Mapping quantitative trait loci affecting sternopleural bristle number in *Drosophila melanogaster* using changes of marker allele frequencies in divergently selected lines

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Summary

Quantitative trait loci (QTLs) responsible for variation in sternopleural bristle number in crosses between the laboratory lines of *Drosophila melanogaster OregonR* and *CantonS* were mapped using information from allele frequency changes of two families of retrotransposon markers in divergently selected populations. QTL effects and positions were inferred by likelihood, using transition matrix iteration and Monte Carlo interval mapping. Individuals from the selected populations were genotyped for markers spaced at an average distance 4.4 cM. Four QTLs of moderate effect ranging from 0·6 to 1·32 bristles accounted for most of the selection response. A permutation test of the correspondence between the mapped QTLs and the positions of bristle number candidate genes suggested that alleles at these candidate genes were no more strongly associated with selected changes in marker allele frequency than were randomly chosen positions in the genome.

1. Introduction

A description of the nature of variability among individuals for quantitative traits and the mechanisms of the maintenance of variability in natural populations represents one of the major challenges in evolutionary genetics (Charlesworth, 1996). The number of segregating loci responsible for the trait variance, their additive, dominance and pleiotropic effects, allele frequencies, linkage relationships, and the fitness effects of segregating alleles (Mackay, 1996) have to be known to understand how variability is maintained; to predict the selection response in a changing environment; and to learn what fraction of standing variation plays a role in evolutionary change during speciation (Lynch & Walsh, 1998). However, the first step in this description, the mapping of quantitative trait loci (QTLs) segregating for the trait in natural populations, is very difficult. The progress in QTL mapping that has been achieved relatively recently is based on the availability of multiple molecular markers densely spaced on the chromosomes.

Usually mapping is started from two homozygous lines that are distinguishable by fixed markers (Sax, 1923; Lander & Botstein, 1989). The lines are crossed, and the association between quantitative trait phenotypes and marker locus genotypes is analysed in backcross, F2 or recombinant inbred lines derived from the parental strains. These designs have obvious limitations, however. Backcross and F2 designs may be employed only for studying traits that can be measured on single individuals, for example morphological traits (e.g. bristle number, Long *et al.*, 1995; genitalia shape, Liu *et al.*, 1996). The large sample sizes required for mapping traits strongly influenced by environmental and developmental noise may render the experiment prohibitively expensive (Lander & Botstein, 1989). The recombinant inbred line design is more powerful for traits with low heritability, and is useful for studying traits for which measurement of a group of individuals of the same

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Table 1. *Marker positions*

| Line | Transposable element | Chromosome | | |
		X	II	III
OregonR-iso	*412*	4D (9[a])	22F (5), 34F (49), 35B (50), 47E (60), 56F (87), 57C (97)	77E (47), 79F (47), 98A (94)
	297	6A (16), 9C (31), 15A (55), 16C (58), 18A (60)	39D (54), 45E (59), 57B (92)	63E (7), 67F (34), 69F (39), 70F (42), 72A (43), 76C (46), 95C (81), 98A (94), 100EF (102)
CantonS-iso	*412*	5A (12·5)	21A (0), 23D (6), 26F (20), 27A (20), 34A (47), 41F (55), 50A (68), 57D (98), 57E (99)	64D (19), 78D (47), 84B (47), 86B (50), 88C (55), 92C (66), 94E (79), 97B (91), 98E (98), 99C (100), 99F (101)
	297	4B (6), 10A (33), 16A (57), 16F (58), 18C (62), 19A (64)	23C (6), 26D (20), 36F (53), 38E (54)	61A (0), 63A (3), 77D (47), 85A (48), 85C (48)

[a] Genetic positions are given in accordance with Lindsley & Zimm (1992). See text for further explanations.

genotype is necessary, for example some life-history traits, viability or fitness (Crabbe *et al.*, 1994; Long *et al.*, 1995). However, the design is limited to species with short generation intervals since many generations are necessary to establish homozygosity.

An alternative experimental strategy is to follow frequency changes at marker loci in selected populations derived from a cross of parental lines (Dumouchel & Anderson, 1968; Garnett & Falconer, 1975). Selection changes the frequencies of the molecular markers because of hitch-hiking with alleles of QTLs of the selected trait (Maynard Smith & Haigh, 1974; Thompson, 1977), allowing inference of the linkage between the markers and QTLs (Keightley & Bulfield, 1993; Nuzhdin *et al.*, 1993; Keightley *et al.*, 1996). Mapping of QTLs by following frequencies of marker alleles is a potentially powerful approach, as QTLs with relatively small effects can be detected by genotyping a small number of individuals (trait-based mapping approach, Lebowitz *et al.*, 1987). This approach can be successfully used to study fitness-related traits, since the selection for fitness-related traits is usually simple, the approach is powerful for low heritability traits, and it does not require recombinant inbred lines for scoring the trait.

Here we used the trait-based design to map QTLs of a model quantitative trait: sternopleural bristle number in *Drosophila melanogaster*. Bristle inheritance has been studied for many years, since bristles are easy to count, and the genetic variance for bristle number is primarily additive (Thoday, 1979; Mather & Jinks, 1982; Shrimpton & Robertson, 1989 *a*, *b*). The environmental and developmental noise for bristle QTL expression is low relative to the effects of QTLs

segregating in natural populations, leading to high heritabilities of bristle number (Falconer & Mackay, 1996). Additionally, a detailed knowledge of the developmental genetic basis of bristle formation has been acquired. There are many 'candidate' genes, mutations in which have a strong effect on bristle phenotype (Jan & Jan, 1994). Segregation of alleles with smaller effects at these candidate loci may be responsible for a proportion of bristle number variability in natural populations (Mackay & Langley, 1990; Lai *et al.*, 1994; Long *et al.*, 1996; Mackay & Fry, 1996).

The aim of this experiment was to map bristle number QTLs that segregate between unselected *D. melanogaster* lines. The design of the experiment was chosen to maximize the probability of detecting bristle number QTLs irrespective of effects of segregating alleles. However, the set of mapped QTLs is perhaps biased towards alleles with larger effects due to the limited power of QTL analysis. We compare the QTL locations and allelic effects with those obtained in previous mapping studies, and test whether or not the QTLs affecting bristle number are likely to be alleles of bristle number candidate genes.

2. Materials and methods

(i) Drosophila melanogaster *strains*

The unrelated lines *OregonR* and *CantonS* (Lindsley & Zimm, 1992) were used as parental strains for QTL mapping. Isogenic derivatives of both lines (*OregonR-iso* and *CantonS-iso* respectively) were made by crossing the females to the balancer stock *In(1)sc[sL] sc[8R+1]9n(1)s, sc[s1] sc[8] w[a] B; In(2LR)SM1, al Cy cn[2] sp[2]/*

In(2LR) *Pm*; *In(3LR)Ubx^{130}*, *Ubx^{130} e^s/Sb*; *spa^{pol}*, backcrossing F1 males to the balancer stock, intercrossing the progeny of individual males, and collecting their *spa^{pol}* offspring. The fourth chromosomes of the lines were substituted with a chromosome containing *spa^{pol}* to control against contamination. Subsequently each fly was checked for this marker throughout the experiment. All strains used in the experiment were *M* and *I* with respect to the *P-M* and *I-R* systems of hybrid dysgenesis respectively. Therefore, transpositions of these transposable elements (TEs) is not induced by crossing the lines. All crosses and stock maintenance were carried out at 25 °C on cornmeal–agar–molasses medium.

