

Mapping multiple QTL of different effects: comparison of a simple sequential testing strategy and multiple QTL mapping

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Abstract

The aim of this study was to explore, by computer simulation, the mapping of QTLs in a realistic but complex situation of many (linked) QTLs with different effects, and to compare two QTL mapping methods. A novel method to dissect genetic variation on multiple chromosomes using molecular markers in backcross and F₂ populations derived from inbred lines was suggested, and its properties tested using simulations. The rationale for this sequential testing method was to explicitly test for alternative genetic models. The method consists of a series of four basic statistical tests to decide whether variance was due to a single QTL, two QTLs, multiple QTLs, or polygenes, starting with a test to detect genetic variance associated with a particular chromosome. The method was able to distinguish between different QTL configurations, in that the probability to 'detect' the correct model was high, varying from 0.75 to 1. For example, for a backcross population of 200 and an overall heritability of 50%, in 78% of replicates a polygenic model was detected when that was the underlying true model. To test the method for multiple chromosomes, QTLs were simulated on 10 chromosomes, following a geometric series of allele effects, assuming positive alleles were in coupling in the founder lines. For these simulations, the sequential testing method was compared to the established Multiple QTL Mapping (MQM) method. For a backcross population of 400 individuals, power to detect genetic variance was low with both methods when the heritability was 0.40. For example, the power to detect genetic variation on a chromosome on which 6 QTLs explained 12.6% of the genetic variance, was less than 60% for both methods. For a large heritability (0.90), the power of MQM to detect genetic variance and to dissect QTL configurations was generally better, due to the simultaneous fitting of markers on all chromosomes. It is concluded that when testing different QTL configurations on a single chromosome using the sequential testing procedure, regions of other chromosomes which explain a significant amount of variation should be fitted in the model of analysis. This study reinforces the need for large experiments in plants and other species if the aim of a genome scan is to dissect quantitative genetic variation.

Introduction

Most methods to map single or multiple QTLs have been demonstrated using simulation studies. Power for QTL detection is low for low heritability traits in small populations [16], so in order to be able to detect any QTL activity, either population size or heritability (h^2) of QTL has been set to a large value in previous simulation studies [4, 5, 7, 21, 22]. Amongst the more realistic studies, Jansen [4] simulated 11

QTLs on 10 chromosomes with population size of 500 backcross individuals and 70% of the phenotypic variance induced by QTLs, Zeng [23] simulated 10 QTLs on 4 chromosomes with backcross population size of 300 individuals and also with 70% of the phenotypic variance induced by QTLs, and Jansen [5] simulated multiple chromosomes (1 to 17) for a small but realistic backcross population size of 100 with 1 or 2 QTLs explaining 5 to 90% of the phenotypic variance. The choice of genetic settings in these studies is logical

and understandable, because their aim was primarily to investigate and demonstrate statistical properties of the QTL mapping methods. In real life we usually are dealing with multiple chromosomes and no or hardly any prior information on the number and location of QTLs and the distribution of their effects. Depending on this unknown genetic situation (e.g. few QTLs of large effects or many QTLs of small effects), some methods are likely to perform better than others. The question remains what a 'reasonable' genetic situation would look like and, if we generated data by computer under such a 'reasonable' genetic model, what the properties of the QTL mapping methods are.

From mutational effects studies in *Drosophila* [8, 13, 14, 15] it appears that an appropriate genetic model for a quantitative trait is one which has few mutations of large effects, and many mutations of small effects. To mimic a genome scan, we have chosen to study a QTL mapping strategy for a geometric series model of QTL effects [11, 12], because of its simplicity (see Appendix). In particular, we have taken the QTL map of Gimelfarb and Lande [2], in their paper on marker assisted selection. Thus, we simulated a large number of QTLs (25) with different magnitudes of effects, with the QTLs jointly explaining 40–90% of the phenotypic variation. Population size was set to 400. QTLs on the same chromosomes were generated either in coupling or in repulsion.

In this study we explore a sequential strategy to map multiple QTLs under a genetic model of many QTLs with varying effects distributed over multiple chromosomes. The sequential QTL mapping strategy follows a suite of alternative tests proposed by Visscher and Haley [18] and partial implementations by Knott *et al.* [9, 10] and de Koning *et al.* [1]. We first explore the properties of the sequential QTL mapping approach using simulations in backcross and F_2 populations, using different QTL configurations and considering simple single chromosome simulated data sets. These simulations were performed to validate the method and to investigate the appropriate degrees of freedom for multiple QTL tests. Subsequently, the method is compared to the 'MQM' (multiple QTL mapping) approach of Jansen and Stam [6] for the multiple chromosome implementation.

Material and methods

Sequential testing method

The proposed method consists of a series of linear regression models which are tested sequentially to determine the most likely QTL configuration on each chromosome. Each model corresponds to biologically plausible QTL configurations on a single chromosome, i.e. a single QTL, two QTLs, an oligogenic configuration, and many linked genes of small effects. Up to four sequential tests are carried out for a particular data set. The rationale behind these tests is to have a logical and consistent (and automatic) approach to mapping multiple QTLs on multiple chromosomes and, above all, to limit the loss of power due to the use of (too) many tests: we use only four tests (TESTc, TESTp, TEST1 and TEST2) and control of error rate is straightforward. TESTc, TEST1 and TEST2 are very similar to the tests performed by Jansen [4], who showed that interval mapping likelihoods when fitting linked or unlinked QTLs in the model of analysis were similar to fitting the flanking markers of those QTLs.

