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Aspects of maximum likelihood methods for the mapping of quantitative trait loci in line crosses

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Summary

Maximum likelihood methods for the mapping of quantitative trait loci (QTL) have been investigated in an F_2 population using simulated data. The use of adjacent (flanking) marker pairs gave improved power for the detection of QTL over the use of single markers when markers were widely spaced and the QTL effect large. The use of flanking marker loci also always gave more accurate and less biassed estimates for the effect and position of the QTL and made the method less sensitive to violations of assumptions, for example non-normality of the distribution. Testing the hypothesis of a linked QTL against that of no QTL is not biassed by the presence of unlinked QTL. This test is more robust and easier to obtain than the comparison of a linked with an unlinked QTL. Fixing the recombination fraction between the markers at an incorrect value in the analyses with flanking markers does not generally bias the test for QTL or estimates of their effect. The presence of multiple linked QTL bias both tests and estimates of effect with interval mapping, leading to inflated values when QTL are in association in the lines crossed and deflated values when they are in dispersion.

1. Introduction

The identification and mapping of loci having an effect on a quantitative trait of interest (quantitative trait loci or QTL) is valuable for several reasons. Firstly, it provides fundamental knowledge of individual gene actions and interactions, allowing the building of more realistic models of phenotypic variation, responses to selection and evolutionary processes. This should allow more effective methods to be developed for predicting breeding values and implementing selection. Secondly, marker information can be used directly to improve breeding value estimation and marker assisted selection may be an effective means of introgressing a few genes of value from one breed or line to another or of improving selection responses within a breed. Thirdly, the mapping of a QTL provides a route for the eventual cloning of the locus.

Until recently it has been impossible to identify the majority of QTL. Using only phenotypic data it is possible to identify genes of large effect on quantitative traits with various techniques (for a review see Hill & Knott, 1990), but even the best of these techniques, segregation analysis, is relatively lacking in power and also sensitive to failure of normality assumptions in the data (Knott, Haley & Thompson, 1992). The detection of linkage between a QTL and a genetic marker provides a more powerful method of identifying QTL. It has become apparent in recent years that there is an abundance of polymorphism at the DNA level which can be harnessed to produce genetic markers and genetic maps based on such markers have been developed or are under development for major plant and animal species.

The purpose of this paper is to look at maximum likelihood (ML) methods for the detection of QTL and the estimation of their effect under different situations, with the application of the techniques to crop plant and animal species in mind. For this purpose an F_2 population derived from a cross between two inbred lines will be used. Crosses between genetically divergent inbred lines provide the most powerful means for detecting QTL by linkage. QTL and markers for which the F_2 are segregating must have been fixed for different alleles in the parental lines and hence the F_1 individuals are completely heterozygous for these loci. In addition, all parents

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are genetically identical and hence there is no between-family variance to be included in the model and family structure can be ignored. Linkage phase between the QTL and markers is the same in all the founder individuals and need not be inferred. Inbred line crosses provide a benchmark for the assessment of the methods, for example, considering the power to detect QTL and accuracy of estimates, and against which other population structures can be assessed. In this paper we examine alternative test statistics and their distributions under the null hypothesis, investigate how robust the methods are to the estimate for recombination fraction between markers, look at violations of assumptions about the distribution of the residual component and the effects of having multiple QTL segregating in the population.

2. Methods

Several methods have been suggested for the detection of QTL using a population derived from a cross. Some of these have been based on least squares methods (for example, Soller, Brody & Genizi, 1976) and others have used ML (for example Knapp, Bridges & Birkes (1990), Knapp (1991) and Lander & Botstein (1989)). In both cases the possibility of using markers which flank the postulated QTL has been discussed. Lander & Botstein (1989) suggest that, for the single marker analyses they consider, there is little difference in power between the least squares method and the ML method. Haley & Knott (1992) give a least squares method using flanking markers that gives virtually equivalent results to a ML interval mapping method. Lander & Botstein (1989) compare the use of flanking and single markers using expected LOD ($\log_{10}$ of the likelihood ratio) scores but do not present a comprehensive study.

ML methods can be computationally demanding, but have several advantages as they enable both hypothesis testing and parameter estimation simultaneously and allow a model to be fitted which takes into account and estimates the recombination fraction. They also provide greater versatility allowing, for example, different residual variances to be fitted for the different QTL genotypes. Two ML methods will be considered in this paper. One uses information from a single marker at a time and investigates linkage between the postulated QTL and the marker. The other considers markers flanking the postulated QTL.

(i) *Flanking marker likelihood*

The likelihood for a population of F_2 individuals assuming linkage of a QTL to two flanking markers is derived by Paterson *et al.* (1991) as an extension of the work by Lander & Botstein (1989). It can be written as follows:

$$L = \prod_{i=1}^{N} \frac{1}{(2\pi\sigma_w^2)^{\frac{1}{2}}} \sum_{g=1}^{G} \text{trans}(g \mid A_i, B_i) \exp\left[\frac{-(y_i-\mu-a_g-d_g)^2}{2\sigma_w^2}\right]. \quad (1)$$

Where: N, is the number of F_2 individuals; y_i, is the phenotypic score for individual i; μ, is the mid-homozygote value (i.e. the mean of the two homozygotes); G, is the number of QTL genotypes (i.e. $g = 1, 2, 3$); a_g, is the additive deviation from μ for QTL genotype g (i.e. half the difference between homozygotes); d_g, is the dominance deviation from μ for QTL genotype g; σ_w^2, is the residual variance (i.e. that not due to the QTL); $\text{trans}(g \mid A_i, B_i)$ is the transmission probability of offspring i being genotype g given that the flanking marker genotypes are A_i and B_i.

The use of inbred lines means that the F_1 parents are identical and $\text{trans}(g \mid A_i, B_i)$ is the same for all offspring with the same marker genotype. This probability can be obtained from the recombination fractions between the QTL and markers A and B (r_A and r_B respectively) with an assumption about interference between recombination events. When offspring are heterozygous at both markers, the phase of linkage between the two loci is not known and two alternatives have to be considered (i.e. either with no recombination events, or with a recombination event in each parent) weighted according to their expected frequency of occurrence. Lander & Botstein (1989) assume that the recombination fraction (r) between A and B is known without error, in which case, with an assumption about interference, the transmission probabilities can be written in terms of r and a single unknown parameter, either r_A or r_B (with r_A and $r_B \leqslant r$). In the model described above (and all subsequent models), we have assumed that the variance within a QTL genotype is the same across all genotypes, but this assumption could be relaxed and a variance for each genotype estimated.

