Theor Appl Genet (1998) 96 : 628–633

B. Goffinet · B. Mangin

Comparing methods to detect more than one QTL on a chromosome

Received: 15 March 1997 / Accepted: 19 September 1997

Abstract The aim of this paper was to compare different methods for testing the presence of one versus multiple QTLs on a same chromosome. We describe different methods that have partially been taken out of the literature. We perform simulations covering different situations to compare the power of these methods for detecting more than one QTL. None of the tests considered appear to be similar; that is, the first-type error depends on the value of the parameters concerning the first QTL. The method starting with a two-QTL model is the most powerful in many situations.

Key words QTL detection · Mapping · Simultations

Introduction

Following the investigation of Sax (1923), many methods have been developed for detecting quantitative trait loci (QTLs) using marker information. It is possible to estimate the map location of QTLs and the additive or dominant effect of a QTL using "interval mapping" procedures based on maximum likelihood estimation (Lander and Botstein 1989) or on linearized approximation of maximum likelihood (Knapp et al. 1990; Haley and Knott 1992). However, one difficult problem is to test if one or more than one QTLs are present on a same chromosome.

A real issue in QTL analysis would be to estimate the number of QTLs and not only if there is more than one QTL on a chromosome. Our reasoning suggests that this search is possible with QTLs with large effects using, for example, the methods developed by Zeng (1993, 1994), but that for the effect usually observed in real data analysis, it is frequently only possible to answer the question "one or more than one QTL". In the following we will focus our attention on this question, and we will consider essentially QTLs with small or moderate effect; that is, for example, a QTL explaining less than 25% of the total phenotypic variance in an experiment with 150 individuals or less than 10% for 500 individuals.

Different authors have proposed a variety of methods that can be used to build a test answering such a question. Lander and Botstein (1989) proposed using an interval mapping test to determine the more likely position of the QTLs and then computing a least-square method that included these two QTLs in the model. Jansen (1993, 1994) proposed using some marker genotypes chosen on the basis of value of Akaike criteria as covariates in the interval mapping test. The principle of this criterion is to give a penalty for fitting more parameters. Zeng (1993, 1994) considered a somewhat similar method. He proposed looking for QTL in each interval between two markers with an interval mapping test, using all the other marker genotypes as covariates. Whittaker et al. (1996) proposed to use a linear model that included the effect of several QTL and to use ANOVA tests.

In this paper, we will make use of these ideas and of new ideas to construct different tests. In an attempt to compare fairly the power of these tests, we propose to calculate a threshold for each test such that the first-type error is less than or equal to 5% for all possible locations and effects of the first QTL present under the H_0 hypothesis. We then compare the power of the different tests using simulations in different situations including two QTLs. To simplify the computation, we make all the comparisons in a back-cross population.

Communicated by J. Beckmann

B. Goffinet (✉) · B. Mangin
Unité de Biométrie et Intelligence Artificielle, Institut National de la Recherche Agronomique (INRA) Centre de recherches de Toulouse, Chemin de Borde Rouge, Auzeville,
31326 Castanet Tolosan, France
E-mail: goffinet@toulouse.inra.fr

The following section describes the notations and models which will be used for the different tests.

Models for one or two QTLs

We consider a backcross population of size n and a chromosome of length L and M markers located at position $d_1^{\mathcal{M}}, \ldots, d_m^{\mathcal{M}}, \ldots, d_M^{\mathcal{M}}$. Consider a first QTL Q_1 with two alleles Q_1^A and Q_1^B, and therefore two genotypes $Q_1^A \times Q_1^A$ and $Q_1^A \times Q_1^B$, located at a position d_1 from the beginning of the chromosome and a second QTL Q_2 with two alleles Q_2^A and Q_2^B, and therefore two genotypes $Q_2^A \times Q_2^A$ and $Q_2^A \times Q_2^B$, located at a position d_2. The mean difference between genotypes $Q_i^A \times Q_i^A$ and $Q_i^A \times Q_i^B$ is denoted a_i, for $i = 1, 2$. We assume no epistasis between the two QTLs. The genotypic value of the four genotypes at both loci is therefore $\mu + (\mathbb{1}_{Q_1^A} - \frac{1}{2})a_1 + (\mathbb{1}_{Q_2^A} - \frac{1}{2})a_2$, where $\mathbb{1}_{Q_i^A}$ is the indiocator variable for genotype $Q_i^A \times Q_i^A$ for $i = 1, 2$.

