Multiple Trait Analysis of Genetic Mapping for Quantitative Trait Loci

Changjian Jiang* and Zhao-Bang Zeng†

**Department of Agronomy, Jiangsu Agricultural College, Yangzhou, Jiangsu 225001, People's Republic of China and †Program in Statistical Genetics, Department of Statistics, North Carolina State University, Raleigh, North Carolina 27695-8203*

Manuscript received September 22, 1994
Accepted for publication March 30, 1995

ABSTRACT

We present in this paper models and statistical methods for performing multiple trait analysis on mapping quantitative trait loci (QTL) based on the composite interval mapping method. By taking into account the correlated structure of multiple traits, this joint analysis has several advantages, compared with separate analyses, for mapping QTL, including the expected improvement on the statistical power of the test for QTL and on the precision of parameter estimation. Also this joint analysis provides formal procedures to test a number of biologically interesting hypotheses concerning the nature of genetic correlations between different traits. Among the testing procedures considered are those for joint mapping, pleiotropy, QTL by environment interaction, and pleiotropy *vs.* close linkage. The test of pleiotropy (one pleiotropic QTL at a genome position) *vs.* close linkage (multiple nearby nonpleiotropic QTL) can have important implications for our understanding of the nature of genetic correlations between different traits in certain regions of a genome and also for practical applications in animal and plant breeding because one of the major goals in breeding is to break unfavorable linkage. Results of extensive simulation studies are presented to illustrate various properties of the analyses.

MANY data for mapping quantitative trait loci (QTL) contain observations on multiple traits or on one or several traits in multiple environments. With such data, we can ask questions like the following: Does a QTL have pleiotropic effects on multiple traits? Does a QTL show genotype-environment interaction? What is the nature of genetic correlation between different traits? Is the correlation due to pleiotropy or linkage in certain regions of a genome? Statistically this involves multiple trait analysis, because the expression of a trait in different environments can be regarded as different traits or different trait states (FALCONER 1952).

Currently, the many statistical methods developed for mapping QTL (*e.g.*, LANDER and BOTSTEIN 1989; HALEY and KNOTT 1992; JANSEN and STAM 1994; ZENG 1994) are for analysis on one trait only and have not been extended specifically for multiple trait analysis yet. With that omission, a number of studies on mapping QTL (*e.g.*, PATERSON *et al.* 1988, 1991; STUBER *et al.* 1992) analyzed different traits separately. This approach does not take advantage of the correlated structure of data and has a number of disadvantages for mapping QTL and also for understanding the nature of genetic correlations. The statistical powers of hypothesis tests tend to be lower and the sampling variances of parameter estimation tend to be higher for separate analyses. Also, it would be difficult to test a number of biologically interesting questions involving multiple traits by analyzing different traits separately.

Corresponding author: Zhao-Bang Zeng, Department of Statistics, North Carolina State University, Raleigh, NC 27695-8203.
E-mail: zeng@stat.ncsu.edu

Genetics **140:** 1111–1127 (July, 1995)

Different traits are correlated genetically due to pleiotropy and linkage. With observations on a number of polymorphic genetic markers and on a number of quantitative traits, it is possible to dissect a portion of genetic variation and covariation among traits by localizing and estimating responsible QTL. It is also possible to test whether the genetic correlation is due to pleiotropy or linkage for certain regions of a genome. In this paper, we extend the composite interval mapping method (ZENG 1993, 1994) to multiple trait analysis. We demonstrate how the joint analysis on multiple traits can improve the power and precision of mapping QTL; we show how to test some biologically interesting hypotheses involving multiple traits, such as pleiotropy and QTL × environment interaction, and how to test whether significant associations for different traits in certain regions of a genome are due to pleiotropy or close linkage. Properties and behavior of the test statistics are examined by analyses and also by simulation studies.

STATISTICAL MODELS AND LIKELIHOOD ANALYSES

Composite interval mapping model for multiple traits: In this section, we formulate statistical models and likelihood analyses for mapping QTL that affect multiple traits, using the composite interval mapping method.

Suppose that we have a sample of n individuals from an F_2 population crossed from two inbred lines, with observations on m quantitative traits and on a number of codominant genetic markers. Let the

TABLE 1

Probability of QTL genotype given flanking marker genotype

Marker genotype	QTL genotype		
	QQ (2)	Qq (1)	qq (0)
$M_1M_1M_2M_2$	1	0	0
$M_1M_1M_2m_2$	$1-p$	p	0
$M_1M_1m_2m_2$	$(1-p)^2$	$2p(1-p)$	p^2
$M_1m_1M_2M_2$	p	$1-p$	0
$M_1m_1M_2m_2$	$\delta p(1-p)$	$1-2\delta p(1-p)$	$\delta p(1-p)$
$M_1m_1m_2m_2$	0	$1-p$	p
$m_1m_1M_2M_2$	p^2	$2p(1-p)$	$(1-p)^2$
$m_1m_1M_2m_2$	0	p	$1-p$
$m_1m_1m_2m_2$	0	0	1

$p = r_{M_1Q}/r_{M_1M_2}$, $\delta = r^2_{M_1M_2}/[(1-r_{M_1M_2})^2 + r^2_{M_1M_2}]$, where r_{M_1Q} is the recombination frequency between marker M_1 and QTL Q, and $r_{M_1M_2}$ is the recombination frequency between markers M_1 and M_2. Double recombination is ignored.

value of each marker be recorded as 2, 1 and 0 for the homozygote in one parental line, heterozygote and homozygote in the other parental line, respectively. These markers can be mapped in linkage groups or mapped on chromosomes if the locations of some of them are known.

