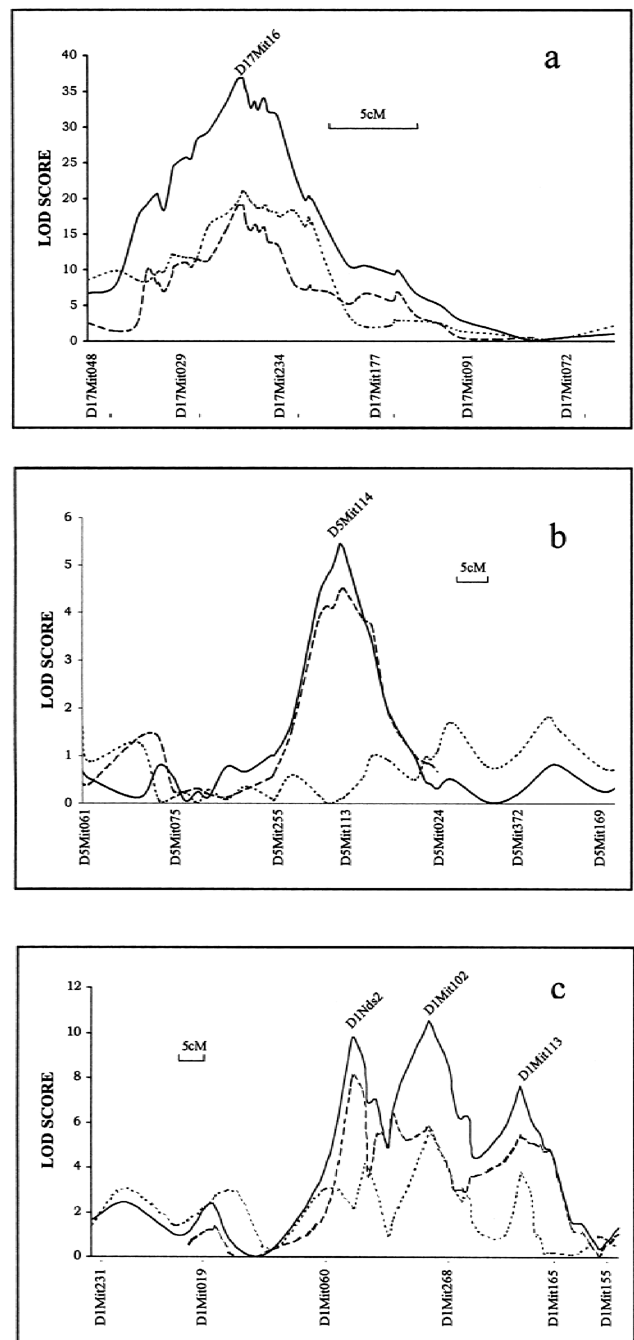

Fig. 1. LOD scores of association of survival with SSLP marker genotype in murine advanced intercross lines at the 6th generation, following challenge with *T. congolense.* Scores are derived from analyses of three populations: (BxB6)G6 ······ , (AxB6)G6 - - - , and the two G6 populations combined ——— . Figs. 1a, b, and c show scores on parts of Chrs 17, 5, and 1, respectively. The positions of relevant SSLP markers are shown on the Y axis. Analyses were according to Haley and Knott (1992).

Genetic and phenotypic data were analyzed by using both the MAPMAKER/EXP/QTL package (Lander et al. 1987; Lincoln and Lander 1992) and the regression method of Haley and Knott (1992). However, for clarity, LOD values throughout are derived from the analysis performed according to Haley and Knott. The data generated by the (AxB6)G6 and (BxB6)G6 populations were analyzed separately and also in combination. The genetic distances between markers were calculated by using CRI-MAP version 2.4 (Lander and Green 1987).