The trait value has a normal distribution with residual variance σ^2. For each individual $k = 1, \ldots, n$, we measure the value of a phenotypic trait y_k and a set of markers $M_{m,k}$, $m = 1, \ldots, M$, that take the value A or B corresponding to the two alleles of the marker. The vector of phenotypic observation will be denoted by Y, and the vector of all marker information by $\mathcal{M}$. The probability, for the individual k, to carry the alleles Q_i^A conditional on the marker information $\mathcal{M}$ will be denoted by $G_{\mathcal{M}}(k, i, d_i)$, for $i = 1, 2$.

In the case of one QTL, this leads to the mixture model:

y_k is normally distributed with expectation $\mu + a_1/2$ and variance σ^2 with a probability $G_{\mathcal{M}}(k, 1, d_1)$, and normally distributed with expectation $\mu - a_1/2$ and variance σ^2 otherwise.

The mixture model can be approximated by the following linear model for each observation y_k (Knapp et al., 1990 or Haley and Knott 1992)

$$y_k = \mu + a_1(G_{\mathcal{M}}(k, 1, d_1) - \tfrac{1}{2}) + \varepsilon_k$$

where ε_k is normally distributed, with null expectation and variance σ^2.

In the case of two QTLs, the approximate linear model is

$$y_k = \mu + a_1(G_{\mathcal{M}}(k, 1, d_1) - \tfrac{1}{2}) + a_2(G_{\mathcal{M}}(k, 2, d_2) - \tfrac{1}{2}) + \varepsilon_k.$$

In the following we will consider that no interference is present and therefore will use the Haldane's mapping function. This function will associate the distance d with the recombination probability $r(d) = 0.5(1 - \exp(-2d))$.

The log-likelihood will be denoted by $L(a_1, d_1, \mu, \sigma)$ for the one-QTL linear model, and by $L(a_1, d_1, a_2, d_2, \mu, \sigma)$ for the two-QTLs linear model. The interval mapping test for one QTL is the log-likelihood ratio test $T^{[1]}$ of the hypothesis H_1 "$a_1 \neq 0$" against the null hypothesis H_0 "$a_1 = 0$"

$$T^{[1]} = 2\left(\sup_{(a_1, d_1, \mu, \sigma)} L(a_1, d_1, \mu, \sigma) - \sup_{(a_1 = 0, \mu, \sigma)} L(a_1, d_1, \mu, \sigma) \right).$$

Methods starting with a single-QTL model

Consider an interval mapping test $T^{[1]}$. The position of the maximum of the test is denoted $\hat{d}_1$ and the marker interval enclosing this position $\hat{j}_1$. Now, we consider two possible tests.

In the first, denoted $T_1^{[2]}$ and called "fixed first QTL", we consider the position $\hat{d}_1$ as fixed and compute a log-likelihood ratio test for the second QTL

$$T_1^{[2]} = 2\left(\sup_{a_1, a_2, d_2, \mu, \sigma} L(a_1, \hat{d}_1, a_2, d_2, \mu, \sigma) - \sup_{a_1, a_2, d_2, \mu, \sigma} L(a_1, \hat{d}_1, a_2 = 0, \mu, \sigma) \right)$$

In the second, we consider the interval containing the first QTL as the correct one, and therefore use a linear model where the two flanking markers of the interval $\hat{j}_1$ are used as covariates X_1 and X_2, that is for $i = 1, 2$, $X_i = 1$ if the marker alleles is A and 0 otherwise

$$y_k = \mu + a_2(G_{\mathcal{M}}(k, 2) - \tfrac{1}{2}) + X_1 b_1 + X_2 b_2 + \varepsilon_k.$$

The test $T_2^{[2]}$, called "fixed first-QTL interval", is the log-likelihood ratio test of the hypothesis "$a_2 \neq 0$" against "$a_2 = 0$" under this model.

The procedure for the two tests is the following

- compute $T^{[1]}$. If $T^{[1]} < \lambda$ decide "there is no QTL". The threshold λ is chosen such that the first-type error is α_1.
- if $T^{[1]} > \lambda$, then compute $T_i^{[2]}$ for tests $i = 1$ or $i = 2$. If $T_i^{[2]} > \lambda_i$ then decide that more than one QTL exists.

For $i = 1, 2$, the threshold λ_i is chosen such that

$$\Pr(T_i^{[2]} > \lambda_i \text{ and } T^{[1]} > \lambda) \leq \alpha_2$$

for $a_2 = 0$ and all possible values of a_1, d_1, μ, σ.

