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Published in:
Genetics

Publication date:
2001

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Manuscript received March 26, 2000
Accepted for publication October 9, 2000

ABSTRACT

The discovery of epistatically interacting QTL is hampered by the intractability and low power to detect QTL in multidimensional genome searches. We describe a new method that maps epistatic QTL by identifying loci of high QTL by genetic background interaction. This approach allows detection of QTL involved not only in pairwise but also higher-order interaction, and does so with one-dimensional genome searches. The approach requires large populations derived from multiple related inbred-line crosses as is more typically available for plants. Using maximum likelihood, the method contrasts models in which QTL allelic values are either nested within, or fixed over, populations. We apply the method to simulated doubled-haploid populations derived from a diallel among three inbred parents and illustrate the power of the method to detect QTL of different effect size and different levels of QTL by genetic background interaction. Further, we show how the method can be used in conjunction with standard two-locus QTL detection models that use two-dimensional genome searches and find that the method may double the power to detect first-order epistasis.

TRAITS that show continuous variation among individuals (quantitative traits) are affected by the environment and by many genes [quantitative trait loci (QTL)] that act singly and in interaction with each other. Interaction among QTL, or epistasis, is implicated in a number of important processes. Epistatic variance, as its fraction of the total genetic variance increases, may reduce the resemblance of offspring to their parents (Lynch and Walsh 1998), affect the importance of genetic drift and population structure in evolution (Wright 1980; Wade 1992), increase the genetic variance remaining in a population after a bottleneck (Goodnight 1987, 1988), and lead to either heterosis (Lynch and Walsh 1998) or its reverse, outbreeding depression, which can cause speciation events (Parker 1992; Orr 1995).

Methods to study epistasis using quantitative genetic methods that are based strictly on individual phenotypes lack power because epistasis contributes little to the resemblance among relatives (Cheverud and Routman 1995). The advent of complete DNA-marker linkage maps offers a new possibility for the study of epistasis: QTL mapping allows for the direct evaluation of interaction among identified loci (*e.g.*, simulation model 4 of Haley and Knott 1992; Chase *et al.* 1997). Nevertheless, such an approach confronts two important problems: low power to detect first-order epistasis (between just two QTL) and the intractability of seeking higher-order epistasis (involving multiple QTL).

To evaluate QTL interactions, methods must search for multiple QTL simultaneously. Such a multidimensional search (Kao *et al.* 1999) necessitates many statistical tests, and a high statistical threshold must be adopted to avoid false-positives among those tests. As an indication, Lander and Botstein (1989, Appendix 6) suggest that an m-fold higher threshold be used to declare significance for an m-dimensional search as for a one-dimensional search. This high threshold would drastically reduce power to find significant QTL interactions. To date, researchers have implemented two partial solutions to this quandary. First, they have increased detection power by restricting the search for QTL interactions to portions of the genome (Fijneman *et al.* 1996). This restriction ensures a feasible number of tests but leaves other portions of the genome unexplored. Second, they have sought QTL interactions that affect only those QTL that have detectable main effects (Lark *et al.* 1995). This option also drastically reduces the number of tests but fails to discover interacting QTL that have no strong individual effect (Fijneman *et al.* 1996; Holland *et al.* 1997).

Despite the problem of an appropriate statistical threshold, methods for two-dimensional searches have been developed (Haley and Knott 1992; Chase *et al.* 1997; Holland 1998; Wang *et al.* 1999). These methods will allow the detection of first-order interactions but not necessarily of higher-order interactions. The ability to detect higher-order interactions is desirable because such interactions have been documented (Allard

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1988, 1999; Doebley *et al.* 1995; Alonso-Blanco *et al.* 1998). Furthermore, the metabolic pathways that presumably underlie quantitative traits involve multiple interacting gene products (enzymes) and regulatory loci that could generate higher-order epistasis (McMullen *et al.* 1998).

A possible resolution to both the search dimension problem and the higher-order interaction problem would be to perform a one-dimensional search for QTL that interact with the genetic background. In the simplest case of two loci, the alleles present at one locus form the genetic background for alleles present at the other locus. Thus, a first-order interaction between these two loci would cause each locus to interact with its genetic background. Higher-order epistasis may also cause each locus to interact with its genetic background. Higher-order epistasis may also cause QTL-by-genetic-background interaction (Doebley *et al.* 1995; Alonso-Blanco *et al.* 1998). Detecting such interactions has become more important given improvements in methods to transfer alleles from one background to another using marker-assisted selection (Hospital and Charcosset 1997). Such efforts could be fruitless if the transferred alleles failed to affect the phenotype in a new genetic background as they had in their background of origin. Charcosset *et al.* (1994) and Rebaï *et al.* (1994, 1997) developed methods to detect QTL-by-genetic-background interaction that employ genotyped progeny resulting from a diallel mating design. These methods use least-squares tests applied directly to DNA marker data.

In this article, we develop a new method that maps within marker intervals the loci of greatest QTL-by-genetic-background interaction by simultaneous analysis of multiple related inbred-line crosses. Using maximum likelihood, the method contrasts models in which QTL allelic values are either nested within populations or are fixed over populations. High likelihood ratios between these models indicate QTL-by-genetic-background interaction. As a further benefit, the method allows statistical control of genetic noise due to other, nonfocal, QTL using multiple regression on marker data [Jansen's multiple-QTL model (MQM) method; Jansen and Stam 1994]. We apply the method to simulated doubled-haploid populations derived from a diallel among three parents and evaluate its power to detect QTL that interact with either the polygenic background or with each other. Finally, we show how the method can be used in conjunction with standard two-locus models.

