Genet. Res., Camb. (2002), **79**, *pp.* 175–184. *With 2 figures.* © 2002 Cambridge University Press
DOI: 10.1017/S001667230200558X Printed in the United Kingdom

Use of randomization testing to detect multiple epistatic QTLs

ÖRJAN CARLBORG AND LEIF ANDERSSON
Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, BMC, Box 597, S-751 24 Uppsala, Sweden

(*Received 8 May 2001 and in revised form 28 September and 10 December 2001*)

Summary

Here, we describe a randomization testing strategy for mapping interacting quantitative trait loci (QTLs). In a forward selection strategy, non-interacting QTLs and simultaneously mapped interacting QTL pairs are added to a total genetic model. Simultaneous mapping of epistatic QTLs increases the power of the mapping strategy by allowing detection of interacting QTL pairs where none of the QTL can be detected by their marginal additive and dominance effects. Randomization testing is used to derive empirical significance thresholds for every model selection step in the procedure. A simulation study was used to evaluate the statistical properties of the proposed randomization tests and for which types of epistasis simultaneous mapping of epistatic QTLs adds power. Least squares regression was used for QTL parameter estimation but any other QTL mapping method can be used. A genetic algorithm was used to search for interacting QTL pairs, which makes the proposed strategy feasible for single processor computers. We believe that this method will facilitate the evaluation of the importance at epistatic interaction among QTLs controlling multifactorial traits and disorders.

1. Introduction

Efficient methods for detecting epistatic quantitative trait loci (QTLs) are needed to gain a better understanding of the genetics underlying complex traits. Several lines of evidence indicate the importance of epistasis. For instance, ~ 40 % of the genes in yeast do not yield an aberrant phenotype when ablated (Wolfe, 2000) and the same alleles can cause a strain-specific autoimmune disease in mice (Bolland & Ravetch, 2000). Epistasis has been reported in QTL mapping studies (Fijneman *et al.*, 1996; Long *et al.*, 1996; Li *et al.*, 1997; Shook & Johnson, 1999; Lieps & Mackay, 2000; Mackay, 2001) and in various basic biological processes that are expected to affect the expression of most traits. Biological processes in which epistasis has been shown to be important include signaling pathways in both plants (Beaudoin *et al.*, 2000) and animals (Araujo & Bier, 2000; Scanga *et al.*, 2000; Luschnig *et al.*, 2000), and differential crossing-over and segregation (Khazanehdari & Borts, 2000).

* Corresponding author. Tel: +46 18 471 4589. Fax: +46 18 471 4833. e-mail: orjan.carlborg@hgen.slu.se

Several methods for mapping quantitative trait loci have been proposed in the literature. The first interval mapping methods were aimed at detecting marginal additive and dominance effects of individual QTLs (e.g. Lander & Botstein, 1989; Haley & Knott, 1992; Martinez & Curnow, 1992), and several authors have developed methods for mapping multiple non-interacting QTLs (e.g. Jansen, 1992, 1993; Jansen & Stam, 1994; Zeng, 1993*a*, *b*). Strategies have been proposed for mapping multiple interacting QTLs using a non-orthogonal two-locus linear epistatic model (Jansen, 1992; Haley & Knott, 1992). Recently, Zeng and co-workers described Multiple Interval Mapping (MIM) for mapping multiple interacting QTLs using Cockerham's genetic model (Kao & Zeng, 1997; Kao *et al.*, 1999; Zeng *et al.*, 1999; Zeng *et al.*, 2000).

Churchill & Doerge (1994) proposed the use of randomization testing to derive empirical significance thresholds for detection of single QTL and then expanded this approach to multiple non-interacting QTLs (Doerge & Churchill, 1996). These methods are based on forward selection of one QTL at a time. This approach fails to detect epistatic QTLs unless at least one of the epistatic QTLs has significant marginal

effects. We have previously shown that a simultaneous search for epistatic QTL pairs is more efficient than forward selection to find epistatic QTL pairs for all epistatic models tested (Carlborg *et al*., 2000). Residual randomization or residual bootstrap testing have been suggested by Zeng *et al*. (1999) as a means for final model selection when mapping multiple epistatic QTLs by MIM, but no integrated use of randomization testing in the search procedure has yet been described.

This report describes a forward selection strategy in which non-interacting QTLs and subsequently simultaneously mapped interacting QTL pairs are added to a total genetic model. Randomization testing is used to test for marginal QTL effects, for epistasis between all pairs of QTL with significant marginal effects and for pairs of epistatic QTLs in which at least one did not have significant marginal effects. The strategy is designed to use the increased efficiency of a simultaneous search in detecting interacting QTL pairs (Carlborg *et al*., 2000). Simulations were used to evaluate how much statistical power can be gained by simultaneously mapping two epistatic QTLs for different types of epistasis and to evaluate the statistical properties of the proposed randomization tests.

2. Methods

(i) *Motivation*

Detection of QTLs is normally based on the marginal additive and dominance effects of individual QTLs. The strategies to search for the QTLs vary from a one-dimensional genome scan (e.g. Churchill & Doerge, 1994) to forward selection and stratification procedures (e.g. Doerge & Churchill, 1996). The reasons for type I (falsely detecting nonexistent QTLs) and type II (not detecting real QTLs) errors in these studies are thoroughly discussed by Doerge & Churchill (1996). Epistasis can increase the type II error in the forward selection procedure of Doerge & Churchill (1996) by decreasing marginal QTL effects and thereby causing premature convergence of the search procedure. By simultaneously mapping QTLs using an epistatic QTL model, the type II error can be decreased. To address this, we suggest an alternate forward selection testing procedure with additional step in which pairs of QTLs are mapped simultaneously using an epistatic QTL model. In the forward selection strategies based on randomization tests, the type I error for the inclusion of one (or two) additional QTL in the total genetic model is controlled by setting a desired significance threshold based on the empirical distribution obtained in the randomization test. One of the difficulties when using a forward selection strategy is to control the total experimental type I error of the entire search procedure. The correct way to correct the significance threshold to obtain the desired type I error level still needs to be explored further.