(ii) *Molecular markers*

Cytological positions of the TE families *297*, *412*, *copia*, *Doc* and *mdg-1* (Berg & Howe, 1989) were probed as molecular markers. The positions were determined by *in situ* hybridization of biotin-labelled DNA of plasmids carrying each corresponding element to polythene salivary chromosomes of third instar larvae raised at 25 °C (Shrimpton *et al.*, 1986). The plasmids were labelled with biotinylated dATP (bio-7-dATP, BRL) by nick translation. Hybridization was detected using the Elite Vectastain ABC kit (Vector Laboratories) and visualized with diaminobenzidine. The element locations were determined at the level of cytological bands on the standard Bridge's map of *D. melanogaster* (Lefevre, 1976).

Homozygosity of the strains was verified by scoring *copia* locations in 52 larvae of the *OregonR-iso* strain and 10 larvae of the *CantonS-iso* strain (data not shown). Among the TE families tested, *412* and *297* showed the most even spacing on the chromosomes. A total of 63 TEs situated on average 4·4 cM apart were polymorphic between the lines and were used for mapping purposes (Table 1). No new *297* and *142* insertions were found during the course of the experiment, suggesting the absence of detectable transposition activity of these TEs and confirming the absence of contamination by unrelated lines.

(iii) *Experimental population and selection procedure*

The lines *OregonR-iso* and *CantonS-iso* were reciprocally crossed, and equal numbers of progeny from both crosses were mixed together to start a random mating population (generation F1, Fig. 1). Each generation, vials were randomly subdivided into 10 groups, and about 200 progeny collected from the vials within each group were mixed and transferred into 10 new vials. The purpose of this 'linkage breakdown phase' of the experiment was to reduce initial large-scale linkage disequilibrium between the markers and the QTLs for bristles (Darvasi & Soller, 1995).

At generation F20, 1000 virgin females and 1000 males were collected from the first to the seventh day after eclosion, and the numbers of sternopleural bristles on both sides of the body were scored. Populations selected for higher and lower bristle number were started by crossing 200 virgin females and 200 males with the highest and the lowest bristle numbers, respectively. Selected parents were put in a population cage of about 10 l capacity containing 40 open shell vials with fresh medium. The vials were removed from the cage on the seventh day, covered, and 1000 virgin females and males of the next generation collected from them and selected for higher or lower bristle number as described above for nine generations more (generations S1–S9, Fig. 1). Subsequently, flies were kept without artificial selection as in the initial random mating population for about 40 generations, when their sternopleural bristle number was scored again (generation R, Fig. 1).

(iv) *Effective sizes of the random mating population and populations under selection*

We did not estimate the effective sizes of the experimental populations directly, but rather conservative assumptions were applied for the following data analysis. Crow & Morton (1955) estimated the reduction of the effective size of *Drosophila melanogaster* cage populations in comparison with the census size by a factor of about 1.4. Correspondingly, we assumed that the effective population size during the linkage breakdown phase was $T = 1400$. The effective size of a population under selection is reduced since the variance of the number of progeny increases. Since the base population was randomly mated for 20 generations before selection started, most loci are unlinked with selected QTLs. Then, if the correlation between genes of male and female parents is zero, and N is large, in which case deviations from Hardy–Weinberg proportions approach zero, N_e may be calculated as

$$N_e = \frac{4N}{2 + S_k^2 + 4Q^2C^2},$$

where N is the census size, S_k^2 is the variance of the family size due to sampling without selection, C^2 is the variance of selective advantage among families, and Q^2 is a term accounting for the cumulative effect of selection on an inherited trait. For the case of the mass truncation selection

$$S_k^2 = 2, \quad C^2 \approx \tfrac{1}{2}i^2\left(\frac{1}{h^2} - i(i-x)\right),$$

and

$$Q = \frac{2}{1 + i(i-x)h^2},$$

where i is the intensity of selection ($\approx 1\cdot7$ across different generations of selection, data are not shown), h^2 is the heritability ($\sim 0\cdot1$, see below), and $x(\approx 0\cdot84)$ is the truncation point (Santiago & Caballero, 1995). Estimates of $N_e = 247$ for autosomes and $N_e = 185$ for X chromosomes were obtained, and used in the likelihood calculations. Although residual linkage between markers in the close proximity of selected QTLs may further decrease the estimates of N_e, currently there is no approach to account for that.

(v) *Likelihood analysis*

The observed phenotype frequencies at single marker loci were used in a method based on transition matrix iteration to compute maximum likelihood estimates of QTL effects under the assumptions of additive QTL effects and complete linkage between markers and QTLs. A vector $\mathbf{f(t)}$ of dimension $2N_e + 1$ was defined whose elements contained the probability distribution of marker allele frequency at generation t (the probability that the population has a frequency $q = j/(2N_e)$). To allow for spread of marker allele frequency during the 20 generation linkage breakdown phase, at the start of the selection f(0) was initialized by iterating a square transition matrix of dimension T for 19 generations with initial allele frequency $0\cdot5$, zero expected change of allele frequency, and incorporating a change of population size from T to N_e at generation 20 using a rectangular transition matrix of dimensions $T \times N_e$.

A transition probability matrix $\mathbf{M}$, also of dimension $2N_e + 1$, was set up by standard methods (Ewens, 1979), with the gene frequency change, Δq, each generation given by $\Delta q = [sq(1-q)]/2$, where s is the selective advantage/disadvantage associated with the marker assuming opposite signs of s for the opposite directions of selection. s is related to the magnitude of the effect on the trait according to $s = ia/\sigma_P$ (Falconer & Mackay, 1996). The probability distribution of q at generation 6 was obtained by iterating $\mathbf{f(t+1)} = \mathbf{f(t)M}$ for six generations. In the final generation, a change of population size from N_e to N was modelled using a rectangular transition matrix $\mathbf{M}$ of dimension $N_e \times N$ with the same expected change of allele frequency as above, and obtaining $\mathbf{g(7)} = \mathbf{f(6)M}$, where $\mathbf{g(7)}$ is a vector of dimension $2N + 1$.