Further, let y_{jk} denote the value of the kth trait in the jth individual. To test for a QTL on a marker interval $(i, i+1)$, the statistical model for mapping QTL for one trait (ZENG 1994) can be readily extended for mapping QTL for multiple traits as follows:

$$\begin{aligned}
y_{j1} &= b_{01} + b_1^* x_j^* + d_1^* z_j^* + \sum_l^t (b_{l1}x_{jl} + d_{l1}z_{jl}) + e_{j1},\\
y_{j2} &= b_{02} + b_2^* x_j^* + d_2^* z_j^* + \sum_l^t (b_{l2}x_{jl} + d_{l2}z_{jl}) + e_{j2},\\
&\vdots \quad \vdots\\
y_{jm} &= b_{0m} + b_m^* x_j^* + d_m^* z_j^* + \sum_l^t (b_{lm}x_{jl} + d_{lm}z_{jl}) + e_{jm},\\
& \qquad j = 1, \ldots, n, \qquad (1)
\end{aligned}$$

where y_{jk} is the phenotypic value for trait k in individual j; b_{0k} is the mean effect of the model for trait k; b_k^* is the additive effect of the putative QTL on trait k; x_j^* counts the number of the allele at the putative QTL from one of the two parental lines, say parent P_1 [taking values of 2, 1 and 0 with probabilities depending on genotypes of the markers i and $i+1$ flanking the putative QTL and the recombination frequencies between the QTL and the markers (Table 1)]; d_k^* is the dominance effect of the putative QTL on trait k; z_j^* is an indicator variable of the heterozygosity at the QTL taking values 1 and 0 for heterozygote and homozygotes (Table 1); x_{jl} and z_{jl} are corresponding variables for marker l, assuming t markers are selected for controlling residual genetic variation; b_{lk} and d_{lk} are partial regression coefficients of y_{jk} on x_{jl} and z_{jl}; and e_{jk} is the residual effect on trait k for individual j.

It is assumed that the residual effects e_{jk}s (the error terms) are correlated among traits within individuals with covariance $\mathrm{Cov}(e_{jk}, e_{jl}) = \sigma_{kl} = \rho_{kl}\sigma_k\sigma_l$ but are independent among individuals. For likelihood analysis, it will be further assumed that e_{jk}s are multivariate normally distributed among individuals with means zero and covariance matrix

$$\mathbf{V} = \begin{pmatrix} \sigma_1^2 & \sigma_{12} & \cdots & \sigma_{1m} \\ \sigma_{21} & \sigma_2^2 & \cdots & \sigma_{2m} \\ \vdots & \vdots & \ddots & \vdots \\ \sigma_{m1} & \sigma_{m2} & \cdots & \sigma_m^2 \end{pmatrix}. \qquad (2)$$

In matrix notation, model (1) can be expressed as

$$\underset{n\times m}{\mathbf{Y}} = \underset{n\times 1}{\mathbf{x}^*}\,\underset{1\times m}{\mathbf{b}^*} + \underset{n\times 1}{\mathbf{z}^*}\,\underset{1\times m}{\mathbf{d}^*} + \underset{n\times(2t+1)}{\mathbf{X}}\,\underset{(2t+1)\times m}{\mathbf{B}} + \underset{n\times m}{\mathbf{E}}, \qquad (3)$$

where $\mathbf{Y}$ is a $(n \times m)$ matrix of y_{jk}; $\mathbf{x}^*$ is a $(n \times 1)$ column vector of x_j^*; $\mathbf{z}^*$ is a $(n \times 1)$ column vector of z_j^*; $\mathbf{b}^*$ is a $(1 \times m)$ row vector of b_k^*; $\mathbf{d}^*$ is a $(1 \times m)$ row vector of d_k^*; $\mathbf{X}$ is a $[n \times (2t+1)]$ matrix of data on t markers, x_{jl}s and z_{jl}s, fitted in the model as background control, including also the mean effect; $\mathbf{B}$ is a $[(2t+1) \times m]$ matrix of b_{lk}, d_{lk}, and b_{0k}; and $\mathbf{E}$ is a $(n \times m)$ matrix of e_{jk}.

The reasons to include multiple markers in the regression model for testing and estimating the putative QTL effects are mainly twofold. First, these markers can control and eliminate much of the background genetic variation from the residual variances and covariances of the model and thus can increase the statistical power for mapping QTL. Second, if some linked markers are also fitted in the model as background control, these markers can block the effects of other possibly linked QTL in the test and thus can reduce the chance of interference of possibly multiple linked QTL on hypothesis testing and parameter estimation. These properties of multiple regression analysis have been discussed in detail by ZENG (1993, 1994) in reference to QTL mapping for one trait and can be directly applied to QTL mapping on multiple traits.

In model (1), for simplicity it is assumed that t markers are each fitted with additive and dominance effects for genetic background control. Since many different markers can be fitted in the model, the question of selecting how many and what markers to be fitted in the model becomes a major issue for mapping QTL. A number of considerations have to be taken into account for such a selection process, and many relevant issues involved have been discussed in ZENG (1994). Here, we concentrate our discussion on the extension of the method to multiple trait analysis and avoid lengthy discussion on this issue.

Likelihood analysis: Given model (1), which is defined as a mixture model, and the assumption of multi-

variate normal distribution of error terms, the likelihood function of the data is defined as

$$L_1 = \prod_{j=1}^{n} [p_{2j} f_2(\mathbf{y}_j) + p_{1j} f_1(\mathbf{y}_j) + p_{0j} f_0(\mathbf{y}_j)], \quad (4)$$

where p_{2j}, p_{1j} and p_{0j} denote the prior probability of x_j^* taking values 2, 1 and 0, respectively, for the three genotypes of the putative QTL (Table 1), and $f_2(\mathbf{y}_j)$, $f_1(\mathbf{y}_j)$ and $f_0(\mathbf{y}_j)$ represent the multivariate normal density functions of the vector variable $\mathbf{y}_j$ (the jth row of $\mathbf{Y}$) with means $\mathbf{u}_{j2} = \mathbf{x}_j\mathbf{B} + 2\mathbf{b}^*$, $\mathbf{u}_{j1} = \mathbf{x}_j\mathbf{B} + \mathbf{b}^* + \mathbf{d}^*$, and $\mathbf{u}_{j0} = \mathbf{x}_j\mathbf{B}$, respectively, and the covariance matrix (2).