We will describe the randomization testing strategy by first describing the search strategies used for detection of marginal and interaction effects of QTLs and the genetic models used in the mapping procedure, and then introducing the principle of model selection strategy and of our proposed randomization tests.

(ii) *Genomic search strategies*

A one-dimensional (1D) exhaustive enumerative search for QTLs using a 1 cM step size was used to detect marginal QTL effects. A genetic algorithm, as implemented by Carlborg *et al*. (2000), was used to search for pairs of epistatic QTLs. In the randomization testing procedure, a conditional QTL search has also been used. This is a 1D, forward selection strategy that is used when one of the QTLs in the epistatic QTL pair proposed by the genetic algorithm has already been declared significant and included in the genetic model. During this search, a 1D genome scan for an epistatic QTL pair is performed where the location of the already significant QTL in the pair is held fixed.

(iii) *Genetic modelling*

A full genetic model including marginal additive and dominance effects of all QTL as well as all possible pairwise interaction effects can be written as

$$y = \mu + \sum_{j=1}^{n} (a_j + d_j) + \sum_{k=1}^{n-1} \sum_{l=k+1}^{n} (aa_{kl} + ad_{kl} + da_{kl} + dd_{kl}) + e,$$

where a and d are the single-locus marginal additive and dominance effects, aa, ad, da and dd are the interaction terms for a specific QTL pair, and n is the number of QTLs in the model.

In linear regression form, this model becomes

$$y_i = \beta_0 + \sum_{j=1}^{n} (\beta_{1j} A_j + \beta_{2j} D_j) + \sum_{k=1}^{n-1} \sum_{l=k+1}^{n} (\beta_{1kl} A_k A_l + \beta_{2kl} A_k D_l + \beta_{3kl} A_l D_k + \beta_{41kl} D_k D_l) + \epsilon_i,$$

where y_i is the phenotype of an individual i; $\epsilon_i \sim N(0, \sigma^2)$ (where σ^2 is the environmental variance independent of the expected value of the mean); A_j and D_j are regression indicator variables for the marginal additive and dominance effect of the jth QTL as given by Haley & Knott (1992); AA_{kl}, AD_{kl}, AD_{lk} and DD_{kl} are regression indicator variables for

https://doi.org/10.1017/S001667230200558X Published online by Cambridge University Press

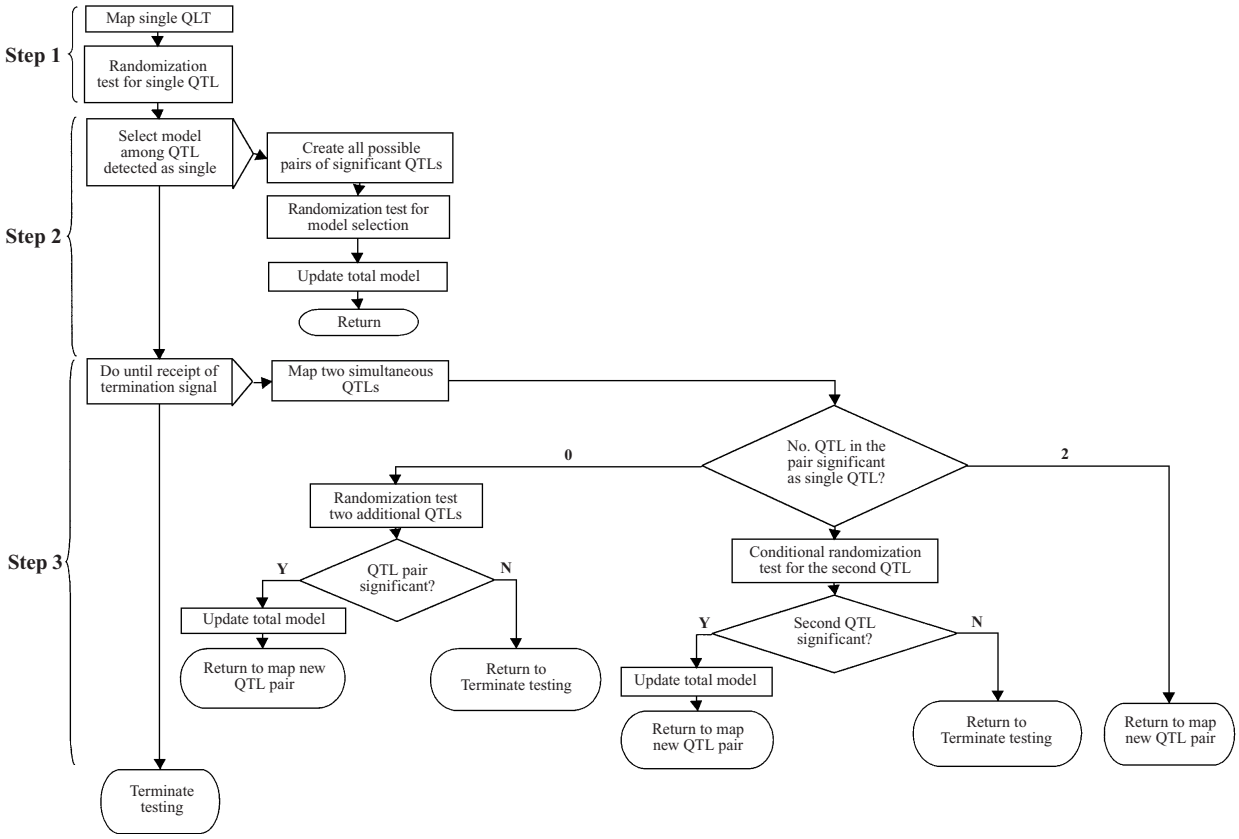

Fig. 1. Flow chart for the randomization testing strategy used to detect multiple QTLs.

the combinations of the additive and dominance effects for QTLs k and l in the model, which are obtained by multiplying the respective additive and dominance regression variables for QTL k and l (Haley & Knott, 1992); and the β values are the partial regression coefficients for the genetic parameters corresponding to the indicator regression variables.