The likelihood of the observed phenotype frequencies for the dominant autosomal markers was

$$L = \sum_{j=0}^{2N} \mathbf{g(7)}$$
$$\times [(2q - q^2)^{n1}((1-q)^2)^{n2}(2p - p^2)^{n3}((1-p)^2)^{n4}], \quad (1)$$

where $p = 1 - q$, $n1$ is the observed number of individuals with the dominant marker in the high line, $n2$ the observed number without the dominant

marker in the high line, $n3$ the observed number with the dominant marker in the low line, and $n4$ the observed number without the dominant marker in the low line. The likelihood was maximized as a function of s. In the case of X-linked markers, the likelihood was calculated as follows

$$L = \sum_{j=0}^{2N} \mathbf{g(7)} L_f [(q)^{n5+n8}(p)^{n6+n7}],$$

where L_f is the term for females, identical to the term inside the square brackets in equation (1). The $n1$–$n4$ ($n5$–$n8$) terms are the numbers of females (males) in the different phenotype classes defined above.

A procedure to compute the likelihood of data along the interval flanked by two linked markers has been described in detail by Keightley *et al.* (1996). Briefly, Monte Carlo simulation was used to simulate the effects of selection on a locus with an additive fertility effect s on the frequency changes at a pair of linked flanking markers. Fertility selection was simulated for computational efficiency. The population size was the same as assumed for the transition matrix likelihood evaluation method. The starting population for the simulation was an F1 population between two lines differing for the QTL alleles and flanking marker alleles. A 20 generation period with no selection was simulated in order to model the recombination breakdown phase, then seven generations of selection were simulated. For one replicate of the simulation a vector of the proportions of the four phenotype classes for the autosomal markers at generation 7 was generated. A sampled likelihood for an observed vector of autosomal marker phenotype numbers from one of the selection lines, given the simulated expected frequency vector, was obtained under the assumption that the observed numbers have probabilities from the multinominal distribution with expected frequencies those simulated. A sampled likelihood for both directions of selection was the product of sampled likelihoods computed separately for the high and low lines, using the same linkage breakdown phase population for each replicate, but assuming opposite signs of s for the opposite directions of selection. The overall likelihood of the data was calculated by integrating over at least 150000 sample likelihoods. Likelihoods for X-linked markers were computed in a similar manner, except in this case the simulation kept track of two sexes, and a sampled likelihood was the product of a sampled likelihood for each sex. Profile likelihoods as a function of s were computed at the marker positions and at one position in between, and the s value which maximized likelihood obtained at each position. In cases where a strong QTL effect was detected in an interval, likelihoods were calculated at five evenly spaced positions in the interval. The likelihood ratio was obtained from the ratio of the maximum likelihood to the likelihood for s set to zero.

We used two different marker systems (i.e. positions of TE families *297* and *412*) for which different individuals were scored. Correspondingly two interval mappings were done, with intervals bracketed by one of the TE family markers. Likelihood ratios obtained for the above procedure, but with a single marker, were very similar to likelihood ratios obtained with the transition matrix method.

3. Results

(i) *Selection response*

The lines *OregonR-iso* and *CantonS-iso* had mean sternopleural bristle numbers summed over the two sides of the thorax of 18·6 and 17·4 respectively (Fig. 1). These values are in the range characteristic of wild-caught flies. The bristle number of F1 progeny was higher than that of either parent, suggesting heterosis. However, bristle number varied strongly between generations so the appearance of heterosis may have been an environmental effect. We assumed additivity of genes affecting bristle number in the data analysis. The response to divergent selection starting at F20 (generation of selection S0) is shown in Fig. 1. The between-population difference was 3·5 bristles after seven generations of selection, and did not change markedly after another two generations of selection. The estimated realized heritability from unweighted least-squares regression of cumulative response on cumulative selection differential (Falconer & Mackay, 1996) over nine (seven) generations of selection was
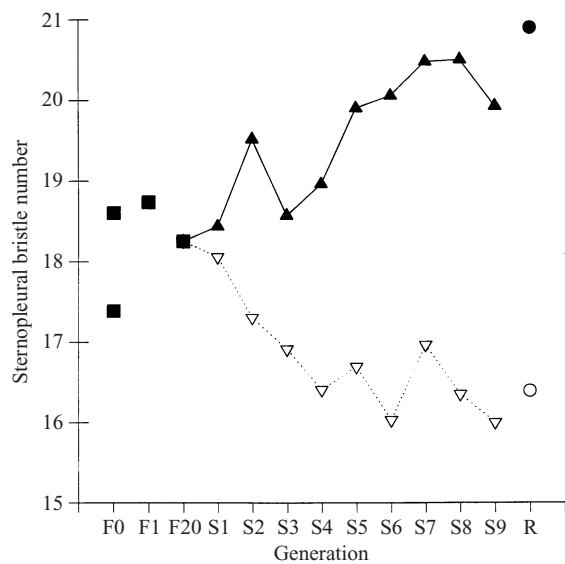

Fig. 1. Selection response. The squares represent bristle scores in parental lines, F1 and F20 populations. Filled triangles show scores in the lines selected for high bristle number and open triangles the scores for low bristle number. Circles represent bristle scores in selected lines after relaxed selection for 40 generations.

0·10 (0·12) for selection for higher bristle number, and 0·11 (0·11) for selection for lower bristle number. Since the two parental lines were homozygous, all F1 progeny from one direction of their cross were genetically identical, and bristle number variance between them originated from environmental and developmental fluctuations (V_E). V_E for generation F1 was equal to 2·6. Bristle number QTLs segregated between flies in F20, and the variance of bristle numbers between flies (V_p) included V_E plus a genetic component (V_G). The estimate of $V_P = V_E + V_G$ for generation F20 when selection started was 3·4. Thus, the heritability of bristle number has an upper limit of

$$h^2 = \frac{V_A}{V_P} < \frac{V_G}{V_P} = 0.24,$$

where V_A is an additive genetic variance. A similar estimate calculated from

$$\frac{\operatorname{cov}(L, R)}{\operatorname{var}(L) + \operatorname{var}(R) + \operatorname{var}(error)}$$

with the SAS VARCOMP procedure (SAS, 1988), where L and R refer to bristles on the right and left sides of flies, was obtained from the repeatability of bristle scores in generation F20:

$$h^2 < \frac{V_G}{V_P} < \frac{(V_G + V_{Eg})}{V_P} = 0.35,$$

where V_{Eg} represents developmental similarity between bristle numbers on two body sides. Bristle number did not change substantially after relaxation of selection for approximately 40 generations (generation R, Fig. 1), implying that genes responsible for selection response were selectively neutral under our experimental conditions or were fixed by generation 9.

(ii) *Marker allele frequency divergence*

Since markers were fixed in the parental lines, the frequency of each marker in the F1 is expected to be 0·5. During the first 20 generations, when artificial selection was not applied, marker alleles may have changed their frequencies due to drift, and due to hitch-hiking with genes segregating for fitness. Unfortunately, the slides prepared from F20 larvae to estimate marker frequencies at the beginning of selection were not protected from the light, and could not be analysed due to weak hybridization signals.