The tests are as follows:

TESTc Chromosomal test. A multiple regression on all m markers on a single chromosome is performed. Under the null hypothesis of no genetic variance associated with that chromosome, the test statistic should approximately be distributed as an F with $\{m\}$ and $\{N-m-1\}$ degrees of freedom. The number of degrees of freedom is the same for backcross and F_2 populations if the aim is to detect additive genetic variation, as is assumed in the present study. If TESTc is not significant, no other tests are carried out. The statistical power of the experiment is based on this test, i.e. the power that genetic variation associated with a particular chromosome will be detected. The optimal number of markers to be used in this test was investigated by Visscher and Haley [19].

TESTp Polygenic test. The null hypothesis of a large number QTLs of equal size all in coupling is tested by comparing the fit of a model with a multiple regression on all markers to the fit of a model with genomic proportion as explanatory variable. Genomic proportion for a particular chromosome is the fraction of that chromosome which originates from one of the founder population; for example, the proportion of the chromosome which is from the recurrent line in a backcross population. Genetic markers can be used

to estimate the genomic proportion [17, 18]. The resulting test is approximately distributed as an F with $\{m-1\}$ and $\{N-m-1\}$ degrees of freedom.

TEST1 Single-QTL test. The null hypothesis of a single QTL being responsible for the genetic variance associated with that chromosome is tested by comparing the residual sum of squares for fitting the two markers flanking the best position of a single QTL to fitting all markers on that chromosome. The resulting test should approximately be distributed as an F with $\{m-2\}$ and $\{N-m-1\}$ degrees of freedom. In essence, the null hypothesis is that the regression coefficients for markers other than those flanking the QTL are zero. Hence, it is a test that the remaining markers do not explain a significant amount of the variance associated with this chromosome.

TEST2 Two-QTL test. The null hypothesis of two QTLs being responsible for the genetic variance associated with that chromosome is tested by comparing the residual sum of squares for fitting two QTLs at their best positions to fitting all markers on that chromosome. The resulting test should approximately be distributed as an F with $\{m-4\}$ and $\{N-m-1\}$ degrees of freedom for isolated QTLs (i.e., QTLs not in adjacent intervals; see [20]), because the variation for each isolated QTL is absorbed by fitting its flanking markers [20]. This test is only performed if the single QTL test was significant, i.e. there was evidence to reject the null hypothesis of a single QTL. The method of transforming estimated regression coefficients of flanking markers to estimates of QTL location and effect [20] was used in this study. This transformation method is equivalent to a two-dimensional search procedure, but computationally much faster [20].

If the tests for a single QTL, two QTLs, and many QTLs in coupling (i.e. the polygenic model) are all significant, i.e. all these hypotheses can be rejected, the most likely QTL configuration is an unspecified oligogenic model (i.e. > 2 QTL).

It is possible that the null hypothesis for two or more tests are not rejected. For example, there may be insufficient evidence to reject the null hypothesis of 2 QTLs and the null hypothesis of many QTLs in coupling. In that case, the hypothesis which gives the highest P -value, i.e., the hypothesis for which there was the least evidence for rejection, was considered to be the more likely one. It is possible in principle to end up with a single ‘best’ hypothesis, by using the parsimony argument that models with fewer parameters

should be preferred if the level of significance is equal between two hypotheses. For this study, this kind of model selection was not applied.

Significant thresholds and degrees of freedom

For backcross populations, each QTL configuration, i.e. the true underlying genetic model on a single chromosome, is tested separately, so that the overall type-I error (α) for the chromosomal test (*TESTc*) coincided with the type-I error for a single chromosome. For all QTL maps which are considered in this study, $\alpha = 0.05$. For the chromosomal test for F_2 populations (with 10 chromosomes), each chromosome is tested with a type-I error of $\{1 - (1 - 0.05)^{1/10}\} = 0.0051$. If there is evidence of genetic variation associated with a particular chromosome, then each subsequent test was tested with $\alpha = 0.05$. This is not strictly correct because additional tests are carried out, but the increase in type-I errors because of multiple testing is small because a maximum of only three additional tests are performed.

For isolated QTLs, regression coefficients for pairs of markers can be transformed to give estimates of position and QTL effects [20]. However, in some cases the estimate of the regression coefficients is not consistent with a one or two QTL model. For example, if regression coefficients of two flanking markers are of opposite sign, this is inconsistent with a single QTL being within this marker interval. In this case, there could be two QTLs in repulsion, or the results could be due to chance (statistical noise). In practice this means that for this interval, the best position for a QTL is at one of the two markers. If this was encountered, the degrees of freedom for the test for > 1 QTL (*TEST1*) was modified from $\{m-2\}$ to $\{m-1\}$. Essentially, we are testing the significance of the remaining $\{m-1\}$ markers. When fitting two QTLs, more possibilities exist. Here the degrees of freedom taken out by fitting the two QTLs can take the values 4 (two isolated QTLs), 3 (one isolated QTL plus the remaining QTL at a marker, or two QTLs in adjacent intervals), or 2 (both QTL at marker positions). Again, the degrees of freedom for testing the remaining markers was modified to take account of this. To check this adjustment, simulations were performed with two QTLs of the same absolute effect located with various combinations of location on a single chromosome. The test under investigation is the 2 QTL vs. > 2 QTL test (*TEST2*).