If the value of the parameter a_1 is sufficiently large (say $a_1 > \mathcal{A}$), the information matrix is definite-positive and therefore the parameters can be consistently estimated that is the estimation converges toward the true values of the parameters. In this case, it is possible to find a threshold λ_i such that

$$\Pr(T_i^{[2]} > \lambda_i \text{ and } T^{[1]} > \lambda) = \alpha_2$$

for $a_2 = 0$, $a_1 > \mathcal{A}$, and all possible values of the parameters.

For QTL with moderate effects, this is not true. The parameters μ and σ^2 can be estimated consistently, but it is not the case for a_1 and d_1 (Mangin et al. 1994). We therefore propose to calculate a threshold $\lambda_i(a_1, d_1)$ by Monte-Carlo simulations for different values of the parameters a_1 and d_1 and to take the threshold λ_i as the maximum of these thresholds.

Method starting with a multiple-QTL model

Consider now an interval mapping test for each interval j between two markers, where all of the $M - 2$ other markers are used as covariates in the model. The test statistic in interval j is denoted by $T_3^{(j)}$ and called "multiple-QTL model". One possible procedure to test whether or not there is more than one QTL is the following:

- Compute $T_3^{[1]} = \max_j T_3^{(j)} = T_3^{(j_1)}$ and $T_3^{[2]} = \max_{j \neq j_1 - 1, j_1, j_1 + 1} T_3^{(j)}$

– If $T_3^{[1]} > \lambda_3$ and $T_3^{[2]} > \lambda_3$ decide there is more than one QTL.
The threshold λ_3 is chosen such that

$$\Pr(T_3^{[2]} > \lambda_3 \text{ and } T_3^{[1]} > \lambda_3) = \Pr(T_3^{[2]} > \lambda_3) \leq \alpha_2$$

for $a_2 = 0$ and all possible values of a_1, d_1, μ, σ.

As for tests $T_1^{[2]}$ and $T_2^{[2]}$, the threshold λ_3 is taken as the maximum of the thresholds obtained by simulations for different values of the parameters a_1 and d_1.

Method starting with a two-QTL model

Let $L^{[2]}$ be the maximum of the log-likelihood $L(a_1, d_1, a_2, d_2, \mu, \sigma)$ corresponding to the two-QTL model and $\hat{d}_1$ and $\hat{d}_2$, the maximum likelihood estimator of the parameters d_1 and d_2. This maximization is done under the constraint that the two QTLs locations are not in adjacent intervals. The same kind of constraint is used by Whittaker et al. (1996) who found that the two locations and effects of two QTLs in adjacent marker intervals are not jointly estimable. Note that $\hat{d}_1$ and $\hat{d}_2$ are consistent estimates of d_1 and d_2 if the effect a_1 and a_2 are large, but not if these effects are small or moderate.

Let $L^{[1]}(\hat{d}_1)$ (resp. $L^{[1]}(\hat{d}_2)$) be the maximum of the log-likelihood $L(a_1, d_1 = \hat{d}_1, \mu, \sigma)$ where the parameter d_1 is fixed at the value $\hat{d}_1$ (resp. $d_2 = \hat{d}_2$). We propose the following procedure to test whether or not there is more than one QTL:

– Compute $T_4(\hat{d}_1) = 2(L^{[2]} - L^{[1]}(\hat{d}_1))$ and $T_4(\hat{d}_2) = 2(L^{[2]} - L^{[1]}(\hat{d}_2))$
and let
$T_4^{[1]} = \max(T_4(\hat{d}_1), T_4(\hat{d}_2))$ and $T_4^{[2]} = \min(T_4(\hat{d}_1), T_4(\hat{d}_2))$
– If $T_4^{[2]} > \lambda_4$, then decide there is more than one QTL.

This test $T_4^{[2]}$ is called "two-QTL model".

The threshold λ_4 is chosen such that

$$\Pr(T_4^{[2]} > \lambda_4 \text{ and } T_4^{[1]} > \lambda_4) = \Pr(T_4^{[2]} > \lambda_4) \leq \alpha_2$$

for $a_2 = 0$ and all possible values of a_1, d_1, μ, σ. As for tests $T_1^{[2]}$, $T_2^{[2]}$ and $T_3^{[2]}$, the threshold λ_4 is taken as the maximum of the thresholds obtained by simulation for different values of the parameters a_1 and d_1.