Although unknown, the initial allele frequencies at any marker before selection were the same in both populations since they originated from one sample of 2000 flies. The frequencies of alleles at bristle number QTLs and the frequencies of linked markers are expected to change in opposite directions as a result of divergent selection for bristle number. To determine
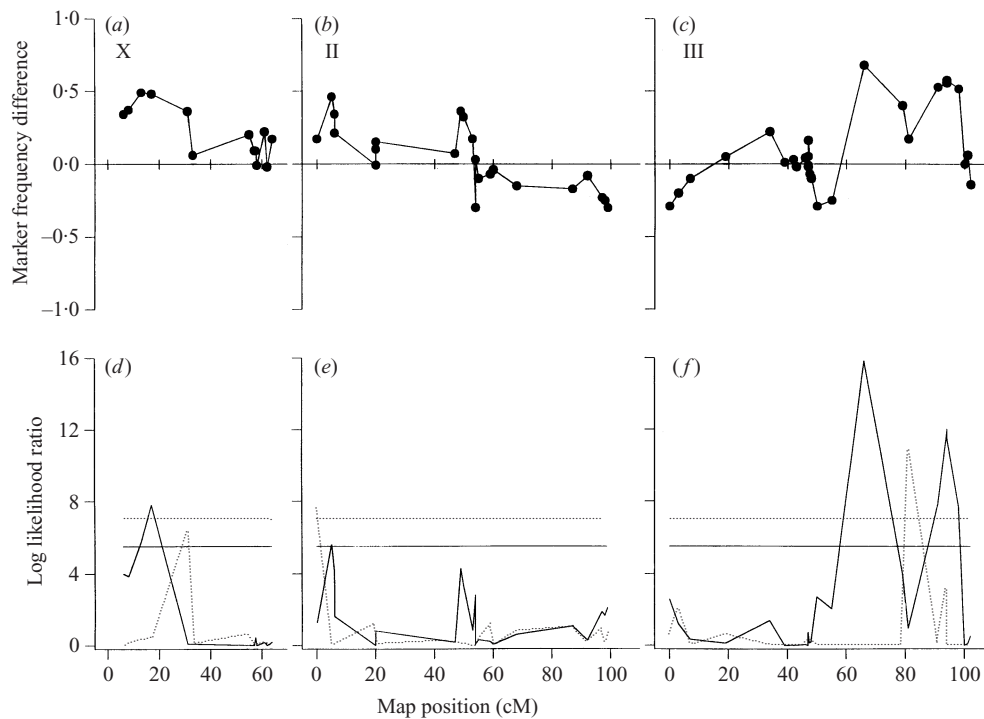
Fig. 2. Plots (*a*), (*b*) and (*c*) represent the estimated difference in the frequency of markers between low and high selected lines in generation F7 for markers situated on X, second and third chromosomes respectively. Continuous lines in plots (*d*), (*e*) and (*f*) are the LLR1, and dotted lines are LLR2 estimates.

the difference in marker frequencies between selection lines we analysed between 54 and 75 individuals from each selection line for each marker. The estimates of marker frequencies assuming Hardy–Weinberg proportions, after 7 generations of selection are shown in the Appendixes (Tables A1–A3). A genetic map depicting allele frequency differences is shown in Fig. 2.

(iii) *Transition matrix single-marker analysis assuming* $E(q_0) = 0.5$

We first assumed that the allele frequencies at a marker were affected only by drift and by selection on a bristle number QTL completely linked to the marker – a valid approximation for a high marker density. We assumed that the bristle number QTL had the same value of the selection coefficient, s, but with different signs for the two directions of selection. The natural log likelihood ratio (LLR1) calculated for each marker is the difference between the natural log maximum likelihood of the data and the log likelihood for the selection coefficient set to zero. In several regions of the genome peaks of LLR1 values occur (Fig. 2). For the X chromosome, one group of four markers (4B, 4D, 5A and 6A) showed LLR1 values between 3·8 and 7·7 (Table A1). For the second chromosome, two markers (22F and 23C) represented one peak with LLR1 values 5·6 and 3·9, and the other

two markers (34F and 35B) represented the second peak with LLR1 values 4·2 and 3·2, respectively (Table A2). Two peaks were also characteristic for the third chromosome, with the markers 92C and 94E with LLR1 values 15·5 and 3·9 in one of them; and the markers 97B, 98A, 98A and 98E with LLR1 values 7·7, 11·8, 11·2 and 7·5 respectively in the other (Table A3).

The likelihood ratio threshold at which to accept or reject a QTL marker association in our experiment is difficult to set since genetic linkage between markers was present but moderated by random mating for 20 generations prior to selection. We used a Bonferroni corrected LLR1 threshold value of 5·5 obtained for $\alpha = 0.05$ and 60 independent tests. This correction represents a conservative threshold boundary in a marker-based design since it assumes sparse uncorrelated markers (Lander & Botstein, 1989). To verify that this is a conservative assumption for the trait-based design, 1000 replicates of a population of $N = 100$ with 10 markers, and no selection for 20 generations, were simulated. For each replicate the LLR of the estimated selection coefficient was calculated at each marker individually. LLR_{MAX} is the highest LLR occurring at any of the 10 markers in any one replicate, and the 50th highest LLR_{MAX} value was taken as the experimentwise threshold (Churchill & Doerge, 1994; Doerge & Churchill, 1996). With no recombination between markers ($c = 0$) there is

Table 2. *Log likelihood ratio (LLR) threshold as a function of recombination fraction between the markers*

Recombination fraction between markers	LLR threshold
0·000	1·77
0·005	2·64
0·010	2·64
0·020	3·41
0·050	3·43
0·100	3·56
0·200	3·72
0·300	3·41
0·400	3·72
0·500	3·56

effectively one marker, and the LLR threshold is close to the value expected (Table 2), if $2 \times$ LLR is distributed at chi-square with one degree of freedom (1·92). With $c = 0.5$, there are 10 independent markers, and the value is again close to that expected for $P = 0.005$ (3·94). The simulation illustrates that assuming unlinked markers to set the significance threshold is conservative: if the simulation invokes linkage the expected LLR threshold is smaller.

In the experiment, four groups of markers were associated with QTLs, since at least one marker in each group exceeded the threshold. An additional region on chromosome 2 represents a likely candidate for further analysis.

(iv) *Transition matrix single marker analysis with variable initial gene frequency*

Although the frequency of each marker in the F1 was 0·5, it may have changed by F20 due to hitch-hiking with genes segregating for fitness. Indeed, the frequencies of several markers changed in the same direction for both directions of selection (see for instance 95C, Table A 3). To account for this we set q_0 variable, and maximized the log likelihood ratio for the selection coefficient and q_0 simultaneously. The log likelihood ratio (LLR2) is the difference between the natural log maximum likelihood and the log likelihood for q_0 set to 0·5. The LLR2 for the two markers 21A and 95C was above the threshold value 7·0 for $\alpha = 0.05$, two degrees of freedom and 60 independent tests (log likelihood ratios were 8·3 and 11·0 respectively), changes of frequency at these markers may be interpreted as significant joint effects of bristle number selection and fitness selection. These markers did not point to additional bristle number QTLs, but rather extended the regions of QTLs inferred with $E(q_0)$ was set to 0·5 (Fig. 2, Tables A 2, A 3).