MQM

Jansen [4, 5] and Jansen and Stam [6] proposed a method called MQM (Multiple QTL Mapping) to dissect the QTL configuration on single or multiple chromosomes. Essentially, the method is a two-step procedure. Firstly, a multiple linear regression method is employed in which the phenotypic observations are regressed onto multiple markers to detect and select markers in plausible QTL regions on the basis of a 2% significance per marker test. Secondly, a maximum likelihood interval mapping approach is used, in which the presence of a QTL for a particular genomic marker interval is tested at a genome-wide 5% significance level, while simultaneously fitting the selected markers from the first step in the model of analysis. Hence, the selected markers in the first step function as cofactors in the model used in the second step. The proposed method of Jansen is similar to the composite interval method (CIM) method [22, 23].

For the present study, an approximation of the second step in the full MQM method was used, by only testing at marker positions instead of within marker intervals. Given the relatively dense marker map used in this study, and the simulation of genome-wide thresholds, we assume that the number of significant markers per chromosome closely corresponds to the number of significant QTLs in a full MQM analysis. The outcome of steps one and two of such an analysis is the number of selected markers per chromosome, and the number of significant markers per chromosome, respectively. Significant markers are obtained by testing a marker against a 5% genome-wide significance, conditional on other selected markers which are in the final model. Genome-wide significance thresholds for MQM mapping were obtained by simulation ('parametric bootstrapping') as in Jansen [5].

Simulation

Backcross populations

Backcross populations derived from inbred lines were simulated. Six different QTL configurations were simulated, each on a single chromosome. Each simulated population consisted of phenotypes on N individuals ($N = 200$ or 1000), and marker genotypes on a single chromosome. Chromosomes were of length 100 cM, and had six fully informative markers spaced at 20 cM intervals. Recombination events were simulated using

Haldane's mapping function. The QTL maps were as followed:

MAP0: no QTL on the chromosome.

MAP1: a single QTL was simulated at position 30.

MAP2c: two QTLs of equal effect in coupling were simulated at positions 30 and 90.

MAP2r: two QTLs of equal effect in repulsion were simulated at positions 30 and 90.

MAPoligo: 3 QTLs of equal effects in repulsion (i.e. + - +) were simulated at positions 10, 50, and 90.

MAPpoly: 11 QTLs of equal effect in coupling were simulated at 10 cM intervals.

Given the effect of the n QTLs, and their relative position, the expected genetic variance in the backcross population was calculated as:

$$\text{var}_g = (1/4)\Sigma^n \Sigma^n (1 - 2r_{ij}) \alpha_i \alpha_j$$

with r_{ij} the recombination rate between QTLs i and j , and α_i the effect of QTL i . The environmental variance was calculated from the expected genetic variance and the heritability in the backcross population.

To investigate the ability of the suite of tests to pick up different QTL configurations, a powerful experiment was simulated by setting the heritability to 0.5. For each QTL map, 1000 replicate populations were simulated.

F_2 populations

To investigate the sequential testing method under a genome wide scan, we used an F_2 population derived from inbred lines. In particular, we used the QTL map which was used by Gimelfarb and Lande in a marker-assisted selection simulation study [2]. The map of Gimelfarb and Lande [2] consisted of 25 QTLs whose effects follow a geometric series in an F_2 population derived from inbred lines. The QTLs are randomly allocated to 10 chromosomes. Depending of the phase of the QTLs, the contribution of the chromosomal variance to the total genetic variance can be determined. Two phases were studied, one where all QTLs were in coupling, i.e. one of the founder inbred lines had all positive alleles whereas the other founder population contained only negative alleles, and one where are QTLs were in repulsion, i.e. adjacent QTLs were of opposite sign in each of the founder populations. Properties of the QTL maps are shown in Table 1. Two marker maps were studied, one with markers spaced every 20 cM, and one with a marker spacing of 10 cM.

Table 1. Properties of QTL map used in the analysis of 10 chromosomes. The QTL map was used to simulate F₂ populations which were analysed with a sequential testing method and with MQM. The effects of the QTLs follow a geometric series.^a

Chromosome	Location (cM), order k in parenthesis ^b						% genetic variance explained	
	1	2	3	4	5	6	coupling	repulsion
1	8 (14)	22 (3)	45 (13)				15.3	11.2
2	23 (25)	73 (24)					0.2	0.5
3	65 (22)	85 (10)	93 (9)				8.0	2.8
4	38 (17)						0.4	1.7
5	29 (12)	45 (20)					2.0	2.6
6	18 (4)	23 (5)					18.6	4.2
7	14 (2)	17 (6)	27 (15)				28.1	13.2
8	22 (1)	75 (16)					11.8	37.8
9	14 (11)	34 (23)	63 (19)	73 (21)	77 (8)	98 (18)	12.6	13.3
10	54 (7)						<u>3.0</u>	<u>12.7</u>
							100.0	100.0

^aQTL map from Gimelfarb and Lande (1994). All QTLs acted additively.