The proposed mapping strategy is a model selection strategy in which the QTLs to be included in the model are initially proposed by a standard interval mapping procedure (Haley & Knott, 1992) and the model parameters for marginal effects of the proposed QTLs are included in the model based on a randomization test. Inclusion of epistasis parameters for all pairs of QTLs that have marginal effects in the model is based on a randomization test performed for each pair. Subsequently, marginal and epistasis parameters for pairs of QTLs, indicated by a genetic-algorithm-driven simultaneous search for interacting QTL pairs (Carlborg *et al.*, 2000), are included based on a randomization test. The final model thus includes parameters for the marginal effects of all selected QTLs and epistatic parameters for a number of all the possible pairs of QTLs with marginal effects in the model. A more thorough description of the decision rule for selecting the genetic parameters to be included in the model is given below.

(iv) *Model selection strategy*

We propose that simultaneous mapping of epistatic QTL pairs is included in the following model selection strategy, based on randomization testing (Fig. 1). The first step aims to find the QTLs that can be detected by their marginal effects, and the second step then tries to select the best pairwise model (that is, to evaluate whether epistasis is important for any pairs that exist among the detected QTLs). The third step in the procedure is to search simultaneously for pairs of QTLs using an epistatic QTL model. This step is included to detect QTLs without significant marginal effects but for which, instead, marginal effects together with interaction effects are sufficient to indicate QTL activity. In step 1, QTLs are mapped, without regard for epistatic interactions, by a standard interval mapping method using Model I from Table 1 (Haley & Knott, 1992). Putative QTL locations are evaluated using a randomization test (Churchill & Doerge, 1994). Additive and dominance parameters for the detected QTLs are added to a total genetic model. In step 2, all pairs of QTLs are tested for digenic epistasis by comparing the total genetic model with an updated total genetic model that also contains the four interaction parameters for the QTL pair (Table 1, Model II). Model selection is based on a randomization test described below. For QTL pairs for which

https://doi.org/10.1017/S001667230200558X Published online by Cambridge University Press

Table 1. *Genetic models used for QTL detection by stepwise model selection and randomization testing*

Search strategy	Genetic model
I n QTLs without epistatic interactions	$y_i = \beta_0 + \sum_{j-1}^{n} (\beta_{1j} A_j + \beta_{2j} D_j)$
II Epistatic interaction for QTLs x and y detected using model I	$y_i = \beta_0 + \sum_{j-1}^{n} (\beta_{1j} A_j + \beta_{2j} D_j) + \sum_{(k,\, l) \subset Q_{ep}} (\beta_{1kl} A_k A_l + \beta_{2kl} A_k D_l + \beta_{3kl} A_l D_k + \beta_{4kl} D_k D_l) + (\beta_{1xy} A_x A_y + \beta_{2kl} A_x D_y + \beta_{3xy} A_y D_x + \beta_{41xy} D_x D_y)$
III Simultaneous search for QTL $(n+1)$ and $(n+2)$ not detected by their marginal effects	$y_i = \beta_0 + \sum_{j-1}^{n} (\beta_{1j} A_j + \beta_{2j} D_j) + \sum_{(k,\, l) \subset Q_{ep}} (\beta_{1kl} A_k A_l + \beta_{2kl} A_k D_l + \beta_{3kl} A_l D_k + \beta_{4kl} D_k D_l) + \left(\sum_{j-n+1}^{n+2} (\beta_{1j} A_j + \beta_{2j} D_j) + \sum_{k-n+1}^{n+1} \sum_{l-k+1}^{n+2} (\beta_{1kl} A_k A_l + \beta_{2kl} A_k D_l + \beta_{3kl} A_l D_k + \beta_{4kl} D_k) \right)$

A_j and D_j are regression indicator variables for the marginal additive and dominance effect of the jth QTL, and AA_{kl}, AD_{kl}, AD_{lk} and DD_{kl} are regression indicator variables for the combinations of the additive and dominance effects for all pairs of the n QTLs in the model (Haley & Knott, 1992). The β values are the partial regression coefficients for the genetic parameters corresponding to the indicator regression variables; n is the number of already significant QTLs in the model, $(k, l) \subset Q_{ep}$ are all QTL pairs with significant interactions that are contained in the set Q_{ep} and $1 \leqslant x \leqslant n$, $y > x$.

the epistatic model is selected, their interaction parameters are added to the total genetic model. Testing for digenic epistasis is repeated until all QTL pairs have been evaluated using a genetic model including all selected interaction terms.

In step 3, a simultaneous search is performed for an epistatic QTL pair, in the same or in different linkage groups. QTLs in the same marker bracket are not tested for because their detection is based on the same genetic and phenotypic information. The total genetic model is used with additional parameters for the epistatic QTL pair (Table 1, Model III). During the search, a QTL is defined as a ± 10 cM interval surrounding the best map position. Based on this, a QTL mapped within 10 cM of a previously mapped QTL is considered to reflect the same QTL. Thus, the indicated locations for an epistatic QTL pair include no or one already detected QTLs. If a less dense genetic map used is used, the region covered by the QTL might have to be expanded in order to avoid dependencies in the QTL analysis. Inclusion of a second interacting QTL or an interacting QTL pair in the total genetic model is based on randomization testing. Additional pairs of epistatic QTL are sought until the last pair indicated by the genetic algorithm is non-significant, which terminates the search procedure.