Method using the shape of the likelihood

In this section, we propose to build a test by combining two different estimates of a_1^2, the square of the effect of the first QTL. One estimate will be the usual maximum likelihood estimate $\hat{a}_1^2$ obtained with an interval mapping procedure under the one-QTL model. The second estimate is described in the following.

A new estimator of a_1^2

Consider the model with one QTL located at d_1. We can compute the estimate $\hat{a}_1(m)$ of the effect a_1 at each marker position d_m'', for $m = 1, \ldots, M$. The expectation of the square of this estimation is, neglecting terms of order n^{-2}

$$E(\hat{a}_1^2(m)) = (1 - 2r(|d_m'' - d_1|))^2 a_1^2 + \frac{\sigma^2}{4n}.$$

Consider now the family of estimator $\hat{a}_1^2(\lambda)$

$$\hat{a}_1^2(\lambda) = \sum_{m=1}^{M} \hat{a}_1^2(m)\lambda(m) - \frac{\sigma^2}{4n} \sum_{m=1}^{M} \lambda(m)$$

where $\lambda(m), m = 1, \ldots, M$ are real constants. The aim is to find a set of weights $\lambda(m)$ $m = 1, \ldots, M$ such that the estimator $\hat{a}_1^2(\lambda)$ is unbiased for all possible locations of the QTL. It is not possible to find such a weighting, but we propose to use a weighting such that the estimator is unbiased for all the locations of the QTL which are on a marker. It is easy to find this weighting by solving the linear system:

$$\sum_{m=1}^{M} \lambda(m)(1 - 2r(|d_m'' - d_1|))^2 = 1$$

for $d_1 = d_1'', \ldots, d_m'', \ldots, d_M''$.

For a chromosome of 100 cM, with six equally spaced markers, we found

$$\lambda(1) = \lambda(6) = 0.69 \text{ and } \lambda(2) = \lambda(3) = \lambda(4) = \lambda(5) = 0.38$$

Simulations (not shown) showed that this estimator remains relatively unbiased for the location of the first QTL which is between two markers, when the effect of the QTL is not too large.

Test construction

Now, if there is only one QTL on a chromosome, the maximum likelihood estimate $\hat{a}_1$ obtained with an interval mapping method, can be used to build an approximate unbiased estimator of a_1^2

$$\tilde{a}_1^2 = (\hat{a}_1)^2 - \widehat{var}(\hat{a}_1) = (\hat{a}_1)^2 - 4\frac{\sigma^2}{n}$$

where $\widehat{var}(\hat{a}_1)$ is an approximate estimate of the variance of a_1^2, obtained as if the location of the QTL was known and on a marker.

Now, we have two different estimates $\hat{a}_1^2(\lambda)$ and $\tilde{a}_1^2$, of a_1^2 whose sensibility to a second putative QTL is different. The joint distribution of these estimates is very difficult to establish analytically. Nevertheless, different test statistics for a second QTL can be considered with the general form:

$$T(\eta) = \frac{\hat{a}_1^2(\lambda) - \tilde{a}_1^2}{(\tilde{a}_1^2)^\eta}.$$

The choice of the parameter η can be made empirically in such a way that this test can be similar and powerful. Another required quality is that the distribution of the test statistic does not depend heavily on the marker density and on the number of individuals. The value $\eta = 0.75$ was found to give the better results and will be used in the next section. The corresponding test statistic will be denoted $T_5^{[2]} = T(0.75)$, and called "likelihood shape".

We propose the following procedure to test whether or not there is more than one QTL:

– compute $T^{[1]}$. If $T^{[1]} < \lambda$ decide "there is no QTL". The threshold λ is chosen such that the first-type error is α_1.
– if $T^{[1]} > \lambda$, then compute $T_5^{[2]}$. If $T_5^{[2]} > \lambda_5$ then decide that there exist more than one QTL.

The threshold λ_5 is chosen such that

$$\Pr(T_5^{[2]} > \lambda_5 \text{ and } T^{[1]} > \lambda) = \alpha_2$$

for $a_2 = 0$ and all possible values of a_1, d_1, μ, σ.

As for the previous tests the threshold λ_5 is taken as the maximum of the thresholds obtained by simulations for different values of the parameters a_1 and d_1.