(ii) *Single marker likelihood*

The likelihood involving a single marker has been given by Weller (1986) and can be obtained by simple alteration of the flanking marker likelihood given above. The probability of being a particular QTL genotype given the marker genotype now depends on the recombination fraction between the marker and the postulated QTL, and can take any value between 0 and 0·5. The likelihood can be written as follows:

$$L = \prod_{i=1}^{N} \frac{1}{(2\pi\sigma_w^2)^{\frac{1}{2}}} \sum_{g=1}^{G} \text{trans}(g \mid A_i) \exp\left[\frac{-(y_i-\mu-a_g-d_g)^2}{2\sigma_w^2}\right],$$

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where: $\text{trans}(g \mid A_i)$ is the transmission probability of offspring i being genotype g given that it has marker genotype A_i at the marker being considered.

(iii) *Unlinked QTL*

One test for the presence of a linked QTL is to compare the likelihood of the data under the assumption of linkage between the marker(s) and a postulated QTL with the likelihood for a QTL not linked to the marker(s). This unlinked QTL likelihood accounts for non-normality of the distribution of phenotypes as might be expected in the presence of a QTL. The likelihood for an unlinked QTL can be written as follows:

$$L = \prod_{i=1}^{N} \frac{1}{(2\pi\sigma_w^2)^{\frac{1}{2}}} \sum_{g=1}^{G} \text{trans}(g) \exp\left[\frac{-(y_i - \mu - a_g - d_g)^2}{2\sigma_w^2}\right],$$

where: $\text{trans}(g)$ is the transmission probability of the offspring being genotype g.

As stated before, the F_1 parents are all assumed to be heterozygous at the QTL, hence the probability of the F_2 offspring being either of the homozygous genotypes is 0·25 and of the heterozygous genotype 0·5. This likelihood is independent of the postulated position of the QTL on the chromosome.

(iv) *No segregating QTL*

An alternative hypothesis is one assuming either no genetic component or a genetic component due to many independent genes of small effect. In this case the single random effect is assumed to be normally distributed. The likelihood can be written as follows:

$$L = \prod_{i=1}^{N} \frac{1}{(2\pi\sigma_w^2)^{\frac{1}{2}}} \exp\left[\frac{-(y_i - \mu)^2}{2\sigma_w^2}\right],$$

where: μ is now the population mean.

(v) *Testing for a QTL*

To test for a linked QTL using flanking markers Lander & Botstein (1989) suggest moving the position of the putative QTL along the chromosome. For a given relationship between distance and recombination fraction the position can be converted into a recombination fraction with one marker (e.g. r_A). Given this, the likelihood can be maximized with respect to the remaining parameters (μ, a, d, σ_w^2) at each position. This likelihood is compared with the likelihood maximized assuming that a QTL is not segregating, the logarithm to base ten of the ratio of these likelihoods being plotted against the position to give the characteristic likelihood curve. Lander & Botstein (1989) suggest that the alternative hypothesis ought to be one of an unlinked QTL rather than no QTL, to take account of non-normality caused by unlinked QTL. However, they suggest that the QTL found in practice would not contribute greatly to any non-normality in the distribution of phenotypes and, hence, use a 'no QTL' model for their null hypothesis. From the use of regression models with flanking markers we (Haley & Knott, 1992) suggest that the evidence for a QTL is primarily obtained from differences in the mean effects of different marker genotypes, differences which will only be observed for QTL linked to markers being considered. This suggests that the use of 'no QTL' as the null hypothesis will not bias the results when an unlinked QTL is present.

We use twice the natural logarithm of the likelihood ratio (rather than the logarithm to base ten of the likelihood ratio) to indicate the presence of a QTL. With data under the null hypothesis this test statistic is expected to be asymptotically distributed as χ^2 with degrees of freedom equal to the number of parameters estimated in the full model and fixed in the reduced model (Wilks, 1938). For the test of linkage (linked QTL versus unlinked QTL) this would be 1 D.F. because the recombination fraction is estimated in the alternative model (linked QTL) and effectively fixed at 0·5 in the null hypothesis model (unlinked QTL). We have also looked at the test of a linked QTL versus no QTL. In this case, twice the natural logarithm of the likelihood ratio is expected to be distributed as χ^2 (3 D.F.) under the null hypothesis, as three parameters (a, d, r_A) are estimated in the full model (linked QTL) but not in the reduced model (no QTL).

The use of the null hypothesis of no QTL, compared with using an unlinked QTL, increases the test statistic by a constant along the chromosome, this constant being equal to the test statistic obtained from the comparison of a segregating QTL versus no QTL. With flanking markers the test of a linked versus an unlinked QTL provides a statistic that may not be expected to be distributed as χ^2 under the null hypothesis. For the linked hypothesis the recombination fraction between the marker and QTL is constrained to be less than or equal to the recombination fraction between the two flanking markers; hence the flanking marker likelihood cannot collapse to the unlinked QTL likelihood unless the QTL effect is zero in both models. This results in the situation where the likelihood given close linkage might be less than that given no linkage and hence the test statistic can be negative despite the estimation of one more parameter. That is, the two hypotheses are not nested unless areas outwith the interval are considered. A comparison of linked QTL versus no QTL does not have this problem.

Examples of log likelihood curves obtained as suggested by Lander & Botstein (1989) are given later. However, for most of this work we have maximized the likelihood for each interval in turn, estimating the recombination fraction between the postulated QTL and one of the markers (e.g. r_A) and constraining it to be less than the recombination fraction between the two markers (r).

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(vi) *Estimation of the recombination fraction between markers*

In most analyses presented in the literature it has been assumed that r (the recombination fraction between the markers) is known without error. For analyses including a single marker, the uncertainty of the position of the marker will not affect the detection of the QTL or estimates of its effect as in this situation the QTL is being located relative to the position of the marker. The analyses using flanking markers, however, take account of the distance between the markers. There are several possibilities for the best way to proceed. If an estimate of r is known from previous analysis with additional data this value could be used in the QTL detection analyses or r could be estimated from the data being used for QTL detection. If the same data are being used, both to estimate r and the QTL parameters, then this could be achieved either in a two step procedure, where r is estimated using only the marker information on the F_2 individuals and then this value used in the QTL analysis, or a one-step procedure, where all the parameters are estimated simultaneously.

Estimating r prior to the analysis for QTL detection is relatively fast and simple, requiring only the marker genotypes of the F_2 individuals. The likelihood is the product of the probability of the marker genotype for each individual which has only one unknown parameter, r. Estimating r at the same time as the QTL parameters, however, makes use of all the information available. The likelihood is similar to that for flanking markers when r is known (equation 1) but with trans $(g \mid A_i, B_i)$ redefined to include the probability of the observed marker class (i.e. the probability of g, A_i, B_i). To compare this likelihood with alternative models it has to be adjusted by dividing by the likelihood of the observed marker genotypes calculated at the ML estimate of r from the full model. Estimating all the parameters in one analysis, therefore, requires more computations, including the optimisation of a likelihood with an additional, unknown parameter.