(v) *Randomization testing for detecting epistatic QTLs*

The randomization test used to select between an additive-dominance and an epistatic QTL model for QTLs detected by their marginal effects is based on the following principle. Many data permutations are generated in which the interaction regression variables (AA_{12}, AD_{12}, AD_{21} and DD_{12}) for the QTL pair are permuted with regard to the phenotypes and the variables in the total genetic model (Fig. 2). In each permuted sample, the model fit evaluation is calculated (we used the residual sum of squared errors) and retained. The number of permutations needed is determined by calculation of the efficient sample size (Nettleton & Doerge, 2000). When all permuted data sets have been evaluated, an empirical model selection criterion can be calculated.

Two alternate randomization tests have been developed to handle model selection for the QTL pairs indicated in the simultaneous search. If one of the QTLs in a QTL pair has already been detected in step 1 or by its interaction with another QTL, its marginal genetic effects are already in the total genetic model. A conditional randomization test is then used to test whether the second QTL and the interaction parameters should be added to the model (Fig. 1). For this test, data permutations are generated until an efficient sample size has been obtained.

- The location of the already-significant QTL is held as a fixed parameter in the model and a 1D genome scan is performed to propose locations for the second QTL in the model. For each proposed location, the regression variables for the second QTL (A_2 and D_2) and the interaction regression

https://doi.org/10.1017/S001667230200558X Published online by Cambridge University Press

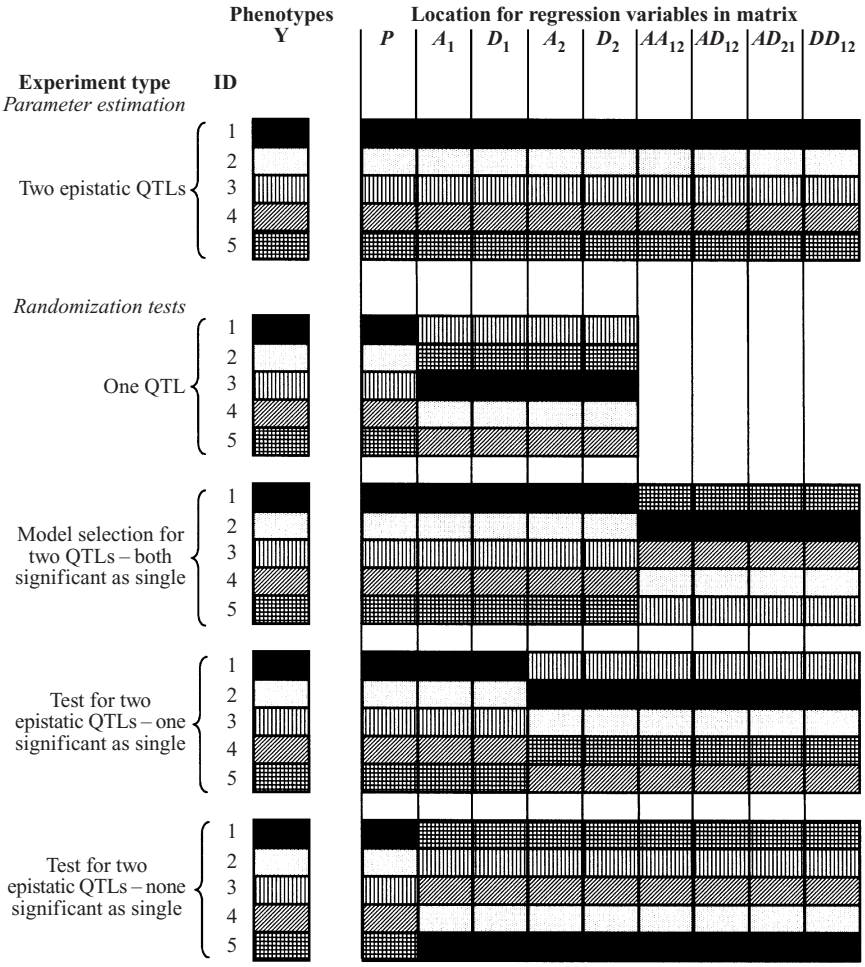

Fig. 2. One possible data permutation in four different types of randomization test based on a dataset of five individuals (ID 1–5). The randomization tests are for: 1, one QTL; 2, model selection between an epistatic and an additive/dominance model; 3, a second QTL interacting with a QTL already in the model; 4, two additional interacting QTLs. Each column represents a parameter in the model and each row represents an individual entry. The shadings indicates the individual origins of the regression variables.

variables (AA_{12}, AD_{12}, AD_{21} and DD_{12}), are calculated and permuted with regard to the phenotypes and the variables in the total genetic model (Fig. 2).

- The best model fit evaluation from the genome scan for the second QTL is retained.

After all permutations have been obtained, the model fit evaluations are used to calculate an empirical significance threshold for model selection. If the QTL pair proposed by the genetic algorithm is significant, the marginal genetic parameters for the second QTL and the interaction parameters of the QTL pair are added to the total genetic model. If the second proposed QTL is not significant, the testing strategy has converged. This test is used to obtain a test for a second interacting QTL that is conditional on the marginal effects of an already selected QTL.

If neither of the QTLs in the detected pair has previously been included in the total genetic model, a randomization test is used to test whether the interacting QTL pair should be added to the total genetic model. Principally, the randomization test is performed as above but with the following exceptions. A simultaneous search for an epistatic QTL pair is performed in each permuted sample and, for each pair of proposed genomic locations, the regression variables for both QTLs to be added to the model (A_1, D_1, A_2, D_2, AA_{12}, AD_{12}, AD_{21} and DD_{12}) are calculated. These are then permuted with regard to the phenotypes and the already selected effects in the model (Fig. 2). The model fit evaluations are retained and an empirical significance threshold is calculated.