^bAbsolute effect of the k^{th} QTL is $[(9/11)^{k-1}]^{0.5}$.

To investigate the behaviour of the method under the most favourable conditions we could reasonably expect, a very powerful design was simulated of 1000 individuals and a heritability of 0.9. Such heritabilities can be achieved in certain plant experiments, where the error variance is reduced through replication. One thousand replicate populations were simulated.

Comparison with MQM

To compare the sequential test method with the method of Jansen, some of the BC populations were analysed using MQM mapping. For practical reasons (different simulations were performed by different co-authors), separate BC populations (data sets) were simulated for the two methods for $N = 400$ and $h^2 = 0.4$ and 0.9 , i.e. the analyses were done on different data sets which were sampled with the same population parameters. Given the marker density, only selected markers and significant markers are reported for the MQM method, i.e. there is no attempt to

map the QTLs within marker intervals. This was done for computational reasons. However, since the aim is to distinguish between competing genetic models (number of QTLs per chromosome), the selection of significant markers should be similar to identifying the number of QTLs.

Results

Degrees of freedom adjustment

The adjustment for the degrees of freedom for TEST2, i.e. testing the null hypothesis of 2 QTLs which explain the genetic variance, was checked using simulation. In Table 2 the results are shown for an approximate likelihood ratio test (Haley and Knott, 1992) and an F-test with numerator degrees of freedom depending on the estimated locations of the QTLs. Results are for a backcross population of $N = 1000$ and $h^2 = 0.9$, and 1000 replicates. Since the residual

Table 2. Mean and variance of the test statistic in likelihood ratio tests (LR) and F-tests for various combinations of positions of two QTLs, for a population size (N) of 1000, and a heritability (h^2) of 0.9. A single chromosome was simulated with 6 equally spaced fully informative markers. Results from 1000 replicates.

Location of two QTLs		Signs ^a	LR test		F test	
QTL ₁ (CM)	QTL ₂ (CM)		mean	variance	mean	variance
30	90	+/+	2.1	4.1	1.0	1.0
30	90	+/-	2.0	4.2	1.0	1.0
30	50	+/+	2.1	4.4	0.9	0.8
30	50	+/-	2.4	5.7	1.0	0.9
40	60	+/+	1.6	2.2	0.6	0.3
40	60	+/-	3.1	6.4	1.0	0.6

^aSign of the effect of the first QTL relative to the second one in the founder lines, i.e. coupling (+/+) or repulsion (+/-).

degrees of freedom are large, the F statistic is approximately distributed as $[\chi^2(k)]/k$, with k being the degrees of freedom for the numerator in the F-test. Hence, the expected mean and variance are 1.0 and $2/k$, respectively. For the approximate likelihood ratio test, no adjustments were made for the degrees of freedom. Hence, this test is expected to be a mixture of χ^2 distributions depending on the locations of the QTLs. The degrees of freedom should be $(m-4)$ for isolated QTLs, $(m-3)$ for QTLs in adjacent intervals, and $(m-2)$ for both QTLs on markers positions.

From Table 2 it appears that the adjustment is working reasonably, in that the mean and variance of the test statistic are close to expectation for the null hypothesis of two QTLs (2.0 and 4.0 for the LR test, and 1.0 and 1.0 for the F-test), in particular for QTLs of opposite signs. If the QTLs are of the same sign, any marker in the chromosome region should take out most of the variance due to both QTLs, and the test behaves less well. This is most clearly seen from the case where both QTLs were simulated at marker positions (40 and 60 cM). The average likelihood ratio is only 1.6, whereas a value of $(m-2)=4$ was expected under the null hypothesis of two QTLs, fitting 6 markers for the full model and fitting both QTLs (markers) for the reduced model. The results indicate that, on average, $(6-1.6)=4.4$ ‘markers’ were fitted in the reduced model. Presumably this is because the second (and third and fourth) marker fitted to absorb the QTL effects are essentially randomly located on the chromosome, and hence are ‘selected’ because they account for variation by chance. This was corroborated by looking at the results from two further scenarios. (The following results are not shown in ta-

bles.) Firstly, the average ML and F test statistic were compared when testing 2 QTLs vs. no QTL, i.e. the null hypothesis is that there is no genetic variance, in the case of no simulated genetic variation. In that case, the average mean and variance for the ML test were 4.8 and 9.1 (and for the F-test, 2.4 and 2.3, respectively), implying that, on average, the degrees of freedom were close to 5. Secondly, the test 2 QTLs vs. > 2 QTLs (TEST2) was performed for the case of a single simulated QTL at position 30, explaining 50% of the variance. This resulted in a mean ML test statistic of 1.6 (variance 2.6), and an average F-statistic of 0.6 (variance 0.4). Essentially, the second QTL was fitted at random, which caused the degrees of freedom for the test to decrease. This was clearly seen by looking at the estimated positions and their standard deviations of the two fitted QTLs: 24 ± 8 cM for the first QTL, and 63 ± 26 cM for the second QTL. Hence, the first fitted QTL takes out most of the genetic variance, while the position of the second one is essentially randomly distributed on the chromosome.