METHODOLOGY

Consider three doubled-haploid parents, denoted A, B, and C, a diallel of the three possible crosses between them, A × B, A × C, and B × C, and the doubled-haploid populations derived from each of these crosses. Using these populations, QTL may be mapped using the MQM procedure (Jansen and Stam 1994) within each population. The linear model for such a procedure is

$$y_{ij} = \mu_i + \alpha_i x^q_{ij} + \sum_{c=1}^{f_i} \beta_{ic} x^c_{ij} + \varepsilon_{ij}, \qquad \text{(FULL)}$$

where y_{ij} is the phenotypic value of individual j in population $i = 1 \ldots 3$, μ_i is the mean for population i, and $\varepsilon_{ij} \sim N(0, \sigma^2_i)$ is a residual error for individual y_{ij}. The independent variables x^q_{ij} and x^c_{ij} depend on the genotype at the QTL analyzed and at marker cofactor $c = 1 \ldots f_i$, respectively, where f_i is the number of cofactors used in population i. These independent variables take on the values given in Table 1. The regression coefficient α_1 estimates $\frac{1}{2}(g_{A1} - g_{B1})$, where g_{X1} is the genetic value of the homozygote of the allele derived from parent X at the QTL locus analyzed in the genetic background of population 1. Equivalently, α_1 estimates the substitution effect between the alleles derived from parent A and parent B in the genetic background of population 1 and under the assumption of no dominance. The regression coefficients α_2, α_3, and β_{ic} have similar interpretations. Note that in (FULL), the allelic values g_{Xi} are nested within populations.

TABLE 1

Linear model coefficients from (FULL) and (REDUCED) as determined by the population being analyzed and the genotype at either a QTL or a marker cofactor

		(FULL) (x^q_{ij} and x^c_{ij})			(REDUCED) (x^{q*}_{ij})	
Population	Genotype	x_{1j}	x_{2j}	x_{3j}	x^{q*}_{1j}	x^{q*}_{3j}
1. A × B	AA	+1	0	0	+1	0
	BB	−1	0	0	−1	0
2. A × C	AA	0	+1	0	+1	+1
	CC	0	−1	0	−1	−1
3. B × C	BB	0	0	+1	0	+1
	CC	0	0	−1	0	−1

For missing QTL or marker information, Jansen and Stam (1994) have shown that maximum-likelihood estimates for the parameters μ_i, α_i, β_{ic}, and ε_{ij} can be obtained within each population by an expectation-maximization procedure using weighted multiple regression. Given this procedure, the support level for the presence of a QTL at a map location, using information from all populations simultaneously, derives from the likelihood ratio between (FULL) and a no-QTL null model. (FULL) contains three more estimated parameters than the no-QTL model, that is, one QTL effect per population.

A reduction in the number of estimated parameters is possible if we assume the QTL does not interact with genetic background. In that case, we may consider the allelic value of a QTL fixed over populations and repre-

sent the value of the homozygote of the allele from parent X at the QTL locus analyzed as g_X, irrespective of genetic background (*i.e.*, population) in which this genotype occurs. The regression coefficients α_1, α_2, and α_3 then respectively estimate $\frac{1}{2}(g_A - g_B)$, $\frac{1}{2}(g_A - g_C)$, and $\frac{1}{2}(g_B - g_C)$, which we denote α_1^*, α_2^*, and α_3^*. Using the identity $(g_A - g_C) = (g_A - g_B) + (g_B - g_C)$, that is, $\alpha_2^* = \alpha_1^* + \alpha_3^*$, we can develop a second model,

$$y_{ij} = \mu_i + \alpha_1^* x_{1j}^{q*} + \alpha_3^* x_{3j}^{q*} + \sum_{c=1}^{f_i} \beta_{ic} x_{ij}^c + \varepsilon_{ij}, \qquad \text{(REDUCED)}$$

where the variables x_{1j}^{q*} and x_{3j}^{q*} take values that cause α_1^* and α_3^* to be summed to estimate α_2^* (Table 1). Other parameters are the same as in (FULL). (REDUCED) contains two more estimated parameters than the no-QTL model, that is, one parameter less than FULL.

Having defined (FULL) and (REDUCED), we see that a QTL-by-genetic-background interaction would cause a difference in their likelihoods. Thus, when using the models to fit a QTL at a locus, a large likelihood ratio between the models provides evidence of a QTL at that locus that interacts with genetic background, in other words, a QTL that interacts epistatically with other loci. In the presence of epistasis, a general relationship between the regression coefficients of (FULL) can be expressed

$$\alpha_1 - \alpha_2 + \alpha_3 = d, \qquad (1)$$

where d represents a deviation from the identity $\alpha_1^* - \alpha_2^* + \alpha_3^* = 0$ used to develop (REDUCED). Interaction between a QTL and genetic background would cause a nonzero deviation contrast: in the presence of epistasis between the locus under consideration and other loci, $|d| > 0$. Charcosset *et al.* (1994) proposed a similar contrast but did not use it in developing their linear models.

Further analytical exploration of d reveals its importance in mapping epistatic QTL. We address two questions: first, what is the maximal value of d relative to the substitution effect of the QTL in which it occurs, and second, for a first-order interaction between two loci, locus 1 and locus 2, how are the corresponding values of d_1 and d_2 related?

To answer the first question, consider that in a doubled-haploid population, a QTL with allele-substitution effect α induces an additive genetic variance of α^2. Assuming that α_1, α_2, and α_3 are not equal (as is indeed impossible if at least one is nonzero and epistasis is absent), we ask what the maximal value of d may be, given a mean QTL variance over the three populations of σ^2_{QTL}, that is, given

$$\alpha_1^2 + \alpha_2^2 + \alpha_3^2 = 3\sigma^2_{QTL}. \qquad (2)$$

To use the Lagrange multiplier theorem to solve this constrained maximization problem, define the auxiliary function $h(\alpha_1, \alpha_2, \alpha_3, \lambda) = \alpha_1 - \alpha_2 + \alpha_3 - \lambda(\alpha_1^2 + \alpha_2^2 + \alpha_3^2 - 3\sigma^2_{QTL})$, differentiate it with respect to $(\alpha_1, \alpha_2, \alpha_3, \lambda)$, and set the partial derivatives to zero. One obtains $1 - 2\lambda\alpha_1 = 0$, $-1 - 2\lambda\alpha_2 = 0$, $1 - 2\lambda\alpha_3 = 0$, and $\alpha_1^2 + \alpha_2^2 + \alpha_3^2 = 3\sigma^2_{QTL}$. Solving gives $\alpha_1 = -\alpha_2 = \alpha_3$ so that $3\alpha_1^2 = 3\sigma^2_{QTL}$. Therefore the allele substitution effects $(\alpha_1, \alpha_2, \alpha_3) = (\sigma_{QTL}, -\sigma_{QTL}, \sigma_{QTL})$ yield a maximal d of $3\sigma_{QTL}$, that is, a maximal ratio $|d|/\sigma_{QTL}$ of three. Note that these are indeed odd substitution effects: fixing α_1 and α_3 to σ_{QTL}, α_2 would be $2\sigma_{QTL}$ in the absence of epistasis; instead it is $-\sigma_{QTL}$. We refer below to the ratio between d and σ_{QTL} as the "deviation ratio."