(vi) *Simulation study*

Complementary epistasis has an expected mendelian segregation ratio of 9:7 and is observed when homozygosity for a recessive allele in either of two genes gives the same mutant phenotype. Duplicate epistasis is observed when homozygosity for two recessive alleles is required to give a mutant phenotype, and the expected segregation ratio is 15:1. Dominant and inhibitory epistasis occur when one gene blocks

https://doi.org/10.1017/S001667230200558X Published online by Cambridge University Press

Table 2. *Genetic effects for two QTL with two alleles each* (Q *and* q) *for the different genetic models evaluated*

	Genotypic values									Variance (Tot/Q_1/Q_2) (%)		
Genetic models	$Q_1Q_1Q_2Q_2$	$Q_1Q_1Q_2q_2$	$Q_1Q_1q_2q_2$	$Q_1q_1Q_2Q_2$	$Q_1q_1Q_2q_2$	$Q_1q_1q_2q_2$	$q_1q_1Q_2Q_2$	$q_1q_1Q_2q_2$	$q_1q_1q_2q_2$	V_a	V_d	V_i
Non-epistatic												
One dominant	0·2	0·2	0·2	0·2	0·2	0	0	0	0	0·5/0·5/–	0·3/0·3/–	0·0
Two additive	0·4	0·2	0	0·2	0	−0·2	−0·2	−0·4	4·0/2·0/2·0	0·0/0·0/0·0	0·0	
Epistatic												
Complementary	0·6	0·6	−0·2	0·6	0·6	−0·2	−0·2	−0·2	−0·2	9·0/4·5/4·5	4·5/2·3/2·3	2·3
Dominant	0·15	0·15	0·15	0·15	0·15	0·15	−0·55	−0·55	0·25	3·8/3·5/0·4	1·9/1·7/0·2	1·7
Duplicate	0·2	0·2	0·2	0·2	0·2	0·2	0·2	0·2	−0·6	1·0/0·5/0·5	0·5/0·3/0·3	2·3
Inhibitory	0·2	0·2	0·2	0·2	0·2	0·2	−0·6	−0·6	0·2	5·0/4·5/0·5	2·5/2·3/0·3	2·3

V_a, additive genetic variance; V_d, dominance genetic variance; V_i, epistatic genetic variance defined as $V_i = V_g - (V_a + V_d)$, where V_g is the total genetic variance explained by the QTL pair. For all genetic variances (V_a, V_d, V_i), the variances are reported as: Tot, total variance explained by all QTLs in the simulated model; Q_1, variance explained by the first QTL; Q_2, variance explained by the second QTL in the two-QTL models.

the phenotypic expression of a second gene. For dominant epistasis, the dominant allele at the first locus is also dominant over the alleles at the second locus. The phenotypic effects of the second locus are therefore only expressed when the individual is homozygous recessive at the first locus. This gives an expected segregation ratio of 12:3:1. Inhibitory epistasis works in the same way as dominant epistasis and is the special case when the two genes have effects of equal size but opposite sign. The expected segregation ratio is here 13:3. All of these segregation ratios are relevant for unlinked loci in an F_2 cross and for the case where both parents are heterozygous at both loci.

A simulation study was performed to evaluate whether simultaneous mapping of pairs of QTLs using an epistatic model is more powerful than mapping single QTLs by their marginal effects. The aim was to find out for which types of epistasis the increase in statistical power is most pronounced and how the method performs when mapping non-interacting QTL pairs or single QTL. In the simulations, 100 replicates of 520 F_2 individuals from a cross between two inbred lines were simulated for six genetic models; 100 replicates were also simulated for a smaller population size of 260 F_2 individuals for one genetic model. The simulated genome consisted of 20 chromosomes, each 100 cM in length, carrying fully informative marker loci at the ends and at 10 cM intervals. Crossovers were generated using Haldane's mapping function without interference (Haldane, 1919).

The individual phenotypes were simulated as

$$y_i = E(G_i) + \epsilon_i,$$

where $E(G_i)$ values are given for all two-locus genotypes in Table 2 and $\epsilon_i \approx N(0, \sigma^2)$; σ^2 is determined by holding the heritability, h^2, fixed and calculating the variance of phenotypes caused by additive genetic effects, V_A, for each dataset. Then, σ^2 is computed using the equation

$$V_A\,(1-h^2) \div h^2.$$

In total, six different combinations of non-interacting and interacting QTLs were evaluated. Four contained two epistatically interacting QTLs, one contained two fully additive QTLs and one a single fully dominant QTL (Table 2). All QTLs were fixed in the parental lines and simulated at random locations in the genome. Only one QTL was allowed on each chromosome. The narrow-sense heritability was set to 0·01 for the evaluation of the randomization testing procedure and heritabilities for the evaluation of the model selection randomization test were 0·01, 0·05 and 0·10. The simulated effects of the QTLs and the heritability were chosen such that the standard interval mapping method of Haley & Knott (1992) would

https://doi.org/10.1017/S001667230200558X Published online by Cambridge University Press

detect at least one QTL in at least 75% of cases. The QTL effects and the variances explained by the QTL are given in Table 2.

(vii) *Computational techniques*

Randomization testing is a computer-intensive method to derive empirical significance thresholds for detection of QTLs. We have developed QTL mapping software that has been performance tuned and written for parallel computing. The simulation study was performed using 24,000 CPU hours on a Cray T3E computer at the National Supercomputing Center in Linköping, Sweden.

3. Results

(i) *Power of QTL mapping*

The statistical power when mapping one or two QTL using standard interval mapping and our proposed randomization testing strategy is presented in Table 3. The simultaneous mapping step increased the ability to map the simulated QTL pairs whether epistasis was present or not. In most of the simulated populations, at least one of the QTLs was detected using standard interval mapping for the dominant, inhibitory and complementary epistatic QTL models. Both QTLs are more rarely detected using standard interval mapping, and the simultaneous mapping step and the test for a second QTL conditional on the significant QTL adds substantial power, with the largest increase for the dominant epistatic QTL model (49%). The largest increase in power from the test for an additional QTL pair is obtained for the duplicate epistatic model, with substantial increases for both the large (36%) and the small (22%) population sizes. When the population size is decreased for the duplicate epistatic model, the total power to detect QTL decreases from 96% to 39%.