Then, by applying standard maximum likelihood procedures, the maximum likelihood estimates of parameters can be found as in the following. As in ZENG (1994), these maximum likelihood estimates can be computed by iteration through an ECM algorithm (MENG and RUBIN 1993) that is a special version of general EM algorithms. In the $(v+1)$th iteration, the *E*-step (*i.e.*, expectation step) calculates the posterior probabilities of individual j being a particular genotype at the putative QTL as

$$\begin{aligned}
q_{2j}^{(v+1)} &= p_{2j} f_2^{(v)}(\mathbf{y}_j) / [p_{2j} f_2^{(v)}(\mathbf{y}_j) \\
&\qquad + p_{1j} f_1^{(v)}(\mathbf{y}_j) + p_{0j} f_0^{(v)}(\mathbf{y}_j)], \\
q_{1j}^{(v+1)} &= p_{1j} f_1^{(v)}(\mathbf{y}_j) / [p_{2j} f_2^{(v)}(\mathbf{y}_j) \\
&\qquad + p_{1j} f_1^{(v)}(\mathbf{y}_j) + p_{0j} f_0^{(v)}(\mathbf{y}_j)], \\
q_{0j}^{(v+1)} &= p_{0j} f_0^{(v)}(\mathbf{y}_j) / [p_{2j} f_2^{(v)}(\mathbf{y}_j) \\
&\qquad + p_{1j} f_1^{(v)}(\mathbf{y}_j) + p_{0j} f_0^{(v)}(\mathbf{y}_j)], \quad (5)
\end{aligned}$$

where $f_2^{(v)}(\mathbf{y}_j)$, $f_1^{(v)}(\mathbf{y}_j)$ and $f_0^{(v)}(\mathbf{y}_j)$ are the corresponding normal density functions with parameters replaced by estimates in the vth iteration. In the *CM*-step (*i.e.*, conditional maximization step), parameters in $f_2(\mathbf{y}_j)$, $f_1(\mathbf{y}_j)$ and $f_0(\mathbf{y}_j)$ are divided into three groups, $(\mathbf{b}^*, \mathbf{d}^*)$, $\mathbf{B}$, $\mathbf{V}$, and estimated consecutively between groups but simultaneously within each group. These estimators can be shown to be as follows:

$$\mathbf{b}^{*(v+1)} = \mathbf{q}_2^{(v+1)\prime}(\mathbf{Y} - \mathbf{X}\mathbf{B}^{(v)}) / (2\mathbf{q}_2^{(v+1)\prime}\mathbf{1}), \quad (6)$$

$$\begin{aligned}
\mathbf{d}^{*(v+1)} &= [\mathbf{q}_1^{(v+1)\prime} / (\mathbf{q}_1^{(v+1)\prime}\mathbf{1}) \\
&\quad - \mathbf{q}_2^{(v+1)\prime} / (2\mathbf{q}_2^{(v+1)\prime}\mathbf{1})](\mathbf{Y} - \mathbf{X}\mathbf{B}^{(v)}), \quad (7)
\end{aligned}$$

$$\begin{aligned}
\mathbf{B}^{(v+1)} &= (\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'[\mathbf{Y} - (2\mathbf{q}_2^{(v+1)} \\
&\quad + \mathbf{q}_1^{(v+1)})\mathbf{b}^{*(v+1)} - \mathbf{q}_1^{(v+1)}\mathbf{d}^{*(v+1)}], \quad (8)
\end{aligned}$$

$$\begin{aligned}
\mathbf{V}^{(v+1)} &= [(\mathbf{Y} - \mathbf{X}\mathbf{B}^{(v+1)})'(\mathbf{Y} - \mathbf{X}\mathbf{B}^{(v+1)}) \\
&\quad - 4(\mathbf{q}_2^{(v+1)\prime}\mathbf{1})\mathbf{b}^{*(v+1)\prime}\mathbf{b}^{*(v+1)} \\
&\quad - (\mathbf{q}_1^{(v+1)\prime}\mathbf{1})(\mathbf{b}^{*(v+1)} + \mathbf{d}^{*(v+1)})' \\
&\qquad \times (\mathbf{b}^{*(v+1)} + \mathbf{d}^{*(v+1)})]/n, \quad (9)
\end{aligned}$$

where $\mathbf{q}_2^{(v+1)}$ and $\mathbf{q}_1^{(v+1)}$ are $(n \times 1)$ vectors of $q_{2j}^{(v+1)}$ and $q_{1j}^{(v+1)}$, and $\mathbf{1}$ is a column vector of ones. A prime represents the transpose of a matrix or a vector.

The calculation begins with $q_{2j}^{(0)} = p_{2j}$, $q_{1j}^{(0)} = p_{1j}$, $q_{0j}^{(0)} = p_{0j}$, and some starting values for $\mathbf{b}^{*(0)}$ and $\mathbf{d}^{*(0)}$ (one possible choice is to set them to zero). Iterations are then made between (5), (6), (7), (8), and (9) and terminated when a predetermined criterion is satisfied. The criterion for termination is set to be that the changes of the parameter estimates, or the increment of the log-likelihood value, at each iteration become less than ϵ (a small positive number, say, 10^{-8}). The final estimates are denoted as $\hat{\mathbf{b}}^*$, $\hat{\mathbf{d}}^*$, $\hat{\mathbf{B}}$ and $\hat{\mathbf{V}}$, which will then be used for the calculation of the maximum likelihood value for hypothesis testing.