Backcross populations

For population sizes of 200 and 1000, and a heritability in the backcross population of 0.5, results are shown in Table 3. The results are summarised as the proportions of significant replicates (from the chromosomal test) which were assigned to the competing QTL configurations.

In general, the results are encouraging, since the correct QTL configuration is usually identified. However, the polygenic coupling model is difficult to separate completely from a two QTL model (78% vs.

Table 3. Most likely QTL configuration (in %) from 1000 replicates for each of six different QTL maps, using the sequential testing method. The heritability in the backcross population was 0.5 for all QTL maps, except for MAP0 ($h^2 = 0$).

N ^a	QTL map ^b	Power ^c (%)	QTL configuration from testing (%)			
			1 QTL	2 QTLs	oligogenic	polygenic
200	MAP0	5	52	22	15	11
	MAP1	100	95	3	2	0
	MAP2c	100	0	90	3	7
	MAP2r	100	0	94	6	0
	MAPoligo	100	0	0	100	0
	MAPpoly	100	0	18	4	78
1000	MAP0	5	58	21	19	2
	MAP1	100	95	3	2	0
	MAP2c	100	0	94	6	0
	MAP2r	100	0	94	6	0
	MAPoligo	100	0	0	100	0
	MAPpoly	100	0	0	25	75

^aPopulation size.

^bMAP0, no QTL; MAP1, a single QTL at 30 cM; MAP2c, two QTLs of equal effect in coupling at 30 and 90 cM; MAP2r, two QTLs of equal effect in repulsion at 30 and 90 cM; MAPoligo, 3 QTLs of equal effects in repulsion at 10, 50, and 90 cM; MAPpoly, 11 QTLs of equal effects in coupling at 10 cM intervals.

^cProportion ($\times 100$) of significant replicates from the chromosomal test.

18%, for $N = 200$) or an oligogenic model (75% vs. 25%, for $N = 1000$).

Simulating alternative oligogenic models with more QTLs showed that it was very difficult to distinguish between the two QTL model and the oligogenic model (results not shown in tables). Indeed, the most frequently identified configuration was the two QTL model when in fact 11 QTL (evenly spaced at 10 cM intervals) in repulsion were simulated. This is presumably because the test compares fitting 4 markers (for 2 isolated QTLs) to fitting 6 markers. If the QTL variance associated with the two remaining markers is already absorbed by the 4 fitted markers, the additional variance due to fitting those remaining markers can be small, so that the test is not significant.

F₂ populations

Tables 4 and 5 show the results for both QTL phases (coupling and repulsion) and marker density maps, for a design of 1000 individuals and a heritability of 0.9 in the experimental F_2 population.

For the coupling QTL model (Table 4), genetic variation was detected (through the chromosomal test) with 100% power in 6 of the 10 chromosomes. The chromosomes with a power of less than 100% are those which explain the least amount of genetic vari-

ation (see Table 1). For those 4 chromosomes, the power was slightly reduced by using a denser map, because the chromosomal test was based on 11 rather than 6 degrees of freedom, and only small parts of the chromosomes contained QTLs, so that the overall F-test was more ‘diluted’ in the case of 11 degrees of freedom. The proportion of phenotypic variance explained by the selected model (the R^2 values) are slightly less than the true proportion of genetic variance explained by each chromosome (Table 1) multiplied by the heritability. This is most likely because not all genetic variance is accounted for by any of the models when using linear regression. Even for a simple QTL mapping model with a single QTL flanked by two markers, all genetic variance is accounted for only if there is a marker at the QTL position. However, the overall R^2 , obtained by adding the R^2 from each chromosome, was 84.0% and 85.9% for both marker spacings, respectively, which is close to the heritability of 90% (Table 4).

Except for chromosome 9, the most frequently obtained result was a single QTL for all chromosomes. The true QTL configuration is different, i.e. not a single QTL, except for chromosomes 4 and 10. The reason for this seemingly poor performance of the sequential testing method to distinguish between competing models, is that a single QTL would explain

Table 4. Most likely QTL configuration (proportion of significant replicates $\times 100\%$) for each chromosome from 1000 replicates for a heritability of 0.9 in an F_2 population of 1000 individuals, using the sequential testing method. The simulated QTL configuration was as in Table 1, in coupling phase.

Marker spacing (cM)	Chromosome	Power (%)	R^2 (%) ^a	QTL configuration from testing (%)			
				1 QTL	2 QTLs	oligogenic	polygenic
20	1	100.0	13.2	89	9	2	0
	2	3.1	0.1	61	16	13	10
	3	100.0	6.7	94	4	2	0
	4	5.7	0.1	55	28	5	12
	5	59.3	1.4	80	5	1	14
	6	100.0	16.1	97	2	1	0
	7	100.0	23.1	93	6	2	0
	8	100.0	10.0	78	20	2	0
	9	100.0	11.0	11	71	3	15
	10	83.3	<u>2.3</u>	84	4	1	11
	total		84.0				
10	1	100.0	13.3	77	21	2	0
	2	1.7	0.0	41	41	6	12
	3	100.0	7.1	95	4	1	0
	4	4.5	0.1	60	27	9	4
	5	54.3	1.4	85	5	1	9
	6	100.0	16.3	95	4	1	0
	7	100.0	24.1	90	8	2	0
	8	100.0	10.1	81	17	1	1
	9	100.0	11.1	9	68	5	18
	10	77.1	<u>2.3</u>	86	3	2	9
	total		85.9				

^aThe average proportion of phenotypic variation explained by the model.

most of the genetic variance on each chromosome because the QTLs tend to be clustered. An exception is chromosome 9, with 6 QTLs spread out, and for this chromosome the most likely configuration is a 2 QTL model. In 15% of the simulated populations (18% for a marker spacing of 10 cM), the most likely configuration was a polygenic model for this chromosome (Table 4). The second QTL on chromosome 8 does not get detected because it explains so little variance (the first QTL explains $(1/0.222)^2 = 20.3$ times as much variation as the second QTL).