To analyze the relationship between the deviation contrasts d_1 and d_2 of two interacting loci, consider the vector of genetic values

$$\mathbf{a}^T = (a_{11}, a_{12}, a_{13}, a_{21}, a_{22}, a_{23}, a_{31}, a_{32}, a_{33},)^T,$$

where a_{ij} is the genetic value of a double homozygote at loci 1 and 2, subscript i takes the value 1, 2, and 3, when parent A, B, or C, respectively, confer the allele present at locus 1, and subscript j does likewise for locus 2 (the superscript ^{T} indicates transpose). With these genetic values, the allele substitution effects at locus 1, $(\alpha_{11}, \alpha_{21}, \alpha_{31})^T$, and at locus 2, $(\alpha_{12}, \alpha_{22}, \alpha_{32})^T$, are given by

$$\begin{bmatrix}\alpha_{11}\\ \alpha_{21}\\ \alpha_{31}\end{bmatrix} = \tfrac{1}{4}\begin{bmatrix}1 & 1 & 0 & -1 & -1 & 0 & 0 & 0 & 0\\ 1 & 0 & 1 & 0 & 0 & 0 & -1 & 0 & -1\\ 0 & 0 & 0 & 0 & 1 & 1 & 0 & -1 & -1\end{bmatrix}\mathbf{a}$$

$$\begin{bmatrix}\alpha_{12}\\ \alpha_{22}\\ \alpha_{32}\end{bmatrix} = \tfrac{1}{4}\begin{bmatrix}1 & -1 & 0 & 1 & -1 & 0 & 0 & 0 & 0\\ 1 & 0 & -1 & 0 & 0 & 0 & 1 & 0 & -1\\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & 1 & -1\end{bmatrix}\mathbf{a}. \qquad (3)$$

Rearranging Equation 3 we find $\alpha_{11} - \alpha_{21} + \alpha_{31} + \alpha_{12} - \alpha_{22} + \alpha_{32} = 0$. In other words $d_1 + d_2 = 0$. This relationship indicates that if we assume that the deviation contrast found at a locus was caused by an epistatic interaction with only one other locus, then we can expect that at that other locus we may find a deviation contrast of similar magnitude but opposite sign. A single genome scan mapping loci with high deviation contrasts could therefore simultaneously identify loci affected by epistasis and suggest which other loci might be interacting with them.

SIMULATIONS

Genome generation: The genome consisted of 10 chromosomes of 200 cM each. Two hundred marker loci were randomly distributed over the genome. Each marker locus was assumed to be triallelic, with allele frequencies, in order of abundance, of 0.5, 0.3, and 0.2. For each of three doubled-haploid parents denoted A, B, and C below, alleles were randomly assigned at each marker locus with the probability of each allele depending on its frequency. Given this procedure, the

TABLE 2

Genetic values conferred by the combination of homozygotes at two loci used in simulations to determine the power to detect first-order epistasis

Parent conferring allele at locus 1	Parent conferring allele at locus 2		
	A	B	C
A	1	−1	−1
B	−1	1	1
C	1	−1	−1

These values would arise if all genetic variance caused by loci 1 and 2 was epistatic in population A × B and parents A and C were identical in state at locus 1 while parents B and C were identical in state at locus 2.

probability that two parents shared an allele at a given marker locus was $(0.5)^2 + (0.3)^2 + (0.2)^2 = 0.38$. Thus, for a given population, the expected number of segregating markers was $200*(1 - 0.38) = 124$.

In a first set of simulations (set 1), we explored the power to detect QTL interacting with the polygenic background. Doubled-haploid populations from all three crosses (A × B, A × C, and B × C) were generated by doubling simulated gametes from F_1's of each of the crosses. We assumed an isolated QTL to be present at the center of each of six of the chromosomes. Each parent carried a different QTL allele and QTL substitution effects (α_1, α_2, α_3) were picked randomly but were subject to the constraints of Equations 1 and 2 to obtain desired deviation contrasts and average QTL effects. On each of two chromosomes, the fraction of the phenotypic variance caused by additive QTL effects, averaged over the three populations, was $h^2_{\text{QTL}} = 0.16$. On each of four other chromosomes, the fraction of the phenotypic variance, averaged over the populations, caused by additive QTL effects was $h^2_{\text{QTL}} = 0.07$. We did not generate QTL-by-genetic-background interaction by simulating epistasis among the six QTL. Rather, QTL allelic values were assumed to be affected by other background loci specific to each population. Averaged over the populations, the fraction of the phenotypic variance due to these six QTL was $h^2 = 0.60$. The genetic value of each individual depended on the simulated allele substitution effects within its population and on which parent contributed each of its QTL alleles. We added a random normal deviate of variance $(1 - h^2)$ to each individual.

In a second set of simulations (set 2) we evaluated how best to combine QTL-by-background interaction information with standard two-locus models to detect first-order epistasis. We simulated two QTL, each at the center of a chromosome. Genetic values for the alleles derived from each parent were generated as given in Table 2, scaled so that the total genetic variance caused by the QTL together was a fraction $H^2_{\text{QTL}} = 0.10$ or $H^2_{\text{QTL}} = 0.20$ of the phenotypic variance. We added a random normal deviate of variance $(1 - H^2_{\text{QTL}})$ to each individual. Generation of the genome and marker information was the same as above. Populations were of 100 doubled-haploid progeny. Note that, in the above, we use h^2_{QTL} to denote the ratio of the *additive* genetic variance of a *single* QTL relative to the phenotypic variance and H^2_{QTL} to denote the ratio of the *total* genetic variance of a *pair* of QTL relative to the phenotypic variance. Thus, for a pair of QTL generated as in Table 2, if $H^2_{\text{QTL}} = 0.20$, then for each QTL, $h^2_{\text{QTL}} \cong 0.07$.