The log-likelihood of (4) is calculated, with the parameters replaced by the estimates, as

$$\begin{aligned}
\ln(L_1) &= K - (n/2)\ln(|\hat{\mathbf{V}}|) \\
&\quad + \sum_{j=1}^{n} \ln\{p_{2j} \exp[-(1/2) \\
&\quad \times (\mathbf{y}_j - 2\hat{\mathbf{b}}^* - \mathbf{x}_j\hat{\mathbf{B}})\hat{\mathbf{V}}^{-1} \\
&\quad \times (\mathbf{y}_j - 2\hat{\mathbf{b}}^* - \mathbf{x}_j\hat{\mathbf{B}})'] + p_{1j} \exp[-(1/2) \\
&\quad \times (\mathbf{y}_j - \hat{\mathbf{b}}^* - \hat{\mathbf{d}}^* - \mathbf{x}_j\hat{\mathbf{B}})\hat{\mathbf{V}}^{-1} \\
&\quad \times (\mathbf{y}_j - \hat{\mathbf{b}}^* - \hat{\mathbf{d}}^* - \mathbf{x}_j\hat{\mathbf{B}})'] \\
&\quad + p_{0j} \exp[-(1/2)(\mathbf{y}_j - \mathbf{x}_j\hat{\mathbf{B}})\hat{\mathbf{V}}^{-1} \\
&\quad \times (\mathbf{y}_j - \mathbf{x}_j\hat{\mathbf{B}})']\} \\
&= K - (n/2)\ln(|\hat{\mathbf{V}}|) - (1/2) \\
&\quad \times \sum_{j=1}^{n} (\mathbf{y}_j - \mathbf{x}_j\hat{\mathbf{B}})\hat{\mathbf{V}}^{-1}(\mathbf{y}_j - \mathbf{x}_j\hat{\mathbf{B}})' \\
&\quad + \sum_{j=1}^{n} \ln\{p_{2j} \exp[2\hat{\mathbf{b}}^*\hat{\mathbf{V}}^{-1}(\mathbf{y}_j - \hat{\mathbf{b}}^* - \mathbf{x}_j\hat{\mathbf{B}})'] \\
&\quad + p_{1j} \exp[(\hat{\mathbf{b}}^* + \hat{\mathbf{d}}^*)\hat{\mathbf{V}}^{-1}(\mathbf{y}_j - \hat{\mathbf{b}}^*/2 \\
&\qquad - \hat{\mathbf{d}}^*/2 - \mathbf{x}_j\hat{\mathbf{B}})'] + p_{0j}\}, \quad (10)
\end{aligned}$$

where $|\hat{\mathbf{V}}|$ is the determinant of the covariance matrix, and $K = -nm\ln(2\pi)/2$.

Numerical procedures of the ECM algorithm: Here we explain how the ECM algorithm involving (5) – (9) can be implemented efficiently. Computationally, the major element of the analysis involves least squares analysis or, more specifically, the calculation of $\mathbf{Y} - \mathbf{X}\mathbf{B}^{(v+1)}$ in each step. Numerically, least squares analysis is usually performed through a *QR* factorization of matrix $\mathbf{X}$. Namely, for an $(n \times m)$ $\mathbf{X}$ matrix (assuming $n > m$), we can decompose $\mathbf{X}$ as

$$\mathbf{X} = (\mathbf{Q}_1 \quad \mathbf{Q}_2)\begin{pmatrix}\mathbf{R}\\ \mathbf{0}\end{pmatrix} = \mathbf{Q}_1\mathbf{R}, \quad (11)$$

where $\mathbf{Q} = (\mathbf{Q}_1, \mathbf{Q}_2)$ is an $(n \times n)$ orthogonal matrix (*i.e.*, $\mathbf{Q}'\mathbf{Q} = \mathbf{I}$) and is partitioned into two parts, $\mathbf{Q}_1$ $(n \times m)$ and $\mathbf{Q}_2$ $(n \times (n-m))$, and $\mathbf{R}$ is an $(m \times m)$ upper triangular matrix. With this factorization, the

usual least squares estimates $\mathbf{B}$ and $\mathbf{Y} - \mathbf{XB}$ (residuals) can be calculated as

$$\mathbf{B} = (\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{Y} = (\mathbf{R}'\mathbf{Q}_1'\mathbf{Q}_1\mathbf{R})^{-1}\mathbf{R}'\mathbf{Q}_1'\mathbf{Y} = \mathbf{R}^{-1}\mathbf{Q}_1'\mathbf{Y}, \quad (12)$$

$$\mathbf{Y} - \mathbf{XB} = \mathbf{Y} - \mathbf{Q}_1\mathbf{R}\mathbf{R}^{-1}\mathbf{Q}_1'\mathbf{Y} = (\mathbf{I} - \mathbf{Q}_1\mathbf{Q}_1')\mathbf{Y}. \quad (13)$$

In our numerical implementation, before entering the ECM loop, $\mathbf{X}$ is first factorized into $\mathbf{Q}$ and $\mathbf{R}$ by Householder transformations using the LINPACK routines (DONGARRA *et al.* 1979). In each loop, $\mathbf{Y} - \mathbf{XB}$ is obtained by first calculating

$$\mathbf{W} = [\mathbf{Y} - (2\mathbf{q}_2 + \mathbf{q}_1)\mathbf{b}^* - \mathbf{q}_1\mathbf{d}^*] - \mathbf{XB} = \tilde{\mathbf{Y}} - \mathbf{XB} = (\mathbf{I} - \mathbf{Q}_1\mathbf{Q}_1')\tilde{\mathbf{Y}} \quad (14)$$

from (13) and then recovering from $\mathbf{W}$

$$\mathbf{Y} - \mathbf{XB} = \mathbf{W} + (2\mathbf{q}_2 + \mathbf{q}_1)\mathbf{b}^* + \mathbf{q}_1\mathbf{d}^*. \quad (15)$$

This is used for calculation in (5), (6), (7), and (9). Equation 8 becomes redundant and is omitted in calculation. These numerical procedures are the same procedures used by ZENG (1994).