(v) *Monte Carlo interval mapping*

Maximum likelihood interval mapping based on pairs of flanking markers was carried out to estimate s for single QTLs at positions between the markers, along with likelihood ratios under the null hypothesis of s set to zero. The analysis assumed that marker recombination rates were known (Lindsley & Zimm, 1992), and used Haldane's (1919) mapping function to relate map distance to recombination rate. Likelihood ratios based on interval mapping are shown in Fig. 3 for two marker systems separately. Interval likelihood estimates for *297* and *412* retrotransposon markers resembled each other except in the middle of the third chromosome. This is trivially explained by the absence of *297* markers there (Table 1). As expected, the graphs are quite similar to those obtained from the analysis in which markers were analysed one at a time (Fig. 2 *d–f*), and the peaks in log likelihood ratio closely correspond to regions of the genome in which the greatest differences in marker allele frequencies occurred (Fig. 2 *a–c*). Natural log likelihood ratios are, in general, higher than for the single marker analysis, presumably because in many cases broad areas of chromosomes show changes of allele frequency in the same direction.

(vi) *QTL effects*

QTL effects may be estimated by single-marker analysis and by interval mapping. With the single marker analyses we assume that each significant peak of LLR value marks only one gene responsible for bristle number selection response, and the frequency of a QTL is the same as the frequency of the marker with the highest LLR value due to complete linkage of this marker and the QTL. Then, QTL effect can be calculated as $a = s\sigma_P/i$, where σ_P is the phenotypic standard deviation 1·84 (from $V_P = 3.4$). The effects of the mapped bristle number QTLs were 0·23 (marker 6A), 0·31 (22F), 0·56 (92C) and 0·47 (98A), where effect refers to the difference between homozygous *OregonR-iso* and *CantonS-iso* originated alleles. The mapped genes accounted for 0·77 bristles, that is 22% of the total divergence of 3·5 bristles obtained as a selection response (Fig. 1). All *OregonR-iso* alleles of mapped QTLs increased the bristle number (Tables A 1–A 3), in agreement with the observation of 1·2 more bristles in the *OregonR-iso* line compared with the *CantonS-iso* line. From the estimates of σ_P in generation F20 and $h^2 = 0.1$, V_A may be calculated as 0·34. Assuming that all mapped factors segregated in F20 with the frequency 0·5, one may approximate their contribution to V_A as $a^2/8$, which is equal to 0·09. Mapped factors therefore account for about 26% of additive variance.
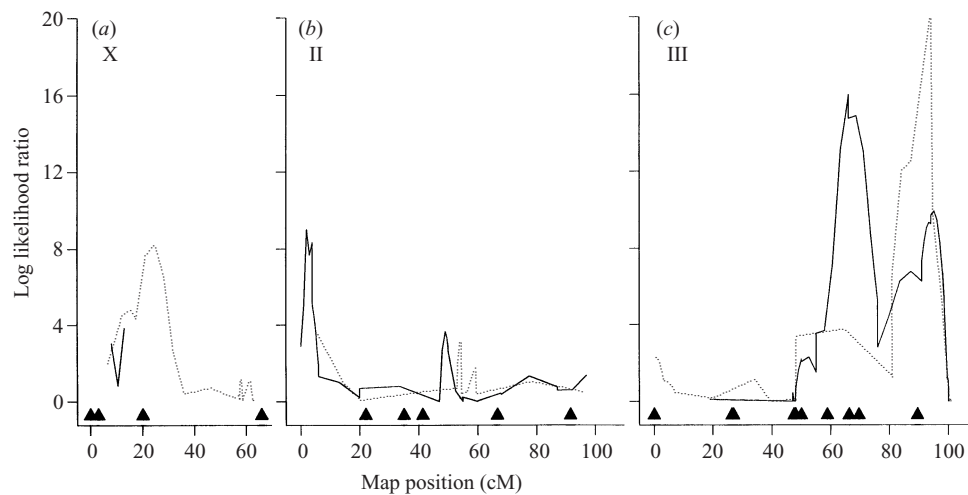
Fig. 3. Log likelihood ratios from interval mappings based on marker types *412* (continuous line) and *297* (dotted line) for the X, second and third chromosomes, shown in plots (*a*), (*b*) and (*c*) respectively. The positions of candidate genes *ASC* (1 0·0), *N* (1 3·0), *ct* (1 20·0), *bb* (1 66·0), *Sp* (2 22·0), *numb* (2 35), *da* (2 41·3), *sca* (2 66·7), *sm* (2 91·5), *emc* (3 0·0), *abd* (3 26·5), *h* (3 27·0), *mab* (3 47·5), *atonal* (3 48), *neu* (3 50), *AbdAB* (3 58·8), *Dl* (3 66·2), *H* (3 69·5) and *E* (*spl*) (3 89·1) (Lindsley & Zimm, 1992), mutations in which are likely to affect bristle number (Mackay, 1995), are represented by triangles.

Table 3. *Bristle QTL positions and effects inferred from interval mapping*

Chromosome	Map position	Estimated *s*	Effects *a* (bristles)
X	24	> 0·9[a]	1·32
II	2	0·49	0·73
III	66	0·50	0·73
III	94	0·41	0·60

[a] The upper limit 0·9 was set on the selection coefficient. The large estimate of the selection coefficient is caused by the very broad likelihood peaks (see text for more explanation).

Since complete linkage of the gene and the marker was assumed in the single marker analysis the inferred effects underestimate the true values. This may be illustrated by the results of interval mapping analysis allowing for recombination between the markers and the genes. Estimated effects of the four major QTLs at the highest LLR values from the interval mapping are shown in Table 3. The estimates were larger than the peak estimates obtained from the single marker analysis and the LLR values were higher, perhaps due to positive correlation of frequencies of markers surrounding mapped QTLs. The first and the third peaks in the chromosome likelihood profiles (Fig. 3) were broader than those simulated. The best fit of the model to the data for the first peak required *s* > 0·9, and only the minimal estimate of the effect of its alleles was obtained. Assuming that the QTL alleles were fixed as a result of selection, which is suggestive from the absence of the selection response for two last

generations, and no bristle number change during selection relaxation, the alleles of mapped QTLs accounted for 3·4 bristles, or the majority of the observed response. Their segregation in F20 accounted also for most of the additive variance (0·39).