QTL analysis, set 1: For each population separately, three markers were chosen per chromosome to be candidate cofactors in the MQM procedure (leading to $3 \times 10 = 30$ candidate cofactors over the entire genome). Segregating markers were chosen that allowed the most uniform chromosome coverage. Because the same markers did not necessarily segregate in each population, different sets of candidate cofactors resulted per population. Using all candidate cofactors we calculated a bias-adjusted residual variance for each population. These variances are unbiased (Jansen 1994) and we used them for all further estimations on the three populations. Again, for each population separately, we used a backward elimination procedure to retain in the model only those cofactors that explained significant proportions of variance. To determine whether to retain a cofactor, we used a threshold T such that $P(F_{1,\text{d.f.}} > T) = 0.02$, with d.f. equaling the number of residual degrees of freedom in the all-cofactor model, that is, d.f. = [number of individuals in population − (number of candidate cofactors + 1)].

To locate QTL we then scanned the full genome in 5-cM steps. We first calculated the likelihood of the data under (FULL) in the absence of a QTL (L_{NoQTL}), but using all retained cofactors with the exception of cofactors within 25 cM of the putative QTL. The likelihood of the data under (FULL) was then calculated in the presence of a QTL (L_{Full}). Finally, we calculated the likelihood of the data under (REDUCED) (L_{Reduced}). From these likelihoods we calculated three likelihood ratios:

$$\begin{aligned} \text{LR}_{\text{Full}} &= 2*\log(L_{\text{Full}}/L_{\text{NoQTL}}) \\ \text{LR}_{\text{Reduced}} &= 2*\log(L_{\text{Reduced}}/L_{\text{NoQTL}}) \\ \text{LR}_{\text{Deviation}} &= 2*\log(L_{\text{Full}}/L_{\text{Reduced}}). \end{aligned} \quad (4)$$

The first two statistics indicate the support level for the presence of a QTL using either (FULL) or (REDUCED). The third statistic increases as the level of QTL-by-genetic-background increases.

We ran simulations with population sizes of 50, 100, and 200 doubled-haploid individuals per population, and with deviation ratios of zero to three in one-half increments. To determine genome-wide significance thresholds for these three statistics we performed 3000 simulation runs on individuals generated without ge-

netic variance. We chose as threshold the 95th percentile value of the genome-wide maxima of the statistics. The power of (FULL) or (REDUCED) to detect a QTL under specified conditions is the fraction of simulated QTL for which LR_{Full} or $LR_{Reduced}$ exceeded their thresholds. Similarly, the power to detect a QTL-by-genetic-background interaction is the fraction of QTL for which $LR_{Deviation}$ exceeded its threshold.

QTL analysis, set 2: We used two methods to detect pairs of interacting QTL, one with information from the deviation contrast method and one without it. In the first, we assumed that population A × B was part of a diallel, as would be typical for a population in an applied plant breeding program. That diallel was analyzed as indicated above and results were used to determine which regions of the genome should be paired for analysis using the two-locus model for epistasis,

$$y_{ij} = \mu_i + \alpha_i^1 x_{ij}^{q1} + \alpha_i^2 x_{ij}^{q2} + \sum_{c=1}^{f_i} \beta_{ic} x_{ij}^c + \varepsilon_{ij} \qquad \text{(BILOC}_a\text{)}$$

$$y_{ij} = \mu_i + \alpha_i^1 x_{ij}^{q1} + \alpha_i^2 x_{ij}^{q2} + \kappa_i^{12} x_{ij}^{q1} x_{ij}^{q2} + \sum_{c=1}^{f_i} \beta_{ic} x_{ij}^c + \varepsilon_{ij}, \qquad \text{(BILOC}_b\text{)}$$

where y_{ij}, μ_i, α_i, β_{ic}, x_{ij}^c, and ε_{ij} have the same interpretation as in (FULL). The $\alpha_i^1 x_{ij}^{q1}$ and $\alpha_i^2 x_{ij}^{q2}$ regressions account for main effects at two QTL loci being analyzed and the $\kappa_i^{12} x_{ij}^{q1} x_{ij}^{q2}$ regression accounts for interaction between the loci. Statistically controlling for possible epistatic interactions between cofactors was not attempted (but see Wang *et al.* 1999 for a possible approach). The likelihood ratio between ($BILOC_a$) and ($BILOC_b$), $LR_{Epistasis}$, indicates the level of support for an epistatic interaction between the loci being analyzed. In a (BILOC) analysis, we scanned the two regions of interest on a 5-cM grid using all retained cofactors with the exception of cofactors within 25 cM of either putative QTL.

The overall analysis proceeded as follows. The maximal $LR_{Deviation}$ (Equation 4) for each chromosome determined the position of maximal support for the presence of an epistatic QTL. If the sum of $LR_{Deviation}$ for two chromosomes exceeded a threshold T, and the signs of the deviation contrast d calculated from Equation 1 were opposite, 90-cM regions surrounding the points of maximal support were analyzed in each population separately using (BILOC). As T increases, the number of locus pairs analyzed using (BILOC) declines and therefore the likelihood ratio necessary to ensure a 5% type I error rate also declines. We obtained thresholds for BILOC at different T from 1000 simulation runs on individuals generated without genetic variance.

In the second method, we assumed that the population A × B was analyzed for epistatic QTL without the benefit of information from a diallel analysis. (BILOC) was therefore applied over the whole genome leading to a much greater number of tests. We report the power to detect both QTL of an interacting pair, mapped to within 25 cM of their simulated positions. For both methods, we used significance thresholds for a 5% type I error rate on a population-wise basis. If several populations were analyzed, a further Bonferroni correction would be applied.