Table 3. *Empirical power and type I errors* (*) *for mapping QTLs simulated under different genetic models using the proposed randomization testing strategy* (*Fig. 1*). *The significant QTLs are reported for the randomization test used to detect them. The total power of the proposed testing strategy is given as well as the gain in power of mapping epistatic QTL pairs compared with mapping single QTLs using a standard least squares interval mapping method*

		Significant QTLs from standard interval mapping		Gain in power by forward selection of:		Two QTLs:	
Genetic model	Population size	At least one	Two	Additional QTL	Additional QTL pair	Total power	Total gain in power
Non-epistatic							
One dominant	520	82%	3%(*)	6%(*)	1%(*)	–	–
Two additive	520	96%	54%	5%	2%	61%	7%
Epistatic							
Complementary	520	94%	56%	14%	1%	71%	15%
Dominant	520	93%	10%	49%	3%	62%	52%
Duplicate	520	60%	25%	35%	36%	96%	71%
	260	31%	3%	14%	22%	39%	36%
Inhibitory	520	97%	14%	27%	1%	42%	28%

Table 4. *A comparison of the type I error and power of the randomization test proposed for model selection. A type I error is for this test defined as selecting an epistatic model when a two-QTL additive model was simulated, and power is defined as the ability to select an epistatic model when an epistatic genetic model was simulated. The results are based on > 500 simulations for each combination of model and heritability*

	$h^2 = 0{\cdot}01$		$h^2 = 0{\cdot}05$		$h^2 = 0{\cdot}10$	
Simulated genetic model	Type I error	Power	Type I error	Power	Type I error	Power
Non-epistatic						
Two additive	4·6%	–	6·7%	–	7·4%	–
Epistatic						
Dominant	–	86·6%	–	98·9%	–	99·6%
Duplicate	–	94·8%	–	99·3%	–	99·6%

https://doi.org/10.1017/S001667230200558X Published online by Cambridge University Press

(ii) *Randomization test for model selection*

The results from the study of the randomization test for model selection are presented in Table 4. The three different genetic models evaluated represent three different proportions of the genetic variance that cannot be accounted for using an additive or a dominance model (Table 2): none for the additive, low for the dominant epistatic and high for the duplicate epistatic genetic models. The type I error when testing for epistasis using an additive genetic model was constant around the selected threshold (5%) for all heritabilities tested. The power to detect epistasis was higher for the duplicate epistatic than for the dominant epistatic model and increased with the heritability for both models.

4. Discussion

Here, we have described and evaluated the properties of a stepwise randomization testing strategy based on three novel randomization tests to be used for mapping of epistatic QTLs. A simulation study showed that the ability to detect interacting QTLs is increased substantially by searching simultaneously for epistatic QTL pairs in a forward selection procedure. The method is applicable to other genetic models and other QTL mapping methods. It is currently limited to mapping pairs of QTLs but could easily be expanded to include epistasis of higher order, for instance triplets of QTLs instead of only pairs.

By simulation, we show that the power is greater for all epistatic models tested than standard least squares interval mapping of single QTLs. No explicit comparisons have been made with other multiple QTL mapping methods but, implicitly, the following comparison can be made. We have previously shown that a simultaneous search for QTL pairs is superior to a forward selection procedure for detecting the locations in the genome, which explains most variation. Therefore, the number of QTLs detected in the conditional test is the highest that can be detected using a forward selection strategy to map epistatic QTLs using an epistatic model. The power of a forward selection strategy based on an additive/dominance model has not been evaluated but we do not expect that the ability to map epistatic QTLs is higher using this model. The most important point with regard to power comparisons with other multiple QTL mapping methods is that an additional step to map an additional pair of QTLs (i.e. step 3) will, once included, add power to any forward selection procedure for mapping QTLs.

We propose a forward selection, randomization testing strategy including a simultaneous mapping step for epistatic QTL pairs. There are three major issues that need to be addressed for this procedure to be applicable in practice. The first is the selection of a genetic model that is appropriate for the aims of the study. When a genetic model has been selected, the statistical properties of the model need to be addressed. Secondly, a QTL search and significance testing strategy must be designed to make statistical inferences about the number of QTLs that exist and the types of interaction among the detected loci. Finally, the computational performance for real experimental data need to be addressed. The first two points are addressed in this report, and the computational issues will be discussed elsewhere.

In the mapping procedure, we used a non-orthogonal, two-locus linear epistatic model for mapping QTLs. This model was chosen because the aim of our investigations is to achieve a physiological and biochemical interpretation of dominance and epistasis. The parameter estimates for this model have more relevant genetic interpretations than the orthogonal models suggested by Cockerham (1954), for example, which have recently been used for mapping interacting QTLs (Kao *et al*., 1999). The advantage with the Cockerham model is that it is possible to test all parameters in the model independently. If this is done, however, it is not possible to transform the data to get the estimates of the model we propose. We have on this basis decided not to use the Cockerham model for our testing procedure. The use of a non-orthogonal model for analyses aimed at understanding the genetics in finite locus models has been suggested by Jana (1971) and others. A difficulty when using this model in QTL mapping is that residual randomization or residual bootstrap tests cannot be used for significance testing or model selection because the genetic parameters in the model are not orthogonal. For model selection and significance testing, we therefore suggest a different randomization testing procedure (as described). The simulation study shows that the procedure has high power for all epistatic models tested and that it produces a correct type I error.