OregonR-iso originated alleles of all mapped QTLs increased bristle number in comparison with *CantonS-iso* originated alleles. The difference in bristle number between the parental lines accounted for by alleles of four mapped QTLs was 3·4 bristles while the experimentally observed difference was 1·2 bristles. *OregonR-iso* originated alleles which decrease bristle number compared with *CantonS-iso* originated alleles were not detected in our experiment, either because they were in the poorly marked chromosome segments (e.g. the genetic distance from the marker 27A to 34A is 27 cM) or because they had effects that were too small. If the latter is correct, slight changes of frequencies of markers situated at 38E, 57E, 61A and 86B may suggest the presence of QTLs with minor effects linked to these markers (Fig. 2, Tables A 1–A 3).

4. Discussion

(i) *Sternopleural bristle number QTLs*

Sternopleural and abdominal bristle numbers in *D. melanogaster* have long served as model traits for studying quantitative trait inheritance. Mapping bristle number QTLs and estimation of allelic effects segregating in natural populations or differing between laboratory lines have been subjects of numerous experiments. Early bristle number QTL mapping studies suffered from problems connected with the

need to use visible markers. More recent advances in statistical approaches coupled with the use of dense molecular markers have provided sophisticated tools for inference on the genetic architecture of quantitative traits (Lynch & Walsh, 1998). QTL alleles that segregated between laboratory lines selected for high and low trait values from an outbred base population were mapped by the recombinant inbred line design (Long *et al.*, 1995; Gurganus *et al.*, submitted). These experiments were designed for the detection of large-effect alleles, and provided limited information about the average effect of alleles segregating in nature (Mackay, 1995, 1996). The frequency of large-effect alleles of bristle number QTLs in natural populations is a subject of debate (Mackay & Langley, 1990; Lai *et al.*, 1994; Keightley, 1995). Inferences about positions and allele effects of QTLs segregating between two unselected lines may provide information about the average effects of QTL alleles segregating in nature.

We have mapped four sternopleural bristle number QTLs, alleles of which segregate between the laboratory lines *OregonR-iso* and *CantonS-iso*. The mapping procedure made use of correlated changes in bristle QTL allele frequencies and linked marker allele frequencies caused by divergent selection on sternopleural bristle number (Table 3). Estimates of the QTL positions and allelic effects were obtained from the single and interval marker analyses. The single marker analysis underestimated the allele effects, because linkage disequilibrium between marker and QTL alleles was not complete. Interval mapping analysis yielded estimated QTL effects of 0·60 to 1·32 bristles, explaining much of the selection response and genetic variance. However, the estimate of the allelic effect is an increasing function of the recombination distance between markers, which is not known for the *OregonR-iso* line, *CantonS-iso* line and their hybrids. We used average estimates of recombination rates across the *D. melanogaster* genome (Lindsley & Zimm, 1992). The rate of recombination within chromosome segments may vary between lines (Brooks & Marks, 1986), introducing inaccuracy in our estimates of allelic effects. For two regions, the likelihood peaks covered broad sections of chromosomes. This may indicate that the rate of recombination in these regions is lower than the values assumed or that there is more than one QTL in each region.

The data obtained here strongly suggest that the most of bristle number differences between the two unrelated stocks can be explained by segregation of a few factors with effects in the range from 0·6 to 1·5 bristles. Since this conclusion is based on the comparison of two laboratory lines that could have accumulated mutations in bristle number QTLs, further efforts are required to describe effects of QTL alleles segregating in nature (Gurganus *et al.*, 1998).

(ii) *Candidate genes*

QTL mapping is the first step in inferring the genetic architecture of quantitative traits. The next step is to proceed from QTL to genetic locus. One approach is to search for candidate genes identified by mutations of large effects in the region to which the QTL maps (Mackay, 1985; Robertson, 1985). Several experiments give evidence for the candidate gene hypothesis for bristle number in *D. melanogaster*. First, segregation of molecular polymorphisms in candidate genes accounted for an appreciable proportion of bristle number variance between chromosomes sampled from nature (Lai *et al.*, 1994), although the robustness of this conclusion has been questioned (Keightley, 1995). Secondly, interactions have been found between mapped QTLs and candidate genes (quantitative complementation test: Long *et al.*, 1996; Mackay & Fry, 1996). Thirdly, in mutational searches for bristle number modifiers, a large proportion of *P*-element insertions were at positions of candidate genes (Lyman *et al.*, 1996; Mackay *et al.*, 1992), although insertional mutations in many other genomic positions also correlated with the change of bristle phenotype (Lyman *et al.*, 1996). Finally, QTL mapping experiments seem frequently to infer QTLs close to candidate genes (Long *et al.*, 1995).

To establish statistically based criteria for the similarity of positions we have tested the hypothesis that mutations in a set of candidate genes contributed to the selection response in our experiment and in the experiment to map abdominal bristle number reported by Long *et al.* (1995). The exact choice of the set of candidate genes is somewhat arbitrary, and we decided to use one possible set of 19 genes (see Mackay, 1995, table 1). The log likelihood ratios for the presence of a QTL along the chromosomes are known from the interval mapping. Therefore, the sum of log likelihood ratios at candidate gene positions can be calculated (Fig. 3). We excluded the candidate genes situated outside the regions mapped with *297* markers (three genes) and *412* markers (five genes) in our experiment, and the candidate genes situated on the X and second chromosomes for the Long *et al.* (1995) experiment (since no mapping data are available for the second chromosome and most of the X chromosome). To conduct a randomization test we sampled 100 000 sets of nine random positions for the analysis of data reported by Long *et al.* (1995); 16 positions for *297* markers, and 14 for *412* markers for the analysis of the results of our experiment; and calculated the distribution of sums of log likelihoods at these positions. If the sum of the log likelihoods at the candidate positions is in the top 5% tail of this distribution, it supports the hypothesis that segregating candidate genes contributed to the selection response. A contribution by candidate genes was
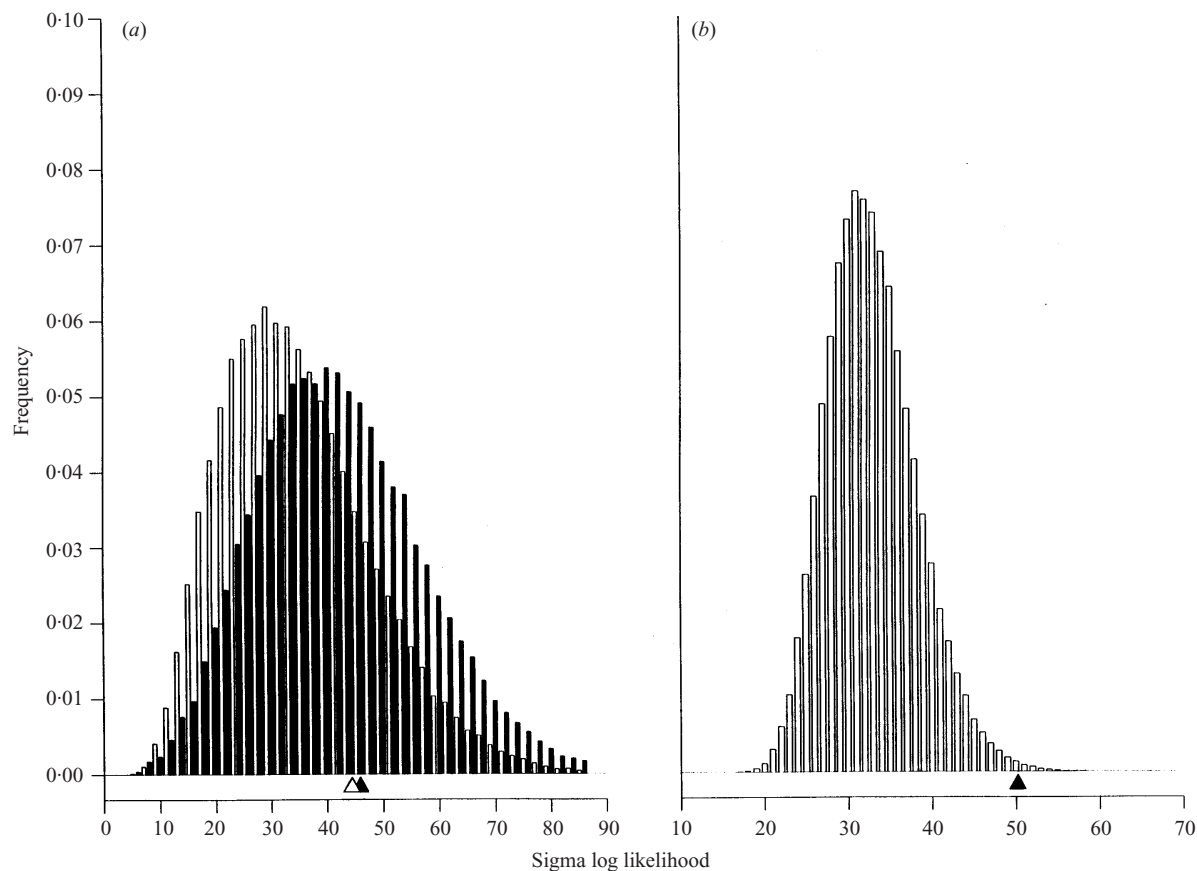
Fig. 4. Histogram showing the distribution of log likelihood sums over randomly chosen positions based on mapping data for the marker type *297* (open bars) and *412* (filled bars) obtained in our experiment (*a*) and on the mapping data of Long *et al.* (1995) (*b*). The triangles indicate the sum of likelihoods at the position of the candidates obtained from the real data.