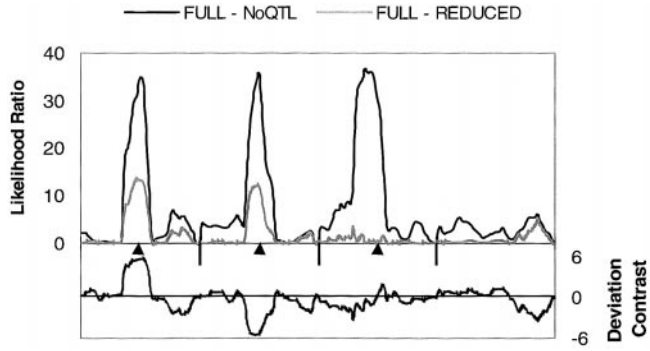

Figure 1.—Diallel analysis results. The top shows the likelihood ratios between (FULL) and a no-QTL model and between (FULL) and (REDUCED) (as described in the text). The bottom shows estimates of the deviation contrast. QTL were simulated at the center of the first three chromosomes (triangles), and none were simulated on the fourth chromosome. The first two QTL were simulated to interact using genetic values given in Table 2, and the third QTL did not interact. Values were scaled to obtain a phenotypic variance of 100 with $h^2_{QTL} = 0.07$ for all QTL. These results derive from an analysis of a diallel of three doubled-haploid populations of 100 individuals each.

RESULTS

QTL-by-background interaction: Analysis results from a single simulation run in Figure 1 illustrate the type of output from the method. The likelihood ratios between (FULL) and a no-QTL model show support for the presence of QTL at their simulated locations, irrespective of whether the QTL is epistatic to others. In contrast, the likelihood ratios between (FULL) and (REDUCED) specifically identify QTL that are epistatic to others segregating in the background of the populations. Using the regression coefficients estimated from (FULL), a deviation contrast can be calculated using Equation 1. Consistent with theory, the estimated deviation contrasts for two QTL involved in first-order epistatic interaction are of similar magnitude but opposite sign. At the loci of maximal $LR_{Deviation}$, the deviation ratios estimated for the two QTL were 2.15 and −2.09, values that exceed in magnitude the ratio of $1.73 = \sqrt{3}$ expected given the genetic values of Table 2.

Empirical powers to detect QTL with nonnull deviation contrasts are graphed in Figure 2. The magnitude of the deviation contrast increases with both the average variation caused by the QTL and with the deviation ratio. For a given h^2_{QTL}, the power to detect a deviation contrast increases with the deviation ratio; conversely,

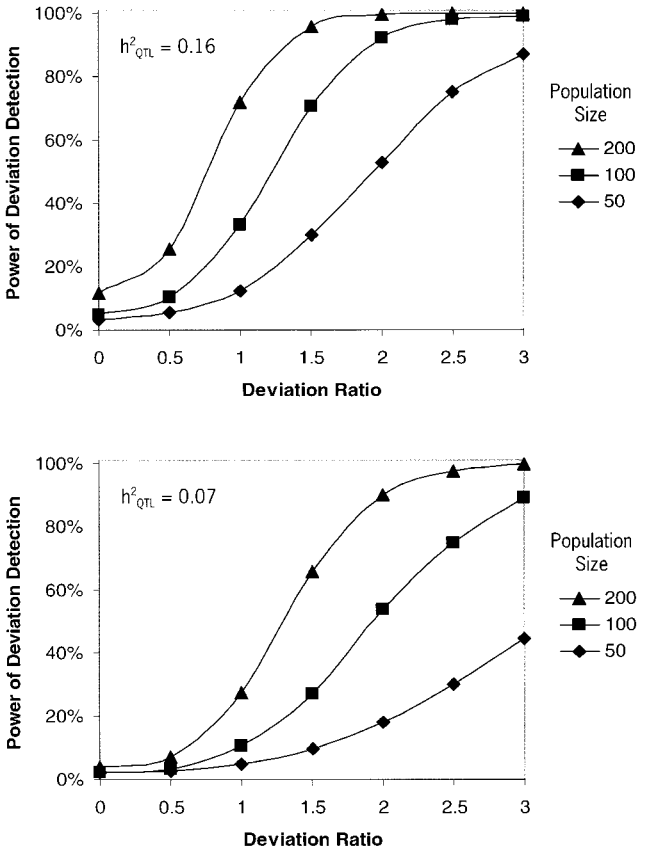

FIGURE 2.—Power to detect nonzero deviation contrasts for QTL of different effects with diallels formed of populations of three sizes, as affected by the deviation ratio (from QTL analysis set 1, see text).

for a given deviation ratio, the power to detect a deviation contrast increases with h^2_{QTL}. Even though the QTL simulated accounted for a fairly large fraction of the phenotypic variance, large population sizes were necessary to obtain adequate power to detect them, unless their deviation ratio was high (>2). What deviation ratio values might occur in real circumstances is an empirical problem that has not been addressed. For the simple configuration of epistatic effects given in Table 2, the deviation ratio is $\sqrt{3} = 1.73$. To get a feel for what deviation ratios might arise from interacting loci under other conditions, consider all double-homozygote genetic values a_{ij} (Equation 3) as independently and identically distributed with $a_{ij} \sim N(0, \sigma^2)$. In that case, manipulation of Equation 3 shows that the deviation contrast $d \sim N(0, [\frac{3}{8}]\sigma^2)$ and α_i from (FULL) is distributed $N(0, \frac{1}{4}\sigma^2)$ so that $E(\sigma^2_{QTL}) = \text{var}(\alpha_i) = \frac{1}{4}\sigma^2$. Analytically we find $E(|d|)/\sqrt{E(\sigma^2_{QTL})}$ is 0.98. The expected deviation ratio, $E(|d|/\sqrt{\sigma^2_{QTL}})$, is more difficult to derive analytically; by simulation we found a mean deviation ratio of 1.16. Given the desire to detect QTL with deviation ratios between 1 and 2, population sizes of 200 individuals per cross in the diallel would seem necessary.