Since $\mathbf{X}$ is unchanged in each loop, $\mathbf{Q}$ is unchanged. The above calculations become very simple updates in each loop. This illustrates the numerical advantage of the ECM algorithm, compared with the full EM algorithm (*e.g.*, JANSEN 1992) that groups $\mathbf{x}^*$ and $\mathbf{z}^*$ (represented by $\mathbf{qs}$) into $\mathbf{X}$ and factorizes or updates factorization of $\mathbf{X}$ in each loop to obtain $\mathbf{B}$ (which includes $\mathbf{b}^*$ and $\mathbf{d}^*$) and $\mathbf{V}$. The convergence of the ECM algorithm to maximum likelihood estimates has been proven by MENG and RUBIN (1993).

HYPOTHESIS TESTS OF QTL EFFECTS

In hypothesis testing, model (1) is usually called the full model. With some parameter values constrained to some specific values, a number of null hypotheses can be constructed and tested. For mapping QTL, we are mostly concerned with testing hypotheses about the additive and dominance effects of QTL. However, before we test other hypotheses, we need first to test for the presence of QTL. Without losing generality, we restrict our discussion to two traits.

Joint mapping for QTL on two traits: With phenotypic observations on two traits, mapping for QTL can be performed for each trait individually or jointly on both traits. Under the joint mapping, the hypotheses to be tested are

$$H_0: b_1^* = 0, \quad d_1^* = 0, \quad b_2^* = 0, \quad d_2^* = 0,$$
$$H_1: \text{At least one of them is not zero.} \quad (16)$$

The log-likelihood under H_0 is then

$$\ln(L_0) = \ln\left[\prod_{j=1}^{n} f_0(\mathbf{y}_j)\right] = K - (n/2)\ln(|\hat{\mathbf{V}}_0|) - nm/2, \quad (17)$$

where $\hat{\mathbf{V}}_0 = (\mathbf{Y} - \mathbf{X}\hat{\mathbf{B}}_0)'(\mathbf{Y} - \mathbf{X}\hat{\mathbf{B}}_0)/n$ and $\hat{\mathbf{B}}_0 = (\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{Y}$. The test is performed with a likelihood ratio statistic

$$LR_1 = -2\ln(L_0/L_1). \quad (18)$$

Under H_0, the likelihood ratio LR_1 will be approximately chi-square distributed. The determination of the critical value for the test is, however, very complicated. The complication is largely due to the fact that the test is usually performed for the whole genome, a situation of multiple tests. With multiple tests, the critical level for each test has to be adjusted. It has been shown (ZENG 1994) that, for the composite interval mapping, tests in different intervals are close to being independent except for those in adjacent intervals that are slightly correlated. Thus, if we choose α' as the genome-wise error rate, the error rate of the test per interval, α, can be approximated by using the Bonferroni correction as $\alpha = 1 - (1 - \alpha')^{1/M}$, or to a good approximation as $\alpha = \alpha'/M$, where M is the number of intervals involved in the test. The maximum of the test statistic within an interval can be roughly approximated by a chi-square distribution with a degree of freedom $2m + 1$ (the number of parameters under the test including one for the position of the putative QTL) [see the simulation study of ZENG (1994) for $m = 1$ and a backcross design]. Thus, in practice, we may use $\chi^2_{\alpha/M,2m+1}$ to approximate the critical value of the test in this situation (for two traits $m = 2$). However, we emphasize that the correct determination of appropriate critical value for this test is a very complicated statistical issue. The above recommendation is suggested only for a very rough approximation. Recently, CHURCHILL and DOERGE (1994) proposed to use a permutation test to empirically estimate the genome-wise critical value for a given data set and a given test. Their method can be extended for multiple trait analysis.

Why do we need to perform joint mapping? First, the joint analysis on two traits can provide formal procedures for testing a number of biologically interesting hypotheses, such as pleiotropic effects of QTL, QTL by environment interaction and pleiotropy *vs.* close linkage, as shown below. Second, if the putative QTL has pleiotropic effects on both traits, the joint mapping on both traits may perform better than mapping on each trait separately.

In APPENDIX A, the relative advantages of the joint analysis as compared to separate analyses are discussed. It is shown that the test statistic for the joint analysis is always higher than that for each separate analysis. However, this does not necessarily mean that the power of the test will be increased by using more traits, because more parameters will be included in the model for test-

ing. When the residual correlation $\rho = 0$, the test statistic under the joint test is approximately the sum of those under separate tests. Otherwise the joint test statistic can be smaller or larger than the sum of separate test statistics, depending on the sign and magnitude of the residual correlation and the differences between QTL effects. The power of the joint test can increase significantly if the relevant QTL has pleiotropic effects on two traits with the product of the effects in different direction to the residual correlation. If the product of pleiotropic effects and the residual correlation are in the same direction, however, the test statistic under the joint test will be smaller than the sum of the test statistics under the separate tests. In this case, the increase of the test statistic for the joint test will be less significant and the power of the joint test may be less than the higher one of the separate tests due to the additional parameters fitted in the model. However, it is generally found that joint mapping is more informative than separate mappings for traits moderately or highly correlated (see simulation studies below).

Testing pleiotropic effects: Given a genome position or region where the presence of a QTL is indicated by joint mapping, statistical tests can proceed to test whether the QTL has pleiotropic effects on both traits. On the assumption that there is only one QTL in the relevant region, which has effects on either one or both of two traits, hypotheses can be formulated as follows:

$$H_{10}: b_1^* = 0,\ d_1^* = 0,\ b_2^* \neq 0,\ d_2^* \neq 0$$

given a position for a QTL,

$$H_{11}: b_1^* \neq 0,\ d_1^* \neq 0,\ b_2^* \neq 0,\ d_2^* \neq 0$$

at the position given by H_{10}; (19)

and

$$H_{20}: b_1^* \neq 0,\ d_1^* \neq 0,\ b_2^* = 0,\ d_2^* = 0$$

at the position given by H_{10},

$$H_{21}: b_1^* \neq 0,\ d_1^* \neq 0,\ b_2^* \neq 0,\ d_2^* \neq 0$$

at the position given by H_{10}. (20)

A test for pleiotropic effects is then equivalent to the test of both (19) and (20). Only rejecting both the null hypotheses (*i.e.*, H_{10} and H_{20}) will suggest the presence of pleiotropic effects.