Numerical comparisons of the test statistics

All the simulations were made with $n = 500$ and $n = 150$ individuals and a residual variance $\sigma^2 = 1$. We considered six equally spaced markers and a chromosome of 1 Morgan length. As in Whittaker et al. (1996), the computation of the test statistics can be done using

the statistics S_m, $m = 1, \ldots, M$

$$S_m = \sum_{k=1}^{n} y_k \mathbb{1}_{M_{m,k}=A} - \sum_{k=1}^{n} y_k \mathbb{1}_{M_{m,k}=B}$$

These statistics S_m, $m = 1, \ldots, M$ are sufficient for the parameters of the two-QTL linear model, if the parameters μ and σ^2 can be considered as known. Actually, they can be viewed as asymptotically sufficient.

Threshold

The threshold λ corresponding to the test $T^{[1]}$ for a level $\alpha = 0.05$ was calculated using the method of Rebaï et al. (1994). In Tables 1 and 2, we give the thresholds λ_i, for $i = 1, \ldots, 5$ for a level $\alpha = 0.05$ and a level $\alpha_2 = 0.05$, and the probabilities $\Pr(T_i^{[2]} > \lambda_i$ and $T^{[1]} > \lambda)$, for $i = 1, 2, 5$ and $\Pr(T_i^{[2]} > \lambda_i)$ for $i = 3, 4$, obtained with 10 000 replications for several values of a_1 and d_1. Table 1 corresponds to the case with 500 individuals and Table 2 to the case with 150 individuals. As its behavior is clearly described in the case $n = 500$, the test $T_1^{[2]}$ "fixed first QTL" is given only in this case.

Values of a_1 used in these simulations do not cover the space of all possible values of this parameter. Nevertheless, we have seen that values of the parameter a_1 larger than those used in Tables 1 and 2 gave approximately the same distribution for the tests statistics as that obtained for the largest value in these tables. More, it indicates that the thresholds could be calculated with only the large values of $\|a_1\|$, cases in which it would be possible to find analytical approximations of the probability distribution functions of the tests.

Table 1 Probabilities $\Pr(T_i^{[2]} > \lambda_i$ and $T^{[1]} > \lambda)$ for $i = 1, 2, 5$ and $\Pr(T_i^{[2]} > \lambda_i)$ for $i = 3, 4$ with $n = 500$ individuals and different values of QTLs position and effect

a	Test	Position					
		0	0.1	0.2	0.3	0.4	0.5
0.2	$T_1^{[2]}$	1.6	1.2	1.4	1.1	1.8	1.1
	$T_2^{[2]}$	1.5	1.2	1.5	1.0	1.5	1.1
	$T_3^{[2]}$	1.9	1.4	1.9	1.1	2.0	1.4
	$T_4^{[2]}$	2.7	2.5	2.7	1.9	3.0	2.2
	$T_5^{[2]}$	1.7	1.3	1.0	1.1	1.0	1.2
0.3	$T_1^{[2]}$	3.6	2.3	3.2	1.7	3.4	1.9
	$T_2^{[2]}$	3.5	2.7	2.6	1.9	2.9	2.3
	$T_3^{[2]}$	3.3	2.7	3.4	2.0	3.0	1.8
	$T_4^{[2]}$	3.4	3.5	3.6	2.7	3.5	2.7
	$T_5^{[2]}$	3.3	2.7	1.8	3.0	2.3	3.2
0.4	$T_1^{[2]}$	5.0	3.4	4.0	2.8	4.8	2.8
	$T_2^{[2]}$	5.0	4.0	3.7	3.0	4.2	3.3
	$T_3^{[2]}$	4.6	3.9	4.1	2.6	3.4	2.4
	$T_4^{[2]}$	4.4	3.8	4.1	2.8	4.4	3.5
	$T_5^{[2]}$	3.9	3.8	2.8	4.0	3.0	4.1
0.5	$T_1^{[2]}$	5.0	3.8	4.6	3.2	4.5	3.4
	$T_2^{[2]}$	5.0	4.2	4.1	3.0	5.0	4.0
	$T_3^{[2]}$	5.0	4.3	4.5	3.5	3.9	3.1
	$T_4^{[2]}$	5.0	4.5	4.3	5.0	4.7	5.0
	$T_5^{[2]}$	3.8	3.8	2.5	4.3	3.2	5.0

$\lambda_1 = 6.03$, $\lambda_2 = 6.19$, $\lambda_3 = 6.64$, $\lambda_4 = 6.82$, $\lambda_5 = 1.41$