3. Simulations and analyses

To investigate the properties of the analytical methods simulated data were used. Offspring were generated from completely heterozygous parents assuming no interference (i.e. that the presence of a recombination event between two loci has no effect on the probability of recombination between the next loci). Hence, $r = r_A + r_B - 2r_A r_B$ and map distances in Morgans are additive. Haldane (1919) has written a mapping function under this assumption relating map distance (x in Morgans) to recombination frequency (r):

$$r = \frac{1-e^{-2x}}{2}.$$

Each data set contained 1000 F_2 individuals. The genotype of each individual was composed of a pair of 'chromosomes' 100 cM in length. Eleven marker loci were simulated at 10 cM intervals and a variety of different models for the QTL were employed (see below).

Analyses either included all eleven markers, or omitted alternate markers leaving six markers at 20 cM intervals or omitted all but three (one at either end of the chromosome and one in the middle) leaving markers 50 cM apart. Data were analysed by ML methods, either interval mapping taking each interval in turn or only using a single marker at a time. In all cases the recombination fraction between the postulated QTL and one marker (r_A) was estimated together with the additive effect (a) and the dominance deviation (d) of the QTL, the mid-homozygote value (μ) and residual variance (σ_w^2). Unless stated otherwise, the recombination fraction between markers (r) was assumed to be known prior to the QTL analysis and fixed at the value used to simulate the data.

All analyses were performed using software written in FORTRAN 77 with maximisation performed using the quasi-Newton routine E04JBF from the NAG library (Numerical Algorithms Group, 1990).

(i) *No linked QTL*

No QTL. To investigate the behaviour of the test statistics, one thousand replicates of data were simulated with a normally distributed residual component only (i.e. without any QTL). These data were analysed under the three hypotheses described previously – no QTL, an unlinked QTL and a QTL linked to the marker(s) being considered. Analyses were repeated using different starting values to maximize the chance of obtaining a global maximum. The three possible test statistics were calculated – linked versus no QTL (*ts1*), linked versus unlinked QTL (*ts2*) and unlinked versus no QTL (*ts3*), which is the test made in segregation analysis (note that, *ts2* + *ts3* = *ts1*). Under the null hypothesis the distribution of *ts1*, *ts2* and of *ts3* over repeated simulation and analyses is expected to be χ^2 (3, 1 and 2 D.F. respectively).

Unlinked QTL. Data sets were simulated with an unlinked QTL of additive effect (a) equal to 0·25 or 2·0 residual standard deviations (i.e. $a = 0{\cdot}25\sigma_w$ or $a = 2{\cdot}0\sigma_w$). Such QTL would explain 3·0% and 67% of the variance in the F_2 population, respectively. Data obtained from 100 replicate simulations were analysed and the three test statistics compared with the appropriate χ^2 distribution.

(ii) *Single linked QTL*

A single QTL, 25 cM from one end of the chromosome was simulated. The following additive effects (a) for the difference between the alleles in residual standard

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deviations were considered: 0·025, 0·05, 0·125, 0·25 of 0·5. These represent half the difference in effect between the homozygous genotypes. QTL of these magnitudes explain approximately 0·03%, 0·12%, 0·78%, 3·0% and 11·1% of the total phenotypic variance in the F_2, respectively. Fifty replicates of each QTL effect were analysed.

Recombination fraction. Analyses of the 50 replicates of data simulated with a QTL with additive effect (a) of 0·25 were repeated with the recombination fraction between the markers fixed at an incorrect value. The simulated recombination fraction (r) was 0·1648 (corresponding to a distance of 20 cM) and the data were analysed assuming this value or 0·0906 (corresponding to a distance of 10 cM) or 0·2256 (corresponding to distance of 30 cM).

The same data were reanalysed, estimating the recombination fraction between the markers (r) from the 1000 F_2 individuals. Two approaches were used, either estimating r using the marker genotypes and fixing it at this estimate in the subsequent QTL analysis, or estimating r at the same time as the QTL parameters (a, d, μ, σ^2_w).

Residual component. Data were also simulated with the residual term being distributed as a χ^2 (4 D.F.). This distribution has an expected coefficient of skewness of 1·4. The distribution was scaled to have the same mean and variance as the normal distribution used previously. Two situations were simulated with 50 replicates each, the first with no QTL, the second with a linked QTL of effect 0·25 residual standard deviations (explaining 3·0% of the F_2 variance). Analyses were carried out assuming that the residual component was from a normal distribution.

(iii) *More than one linked QTL*

The same marked chromosome as described above was used and QTL in association (i.e. all alleles of increasing effect are fixed in the same parental line) were simulated. Two QTL, each of effect (a) of 0·125, were simulated to be 25 cM and 75 cM, or 25 cM and 55 cM, from one end of the chromosome. Ten QTL, each with effect (a) of 0·025, with one in the centre of each interval were simulated. All genes were additive in effect. In each case the mean difference between the inbred lines caused by this chromosome would be one half a residual standard deviation (the same as explained by a single locus with additive effect (a) of 0·25).

Models with two and ten QTL in dispersion (i.e. an inbred line is fixed both for alleles which have an increasing effect and alleles which have a decreasing effect on the trait of interest) were also considered. In both cases the two parental inbred lines would have the same expected effect from this chromosome. In the two-QTL model each locus had an effect (a) equal to 0·125 residual standard deviations. One QTL was simulated to be 25 cM from the end of the chromosome and the other at 75 cM. The ten QTL were distributed for increasing and decreasing effect in the parental lines in alternate positions, and each had an effect (a) of 0·025 residual standard deviations. In all cases fifty replicates were analysed.

4. Results and discussion

(i) *No linked QTL*

No QTL. The mean test statistic for each test over the thousand replicate simulations is given in table 1 for one 20 cM interval and for one marker. Also given is the variance of this statistic over replicates and the percentage significant when compared with the relevant χ^2 distribution. For a χ^2 distribution the mean is expected to be equal to degrees of freedom and the variance equal to two times the degrees of freedom. The numbers of test statistics falling into ten equal regions of the expected χ^2 distribution were compared with the expected number (i.e. 100) using a χ^2 test (9 D.F.). The results are also given in table 1.