Note that two forms of type I error may occur in QTL-by-genetic-background mapping: a significant deviation contrast may be declared either in the absence of true genetic variation or in the presence of a QTL that does not interact with genetic background. We set significance thresholds using simulations of genomes without genetic variance and therefore obtained $\alpha = 5\%$ for the first form of type I error. Figure 2 shows the second form of type I error rate on a per QTL basis as the "detection power" when the deviation ratio is zero. This rate depended on the variation caused by the QTL and on the population size (Figure 2). Because the rate is given on a per QTL basis, it can be <5%, for example, when $h^2_{QTL} = 0.07$ and population size = 100. The genome-wide error rate would depend on the number of segregating nonepistatic QTL. Even on a per QTL basis, however, for QTL of large effect, we found second form type I error rates >5%. CHARCOSSET *et al.* (1994) have pointed out that in the presence of a QTL with simple additive effects a nonnull deviation contrast can be generated by heterogeneous recombination frequencies among the populations between markers and the QTL. In this study, we did not simulate heterogeneous recombination frequencies, but sampling variation in the number of recombinants between a marker and a QTL, irrespective of the expected number of recombinants, could cause a similar nonnull deviation contrast. With more progeny per population such sampling variation effects should decrease, but, in contrast to this prediction, we found the highest type I error rate with the largest population size (Figure 2). As an alternate explanation, consider that because the same markers do not segregate in all populations, marker information content at the simulated QTL position will not be equal over the populations. Low information content at that position in population i will cause a downward bias in the estimation of α_i. If such a bias does not occur in the other populations a nonnull deviation contrast will also result. This mechanism therefore seems better able to explain the increased level of type I error observed in the presence of QTL with large additive effects.

High values for either LR_{Full} or $LR_{Reduced}$ should indicate the presence of a QTL segregating within the diallel. In effect the difference between (FULL) and (REDUCED) is that QTL allele values are nested within populations in (FULL) while they are considered fixed in (REDUCED). Because (REDUCED) estimates one parameter less than (FULL) it may be more powerful to detect a segregating QTL if its assumption of fixed allele effects is correct. Based on our simulations, (REDUCED) is indeed more powerful than (FULL) for QTL with a deviation ratio lower than one (Figure 3). The gain in power, however, appeared relatively small, and for QTL with larger deviation ratios, a possibly large loss in power occurred. In contrast, (FULL) was impervious to changes in the deviation ratio. Methods have been described in the literature that model nested QTL effects over multiple populations (XIE *et al.* 1998; XU

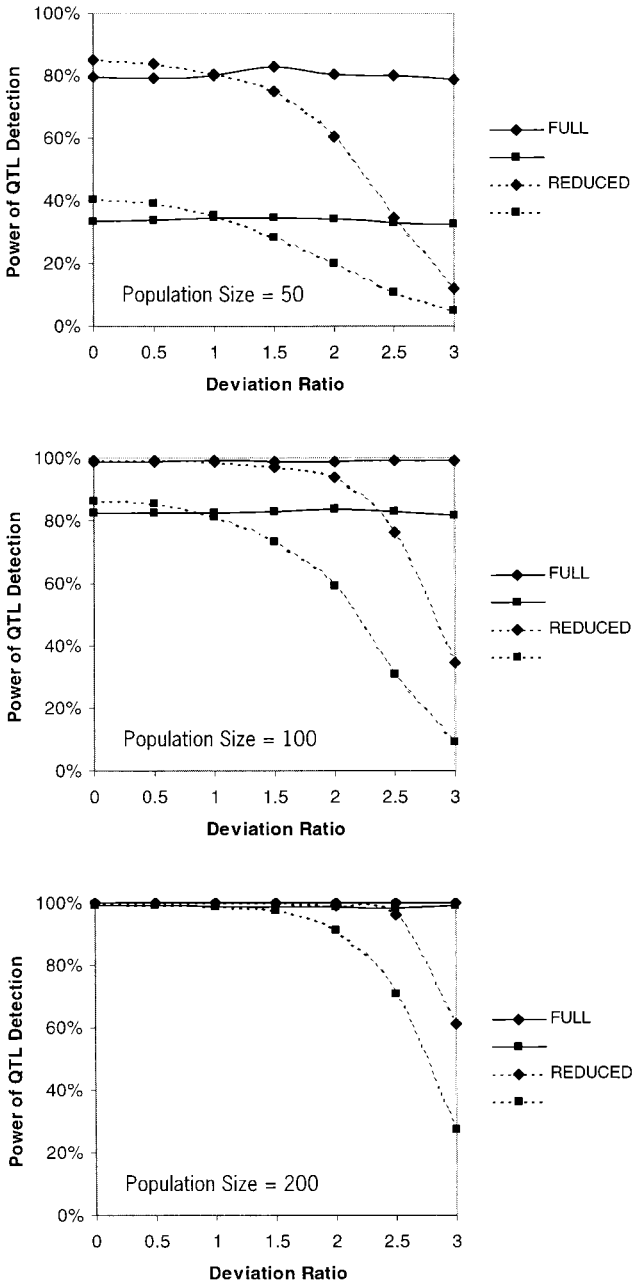

FIGURE 3.—Power to detect segregating QTL *per se*. Diamonds and squares are for QTL with h^2_{QTL} = 0.16 and 0.07, respectively.