Although each of (19) and (20) shows restriction only on one trait, the tests will not be the same as for each trait separately since two traits are correlated. As shown in APPENDIX A, when the two traits are correlated, this test will have more power than separate analyses. The estimates of model parameters under H_{10} and H_{20} can be obtained as in joint mapping of (5) – (9) except that some estimates in (6) and (7) are set to zero. The likelihood ratio test statistics for (19) and (20) can then be calculated correspondingly from (10) in a ratio of the likelihoods with and without constraints. Fixing a testing position or region by the joint mapping for this test is consistent with the pleiotropic hypothesis. Since the testing position is fixed, the likelihood ratio test statistics under the null hypotheses of (19) and (20) will each be asymptotically chi-square distributed with two degrees of freedom.

Testing pleiotropic effects against close linkage: Although rejecting both H_{10} of (19) and H_{20} of (20) is consistent with the hypothesis of pleiotropic effects of a QTL, the test itself does not distinguish whether the significant effect is due to one QTL having pleiotropic effects on both traits, or possibly two (or more) closely linked QTL each having a predominant effect on one trait only. Two closely linked QTL each with an effect on only one trait may behave like one pleiotropic QTL in joint mapping. Also one pleiotropic QTL may be estimated as two QTL at two nearby but different positions if each trait is analyzed separately. Thus in mapping for QTL, for some regions there may exist sufficient interests to distinguish these two possibilities. Undoubtedly, distinguishing these two cases has important implications in genetics and breeding.

Clearly, this test of pleiotropy *vs.* close linkage is for some specific genome regions only. The regions to be tested are first determined by joint mapping. Only those genome regions that are significant under joint mapping may be suitable for this test. Relatively loosely linked pleiotropic or nonpleiotropic QTL (*i.e.*, separated by several relatively large, say 10 cM, marker intervals) may be detected by joint mapping and may not be necessary to this test to distinguish them, although this test can be applied to those situations.

To test the hypotheses of pleiotropy *vs.* close linkage at some significant regions, the likelihood analysis has to be reformulated. Let two QTL, each with an effect on one trait only, have positions symbolically specified by $p(1)$ for the QTL having an effect on trait 1 and $p(2)$ for the QTL having an effect on trait 2 (if the two positions are in the same marker interval, $p(1)$ and $p(2)$ are then defined as the ratios of the recombination frequencies between a marker and the two positions, respectively, and between the two flanking markers). The hypotheses can then be formulated as

$$H_0: p(1) = p(2),$$
$$H_1: p(1) \neq p(2). \quad (21)$$

The H_1 here is a special case of many possible alternatives. A more general alternative may be that both QTL have pleiotropic effects. This alternative is, however, the hypothesis for mapping two closely linked pleiotropic QTL. Although this hypothesis can be tested, we confine our attention here to the alternative of two nonpleiotropic QTL.

The log-likelihood of H_0 in (21) is given by (10). The statistical model for H_1 in (21) is, however, given by

$$y_{j1} = b_{01} + b_1^* x_{1j}^* + d_1^* z_{1j}^* + \sum_{l}^{t} (b_{l1} x_{jl} + d_{l1} z_{jl}) + e_{j1},$$

$$y_{j2} = b_{02} + b_2^* x_{2j}^* + d_2^* z_{2j}^* + \sum_{l}^{t} (b_{l2} x_{jl} + d_{l2} z_{jl}) + e_{j2}. \quad (22)$$

Model (22) should be the same as model (1) for $m = 2$, except that (x_{1j}^*, z_{1j}^*) and (x_{2j}^*, z_{2j}^*) are now defined for two QTL at two different positions in one or some nearby marker intervals. Note that when the test and search cover several marker intervals, the markers inside the search region should not be used for background control, otherwise the models under the null and alternative hypotheses can be inconsistent on the markers used for background control.

Model (22) is actually a mixture model with nine components since recombination can result in nine possible QTL genotypes in an F_2 population for two QTL. Let $p_{i_1 i_2 j}$ (i_1, i_2 = 0, 1 and 2) be the probability of individual j having genotype i_1 for a putative QTL affecting trait 1 and i_2 for another QTL affecting trait 2 for given two testing positions for two QTL. The likelihood function is given by

$$L_2 = \prod_{j=1}^{n} \sum_{i_1=0}^{2} \sum_{i_2=0}^{2} p_{i_1 i_2 j} f_{i_1 i_2}(\mathbf{y}_j), \quad (23)$$

where $f_{i_1 i_2}(\mathbf{y}_j)$ is a bivariate normal density function for $\mathbf{y}_j$ with a mean vector

$$\mathbf{u}'_{i_1 i_2 j} = \begin{pmatrix} \mathbf{x}_j \mathbf{b}_1 + i_1 b_1^* + \delta(i_1) d_1^* \\ \mathbf{x}_j \mathbf{b}_2 + i_2 b_2^* + \delta(i_2) d_2^* \end{pmatrix}$$

and covariance matrix (2), where the indicator function $\delta(i_1) = 1$ if $i_1 = 1$ and 0 otherwise.

The probability $p_{i_1 i_2 j}$ can be inferred from the observed genotypes of the flanking markers. If the two putative QTL are tested in different marker intervals, the probability of QTL genotype can be calculated independently for each QTL from Table 1, *i.e.*, $p_{i_1 i_2 j} = p_{i_1 j} p_{i_2 j}$, assuming that there is no crossing-over interference. If the two putative QTL are tested in the same marker interval, $p_{i_1 i_2 j}$ can be calculated from Table 2.