Table 2 Probabilities $\Pr(T_i^{[2]} > \lambda_i$ and $T^{[1]} > \lambda)$ for $i = 2, 5$ and $\Pr(T_i^{[2]} > \lambda_i)$ for $i = 3, 4$ with $n = 150$ individuals and different values of QTLs position and effect

a	Test	Position					
		0	0.1	0.2	0.3	0.4	0.5
0.4	$T_2^{[2]}$	1.9	1.5	1.7	1.3	1.8	1.3
	$T_3^{[2]}$	2.4	2.2	2.0	1.6	2.4	1.9
	$T_4^{[2]}$	2.9	2.7	3.1	2.5	3.2	2.2
	$T_5^{[2]}$	1.3	1.2	1.3	1.2	1.2	1.4
0.6	$T_2^{[2]}$	3.7	2.9	3.2	2.2	3.7	2.7
	$T_3^{[2]}$	3.8	3.1	3.4	2.2	3.5	2.4
	$T_4^{[2]}$	4.2	3.3	3.8	2.6	3.7	2.9
	$T_5^{[2]}$	3.1	2.8	2.4	3.0	2.6	3.1
0.8	$T_2^{[2]}$	4.8	3.6	3.8	2.8	4.4	3.9
	$T_3^{[2]}$	4.5	4.4	4.4	3.0	4.3	3.0
	$T_4^{[2]}$	4.7	4.1	4.2	3.2	4.2	3.7
	$T_5^{[2]}$	3.9	3.6	2.9	4.4	3.5	4.5
1.0	$T_2^{[2]}$	5.0	4.6	4.1	3.3	5.0	4.0
	$T_3^{[2]}$	4.9	5.0	4.9	3.4	4.2	3.7
	$T_4^{[2]}$	4.0	4.3	4.3	4.8	4.7	5.0
	$T_5^{[2]}$	4.0	4.3	3.5	5.0	3.9	5.0

$\lambda_2 = 6.27$, $\lambda_3 = 6.54$, $\lambda_4 = 6.96$, $\lambda_5 = 0.24$

It appears that none of the tests are similar; that is, with a probability distribution function not depending on the parameters. The most similar test seems to be $T_4^{[2]}$, "two-QTL model". Test $T_5^{[2]}$, "likelihood shape", is the less similar. In general, the thresholds depend on the position of the QTL, and in particular if the QTL is on a marker or between two markers.

The thresholds of test $T_2^{[2]}$, "fixed first-QTL interval", $T_3^{[2]}$, "multiple-QTL model", and $T_4^{[2]}$, "two-QTL model", depend very weakly on the number n of individuals. The threshold of test $T_5^{[2]}$, "likelihood shape", depends on n. This is not a problem as it is always possible to compute the correct threshold for a new value of n, but it can be a practical limitation to the use of this test. A slightly different version of test $T_5^{[2]}$, obtained as the product of $T_5^{[2]}$ with a function of n, could solve this problem.

Power

We consider here that the power is the probability to detect more than one QTL when there are two QTLs; that is, the probabilities $\Pr(T_i^{[2]} > \lambda_i$ and $T^{[1]} > \lambda)$, for

Table 3 Power of test $T_i^{[2]}$ for $i = 1, \ldots, 5$ with $n = 500$ individuals and different values of QTLs position and effect

$\|a_1\|$	$\|a_2\|$	Test	Position			
			(0.1, 0.9)		(0.3, 0.7)	
			Coupling	Repulsion	Coupling	Repulsion
0.3	0.3	$T_1^{[2]}$	39.8	46.9	11.9	17.6
		$T_2^{[2]}$	41.2	46.8	13.4	17.3
		$T_3^{[2]}$	25.1	27.5	7.2	18.1
		$T_4^{[2]}$	37.6	54.9	20.3	39.6
		$T_5^{[2]}$	62.6	< 5.0	29.1	< 5.0
0.5	0.3	$T_1^{[2]}$	64.3	69.4	23.8	39.6
		$T_2^{[2]}$	63.9	69.4	26.6	42.2
		$T_3^{[2]}$	44.6	47.4	20.3	31.9
		$T_4^{[2]}$	62.8	68.2	44.7	50.9
		$T_5^{[2]}$	73.1	< 5.0	38.7	< 5.0
0.4	0.4	$T_1^{[2]}$	79.7	84.1	27.7	44.2
		$T_2^{[2]}$	80.7	83.1	31.8	43.7
		$T_3^{[2]}$	58.1	61.9	24.6	41.5
		$T_4^{[2]}$	79.7	86.1	53.6	68.4
		$T_5^{[2]}$	89.1	14.0	46.3	< 5.0
0.4	0.2	$T_1^{[2]}$	28.2	33.7	9.5	15.9
		$T_2^{[2]}$	27.4	33.5	10.4	16.9
		$T_3^{[2]}$	17.3	20.2	7.4	12.6
		$T_4^{[2]}$	25.2	36.1	17.0	24.4
		$T_5^{[2]}$	44.4	< 5.0	23.5	< 5.0