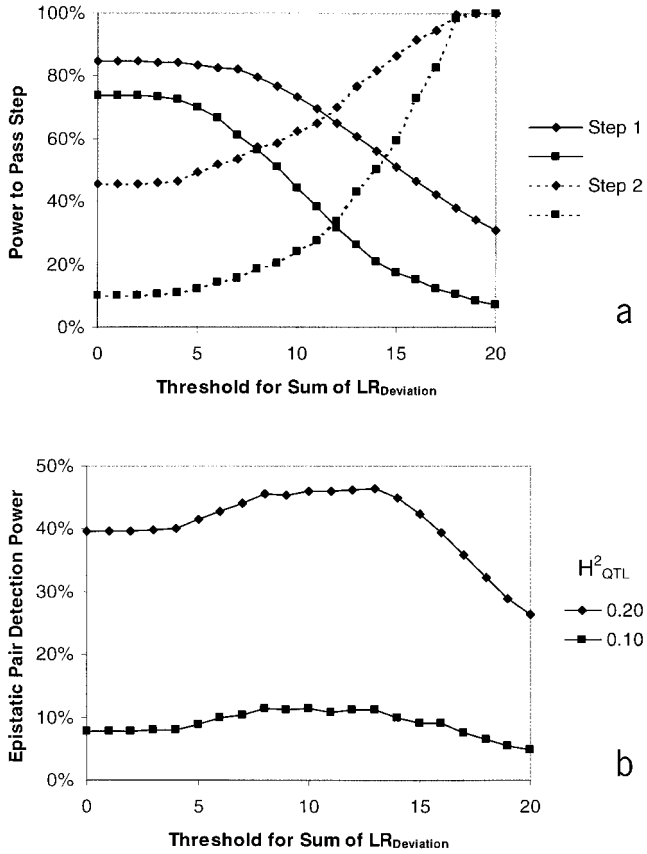

FIGURE 4.—(a) Power to pass each step in a two-step procedure to detect first-order epistasis, as affected by the threshold used to pass step 1, the sum of $LR_{Deviation}$ for the two loci. Diamonds and squares are for QTL with H^2_{QTL} = 0.20 and 0.10, respectively. (b) Overall power to detect first-order epistasis, as affected by the threshold used to pass step 1.

1998). Our simulation results indicate that for methods that seek to gain power by assuming fixed allele effects over multiple populations, a consequence of epistasis might precisely be loss of power. Methods applied to complex human or animal pedigrees that combine segregation and linkage analysis to map QTL (*e.g.*, LIN 1999) often assume fixed allele effects because the sizes of the families they analyze are small. This assumption may also be made for plant populations. In either case, epistasis may reduce the QTL detection power of these methods.

First-order QTL interaction: If a population is embedded within a diallel, information from a prior analysis using (FULL) and (REDUCED) will provide evidence as to which regions of the genome carry epistatic QTL. That information, in turn, may be used to decrease the number of pairwise tests that must be carried out to detect first-order epistasis using (BILOC). Under a null model containing no QTL, reducing the number of tests performed should also reduce the level of highest excursion of (BILOC)'s likelihood ratio. In consequence, a lower threshold could be used to declare significant first-order epistasis and higher power should result. We investigated the effectiveness of such a two-step procedure by simulation.

In a two-step procedure, for an interacting pair of QTL to be detected, they must both pass both steps. Statistical thresholds for both steps must be chosen to attain the desired overall type I error rate (here, α = 5%). High stringency in step 1 reduces the power to pass step 1 but also drastically reduces the number of tests performed in step 2, thereby increasing the power to pass step 2. A trade-off between the powers to pass each step results (Figure 4a). Under the conditions sim-

ulated, the stringency for step 1 that is optimal for obtaining the greatest overall power occurs for a minimal sum of the deviation likelihood ratios (Σ $LR_{Deviation}$) at two loci under consideration between 8 and 13 (Figure 4b). The rationale for using Σ $LR_{Deviation}$ in step 1 derives from the result that even for two loci that are simulated to be interacting, the test statistics associated with each locus were independent. By simulation we found correlations between $LR_{Deviation}$ statistics for two loci of $r = 0.01$ and $r = 0.03$ for $H^2_{QTL} = 0.20$ and 0.10, respectively. That is, for first-order interacting QTL, $LR_{Deviation}$ is distributed as a noncentral χ^2 with the same noncentral parameter but with independent draws for each locus. Presumably for QTL present on different chromosomes, test statistics are independently affected by errors associated with sampling of recombination events and with microenvironment. Because of the test statistic independence, for a given type I error rate in passing step 1, the power to pass that step is higher using Σ $LR_{Deviation}$ than requiring each $LR_{Deviation}$ to exceed a minimal threshold.

With this two-step procedure the powers obtained to pass both steps and map both QTL to within 25 cM of their simulated positions were 46 and 11% for QTL pairs with $H^2_{QTL} = 0.20$ and 0.10, respectively (Figure 4b). Those powers compare favorably to the powers to detect the epistatic pairs in the absence of a prior QTL-by-background analysis, which were 24 and 5% for QTL pairs with $H^2_{QTL} = 0.20$ and 0.10, respectively.

DISCUSSION

Combining across- and within-population information: The two-step procedure that we have explored constitutes a method to combine information obtained across populations (QTL-by-genetic-background interaction) and within populations (QTL-by-QTL interaction). A possible drawback of this combination method is that interacting QTL will not be found unless test statistics for both steps (Σ $LR_{Deviation}$ and $LR_{Epistasis}$) exceed minimal thresholds. Low correlations found by simulation between Σ $LR_{Deviation}$ and $LR_{Epistasis}$ statistics ($r = 0.21$ and $r = 0.10$ for $H^2_{QTL} = 0.20$ and 0.10, respectively) indeed indicate that passing one step is a poor predictor of passing the next step. A rationale we invoked above to justify the use of a minimum for Σ $LR_{Deviation}$ as the criterion for step 1 rather than separate minima for each $LR_{Deviation}$ may therefore apply to these two statistics. That is, using a compound statistic (Σ $LR_{Deviation}$ + $LR_{Epistasis}$) may result in higher power using each separately. In simulations using the compound statistic, we found powers to detect interacting QTL pairs of 61 and 13% for $H^2_{QTL} = 0.20$ and 0.10, respectively. These powers were obtained despite the fact that full-genome two-dimensional searches were performed. In general the two-step and the compound-statistic procedures can be contrasted as alternate methods of combining information from different sources. In one method, across-population information focuses the within-population search, in the other method both information sources contribute to a joint test statistic.

Thus far, we have applied across- and within-population models in separate analyses. As a further refinement, however, it would be possible to combine the linear models used in each analysis. A combined-models approach would test a two-locus extension of (REDUCED) against a two-locus extension of (FULL) combined with (BILOC) interaction regression parameters within each population [call these models $(REDUCED)^2$ and $(FULL)^2$, respectively]. In the case of a three-population diallel, $(FULL)^2$ would differ from $(REDUCED)^2$ by five parameters: one parameter per locus for genetic background interaction and one parameter per population in the diallel for locus-by-locus interaction. Relative to performing separate analyses, the combined-model analysis would gain power by estimating nuisance parameters (the mean and cofactor parameters) only once. It would also more powerfully detect epistatic interactions when they were manifest in all three populations rather than in only a single population as we simulated (Table 2). Finally, we note that while the compound statistic appears to increase the power to detect epistatic QTL, it also creates interpretation problems. If a QTL pair was found to be significant, further analysis would be required to determine what sources contributed to that significance.