The test statistics obtained from the test of a linked QTL using flanking markers versus no QTL (*ts1*) approximately followed the expected distribution. The mean and variance of the test statistics are slightly lower than expected for a χ^2 (3 D.F.) which might be a result of restricting the search area to within the pair of markers.

For the test of a linked versus an unlinked QTL (*ts2*) the mean is higher than expected for a χ^2 distribution with one degree of freedom and the variance much higher when using flanking markers. As expected, some of the test statistics were negative because of the restricted search area, and the proportion of these was high (nearly 30%). In over 14% of the data sets evidence for a linked QTL was found at the 5% significance level, and the top 5% of test statistics were greater than or equal to 6·39 whereas the χ^2 distribution has a 5% quantile of 3·84. Obviously the χ^2 distribution is not the correct one and to ensure that spurious QTL were not detected too frequently a higher value would have to be used to indicate analyses that gave evidence for a QTL.

The test statistics from the comparison of the hypotheses of an unlinked QTL and no QTL (*ts3*) did not follow a χ^2 distribution, with too many test statistics being close to zero. The lack of agreement in this situation is supported by work carried out on mixture distributions which suggests that this sampling distribution for the test statistic is inappropriate (for example, McLachlan & Basford, 1987; Titterington, Smith & Makov, 1985). Local maxima were frequently encountered when maximizing the unlinked QTL likelihood, and although there has been an attempt to prevent this, the results used may not all represent the global maximum. Obtaining local maxima for this hypothesis would also affect the test of linked (with flanking markers) versus unlinked QTL (*ts2*) and would inflate the mean and variance of this statistic.

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Table 1. *Mean and variance of the test statistics obtained from analysis of 1000 replicate simulations without a QTL and two sets of 100 replicate simulations with a QTL unlinked to any markers explaining either 3% or 67% of the F_2 variance. The results are based on a single 20 cM interval or one marker. The percentage significant at the 5% and 1% levels of the relevant χ^2 distribution and the χ^2 value (9 D.F.) obtained comparing the observed with the expected distribution of test statistics are also given*

Simulation	Method	Test†	Mean	Variance	5%	1%	χ^2 value‡
No QTL	Flanking markers	*ts1*	2·901	5·374	4·7	0·8	18·68*
		ts2	1·279	8·857	14·7	4·3	—
		ts3	1·621	3·657	4·1	1·2	158·36***
	Single marker	*ts1*	3·283	6·719	6·2	1·5	21·16*
		ts2	1·661	3·868	12·8	2·9	260·86***
Unlinked QTL (3% of F_2 variance)	Flanking markers	*ts1*	2·784	5·383	3	1	9·80
		ts2	1·138	8·195	11	4	—
		ts3	1·646	2·658	2	0	30·60***
	Single markers	*ts1*	3·169	5·265	3	0	15·20
		ts2	1·524	3·557	8	2	24·00**
Unlinked QTL (67% of F_2 variance)	Flanking markers	*ts1*	2·875	4·661	4	0	7·4
		ts2	−15·35	68·15	0	0	—
		ts3	18·23	62·07	99	93	900·00***
	Single marker	*ts1*	19·43	63·98	98	87	900·00***
		ts2	1·202	3·849	11	2	153·8***

† *ts1* Linked QTL versus no QTL (3 D.F.)., *ts2* Linked QTL versus Unlinked QTL (1 D.F.). *ts3* Unlinked QTL versus no QTL (2 D.F.).
‡ — value cannot be calculated because of negative test statistics.
* indicates significance at 5%, ** at 1% and *** at 0·1%.

Local maxima are a minor problem with the linked QTL hypothesis and not a problem for the no-QTL hypothesis. The test of a linked versus unlinked QTL should be applied with caution when using flanking markers because of these problems.

With a single marker, comparing linked versus no QTL (*ts1*) the mean and variance of the statistics are higher than expected and likewise the number significant, but nonetheless the test statistic distribution is very similar to a χ^2 (3 D.F.). Considering the test of a linked versus unlinked QTL (*ts2*) there is no problem with negative test statistics as the complete space is searched; however, too many data sets gave significant evidence for a QTL when compared with a χ^2 distribution.

We have limited the above results and discussion to a random, single interval for flanking markers and would suggest that, in this situation, the test statistic obtained from the comparison of a model with a linked QTL with no QTL (*ts1*) follows a χ^2 distribution with the expected degrees of freedom sufficiently closely for all practical purposes. Deviations from this distribution are due to the restricted search area. However, in practice, a series of such tests will be made, one for each interval along the chromosome (five in this case). If the interval giving the highest statistic was chosen for each data set the mean test statistic over the thousand replicates was 4·827 and the variance 6·989, with 13·5% significant at the 5% level of a χ^2 distribution (3 D.F.) and 2·4% significant at the 1% level. These are higher than expected for a single test, but not as high as expected if five independent tests had been carried out for each replicate.

Unlinked QTL. Table 1 also contains the results from the analyses of data containing an unlinked QTL. With flanking markers the test of a linked versus no QTL (*ts1*) is not affected by the presence of an unlinked QTL and the distribution of test statistics is not significantly different from a χ^2 (3 D.F.) even when the QTL is responsible for 67% of the F_2 variance. However, the other two statistics (*ts2* and *ts3*) are affected by an unlinked QTL. For the test of an unlinked QTL versus no QTL (*ts3*) this is expected, as the data has been simulated under the alternative hypothesis of an unlinked QTL and, hence, a non-central χ^2 distribution of test statistics is expected. We can see that this test is not very powerful for the QTL of small effect (i.e. 3% of F_2 variance), with very few analyses giving significant evidence of a QTL and a test statistic distribution looking similar to that expected under the null hypothesis. However, when the effect of the segregating gene was large (i.e. 67% of F_2 variance) a QTL was detected in virtually all the replicate data sets with *ts3*. The test of a linked QTL versus an unlinked QTL (*ts2*) is not affected by the gene of small effect; however, when the gene has a large effect there are many negative test statistics when using flanking markers because an unlinked QTL can explain the data better than one between the markers. Searching outside the interval would increase the mean of the test statistics.

From now on we will report results based on the test of a QTL between the markers being considered

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versus no QTL (*ts1*). In contrast to *ts2*, *ts1* is not significantly prone to problems associated with local maxima and the use of χ^2 values provides a suitable criterion against which to test this statistic.