For the purposes of this report, we define the 95% CI for each QTL location as the distance between the points on each side of the peak value

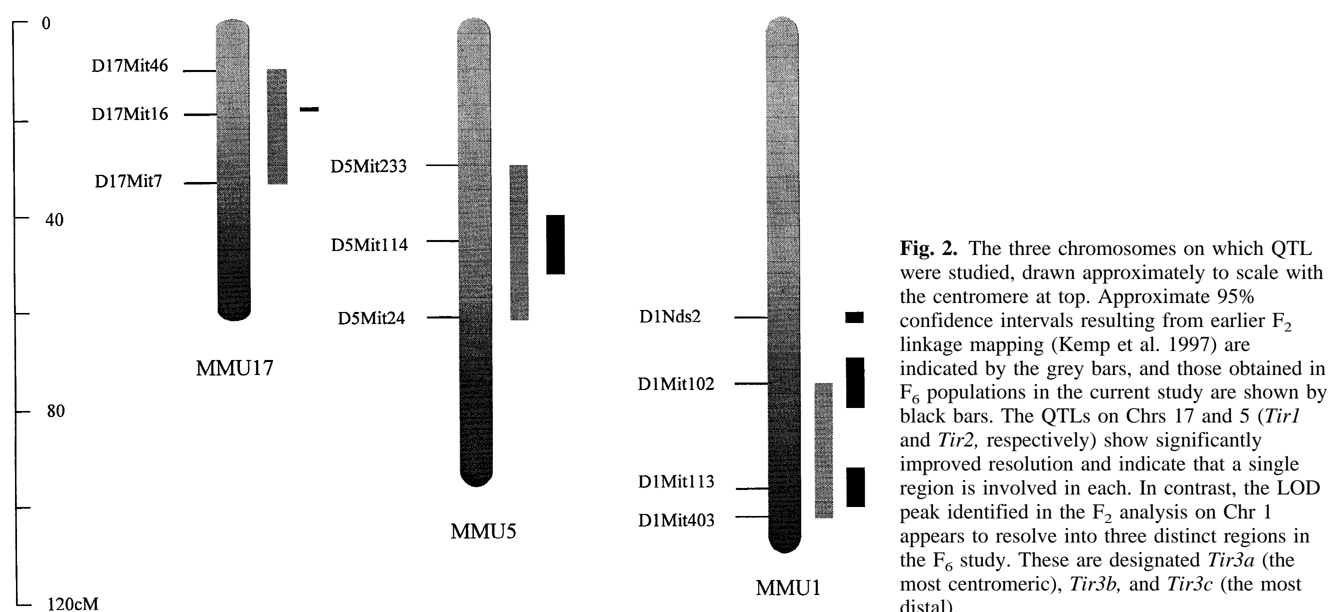

Fig. 2. The three chromosomes on which QTL were studied, drawn approximately to scale with the centromere at top. Approximate 95% confidence intervals resulting from earlier F_2 linkage mapping (Kemp et al. 1997) are indicated by the grey bars, and those obtained in F_6 populations in the current study are shown by black bars. The QTLs on Chrs 17 and 5 (*Tir1* and *Tir2,* respectively) show significantly improved resolution and indicate that a single region is involved in each. In contrast, the LOD peak identified in the F_2 analysis on Chr 1 appears to resolve into three distinct regions in the F_6 study. These are designated *Tir3a* (the most centromeric), *Tir3b,* and *Tir3c* (the most distal).

at which the LOD score falls by 2. Lander and Botstein (1989) defined the 95% confidence interval for the location of each QTL as the distance between points on each side of the peak at which the LOD score falls by 1. Simulation studies (van Ooijen 1992) have shown that this may underestimate the confidence interval under some circumstances and that using an LOD drop of 2 from the peak value provides a confidence interval that is generally conservative.

Results and Discussion

The two analysis methods adopted gave similar and equally informative results. LOD scores, QTL locations, nearest markers, and 95% CIs are summarized in Table 1.

Some minor differences were observed between the published map order of some of the microsatellite markers we used (Hamvas et al. 1998; Kozak and Stephenson 1998; Seldin 1998) and the order resulting from *de novo* mapping on the basis of our genotyping. However, the increase in the apparent genetic distances between markers in the G6 populations was approximately as expected. [These data are not presented here but are available on request.]

The Chr 17 QTL *Tir1* comprised a single locus with LOD scores of 20 and 19 in the (BxB6)G6 and (AxB6)G6 populations, respectively. The same locus was identified in the combined data set with a LOD score of 38 (Fig. 1a). The QTL was mapped to 95% CIs of 2.8 cM and 1.3 cM in the (BxB6)G6 and the (AxB6)G6 populations, respectively. Combining these data resulted in a 95% CI of 0.9 cM.