A denser marker map improved the ability to distinguish between the alternative QTL configuration for chromosome 1, in that the proportion of replicated populations in which more than 1 QTL was detected increased from 11 % to 23% with increased marker density (Table 4).

Results for the repulsion phase, i.e. effects of adjacent QTLs were of opposite sign, are presented in

Table 5. Power for detection of genetic variation was less than 100% for 5 chromosomes. These chromosomes explained the least amount of genetic variation in the repulsion phase (see Table 1). For markers spaced every 20 cM, the total amount of phenotypic variation explained by all chromosomes (70.8%), is significantly lower than the proportion of genetic variation in the population (90%). This is presumably because adjacent QTLs in the same marker interval may not be detected if their effects cancel each other out. A denser map detected more genetic variation (75.2%, Table 5). For chromosomes 5 and 6, the power was increased when adding more markers, most likely because of the same effect, i.e. adding a marker in between two adjacent QTLs whose effects are of opposite sign results in more markers being able to explain more of the variance in the chromosomal test.

Generally, more QTLs are detected when QTLs were in repulsion phase. For the chromosomes which

Table 5. Most likely QTL configuration (in %) for each chromosome from 1000 replicates for a heritability of 0.9 in an F₂ population of 1000 individuals, using the sequential testing method. The simulated QTL configuration was as in Table 1, in repulsion phase.

Marker spacing (cM)	Chromosome	Power (%)	R^2 (%) ^a	QTL configuration from testing (%)			
				1 QTL	2 QTLs	oligogenic	polygenic
20	1	100.0	7.4	14	52	34	0
	2	5.0	0.1	30	66	4	0
	3	37.0	0.7	60	21	16	3
	4	54.0	1.2	83	7	1	9
	5	53.0	1.2	87	9	2	2
	6	13.0	0.2	45	45	10	0
	7	100.0	8.5	90	8	2	0
	8	100.0	31.4	8	89	3	0
	9	100.0	10.5	0	38	62	0
	10	100.0	<u>9.8</u>	94	4	1	1
	total		70.8				
10	1	100.0	8.5	6	49	45	0
	2	4.5	0.1	33	58	9	0
	3	31.3	0.7	54	26	19	1
	4	38.5	0.9	84	4	2	10
	5	63.1	1.6	78	20	2	0
	6	21.8	0.5	19	70	11	0
	7	100.0	9.5	79	19	2	0
	8	100.0	31.7	10	86	4	0
	9	100.0	11.1	0	50	50	0
	10	100.0	<u>10.5</u>	95	4	1	0
	total		75.2				

^aThe average proportion of phenotypic variation explained by the model.

were always further investigated (power = 100%), the correct configurations were usually detected. For chromosome 1, an oligogenic model was determined in 34% of populations, and a two-QTL model in 52% of populations, for a marker spacing of 20 cM, and 45% and 49% for markers 10 cM apart (Table 5).

As expected, the oligogenic QTL configuration did not come up frequently as the most likely configuration when the simulated QTLs were in coupling (Table 4), whereas the polygenic coupling QTL configuration was almost never selected as the best model when simulated QTLs were in repulsion (Table 5).

Comparison with MQM mapping

In Tables 6 and 7 the results are displayed for both sequential testing and MQM mapping, for BC populations of 400 individuals, and a heritability of 0.9 (Table 6) or 0.4 (Table 7). Although both the se-

lected and significant markers are presented for MQM mapping, the best comparison for the likely QTL configuration with the sequential testing method is between rows one (sequential testing) and rows three (significant markers). In the MQM approach we did not explicitly test for the number of QTLs, but we expect the number of significant markers to be close to the number of QTLs.

The power, as defined by the number of replicates in which genetic variation was detected, was significantly higher with MQM, in particular for the most powerful design ($h^2 = 0.9$, Table 6). For example, for chromosome 10 the power of MQM was 941/1000 whereas genetic variation using the chromosomal test was detected in only 196/1000 replicates. This is because the MQM mapping selects markers on all chromosomes simultaneously in the marker selection step, thereby increasing the amount of variance explained by each chromosome after correcting for

Table 6. Most likely QTL configuration for each chromosome from 1000 replicated backcross populations of 400 individuals. The simulated QTL configuration was as in Table 1, in coupling phase. The number of replicates for which different QTL configurations were determined are shown for the sequential testing method and for MQM. QTL configurations in MQM were determined from the number of selected or significant markers. The overall heritability was 0.90.