(ii) *Single linked QTL*

Figure 1 gives an example of the characteristic likelihood curves obtained from interval mapping. The values plotted represent twice the difference between the natural logarithm of the maximum flanking marker likelihood assuming a QTL in the given position and the natural logarithm of the likelihood of a single normal distribution (no QTL). The data were generated with a single QTL with additive effect (a) of 0·5 as described above. The surfaces for the three different interval sizes (i.e. with eleven, six or three markers) for the same set of data are given. The surface can be seen to drop at the position of markers: this is because at this point there can be no recombination between the marker and the QTL so that a given marker genotype must be associated with the same QTL genotype across all individuals and phenotypic values should be independent of the other marker. However, the data provides evidence that recombination has occurred and so the location of the QTL at or near the marker becomes less likely than a location further away. All three curves coincide at common markers. For this simulation the effect of the gene is relatively large and the gene is accurately located by the peak in the test statistic surface. Note that the test statistic is greater than its expected value of three, when no QTL is segregating, along the length of the chromosome, but to a greater extent in the relevant interval. The curve produced from the 50 cM map has a much lower and less obvious peak. The rate at which the test statistic falls as the QTL is moved away from the maximum is a measure of the accuracy with which the QTL is located. Human geneticists often consider a '1 LOD support interval', which is the interval around the peak, bounded by positions where the likelihood has decreased ten-fold from the maximum value. This is used analogously to a confidence interval.

Figure 2 gives the mean test statistic over the 50 replicate analyses for each interval (interval mapping) and for each marker (single marker mapping), where 11 markers were analysed, for the test of a linked QTL versus no QTL. For interval mapping the means have been plotted in the centre of each interval although the estimates for the position of the QTL would vary across the replicates. The lines joining the points are only shown to clarify the trends. On average, the QTL is located in the correct interval and the closest single markers gave the highest test statistic. The results using single markers give lower mean test statistics.

For each data set the interval and the single marker providing the highest test statistic over the whole chromosome was found. The mean and standard deviation of these test statistics over the 50 replicates for each of the five sizes of effect for the QTL and the three interval sizes considered is given in table 2. Table

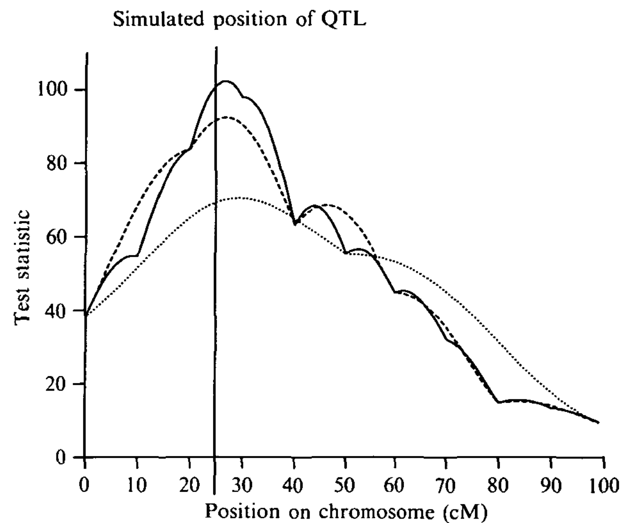

Figure 1. The likelihood curve produced by the analyses of a single set of data. The data were generated with a single QTL of additive effect of one residual standard deviation between homozygotes ($a = 0{\cdot}5$) 25 cM along the chromosome. This QTL explains approximately 11% of the phenotypic variance in the F_2. Eleven markers on a 100 cM chromosome were simulated and in the analyses either all 11 markers (at 10 cM intervals) (shown by a solid line), or six markers (at 20 cM intervals) (dashed line) or three markers (at 50 cM intervals) (dotted line) were used. The putative QTL is moved along the chromosome between each pair of markers and in each position the height of the curve represents twice the natural logarithm of the ratio of likelihoods (QTL in that position/no QTL).

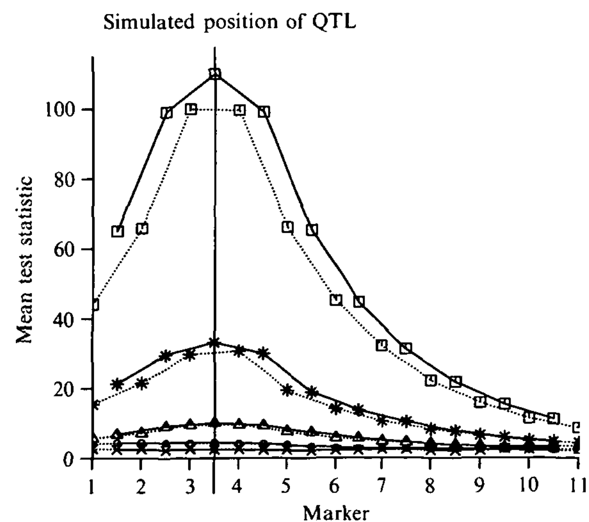

Figure 2. The mean test statistic over fifty replicates for each interval and for each single marker for the analyses where there was a single QTL 25 cM along the chromosome and where all eleven markers were included. The solid line connects results obtained with interval mapping and the dotted line for the same data sets with single marker mapping. The five sizes of gene are indicated as follows: $a = 0{\cdot}5$ – □; $a = 0{\cdot}25$ – *; $a = 0{\cdot}125$ – △; $a = 0{\cdot}05$ – ○; $a = 0{\cdot}025$ – x.

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Table 2. *Mean test statistics (with empirical standard deviation) for the test of a linked QTL versus no QTL (ts1) for the interval or marker that gave the highest test statistic over the 50 replicate simulations*

Simulated QTL effect (a)	Interval mapping: distance between markers 10 cM	20 cM	50 cM	Single marker mapping: distance between markers 10 cM	20 cM	50 cM	Unlinked versus no QTL
0·025	4·94	4·29	3·51	5·18	4·61	3·77	1·55
	(2·13)	(2·20)	(2·20)	(2·56)	(2·62)	(2·20)	(1·63)
0·05	6·42	5·88	4·40	7·08	6·57	4·89	1·26
	(3·46)	(3·51)	(3·05)	(3·54)	(3·35)	(3·06)	(1·30)
0·125	11·21	10·24	7·73	12·00	10·60	7·60	1·76
	(4·69)	(4·26)	(4·53)	(5·04)	(4·30)	(4·29)	(1·99)
0·25	33·65	30·77	20·69	33·63	30·42	18·44	1·78
	(11·97)	(11·93)	(8·60)	(11·56)	(11·50)	(7·99)	(1·79)
0·5	110·01	103·66	64·76	104·72	100·09	50·73	1·39
	(19·59)	(19·90)	(15·28)	(16·92)	(19·12)	(12·12)	(1·89)

Table 3. *The percentage of times that the maximum test statistic was located in the correct interval or linked to the closest marker(s)*

Simulated QTL effect (a)	Interval mapping: distance between markers 10 cM	20 cM	50 cM	Single marker mapping: distance between markers 10 cM	20 cM	50 cM
0·025	12	16	44	24	26	72
0·05	20	24	76	28	26	86
0·125	44	46	82	58	66	88
0·25	72	62	94	86	86	98
0·5	98	92	100	100	98	100

3 indicates the percentage of times this maximum was in the interval simulated to contain the QTL or associated with the marker (or markers) simulated to be adjacent to the QTL.