We also note that we have only discussed combining information in the ideal situation where two loci interact with each other but with no further loci. When a locus interacts with more than one other locus, the simple equation linking deviation ratios among two interacting loci, $d_1 + d_2 = 0$, will no longer hold. For three loci, $d_1 + d_2 + d_3 = 0$ will hold if no three-way interaction occurs among loci. This zero sum, however, does not imply any simple pairwise relationship between, say, d_1 and d_2. As the complexity of interactive sets of QTL increases, therefore, QTL-by-genetic-background interaction information will likely become less useful for the detection of first-order interactions.

Generalization to other population structures: When QTL affecting the same trait are mapped in several populations they are generally not found in the same locations over the populations (*e.g.*, Brummer *et al.* 1997; Orf *et al.* 1999). Beavis (1994) pointed out a number of sources of difference between different QTL experiments that reduce reproducibility. First, when the power to detect a QTL is less than one, it may be detected in some experiments but not others. A QTL's detection is enhanced or reduced by colinearity with error residuals caused by random measurement variation and by colinearity with other QTL caused by random sampling of particular genotypes among segregating progeny. With proper randomization, these sampling variations will not be repeated from experi-

ment to experiment. Second, a QTL will be detected only in populations where it segregates: even in the absence of known coancestry, some parents may carry identical-in-state alleles. Third, QTL-by-environment interaction may affect QTL substitution effects in experiments conducted in different environments. Finally, QTL-by-genetic-background interaction may affect QTL substitution effects in experiments conducted with different populations. This plethora of possible causes leading to discrepancies between QTL mapping results makes the design of experiments to support/reject the QTL-by-genetic-background interaction hypothesis challenging. Clearly, to attribute observed differences in QTL effects to that hypothesis requires mating designs that bring single alleles of known origin into different genetic backgrounds. The diallel mating design achieves this task, but other designs are possible.

For populations produced from matings among N inbred lines a maximum of N alleles may segregate at a given locus. For a (REDUCED) model assuming QTL allelic values g_i ($i = 1 \ldots N$) that are fixed over populations, $N - 1$ values may be estimated with the constraint $\Sigma_{i=1}^{N} g_i = 0$ (Rebaï *et al.* 1997). If crosses are made to produce P populations, a (FULL) model assuming QTL allelic values nested within populations will estimate P QTL allele substitution effects. For $P > N - 1$, the likelihood ratio between (FULL) and (REDUCED) will be distributed as χ^2 with $P - N + 1$ d.f., if there is no QTL-by-genetic-background interaction. We have presented the simple case where $N = 3$ and $P = 3$, which derives from a three-parent diallel, but we briefly describe other possibilities. In a North Carolina design II with two inbred dams (A and B) and sires (C and D), $N = 4$ and $P = 4$, with populations (1) A × C, (2) A × D, (3) B × C, and (4) B × D. Then (FULL) estimates four substitution effects (g_{A1}–g_{C1}), (g_{A2}–g_{D2}), (g_{B3}–g_{C3}), and (g_{B4}–g_{D4}); (REDUCED) arises from the algebraic identity (g_A–g_D) = (g_A–g_C) + (g_B–g_D) − (g_B–g_C), which is obtained under the assumption of no QTL-by-genetic-background interaction.

As a final example consider two inbred parents (A and B) and two recombinant-inbred-line or doubled-haploid populations, one produced from the F_2 generation and the other produced from a BC_1 generation. Here $N = 2$ and $P = 2$, and the populations have different genetic backgrounds because in the first, segregating alleles come from each parent equally, while in the second, segregating alleles come from one parent 75% of the time. Beavis *et al.* (1994) noted such a background difference as a possible factor explaining differences between their results and those of Stuber *et al.* (1992) for QTL affecting yield in maize (*Zea mays* L.). In this case, (FULL) estimates two substitution effects (g_{A1}–g_{B1}) and (g_{A2}–g_{B2}) while the algebraic identity leading to (REDUCED) is particularly simple: (g_{A1}–g_{B1}) = (g_{A2}–g_{B2}) = (g_A–g_B). This example is a special case of the mechanism discussed by Goodnight (1988) whereby founder events convert epistasis into additivity: partial fixation at loci "will tend to incorporate their epistatic interaction into additive genetic variance." Thus, (g_{A1}–g_{B1}) and (g_{A2}–g_{B2}) may differ if an interaction between loci in the F_2-derived population is converted to a main effect in the BC_1-derived population.

As illustrated in Table 2, a similar mechanism can lead to QTL-by-genetic-background interaction when more than two parents are used but the epistatically interacting QTL they carry are only biallelic. When several populations are derived from such parents, one may expect that in some populations some but not all of the interacting QTL will be segregating. In such cases, the epistatic variance will be converted to additive variance at the segregating loci, leading to detectable deviation contrasts. For crop species that have gone through major bottlenecks in the course of their domestication, such as soybean [*Glycine max* (L.) Merrill.] in the United States (Gizlice *et al.* 1993), this mechanism would appear as a simple and possibly frequent explanation for QTL-by-genetic-background interaction.

Implications for marker-assisted selection: Research reports and theory surrounding marker-assisted selection often side-step the issue of QTL-by-genetic-background interaction. For example, Hospital and Charcosset (1997) discuss optimal marker-assisted QTL introgression approaches "provided the expression of the gene is not reduced in the recipient genomic background." However, within a marker-assisted selection program in which progeny from different crosses are routinely genotyped, the methods described would make it possible to systematically uncover QTL that interact with genetic background. Within such a context, the information obtained could refine genotype-based selection indices, either by avoiding QTL that interact with genetic background or by improving the prediction of the genetic value conferred by specific QTL combinations.

We thank two anonymous reviewers for suggestions and improvements. A National Science Foundation North Atlantic Treaty Organization Postdoctoral Fellowship in Science and Engineering, DGE-9902466, supported J.-L.J.'s work on this research.

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Communicating editor: J. A. M. van Arendonk