Chromosome	Method ^a	QTL configuration (out of 1000 replicates)				
		0 QTLs	1 QTL	2 QTLs	oligogenic	polygenic
1	Sequential testing	2	879	69	16	34
	MQM (selected)	0	35	493	472	0
	MQM (significant)	23	433	511	33	0
2	Sequential testing	991	2	4	3	10
	MQM (selected)	504	434	48	14	0
	MQM (significant)	910	88	2	0	0
3	Sequential testing	127	819	26	10	18
	MQM (selected)	0	396	517	87	0
	MQM (significant)	33	830	137	0	0
4	Sequential testing	993	2	2	3	0
	MQM (selected)	260	659	59	22	0
	MQM (significant)	738	258	4	0	0
5	Sequential testing	877	90	13	9	11
	MQM (selected)	0	766	192	42	0
	MQM (significant)	85	899	15	1	0
6	Sequential testing	0	963	26	11	0
	MQM (selected)	0	596	327	77	0
	MQM (significant)	1	937	61	1	0
7	Sequential testing		939	48	12	1
	MQM (selected)	0	4	698	298	0
	MQM (significant)	0	58	916	26	0
8	Sequential testing	18	807	69	10	96
	MQM (selected)	0	237	628	135	0
	MQM (significant)	0	703	289	8	0
9	Sequential testing	5	305	239	11	440
	MQM (selected)	0	0	128	872	0
	MQM (significant)	2	81	582	335	0
10	Sequential testing	804	154	20	5	17
	MQM (selected)	0	739	202	59	0
	MQM (significant)	59	927	12	2	0

^aFirst row, the number of replicates (out of 1000) for which the different QTL configurations were determined using the sequential testing method. Second and third row, the number of replicates (out of 1000) for which the different QTL configurations were determined using the number of selected and significant markers from the MQM method.

QTLs on other chromosomes [6]. In addition, the present implementation of the chromosomal test was done by fitting 11 markers per chromosome, without a selection step, whereas fewer markers could also have picked up the genetic variance [19]. Too many selected markers per chromosome would also decrease the power of MQM mapping, because significance tests are conditional on linked markers.

For those chromosomes which explained a large proportion of the phenotypic variance (i.e. chromo-

somes 7, 6, and 1), the MQM method appears generally better in picking up multiple QTLs. For example, MQM nearly always selects two significant markers on chromosome 7 (916/1000), whereas the sequential testing method decides that there is a single QTL in 939/1000 replicates (Table 6). Again, this is based upon the assumption that the two significant markers correspond to two QTLs. The simulated model had three QTLs, at locations 14, 17, and 27 cM (see Table 1), explaining 28.1% of the genetic variance.

Table 7. Most likely QTL configuration for each chromosome from 1000 replicated backcross populations of 400 individuals. The simulated QTL configuration was as in Table 1, in coupling phase. The number of replicates for which different QTL configurations were determined are shown for the sequential testing method and for MQM. QTL configurations in MQM were determined from the number of selected or significant markers. The overall heritability was 0.40.

Chromosome	Method ^a	QTL configuration (out of 1000 replicates)				
		0 QTLs	1 QTL	2 QTLs	oligogenic	polygenic
1	Sequential testing	219	696	22	7	56
	MQM (selected)	59	715	172	54	0
	MQM (significant)	359	635	6	0	0
2	Sequential testing	993	2	1	4	0
	MQM (selected)	777	136	69	18	0
	MQM (significant)	990	8	2	0	0
3	Sequential testing	680	287	14	16	3
	MQM (selected)	127	718	106	49	0
	MQM (significant)	499	498	3	0	0
4	Sequential testing	993	2	5	0	0
	MQM (selected)	754	159	71	16	0
	MQM (significant)	983	16	1	0	0
5	Sequential testing	972	19	6	1	2
	MQM (selected)	539	359	79	23	0
	MQM (significant)	931	68	1	0	0
6	Sequential testing	101	846	22	10	21
	MQM (selected)	53	764	130	53	0
	MQM (significant)	216	777	7	0	0
7	Sequential testing	10	939	30	13	8
	MQM (selected)	35	670	209	86	0
	MQM (significant)	207	784	9	0	0
8	Sequential testing	437	445	30	11	77
	MQM (selected)	103	684	145	68	0
	MQM (significant)	393	599	8	0	0
9	Sequential testing	414	274	39	7	266
	MQM (selected)	100	548	305	47	0
	MQM (significant)	496	482	22	0	0
10	Sequential testing	951	24	16	4	5
	MQM (selected)	421	465	97	17	0
	MQM (significant)	898	101	1	0	0

^aFirst row, the number of replicates (out of 1000) for which the different QTL configurations were determined using the sequential testing method. Second and third row, the number of replicates (out of 1000) for which the different QTL configurations were determined using the number of selected and significant markers from the MQM method.

For the trait with a lower heritability (Table 7), powers for the two methods are similar, i.e. uniformly low. Hence, even for a modest heritability of 0.4, and a population size of 400, it is difficult to detect genetic variation across chromosomes, let alone to identify the correct QTL configuration.