The mean test statistic is approximately linearly related to the ratio of the variance due to the QTL and the residual variance. The approximation suggested by Lander & Botstein (1989) can be applied for the prediction of the test statistic. This approximation is equivalent to $[(1-2\theta)/(1-\theta)]\ N\log_e[1/1-p]$ for a population of size N with a QTL explaining a proportion, p, of the total variance, located centrally between two markers which are a recombination fraction of θ apart, or $(1-2\theta)^2 N\log_e[1/1-p]$ for the same QTL at a recombination fraction θ from a single marker. These formulae predict test statistics of 106·19 and 96·68 for flanking and single markers respectively for the gene explaining 11% of the variance in a 10 cM map, 63·36 and 43·34 for these two tests in a 50 cM map and 27·76, 25·26, 16·55, and 11·32 respectively for the same four tests for a QTL responsible for 3% of the variance. The predicted values are the non-central portion of the test statistic to which must be added the degrees of freedom (i.e. three) to compare with the values from the simulations shown in table 2. The expected test statistics are very close to the observed, with the tendency of the observed single marker test statistic to be higher than the predicted due to our selection of the highest test statistic over all markers in calculations of the means.

When the QTL have a small effect single marker mapping gives higher mean test statistics than interval mapping. This could reflect the fact that the QTL is only being located (detected) infrequently and in some of the simulations restricting the QTL to be within a given interval (with a restricted value for the maximum recombination fraction from the markers) is less likely than the larger recombination fraction possible with single markers.

For the QTL of larger effect (i.e. $a \geqslant 0·25$) the gain from interval mapping over single marker mapping is small when the markers are closely spaced; however, when the markers are at 50 cM intervals interval mapping is an appreciable improvement over single marker mapping.

Ignoring marker information and using the test of an unlinked QTL versus no QTL (*ts3*) provides virtually no power for the detection of QTL of the size of effect being considered here. The mean test statistics are all lower than 2·0, the expected value for data under the null hypothesis.

The mean estimates for the additive effect and position of the QTL over the fifty replicate simulations are presented in table 4. For the single markers the

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Table 4. *Mean estimates of the additive effect of the QTL and either its mean distance from the end of the chromosome (interval mapping) or its mean distance from the marker † (single markers) (with their empirical standard deviation) over 50 replicate simulations for the interval or marker which gave the maximum test statistic*

Parameter	Simulated additive deviation (a)	Interval mapping: distance between markers			Single marker mapping: distance between markers			Unlinked
		10 cM	20 cM	50 cM	10 cM	20 cM	50 cM	QTL
Additive effect (a)	0·025	0·026	0·025	0·026	0·099	0·099	0·129	0·557
		(0·064)	(0·064)	(0·058)	(0·340)	(0·351)	(0·369)	(0·257)
Distance (cM)		46·46	50·15	57·07	59·74	63·95	71·80	
		(35·18)	(34·24)	(39·58)	(43·44)	(49·89)	(58·44)	
Additive effect (a)	0·05	0·069	0·064	0·055	0·311	0·305	0·271	0·585
		(0·062)	(0·065)	(0·062)	(0·354)	(0·353)	(0·362)	(0·272)
Distance (cM)		33·83	36·46	33·09	58·81	60·79	67·52	
		(29·98)	(31·12)	(32·57)	(39·88)	(40·04)	(53·10)	
Additive effect (a)	0·125	0·130	0·130	0·126	0·375	0·344	0·346	0·624
		(0·055)	(0·054)	(0·063)	(0·273)	(0·281)	(0·297)	(0·300)
Distance (cM)		31·94	30·55	33·45	47·12	45·08	47·66	
		(19·42)	(17·78)	(24·82)	(33·44)	(39·32)	(34·82)	
Additive effect (a)	0·25	0·260	0·257	0·250	0·533	0·499	0·437	0·632
		(0·047)	(0·050)	(0·063)	(0·269)	(0·270)	(0·293)	(0·281)
Distance (cM)		24·22	21·45	25·45	32·62	31·45	35·79	
		(7·54)	(6·74)	(14·34)	(26·96)	(28·80)	(34·26)	
Additive effect (a)	0·5	0·500	0·498	0·497	0·624	0·622	0·601	0·606
		(0·047)	(0·050)	(0·061)	(0·183)	(0·186)	(0·256)	(0·288)
Distance (cM)		24·93	24·19	25·57	13·09	14·05	26·68	
		(1·93)	(3·38)	(4·76)	(13·66)	(14·19)	(21·55)	
Expected distance		25·00	25·00	25·00	5·00	5·00	25·00	

† Does not include analyses that resulted in an unlinked gene. For the 50 cM map and the QTL of effect (a) of 0·025, 0·05 and 0·125, three, one and three analyses were omitted, respectively.

position is given as the mean distance from the marker. For interval mapping the position is given as the mean distance from one end of the chromosome. For the large effect genes (i.e. $a \geqslant 0{\cdot}25$), the results from interval mapping both correctly position the QTL and estimate its effect on average. For example although correctly positioned on average, only thirty-six out of the fifty analyses place the gene of effect (a) 0·25 in the correct 10 cM interval. As the effect of the QTL decreases the average position of the gene moves to around 50 cM which is approximately half way along the chromosome and the value expected, on average, if no QTL was detected. The mean estimated effect of the gene correctly reflects the effect of the simulated gene for genes of all effect. The estimates obtained using single markers are not close to the simulated values, with both the distance from that nearest marker and the additive effect (a) over-estimated. It can be seen that, especially for the small effect QTL, this distance from the nearest marker is large – much larger than the maximum possible when restricting the QTL to be between the markers, providing support for the explanation for interval mapping giving a lower test statistic than single markers for the small effect genes. The empirical standard deviations of both position and effect estimates are much smaller with interval mapping than with single marker mapping and they decline with more closely spaced markers and genes of larger effect. Ignoring marker information and fitting an unlinked QTL gives, on average, an overestimate for the additive effect with a large variance in the estimates over the fifty replicates.