Table 4 Power of test $T_i^{[2]}$ for $i = 2, \ldots, 5$ with $n = 500$ individuals and different values of QTLs position and effect

$\|a_1\|$	$\|a_2\|$	Test	Position			
			(0.1, 0.9)		(0.3, 0.7)	
			Coupling	Repulsion	Coupling	Repulsion
0.5	0.5	$T_2^{[2]}$	24.1	32.7	7.6	11.3
		$T_3^{[2]}$	15.8	18.9	< 5.0	13.5
		$T_4^{[2]}$	20.3	40.0	9.7	29.5
		$T_5^{[2]}$	45.5	< 5.0	21.9	< 5.0
1.0	0.5	$T_2^{[2]}$	45.2	55.4	16.4	34.5
		$T_3^{[2]}$	31.7	37.0	12.2	26.5
		$T_4^{[2]}$	43.8	52.4	29.7	39.7
		$T_5^{[2]}$	58.0	< 5.0	35.5	< 5.0
0.7	0.7	$T_2^{[2]}$	65.1	72.7	20.7	34.0
		$T_3^{[2]}$	44.1	48.6	12.3	33.4
		$T_4^{[2]}$	62.5	76.0	35.1	58.0
		$T_5^{[2]}$	79.3	9.7	42.3	< 5.0
0.7	0.4	$T_2^{[2]}$	26.4	34.3	9.2	16.9
		$T_3^{[2]}$	17.1	20.4	5.4	14.0
		$T_4^{[2]}$	23.4	37.4	13.2	26.7
		$T_5^{[2]}$	43.4	< 5.0	23.3	< 5.0

Table 5 Power of test $T_i^{[2]}$ for $i = 2, 3, 5$ with $n = 500$ individuals and different values of QTLs position and effect

$\|a_1\|$	$\|a_2\|$	Test	Position	
			(0.3, 0.5)	
			Coupling	Repulsion
1.0	1.0	$T_2^{[2]}$	7.8	18.6
		$T_4^{[2]}$	23.4	43.1
		$T_5^{[2]}$	30.2	< 5.0
1.0	0.8	$T_2^{[2]}$	6.1	14.6
		$T_4^{[2]}$	19.3	30.3
		$T_5^{[2]}$	24.7	< 5.0

$i = 1, 2, 5$ and $\Pr(T_i^{[2]} > \lambda_i)$ for $i = 3, 4$. We do not consider situations with more than two QTLs.

In Tables 3–5 we give the power of the different tests for different values and positions of two QTLs obtained with 10 000 replications. Table 3 corresponds to the case with 500 individuals and Table 4 corresponds to the case with 150 individuals. The distances between the two QTLs is greater than 40 cM in Tables 3 and 4 and equal to 20 cM in Table 5.

The first result is that the rank of the three tests $T_1^{[2]}$, "fixed first QTL", $T_2^{[2]}$, "fixed first-QTL interval", and $T_3^{[2]}$, "multiple-QTL model", is almost always the same in all the situations. Test $T_2^{[2]}$ is slightly better than $T_1^{[2]}$, which is better than $T_3^{[2]}$. This is because test $T_3^{[2]}$ is designed to detect the presence of any QTL in any interval and not only to find an effect of a QTL located in another interval. Test $T_4^{[2]}$, "two-QTL model", is slightly less powerful than test $T_2^{[2]}$, "fixed first QTL" for distant QTLs, but is more powerful for QTLs less than 60 cM apart. Test $T_5^{[2]}$, "likelihood shape" is more powerful than the other tests in some situations where the two QTLs are in coupling phase. Test $T_5^{[2]}$ has a very low power when the QTLs are in repulsion. One way for comparing more fairly this test with the other tests in the coupling case is to use a threshold corresponding to a level of 2.5% for test $T_5^{[2]}$. We found, for example in case $n = 500$, $\|a_1\| = \|a_2\| = 0.3$, position 0.1 and 0.9, a power of 54.5%, which remains higher than the power of the other tests.