The randomization testing strategy is a hybrid between a standard interval mapping of single QTLs and simultaneous mapping of epistatic QTLs (Fig. 1). This strategy was chosen to obtain the highest statistical power by using a statistical model that is most appropriate for the data. It has been shown in numerous studies that there are genes that are fully additive, that are dominant to varying degree and that interact. In our testing procedure, we first use an additive-dominance model (Table 1, Model I), to map QTLs that have sufficiently large marginal additive and/or dominance effects to be identified by a standard interval mapping method for single QTLs. For this step, any QTL mapping method could be used to indicate individual QTLs. We have chosen to start (Step 1) with a 1D genome scan mainly for two reasons. In a previous study (Carlborg *et al*., 2000), we

https://doi.org/10.1017/S001667230200558X Published online by Cambridge University Press

have shown that a simultaneous search for pairs of QTLs is superior to a forward selection mapping procedure in mapping multiple QTLs. In the proposed randomization testing procedure, we first perform a single QTL scan and subsequently perform a simultaneous mapping step for multiple QTLs. These two strategies combined will find QTLs that could be detected in a forward selection procedure. The use of a single genome scan for marginal QTL effects also decrease the computational demand by limiting the number of randomization tests to be performed. For some epistatic models (e.g. dominant epistasis), the additive and dominance effects of one QTL can inflate the marginal effects of another QTL, if they are not mapped using an epistatic model. This could lead to the detection of these QTLs as individual QTLs with biased estimates of their genetic effects. The use of the two-locus model is expected to provide more appropriate estimates of the genetic effects of the two interacting QTLs, which led us to test for epistasis among all pairs of individually detected QTLs. Further, to detect interacting QTLs with smaller marginal effects, the mapping procedure also includes a step to search simultaneously for pairs of interacting QTLs. This mapping step uses digenic epistatic variance to map QTLs and thus has a higher power than a conditional search for detecting the most likely genomic locations of interacting QTLs (Carlborg *et al*., 2000).

Owing to the large computational demand involved in evaluating the randomization testing strategy, in which several hundred of each of the described randomization tests are performed, we had to limit the number of genetic models to evaluate. We have chosen only to evaluate various one- and two-QTL models because these models are sufficient to show whether the simultaneous mapping step for an interacting QTL pair adds power and, if it does, for which types of epistatic interactions. They are also sufficient to evaluate the statistical properties of the randomization tests proposed. We also believe that mapping interacting QTL pairs might be of practical importance for traits in which no significant QTL was detected using standard interval mapping methods, despite considerable phenotypic differences between the lines used in the study.

The randomization testing strategy was evaluated using simulations for various one- and two-QTL genetic models with different sizes of genetic effect and two population sizes. The results clearly show that simultaneous mapping of an epistatic QTL pair increases the ability to identify epistatic QTL pairs up to tenfold compared with a standard interval mapping method, depending on the genetic model. The mapping of additional pairs of epistatic QTLs adds power for all genetic models simulated. It is worth noting that the power to identify two non-interacting QTLs also increases. This is probably due to the better ability of the simultaneous search to identify the highest two-dimensional peak. The increase in power is most pronounced for the duplicate epistatic QTL model and is fairly low for the other genetic models.

The model selection randomization test measures whether an epistatic genetic model is preferable to an additive/dominance model for a detected pair of QTLs. The simulations showed that the power to detect epistasis is high and that it increases with the heritability. When an additive model was simulated, there is a weak trend towards increasing type I error at higher heritabilities. Similar results were reported by Goffinet & Mangin (1998) and Visscher *et al*. (2000), who compared methods for multiple QTL mapping. Further simulations need to be performed to evaluate whether this is due to the limited number of simulations or to other factors such as segregation distortion in the simulation.

A one-QTL model was simulated to evaluate the type I error for this multiple testing procedure. The use of a nominal 5% significance threshold for each test, leads to a total type I error of $\sim 10\%$. This is likely to result from the multiple testing performed but, because the number of simulated populations is small, further investigations are needed to investigate whether this result is general or occurred by chance. There is a need for future research on how to handle the multiple testing carried out when evaluating models containing different numbers of QTLs. When this issue has been addressed, a simultaneous search strategy for the full genetic model can be developed. The problem of multiple testing has also been discussed by Zeng *et al*. (1999). One issue that also needs to be investigated further is the statistical implication of using an epistatic or an additive/dominance genetic model in statistical testing for QTLs in various true genetic models. This is in order to evaluate how alternate proposed QTL mapping strategies should be combined to obtain maximum total QTL detection power.

We thank Dietrich von Rosen (Department of Statistics, SLU) and Torgny Faxen (National Supercomputing Center, Linköping, Sweden) for helpful discussions and other support. We also thank the National Supercomputing Center for supplying the computer time used for this study. The National Graduate School in Scientific Computing (NGSSC) and the Food 21 project (MISTRA) supported the work.

References

Araujo, H. & Bier, E. (2000). *sog* and *dpp* exert opposing maternal functions to modify Toll signaling and pattern the dorsoventral axis of the *Drosophila* embryo. *Development* **127**, 3631–3644.

Beaudoin, N., Serizet, C., Gosti, F. & Giraudat, J. (2000). Interactions between abscisic acid and ethylene signaling cascades. *Plant Cell* **12**, 1103–1116.

Bolland, S. & Ravetch, J. V. (2000). Spontaneous autoimmune disease in FcγRIIB-deficient mice results from strain-specific epistasis. *Immunity* **13**, 277–285.

Broman, K. W. (1997). *Identifying Quantitative Trait Loci in Experimental Crosses*. PhD thesis, Department of Statistics, University of California, Berkeley, CA, USA.

Carlborg, Ö., Andersson, L. & Kinghorn, B. (2000). The use of a genetic algorithm for simultaneous mapping of multiple interacting quantitative trait loci. *Genetics* **155**, 2003–2010.

Churchill, G. A. & Doerge, R. W. (1994). Empirical threshold values for quantitative trait mapping. *Genetics* **138**, 963–971.