The *E*-step in this case is to calculate the posterior probabilities of individual j having genotype i_1 for QTL 1 affecting trait 1 at position $p(1)$ and i_2 for QTL 2 affecting trait 2 at position $p(2)$,

$$q_{i_1 i_2 j}^{(v+1)} = p_{i_1 i_2 j} \hat{f}_{i_1 i_2}^{(v)}(\mathbf{y}_j) \Big/ \sum_{k_1=0}^{2} \sum_{k_2=0}^{2} p_{k_1 k_2 j} \hat{f}_{k_1 k_2}^{(v)}(\mathbf{y}_j)$$

$$\text{for} \quad i_1, i_2 = 0, 1, 2. \quad (24)$$

The *CM*-step is to calculate

$$b_1^{*(v+1)} = \{\mathbf{q}_{2\cdot}^{(v+1)\prime} [(\mathbf{y}_1 - \mathbf{X}\mathbf{b}_1^{(v)}) - (\rho^{(v)} \sigma_1^{(v)} / \sigma_2^{(v)}) \times (\mathbf{y}_2 - \mathbf{X}\mathbf{b}_2^{(v)})] + (\rho^{(v)} \sigma_1^{(v)} / \sigma_2^{(v)}) \times [(2\mathbf{q}_{22}^{(v+1)} + \mathbf{q}_{21}^{(v+1)})' \mathbf{1} b_2^{*(v)} + \mathbf{q}_{21}^{(v+1)\prime} \mathbf{1} d_2^{*(v)}]\} / (2\mathbf{q}_{2\cdot}^{(v+1)\prime} \mathbf{1}), \quad (25)$$

$$d_1^{*(v+1)} = \{\mathbf{q}_{1\cdot}^{(v+1)\prime} [(\mathbf{y}_1 - \mathbf{X}\mathbf{b}_1^{(v)}) - (\rho^{(v)} \sigma_1^{(v)} / \sigma_2^{(v)}) (\mathbf{y}_2 - \mathbf{X}\mathbf{b}_2^{(v)})] + (\rho^{(v)} \sigma_1^{(v)} / \sigma_2^{(v)}) [(2\mathbf{q}_{12}^{(v+1)} + \mathbf{q}_{11}^{(v+1)})' \mathbf{1} b_2^{*(v)} + \mathbf{q}_{11}^{(v+1)\prime} \mathbf{1} d_2^{*(v)}]\} / (\mathbf{q}_{1\cdot}^{(v+1)\prime} \mathbf{1}) - b_1^{*(v+1)}, \quad (26)$$

$$b_2^{*(v+1)} = \{\mathbf{q}_{\cdot 2}^{(v+1)\prime} [(\mathbf{y}_2 - \mathbf{X}\mathbf{b}_2^{(v)}) - (\rho^{(v)} \sigma_2^{(v)} / \sigma_1^{(v)}) (\mathbf{y}_1 - \mathbf{X}\mathbf{b}_1^{(v)})] + (\rho^{(v)} \sigma_2^{(v)} / \sigma_1^{(v)}) [(2\mathbf{q}_{22}^{(v+1)} + \mathbf{q}_{12}^{(v+1)})' \mathbf{1} b_1^{*(v+1)} + \mathbf{q}_{12}^{(v+1)\prime} \mathbf{1} d_1^{*(v+1)}]\} / (2\mathbf{q}_{\cdot 2}^{(v+1)\prime} \mathbf{1}), \quad (27)$$

$$d_2^{*(v+1)} = \{\mathbf{q}_{\cdot 1}^{(v+1)\prime} [(\mathbf{y}_2 - \mathbf{X}\mathbf{b}_2^{(v)}) - (\rho^{(v)} \sigma_2^{(v)} / \sigma_1^{(v)}) (\mathbf{y}_1 - \mathbf{X}\mathbf{b}_1^{(v)})] + (\rho^{(v)} \sigma_2^{(v)} / \sigma_1^{(v)}) [(2\mathbf{q}_{21}^{(v+1)} + \mathbf{q}_{11}^{(v+1)})' \mathbf{1} b_1^{*(v+1)} + \mathbf{q}_{11}^{(v+1)\prime} \mathbf{1} d_1^{*(v+1)}]\} / (\mathbf{q}_{\cdot 1}^{(v+1)\prime} \mathbf{1}) - b_2^{*(v+1)}, \quad (28)$$

$$\mathbf{B}^{(v+1)} = (\mathbf{X}'\mathbf{X})^{-1} \mathbf{X}' \mathbf{W}^{(v+1)}, \quad (29)$$

$$\mathbf{V}^{(v+1)} = (\mathbf{W}^{(v+1)} - \mathbf{X}\mathbf{B}^{(v+1)})' \times (\mathbf{W}^{(v+1)} - \mathbf{X}\mathbf{B}^{(v+1)}) / n, \quad (30)$$

where

$$\mathbf{B}^{(v+1)} = (\mathbf{b}_1^{(v+1)} \mathbf{b}_2^{(v+1)}),$$

$$\mathbf{W}^{(v+1)\prime} = \begin{pmatrix} \mathbf{y}_1' - (2\mathbf{q}_{2\cdot}^{(v+1)} + \mathbf{q}_{1\cdot}^{(v+1)})' b_1^{*(v+1)} - \mathbf{q}_{1\cdot}^{(v+1)\prime} d_1^{*(v+1)} \\ \mathbf{y}_2' - (2\mathbf{q}_{\cdot 2}^{(v+1)} + \mathbf{q}_{\cdot 1}^{(v+1)})' b_2^{*(v+1)} - \mathbf{q}_{\cdot 1}^{(v+1)\prime} d_2^{*(v+1)} \end{pmatrix},$$

$$\mathbf{q}_{2\cdot}^{(v+1)} = \mathbf{q}_{22}^{(v+1)} + \mathbf{q}_{21}^{(v+1)} + \mathbf{q}_{20}^{(v+1)},$$

$$\mathbf{q}_{1\cdot}^{(v+1)} = \mathbf{q}_{12}^{(v+1)} + \mathbf{q}_{11}^{(v+1)} + \mathbf{q}_{10}^{(v+1)},$$

$$\mathbf{q}_{\cdot 2}^{(v+1)} = \mathbf{q}_{22}^{(v+1)} + \mathbf{q}_{12}^{(v+1)} + \mathbf{q}_{02}^{(v+1)},$$

$$\mathbf{q}_{\cdot 1}^{(v+1)} = \mathbf{q}_{21}^{(v+1)} + \mathbf{q}_{11}^{(v+1)} + \mathbf{q}_{01}^{(v+1)}.$$