Recombination fraction. Table 5 gives the results from interval mapping analysis of data where an incorrect value for the interval size has been used. In this case using the incorrect value has little effect on the estimates and does not affect the test statistic. The position of the QTL was estimated to be in approximately the same place relative to the two flanking markers (i.e. just less than a quarter of the total distance from the first marker) and the estimate of its effect was the same. This suggests that the use of an incorrect value for the recombination fraction between the markers will not affect the ability to map QTL as long as the order of markers along the chromosome is correct.

Estimating the recombination fraction between the markers (r) using marker information from the 1000 F_2 individuals gave an accurate estimate of the simulated recombination fraction between the markers, and hence the results for the QTL obtained when using this value (results not shown) did not differ significantly from the results using the simulated

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Table 5. *Mean test statistic for the test of a linked QTL versus no QTL (ts1) and mean estimates of the recombination fraction from one of the markers (r_A) and additive effect (a) of the QTL over 50 replicate simulations. The QTL was simulated with additive effect (a) 0·25 residual standard deviations, in a 20 cM interval (r = 0·165) 5 cM from one of the markers (r_A = 0·047) and analysis was carried out assuming an incorrect value for the recombination fraction between markers. Empirical standard deviations are given in brackets*

Assumed interval size, *r*	r_A	*a*	*ts1*
0·091	0·023 (0·018)	0·255 (0·049)	30·15 (12·05)
0·165 (Simulated value)	0·045 (0·035)	0·253 (0·049)	30·16 (12·05)
0·226	0·066 (0·050)	0·256 (0·050)	30·16 (12·04)

value for *r*. Also, when analysing the data estimating *r* at the same time as the QTL parameters, the same estimates were obtained as when estimation of *r* took place prior to the QTL analysis. In the example considered here the QTL has a small effect making genotyping uncertain. Furthermore, the analyses were performed assuming the correct mapping function of no interference. The incorporation of the QTL, which acts as an internal marker, will suggest the same recombination fraction as using the flanking markers alone except for chance deviations from the expected value, and with 1000 observations this effect will be small. Differences between the methods might result, however, if the QTL had a large effect, such that genotyping at this locus was accurate and if the data were analysed with an incorrect interference assumption.

Residual component. Table 6 shows the mean test statistics and mean estimates for the position, additive effect and residual standard deviation (in units of simulated residual standard deviation) when the environmental component was simulated to be skewed and analysed assuming normality. The results illustrate that interval mapping is not sensitive to the use of an incorrect model for the residual component, nor is it sensitive to the distribution of phenotypes. Using the test of a linked QTL versus no QTL (*ts1*) gave little evidence for a QTL when none was simulated and good evidence when a QTL was simulated. In both cases the mean test statistic (i.e. *ts1*) was similar to that observed for the equivalent situations with a normally distributed environmental component. A test of a linked QTL versus an unlinked QTL (*ts2*) gave a negative test statistic on average for flanking markers. The mean parameter estimates

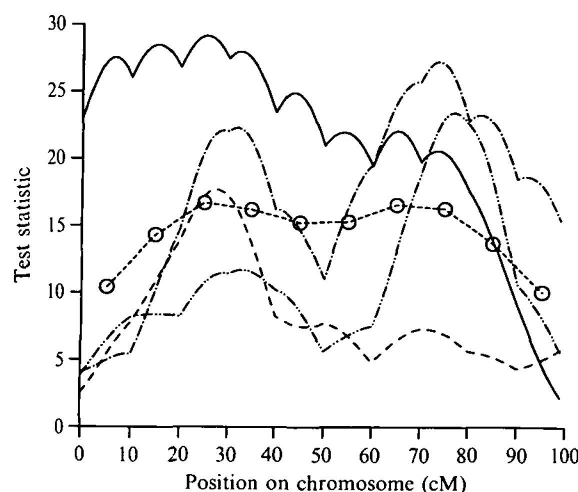

Figure 3. Four likelihood curves, one produced from the analysis of each of four randomly selected replicate data sets simulated with two QTL in association using eleven markers. Each QTL would explain about 1% of the phenotypic variance in the F_2. One is simulated to be 25 cM from the end and the other 75 cM. The dashed line joins the mean test statistics (shown by ○) over 50 replicates of data of this structure from each interval.

Table 6. *Mean test statistics (ts1, ts2 and ts3†) and estimates of the position (cM)‡, additive effect (a) and residual standard deviation (σ_w) for analyses of data simulated either without a QTL or with one QTL with additive effect 0·25 residual standard deviations and a skewed residual component over 50 replicate simulations. The mean is over the highest test statistic for each data set using 6 markers. Empirical standard deviations are given in parentheses. The residual standard deviation was simulated to be 1·0*

Simulation	Method	Position (cM)	*a*	σ_w	*ts1*	*ts2*	*ts3*
No QTL	Flanking markers	51·52 (34·13)	0·017 (0·062)	0·997 (0·036)	4·41 (2·32)	−124·07 (23·98)	128·49 (24·02)
	Single markers	141·30 (30·51)	−0·195 (0·953)	0·667 (0·033)	130·76 (23·90)	2·27 (2·39)	128·49 (24·02)
Linked QTL (*a* = 0·25)	Flanking markers	24·95 (6·32)	0·261 (0·046)	0·997 (0·035)	31·34 (10·59)	−87·83 (23·00)	119·17 (21·57)
	Single markers	90·72 (16·21)	0·988 (0·058)	0·684 (0·027)	130·54 (23·86)	11·37 (6·96)	119·17 (21·57)

† *ts1* linked QTL versus No QTL; *ts2* linked QTL versus Unlinked QTL; *ts3* unlinked QTL versus no QTL.
‡ Given as the distance from the end of the chromosome for flanking markers and from the marker for single markers.

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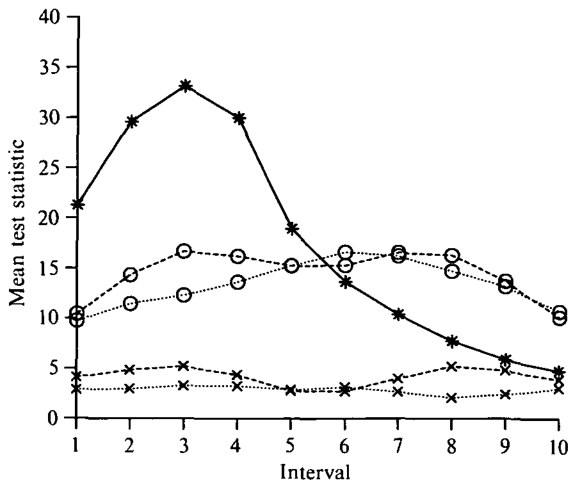

Figure 4. The mean test statistic over fifty replicates for each interval in the analysis of data with more than one QTL. The dashed line joins means from analyses of data with two QTL (each would explain 1% of the F_2 variance) and the dotted line for ten QTL (each would explain 0·03% of the variance). In each case the circle indicates that the loci were in association and the cross that they were in dispersion. The solid line connects means from analyses of data containing a single QTL that explained the same difference between inbred lines as the QTL in association.

obtained with interval mapping are close to the expected values. The methods using single markers and ignoring marker information are sensitive to the distribution of the residual component. In both cases, when the null hypothesis is no QTL (*ts1* and *ts3*), good evidence for a gene is found even when one is not present.