supported for the data described by Long *et al.* (1995); correspondingly the segregation of mutations in candidate genes may be the major source of bristle number difference between homozygous lines obtained as a result of selection from a base outbred population (Long *et al.*, 1996). A significant result of the candidate gene test occurred in this case because two genes, *mab* and *attonal*, map to the most likely position of one of the major QTLs detected. The hypothesis was not supported by the analysis of the data obtained in our experiment (Fig. 4). We conclude that there is no statistical support for the segregation of the candidate gene alleles as a primary source of bristle number variation between *OregonR-iso* and *CantonS-iso* lines. It does not rule out the possibility, however that candidate genes made some contribution to the response.

The disagreements between the two experiments may reflect differences in experimental designs. The distributions of mutational effects differ across bristle number QTLs. For example, among five *P*-element-induced mutations at the *emc* gene, four had effects between 10·8 and 14·8 bristles, while mutational effects at other loci were much smaller on average

(Lyman *et al.*, 1996). Major effect QTL alleles may be more common for candidate genes initially discovered by their large effect mutations (Lindsley & Zimm, 1992). The experiment of Long *et al.* (1995) was designed for the detection of such major effect QTL alleles. The smaller QTL effects mapped in our experiment are perhaps less likely to represent alleles of the candidate genes. The similarity of positions of two QTLs mapped in our experiment (I-24 and III-66, Table 3) to those described by Long *et al.* (1995) argues against this hypothesis, however. Former studies of the distribution of allelic effects in bristle number QTLs are required.

(iii) *Trait-based mapping approach*

Although progress has been achieved in studying the nature of quantitative variation in *Drosophila*, the resolution of QTL mapping experiments is still limited to large effect factors (Lynch & Walsh, 1998). For example, among the QTLs mapped in the most sensitive experiment so far by Long *et al.* (1995), the smallest allelic effect was 0·62 bristles, and the effects of the others ranged from 2·0 to 5·55 bristles. In our

study, the smallest significant QTL allele had an effect of 0·60 with the others distributed between 0·73 and 1·32 bristles. This is a good illustration of the comparable powers of the trait-based and recombinant inbred line mapping approaches, as the sizes of the two experiments, as judged by the total number of markers analysed (approximately 4200 marker genotypes in Long *et al.*, 1995; and 4400 here) and bristles counted (approximately 15000 and 30000 flies scored for bristles respectively). The disadvantage of the trait-based QTL mapping approach is that it misses a large amount of information available from F2, backcross, or recombinant inbred line designs. For example, one cannot estimate the sex-specificity of QTL effects. Dominance of the genes may in principle be estimated from the asymmetry of the selection responses, that is a difference between marker frequency changes in the two directions of selection. However, the asymmetry may be explained equally well by selection of genes that segregate for fitness between the parental lines, and by epistatic interactions between different bristle genes.

Although the trait-based mapping approach misses a large amount of information it can serve as a helpful addition to the other designs (Ollivier *et al.*, 1997). The recombinant inbred line design has been employed successfully for studying traits with low heritabilities, for example life-history traits (Crabbe *et al.*, 1994; Long *et al.*, 1995). This design requires extensive multi-generation genetic manipulations with balancer chromosomes and/or tens of generations of selfing or full-sib mating to create recombinant inbred lines. Either the selective genotyping approach (Darvasi & Soller, 1994) or trait-based mapping approach may help in this case since their power is comparable to that of the recombinant inbred line design, and they do not require the construction of recombinant inbred lines.

Appendixes

Table A1. *Frequencies of markers on the X chromosome of selected lines*

	UP[a]		DOWN								
Band	Males	Females	Males	Females	$q(u)$[b]	$q(d)$	s[c]	a	SE[d]	LLR1	LLR2
4B	6/28	22/46	10/20	29/34	0·72	0·38	0·12	0·16	0·06	3·9	0·0
4D	**28/34**	**38/41**	**15/33**	**19/32**	0·73	0·36	0·11	0·15	0·06	3·77	0·3
5A	7/34	15/41	18/33	29/32	0·80	0·31	0·14	0·18	0·06	5·64	0·6
6A	**26/28**	**44/46**	**10/20**	**18/34**	0·79	0·31	0·17	0·23	0·06	7·7	0·6
9C	**12/28**	**33/46**	**2/20**	**7/34**	0·47	0·11	0·02	0·02	0·06	0·1	5·7
10A	14/28	32/46	15/20	26/34	0·55	0·49	−0·02	−0·02	0·06	0·1	0·0
15A	**9/28**	**33/46**	**7/20**	**16/34**	0·47	0·27	−0·01	−0·01	0·05	0·0	1·0
16A	7/28	34/46	10/20	28/34	0·51	0·42	0·00	0·00	0·06	0·0	0·0
16C	**7/28**	**31/46**	**7/20**	**19/34**	0·43	0·34	−0·04	−0·05	0·06	0·4	0·8
16F	15/28	28/46	9/20	20/34	0·63	0·64	0·00	0·00	0·05	0·0	0·3
18A	**16/28**	**36/46**	**5/20**	**18/34**	0·53	0·31	0·02	0·03	0·06	0·2	0·4
18C	11/28	27/46	12/20	19/34	0·64	0·66	0·00	0·00	0·05	0·0	0·4
19A	16/28	25/46	10/20	25/34	0·68	0·51	0·03	0·03	0·05	0·2	0·0