Cockerham, C. C. (1954). An extension of the concept of partitioning hereditary variance for analysis of covariance among relatives when epistasis is present. *Genetics* **39**, 859–882.

Doerge, R. W. & Churchill, G. A. (1996). Permutation tests for multiple loci affecting a quantitative character. *Genetics* **142**, 285–294.

Fijneman, R. J., De Vries, S. S., Jansen, R. C. & Dermant, P. (1996). Complex interactions of new quantitative trait loci, *Sluc1*, *Sluc2*, *Sluc3*, and *Sluc4*, that influence the susceptibility to lung cancer in the mouse. *Nature Genetics* **14**, 465–467.

Goffinet, B. & Mangin, B. (1998). Comparing methods to detect more than one QTL on a chromosome. *Theoretical and Applied Genetics* **96**, 628–633.

Goldberg, D. E. (1989). *Genetic Algorithms in Search, Optimization and Machine Learning*. Addison & Wesley, Reading, MA, USA.

Haldane, J. B. S. (1919). The combination of linkage values and the calculation of distances between loci of linked factors. *Journal of Genetics* **8**, 299–309.

Haley, C. S. & Knott, S. A. (1992). A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* **69**, 315–324.

Jana, S. (1971). Simulation of quantitative characters from qualitatively acting genes, II. Orthogonal subdivision of hereditary variance in two-locus genetic systems. *Theoretical and Applied Genetics* **42**, 119–124.

Jansen, R. C. (1992). A general mixture model for mapping quantitative trait loci by using molecular markers. *Theoretical and Applied Genetics* **85**, 252–260.

Jansen, R. C. (1993). Interval mapping of multiple quantitative trait loci. *Genetics* **135**, 205–211.

Jansen, R. C. & Stam, P. (1994). High resolution of quantitative traits into multiple loci via interval mapping. *Genetics* **136**, 1447–1455.

Kao, C.-H. & Zeng, Z.-B. (1997). General formulae for obtaining the MLEs and the asymptotic variance-covariance matrix in mapping quantitative trait loci when using the EM-algorithm. *Biometrics* **53**, 653–665.

Kao, C.-H., Zeng, Z.-B. & Teasdale, R. (1999). Multiple interval mapping for quantitative trait loci. *Genetics* **152**, 1203–1216.

Khazanehdari, K. A. & Borts, R. H. (2000). EXO1 and MSH4 differentially affect crossing-over and segregation. *Chromosoma* **109**, 94–102.

Lander, E. S. & Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**, 185–199.

Leips, J. & Mackay, T. F. (2000). Quantitative trait loci for life span in *Drosophila melanogaster*: interactions with genetic background and larval density. *Genetics* **155**, 1773–1788.

Levine, D. (1996). *Users Guide to the PGAPack Parallel Genetic Algorithm Library*. Argonne National Laboratory, Mathematics and Computer Science Division, Argonne, IL, USA.

Li, Z., Pinson, S. R., Park, W. D., Paterson, A. H. & Stansel, J. W. (1997). Epistasis for three grain yield components in rice (*Oryza sativa* L.). *Genetics* **145**, 453–465.

Long, A. D., Mullaney, S. L., Mackay, T. F. & Langley, C. H. (1996). Genetic interactions between naturally occurring alleles at quantitative trait loci and mutant alleles at candidate loci affecting bristle number in *Drosophila melanogaster*. *Genetics* **144**, 1497–1510.

Luschnig, S., Krauss, J., Bohmann, K., Desjeux, I. & Nusslein-Volhard, C. (2000). The *Drosophila* SHC adaptor protein is required for signaling by a subset of receptor tyrosine kinases. *Molecular Cell* **5**, 231–241.

Mackay, T. (2001). Quantitative trait loci in *Drosophila*. *Nature Reviews Genetics* **2**, 11–21.

Martinez, O. & Curnow, R. N. (1992). Estimating the locations and the sizes of effects of quantitative trait loci using flanking markers. *Theoretical and Applied Genetics* **85**, 480–488.

Nettleton, D. & Doerge, R. W. (2000). Accounting for variability in the use of permutation testing to detect quantitative trait loci. *Biometrics* **56**, 52–58.

Scanga, S. E., Ruel, L., Binari, R. C., Snow, B., Stambolic, V., Bourchard, D., Peters, M., Calvieri, B., Mak, T. W., Woodgett, J. R. & Manoukian, A. S. (2000). The conserved PI3′K/PTEN/Akt signaling pathway regulates both cell size and survival in *Drosophila*. *Oncogene* **19**, 3971–3977.

Shook, D. R. & Johnson, T. E. (1999). Quantitative trait loci affecting survival and fertility-related traits in *Caenorhabditis elegans* show genotype–environment interactions, pleiotropy and epistasis. *Genetics* **153**, 1233–1243.

Visscher, P., Whittaker, J. & Jansen, R. (2000). Mapping multiple QTL of different effects: comparison of a simple sequential testing strategy and multiple QTL mapping. *Molecular Breeding* **6**, 11–24.

Wolfe, K. (2000). Robustness – it's not where you think it is. *Nature Genetics* **25**, 3–4.

Zeng, Z.-B. (1993*a*). Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. *Proceedings of the National Academy of Science of the USA* **90**, 10972–10976.

Zeng, Z.-B. (1993*b*). Precision mapping of quantitative trait loci. *Genetics* **136**, 1457–1468.

Zeng, Z.-B., Kao, C.-H. & Basten, C. J. (1999). Estimating the genetic architecture of quantitative traits. *Genetical Research* **74**, 279–289.

Zeng, Z.-B., Liu, J., Stam, L., Kao, C.-H., Mercer, J. M. & Laurie, C. C. (2000). Genetic architecture of a morphological difference between two *Drosophila* species. *Genetics* **154**, 299–310.