These results are supported by the results given previously for an unlinked QTL which, although it caused non-normality of the data, was not detected when looking at the hypothesis of a linked QTL versus no QTL. Also, results using flanking markers in a regression analysis (Haley & Knott, 1992) are very similar to ML results, yet ignore information on the distribution of phenotypes. In practice, the data might be transformed to remove the extremes of non-normality. Our results suggest that any residual non-normality will not greatly bias interval mapping analyses as it might analyses using only a single marker.

(iii) *More than one linked QTL*

Figure 3 gives the mean test statistic for each interval for the analyses of data simulated with two QTL in association, 50 cM apart and the curves obtained from four of the replicate data sets all using eleven markers. Figure 4 gives the mean statistics from analyses of data containing two or ten segregating QTL. Also shown on this graph is the curve obtained from analysis of data where the same difference between inbred lines (0·5 residual standard deviations) was caused by a single gene. The presence of QTL in association inflates the test statistic surface over the whole chromosome due to positive covariances between QTL. However, the average curve is flatter than would be expected if only one locus explained the difference between the homozygotes. When two QTL are simulated, on average it is possible to see two peaks corresponding to the two QTL. When the two QTL are in dispersion the mean test statistic shows the same pattern but it is reduced in value because of the negative covariances between the QTL. If the two QTL are simulated to be closer together then, even on average over the fifty replicates, the two distinctive bumps are no longer visible (data not shown).

Table 7 gives the mean estimates of the additive effect and recombination fraction for the situations with two QTL 50 cM apart analysed using flanking markers. Estimates are given for the two intervals containing the QTL. The results show that the estimates of the additive effect of the QTL are slightly

Table 7. *Mean estimates of the additive effect* (a) *of the QTL and its distance from the marker* (*with their empirical standard deviation*) *over 50 replicate simulations for data simulated with two QTL of effect* (a) *0·125, 50 cM apart. Results are given for the two intervals containing the QTL both for the 10 cM and 20 cM interval size*

		Interval size			
		10 cM		20 cM	
Simulation	Parameter	1st QTL	2nd QTL	1st QTL	2nd QTL
In association	Additive effect (a)	0·179 (0·046)	0·175 (0·051)	0·186 (0·048)	0·182 (0·052)
	Recombination fraction (r_A)	0·056 (0·033)	0·033 (0·029)	0·083 (0·050)	0·085 (0·052)
In dispersion	Additive effect (a)	0·078 (0·044)	−0·081 (0·042)	0·072 (0·043)	−0·063 (0·054)
	Recombination fraction (r_A)	0·042 (0·041)	0·054 (0·038)	0·039 (0·060)	0·011 (0·072)

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overestimated when the genes are in association and underestimated when the genes are in dispersion. When a 20 cM map was used the estimates were slightly more biassed (and the empirical standard deviation increased). The recombination fractions were overestimated in both situations.

For models simulated with two QTL estimates are biassed even when we analyse the data assuming a QTL in the correct interval. In practice estimates will be worse because the intervals containing QTL will be unknown and their position will have to be inferred. To detect more than one QTL on a chromosome a 'Munro' doctrine might be adopted which requires a drop from the highest peak to a likelihood trough and then a minimum increase to a further peak (Munro, 1981). Even setting this minimum increase to as little as a ten-fold likelihood change (equivalent to a change in twice the natural logarithm of the likelihood ratio of 4·6) would result in a second QTL being accepted in only one of the curves in figure 3. Fitting two QTL simultaneously is a much preferable alternative (Haley & Knott, 1992).

5. Conclusions

The use of maximum likelihood methods to map QTL using markers has become well established. However, a number of questions have arisen and we attempt to consider some of them in this paper.

One problem is the choice of test statistic, and in particular whether the use of a 'no QTL' model as the null hypothesis will bias the results. The results here, supported by work using a regression model, indicate that the test of a linked QTL versus no QTL is probably both the easiest and most reliable test for a linked QTL. For practical purposes twice the natural logarithm of the likelihood ratio for this test follows a χ^2 distribution with degrees of freedom equal to the number of parameters different between the models both for single marker and for flanking marker analyses. Furthermore when flanking markers are used (but not for single markers) this test is robust to the effects of segregating unlinked QTL and non-normal residual variance.

The use of flanking markers only provides substantially more power for QTL detection than using markers singly when the markers are widely spaced and the QTL effects large. The former method, however, provides more precise estimates of the effect and position of QTL without substantially increasing the computation time. Estimates from the use of flanking markers are also relatively robust to the effects of the distribution of phenotypes.

A second problem is caused by the presence of multiple QTL. Although the value of the test statistic may be above that expected under the null hypothesis, it will not show the classic peak obtained from a single QTL. When two QTL are segregating, multiple peaks may be observed, with high values for the test statistic both in the intervals that contain the QTL and in intervals that do not. Fitting two QTL to the data can successfully separate these loci (see Haley & Knott, 1992).

We have, to a large extent ignored the problem of determining significance. With multiple, but non-independent, tests being performed the significance threshold will have to be set at a more stringent level than 0·05. For n independent tests the significance level for each test, p, would normally be obtained from $0{\cdot}05 = 1-(1-p)^n$ for an overall significance level of 5%. The number of tests performed is equal to the number of intervals but the number of truly independent tests is equal to the number of chromosomes, thus n might be set somewhere between these two values. The analyses here will often be applied to a cross between lines which differ substantially. If the differences are due to very many genes of small effect distributed through the genome the average likelihood will be inflated. With a total difference of 7·5 S.D. between lines due to 150 loci evenly distributed over fifteen chromosomes, each chromosome would produce a mean surface like that shown for ten associated loci in figure 4. This effect should be taken into account when setting significance levels for the detection of individual QTL (or clusters of QTL). Thus, in crosses between divergent lines one should either consider setting a high significance threshold or be very cautious of likelihood curves which may have a high maximum value but which are not markedly peaked. Further theoretical work is needed in this area, but at present there seems no alternative to the use of simulation, taking into account the genetic map of the organism and line genetic difference, for setting significance thresholds.

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