Discussion

In this paper we studied the power of detection of more than one QTL in the case where there are two QTLs. We did not consider the case of more than two QTLs, and we did not address the problem of locating the QTL. The conclusions of the test are simply that one unique QTL cannot explain the data. It would be possible to continue the process by testing three QTLs against two, and so on. Nevertheless, our opinion is that the power of such tests would be very low.

We do not use here the infinitesimal model of Visscher and Haley (1996) as the null hypothesis. This

model is perhaps a more realistic framework but would have confused the comparisons.

None of the tests considered appears to be similar. Our opinion is that it is not possible to find similar tests for this problem. A "naive" choice would have been to assume that all the tests are distributed as central χ^2 with 1 *df* under the null hypothesis. The χ^2 quantile with tests $T_1^{[2]}$ or $T_2^{[2]}$, for example, would have led to the first-type error attaining 17% for a desired level of 5%. In the situations considered in this paper a χ^2 with 2 *df* as proposed by Wu and Li (1996) would give better results, but it is only by chance as the correct threshold would be different with other chromosome lengths or marker densities. For example, with a chromosome of 2 Morgans and 40 equispaced markers, the first-type error is 22.7% when using the threshold 5.99 corresponding to a χ^2 with 2 *df*. The theoretical justification of such threshold given by Whittaker et al. (1996) is not correct as it considers only one interval and not the complete chromosome and it does not take into account the constraint on the range of the transformed parameters (β_j and β_{j+1} in their notation are not free to take all possible values in $\mathbb{R}^2$).

In most situations, test $T_4^{[2]}$ is the more powerful test, test $T_5^{[2]}$ being better in the other situations. A possible strategy could be to use first $T_4^{[2]}$ and then, in the case where this test is not significant, to use $T_5^{[2]}$. Formally, this sequential way of testing needs further investigation in order to calculate the correct threshold. For practical purposes, tests $T_1^{[2]}$, "fixed first QTL", or $T_2^{[2]}$, "fixed first-QTL interval", are easier to use and present correct powers in cases with distant QTLs. Nevertheless, these tests do not offer correct answers for the problem of non-existing "ghost" QTLs (Haley and Knott 1992).

Jansen (1993, 1994) proposed to use the Akaike criterion to select the markers to be used as covariates in the regression. Basten et al. (1996) proposed a stepwise regression procedure to select the markers. These strategies could be incorporated in the test $T_2^{[2]}$, but we do not use these procedures here to select the markers because it was harder to implement automatically the process of marker selection.

On the other hand, there is no problem using markers belonging to other chromosomes as covariates in all the tests. It would have increased the power of all the tests, but would not have changed the comparisons between them.

The simulations have been made at a 5% level. In general, one would use a more stringent level for each chromosome to obtain a global level of 5% or 10% for the whole genome. In this case, all the powers would be smaller than shown in this study, but the comparison would have led to the same conclusions. The correct threshold corresponding to this level would have been harder to compute because the variance of the estimation of this kind of quantile is very large.

References

Basten CJ, Weir BS, Zeng Z-B (1996) QTL cartographer: a reference manual and tutorial for QTL mapping. North Carolina State University, Raleigh, N.C.

Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci by using molecular markers. Heredity 69:315–324

Jansen RC (1993) Interval mapping of multiple quantitative trait loci. Genetics 135:205–211

Jansen RC (1994) Controlling the type I and type II errors in mapping quantitative trait loci. Genetics 138:871–881

Knapp SJ, Bridges WC, Birkes D (1990) Mapping quantitative trait loci using molecular marker linkage maps. Theor Appl ,Genet 79:583–592

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Mangin B, Goffinet B, Rebaï A (1994) Constructing confidence intervals for QTL location. Genetics 138:1301–1308

Rebaï A, Goffinet B, Mangin B (1995) Comparing power of different methods for QTL detection. Biometrics 51:87–99

Sax K (1923) The association of sizes differencies with seed coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8:552–560

Visscher PM, Haley CS (1996) Detection of putative quantitative trait loci in line crosses under infinitesimal genetics models. Theor Appl Genet 93:691–702

Whittaker JC, Thompson R, Visscher PM (1996) On the mapping of QTL by regression of phenotype on marker-type. Heredity 77:23–32

Wu WR, Li WM (1996) Model fitting and model testing in the method of joint mapping of quantitative trait loci. Theor Appl Genet 92:477–482

Zeng Z-B (1993) Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. Proc Natl Acad Sci USA 90:10972–10976

Zeng Z-B (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468