Analysis of *Tir2* on Chr 5 indicated a single QTL in the (AxB6)G6 population. This QTL was not detected in the (BxB6)G6 population. *Tir2* mapped within a 95% CI of 12 cM with a LOD score of 5.5 in analysis of the combined data set (Fig. 1b). No other QTL were detected above a LOD threshold of 2.5 on Chr 5.

The earlier F_2 study predicted multiple QTL within *Tir3* on the basis that a large region with a high LOD score was detected on Chr 1 (Kemp et al. 1997). The G6 analyses revealed three distinct LOD peaks, each representing one putative QTL (designated *Tir3a, Tir3b,* and *Tir3c*). These loci mapped in an analysis of the combined data set to 95% CIs of 1.8, 10, and 8 cM respectively, with LOD scores of 10, 11, and 8, respectively (Fig.1c).

For comparison, the positions of *Tir* loci obtained in the earlier F_2 study, and in F_6 populations in the current study, are shown in Fig. 2. In all cases, as predicted by Darvasi and Soller (1995), there was marked improvement in locus resolution by comparison with that obtained in an earlier study in F_2 mice. Four of five QTL fell within the corresponding 95% CIs obtained in the earlier study, while one (*Tir3a*) fell outside the CI around the LOD peak previously identified on Chr 1. *Tir3a* may, therefore, be a previously unidentified QTL.

The QTL of greatest effect (*Tir1*) was localized with a 95% CI spanning 17.4–18.3 cM from the centromere on the Chr 17 consensus map (Hamvas et al. 1998), with a LOD score of approximately 38. This represents 10- and 15-fold improvements on the resolutions we reported for the analysis of the (BxB6) F_2 and (AxB6) F_2 populations respectively (Kemp et al. 1997).

A single QTL corresponding to *Tir2* with a LOD score of 4.6 was detected on Chr 5 in the (AxB6)G6 population, at a position corresponding to 42 cM on the consensus map, with a 95% CI of 16 cM. There was, thus, a 2.5-fold decrease in the 95% *Tir2* CI between the F_2 and F_6 generations of the AxB6 line. In contrast to the result obtained in the (BxB6) F_2, no significant QTL was detected at any position on Chr 5 in the (BxB6)G6 population. We believe that this apparent loss of *Tir2* from the BxB6 line may be due to chance loss of one of the alternative alleles in the process of breeding the Liv:B,B6 AIL.

In addition to improving mapping resolution significantly, the AIL analyses revealed a degree of complexity at the *Tir3* locus that was not evident in the F_2 analyses. Whereas the F_2 analysis had shown a single region of significance spanning a CI of some 30 cM, three QTL were revealed at the *Tir3* locus in the G6 analysis. Two of these QTL, *Tir3b* and *Tir3c,* fall within this interval and are, therefore, likely to represent loci that were detected, but could not be separated, in the previous study. However, the third (*Tir3a*) falls centromeric of the previously identified peak and corresponds closely to a non-significant peak (maximum LOD score 2.2) that was seen in the (BxB6) F_2 analysis. This is, therefore, likely to represent an additional QTL on Chr 1.

In summary, QTLs *Tir1* and *Tir3* were confirmed in both AILs, but *Tir2* was confirmed in only one of them. Analysis of the combined AIL data confirmed all three QTLs and reduced confidence intervals by factors of 2.5 to 15. Moreover, a degree of complexity at the *Tir* loci was revealed in the G6 populations which had not been apparent in analyses of F_2 data.

We believe that this is the first successful use of the AIL approach proposed by Darvasi and Soller. It has succeeded in a) significantly increasing the precision with which previously de-

tected QTL were localized, and b) revealing complexity in QTL that were previously only "loosely" defined. In these respects, therefore, our results underline two of the advantages of the AIL approach over congenic approaches to refining gene positions. Beyond that, and very significantly, application of the AIL approach has provided a degree of resolution of at least one murine trypanosomiasis response QTL, *Tir1* on Chr 17, which is now sufficient to make positional cloning a practical possibility.

Finally, it is important to note that while mapping in advanced intercross generations can provide considerable improvement in QTL definition, our studies indicate that, despite care in randomizing matings and maintaining effective population sizes, QTL alleles may be lost in the development of AILs.

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