[a] Number of individuals with the dominant (in bold face) or recessive marker among analysed individuals in populations selected for higher (UP) or lower (DOWN) number of sternopleural bristles.
[b] Marker frequency in population selected for higher $q(u)$ and lower $q(d)$ bristle number.
[c] Inferred selection coefficient s and bristle number effect a associated with marker.
[d] Standard error of the estimated effect.

Table A2. *Frequencies of markers on the second chromosome of selected lines*

Band	UP	DOWN	$q(u)$	$q(d)$	s	a	SE	LLR1	LLR2
21A	16/75	31/65	0·89	0·72	0·11	0·15	0·10	1·3	8·3
22F	**72/75**	**37/65**	0·80	0·34	0·24	0·31	1·10	5·6	0·0
23C	**30/74**	**44/54**	0·77	0·43	0·19	0·25	0·08	3·9	0·2
23D	37/75	49/65	0·71	0·50	0·12	0·16	0·08	1·6	0·2
26D	40/74	28/54	0·68	0·69	0·00	0·00	0·08	0·0	1·6
26F	43/75	46/65	0·65	0·55	0·05	0·07	0·08	0·3	0·2
27A	39/75	46/65	0·69	0·54	0·08	0·11	0·08	0·8	0·4
34A	42/75	42/65	0·66	0·59	0·04	0·05	0·08	0·2	0·6
34F	**65/75**	**30/65**	0·63	0·27	0·20	0·25	0·08	4·2	0·0
35B	**63/75**	**31/65**	0·60	0·28	0·17	0·23	0·08	3·2	0·0

Table A2. *Cont.*

Band	UP	DOWN	$q(u)$	$q(d)$	s	a	SE	LLR1	LLR2
36F	49/74	45/54	0·58	0·41	0·09	0·11	0·08	0·8	0·0
38E	63/74	28/54	0·39	0·69	−0·16	−0·21	0·08	2·7	0·0
39D	**66/74**	**47/54**	0·67	0·64	0·02	0·04	0·10	0·1	0·7
41F	68/75	54/65	0·31	0·41	−0·05	−0·07	0·10	0·3	0·6
45E	**36/74**	**31/54**	0·28	0·35	−0·04	−0·06	0·08	0·2	1·6
47E	**51/75**	**47/65**	0·43	0·47	−0·02	−0·02	0·08	0·1	0·0
50A	70/75	54/65	0·26	0·41	−0·08	−0·10	0·10	0·6	1·0
56F	**33/75**	**43/65**	0·25	0·42	−0·10	−0·11	0·08	1·0	1·5
57B	**42/74**	**36/54**	0·34	0·42	−0·05	−0·06	0·08	0·3	0·3
57C	**31/75**	**46/65**	0·23	0·46	−0·13	−0·17	0·08	1·8	0·3
57D	70/75	48/65	0·26	0·51	−0·13	−0·17	0·10	1·6	0·3
57E	72/75	49/65	0·20	0·50	−0·14	−0·19	0·10	2·1	1·0

Table A3. *Frequencies of markers on the third chromosome of selected lines*

Band	UP	DOWN	$q(u)$	$q(d)$	s	a	SE	LLR1	LLR2
61A	56/74	21/54	0·49	0·78	−0·15	−0·21	0·08	2·5	0·9
63A	48/74	20/54	0·59	0·79	−0·10	−0·13	0·08	1·2	2·4
63E	**48/74**	**41/54**	0·41	0·51	−0·05	−0·07	0·08	0·3	0·0
64D	41/75	40/65	0·67	0·62	0·03	0·04	0·08	0·1	0·9
67F	**61/74**	**32/54**	0·58	0·36	0·11	0·15	0·08	1·4	0·0
69F	**54/74**	**39/54**	0·48	0·47	0·00	0·00	0·08	0·0	0·0
70F	**53/74**	**37/54**	0·47	0·44	0·01	0·01	0·08	0·0	0·0
72A	**56/74**	**42/54**	0·51	0·53	−0·01	−0·01	0·08	0·0	0·0
76C	**60/74**	**42/54**	0·57	0·53	0·02	0·02	0·08	0·0	0·0
77D	**46/74**	**32/54**	0·62	0·64	−0·01	−0·01	0·08	0·0	0·5
77E	**62/75**	**54/65**	0·58	0·59	0·00	0·00	0·08	0·0	0·0
78D	44/75	42/65	0·64	0·59	0·03	0·04	0·08	0·1	0·4
79F	**65/75**	**47/65**	0·63	0·47	0·08	0·11	0·08	0·7	0·0
84B	48/75	36/65	0·60	0·67	−0·03	−0·05	0·08	0·1	0·8
85A	48/74	29/54	0·59	0·68	−0·04	−0·05	0·08	0·2	0·6
85C	49/74	29/54	0·58	0·68	−0·05	−0·06	0·08	0·2	0·5
86B	61/75	31/65	0·43	0·72	−0·15	−0·21	0·08	2·6	0·0
88C	60/75	33/65	0·45	0·70	−0·13	−0·17	0·08	2·0	0·0
92C	19/75	63/65	0·86	0·18	0·42	0·56	0·10	15·5	0·0
94E	47/75	62/65	0·61	0·21	0·20	0·25	0·10	3·9	0·0
95C	**31/74**	**7/54**	0·24	0·07	0·10	0·13	0·10	0·9	11·0
97B	37/75	63/65	0·71	0·18	0·28	0·38	0·10	7·7	0·0
98A	**63/74**	**5/54**	0·61	0·05	0·36	0·47	0·10	11·8	3·1
98A	**70/75**	**19/65**	0·74	0·16	0·34	0·45	0·10	11·2	0·0
98E	35/75	62/65	0·73	0·21	0·28	0·36	0·10	7·5	0·0
99C	58/75	50/65	0·48	0·48	0·00	0·00	0·08	0·0	0·0
99F	55/75	51/65	0·52	0·46	0·03	0·04	0·08	0·1	0·0
100EF	**52/74**	**45/54**	0·49	0·59	−0·07	−0·08	0·08	0·5	0·0

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