Discussion

A sequential testing procedure was presented based upon simple linear regression models to conduct a genome wide scan in a consistent and computationally efficient way. The method starts by asking ‘is there genetic variation associated with a particular chromosome’ and proceeds to estimate the likely QTL configuration (polygenic model, 1 QTL, 2 QTL, >2 QTL) in a sequential manner. Based upon ideal

conditions, i.e. large populations and a trait with a high heritability, the method appears to perform well, in that, on average, the correct QTL configuration is detected (Table 3). However, it is very difficult to distinguish between polygenic models and two-QTL or oligogenic models. Of course, our polygenic model with a large number of QTLs in coupling and with equal effect, may be considered unrealistically simplistic. Moreover, we favoured our polygenic analysis by simulating only QTLs in coupling in the multiple chromosome example (Tables 6 and 7).

In this study, different models to dissect genetic variance were selected through a sequential hypothesis test. An alternative approach would be to use a method to decide on the number of parameters to include in the regression model. These methods, for example Akaike's Information Criterion (e.g. [4]) generally penalise the inclusion of more parameters in the model, and the most parsimonious model is selected.

When comparing the sequential to the MQM method in a multiple chromosome setting, the latter appears to perform better, in terms of detecting genetic variation (power) and selecting numbers of markers which correspond to the number of QTLs. For the difference in power, the likely explanation is that MQM mapping takes out more residual variation by fitting markers on other chromosomes. However, the same approach could be employed by the sequential testing method, i.e. markers could be preselected across chromosomes using a forward or backward selection strategy [10], followed by a chromosome by chromosome approach. In a situation with 10 chromosomes, each chromosome may still be tested with $P = \{1 - (1 - 0.05)^{1/10}\} = 0.0051$. However, because of the use of a preselection stage with many tests or selection steps, it cannot be guaranteed that the genome-wide error rate is still 0.05. The use of preselected markers in the sequential strategy might make it similar to the MQM strategy.

We fitted 11 markers per chromosome in the model of the sequential strategy. For MQM mapping, markers which were fitted in the final model were selected from the total pool of $10 \times 11 = 111$ markers. Depending on the true genetic model on that chromosome, fewer markers could be fitted which should also detect the existing genetic variation. Fitting too many markers reduces the power of the overall F-test in our sequential strategy by adding 'noise' to the numerator of the F statistic. The same applies to MQM mapping. Elsewhere, we show that fitting markers spaced between 40 and 50 cM apart would be most powerful

to detect genetic variance, whether that variance is caused by a single QTL or by polygenes [19]. Therefore, in practice, for a 100 cM chromosome, 2 or 3 fully informative markers should be sufficient for the overall chromosomal test. However, if only, say, 3 markers are fitted for the chromosomal test, there is less scope to explore alternative tests, if tests are to be nested. Hence, a balance needs to be struck between the power of the chromosomal test, and the power of subsequent tests. In practice the detection of a significant amount of variance on a particular chromosome using a sparse marker map may be followed by genotyping individuals for additional markers in the regions of interest. It would be interesting to study the performance of the two methods in more detail under a lower marker density.

There is a slight philosophical difference between the sequential testing approach and the MQM approach. MQM (and CIM) imply that there are large to moderate effect QTLs, but do not explicitly explore alternative genetic models, whereas the sequential testing strategy was developed to explicitly test for polygenic and oligogenic models.

In general, the power to distinguish between alternative genetic models was disappointing, even for powerful designs. In practice many backcross or F_2 populations in livestock have less than 500 individuals, and heritabilities in these populations will seldom exceed 0.5. Hence, for these experiments it will be difficult to detect anything beyond chromosome regions which explain a large proportion of the variance (e.g., as in [10], where QTLs explained $>10\%$ of the F_2 variance). For plant populations, with even smaller experimental populations but larger heritabilities if replication is possible, the same conclusion can be drawn.

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Appendix: Geometric series genetic model

Consider an F_2 population derived from inbred lines, with bi-allelic QTLs each with frequencies of + alleles of 0.5. The total additive genetic variance ($\text{var}(a)$) is the sum of independent contributions of individual loci ($\text{var}(a)_i$ for locus i). For the geometric series model,

$$\begin{aligned}\text{var}(a)_i &= \text{var}(a)(1-a)(a^{i-1}), \text{ and} \\ \text{var}(a) &= \sum^{\infty} \text{var}(a)_i \\ a &= (n_e - 1)/(n_e + 1)\end{aligned}$$

where a is a constant and n_e is termed the effective number of loci. Hence, for the k^{th} largest QTL, the variance due to that locus is

$$2pq\alpha_k^2 = (1-a)a^{k-1}\text{var}(a),$$

with α_k the allele substitution effect of the k^{th} QTL genotype. So ($p = q = 0.5$),

$$\alpha_k = [2(1-a)a^{k-1}\text{var}(a)]^{0.5} = 2[(n_e - 1)^{k-1}/(n_e + 1)^k]^{0.5}\sigma_A$$

The proportion of genetic variance that is explained by the k^{th} locus is,

$$p_k = 2[(n_e - 1)^{k-1}/(n_e + 1)^k]$$

For example, for $n_e = 10$, $p_k = (2/11)(9/11)^{k-1}$, and assuming an arbitrary effect of 1.0 for the first QTL (as in [2]), i.e. $\sigma_A = 1/2(n_e + 1)^{0.5}$, the substitution effect of each locus is, $\alpha_k = [(9/11)^{k-1}]^{0.5}$. These values were presented by Gimelfarb and Lande [2] and are shown in Table 1.