After the convergence of the ECM algorithm, the log-likelihood value of (23) will be calculated from

TABLE 2

Probability of QTL genotype given flanking marker genotype for two QTL within a marker interval

Marker genotype	QTL genotype								
	$Q_1Q_1Q_2Q_2$ (22)	$Q_1Q_1Q_2q_2$ (21)	$Q_1Q_1q_2q_2$ (20)	$Q_1q_1Q_2Q_2$ (12)	$Q_1q_1Q_2q_2$ (11)	$Q_1q_1q_2q_2$ (10)	$q_1q_1Q_2Q_2$ (02)	$q_1q_1Q_2q_2$ (01)	$q_1q_1q_2q_2$ (00)
$M_1M_1M_2M_2$	1	0	0	0	0	0	0	0	0
$M_1M_1M_2m_2$	p_3	p_2	0	0	p_1	0	0	0	0
$M_1M_1m_2m_2$	p_3^2	$2p_2p_3$	p_2^2	0	$2p_1p_3$	$2p_1p_2$	0	0	p_1^2
$M_1m_1M_2M_2$	p_1	0	0	p_2	p_3	0	0	0	0
$M_1m_1M_2m_2$	δp_1p_3	δp_1p_2	0	δp_2p_3	$\delta(p_1^2 + p_2^2 + p_3^2) + (1 - \delta)$	δp_2p_3	0	δp_1p_2	δp_1p_3
$M_1m_1m_2m_2$	0	0	0	0	p_3	p_2	0	0	p_1
$m_1m_1M_2M_2$	p_1^2	$2p_1p_2$	0	$2p_2p_3$	$2p_1p_3$	0	p_2^2	0	p_3^2
$m_1m_1M_2m_2$	0	0	0	0	p_1	0	0	p_2	p_3
$m_1m_1m_2m_2$	0	0	0	0	0	0	0	0	1

It is assumed that the order of markers and QTL are $M_1Q_1Q_2M_2$, and the recombination frequencies between M_1Q_1, Q_1Q_2, Q_2M_1, M_1M_2 are r_1, r_2, r_3, r, respectively. Double recombination is ignored. $p_1 = r_1/r$, $p_2 = r_2/r$, $p_3 = r_3/r$, and $\delta = r^2/[(1 - r)^2 + r^2]$.

$$\ln(L_2) = K - (n/2)\ln(|\hat{\mathbf{V}}|) + \sum_{j=1}^{n} \ln\left\{ \sum_{i_1=0}^{2} \sum_{i_2=0}^{2} p_{i_1 i_2 j} \exp[-(1/2) \times (\mathbf{y}_j - \hat{\mathbf{u}}_{i_1 i_2 j})\hat{\mathbf{V}}^{-1}(\mathbf{y}_j - \hat{\mathbf{u}}_{i_1 i_2 j})'] \right\}. \quad (31)$$

In theory, the search can be made in the two-dimensional space of possible locations of the two putative nonpleiotropic QTL in the testing region. The test statistic is then given by the likelihood ratio

$$LR_2 = -2\ln(L_{20}/L_2), \quad (32)$$

where L_2 is the maximum of the likelihoods in the two-dimensional space, and L_{20} is the maximum of the likelihoods on the diagonal of the two-dimensional space that corresponds to the null hypothesis of $p(1) = p(2)$. In practice, however, the search in the two-dimensional space is unnecessary. It is expected that the likelihood under the alternative hypothesis is maximized in the region near the peak indicated by the separate mappings, whereas the maximum likelihood under the null hypothesis corresponds to the joint mapping under the same model. So instead of searching in the two-dimensional space, the search can be safely concentrated in the areas suggested by the joint and separate mappings. As the hypotheses in (21) are nested hypotheses, the test statistic under H_0 will be asymptotically chi-square distributed with 1 degree of freedom. The performance of this test will be investigated by simulations below.

QTL by environment interaction: Genes expressed in different environments can show different effects. This differential expression is usually called genotype by environment interaction. There are generally two types of experimental designs used in practice to study QTL × environment interaction (PATERSON *et al.* 1991; STUBER *et al.* 1992). In one design, the same set of genotypes recorded on markers is evaluated phenotypically in different environments, which may be called paired comparison or design I. In the other design, different random sets of genotypes (or individuals) from a common population are evaluated phenotypically in different environments, which may be called group comparison or design II.

In design I, since the same set of genotypes recorded on markers are evaluated phenotypically on multiple environments, the same **X** (marker data) matrix is paired to multiple phenotypic vectors in **Y**, and the statistical model for analysis is just the same as model (1). Essentially, we regard different expressions of the same trait in different environments as different traits or different trait states, a concept originally introduced by FALCONER (1952). However, by testing the QTL × environment interaction, we test the hypotheses

$$H_0: b_1^* = b_2^* = b^*, \quad d_1^* = d_2^* = d^*,$$
$$H_1: b_1^* \neq b_2^*, \quad d_1^* \neq d_2^*. \quad (33)$$

This test, of course, is performed only at the chromosome regions where QTL have been suggested by the joint mapping [*i.e.*, the null hypothesis H_0 of (16) has been rejected]. Under H_1 of (33), the model is the full model of (1).

Under H_0, the *E*-step will be similar to the full model except that b^* is substituted for b_1^* and b_2^* and d^* for d_1^* and d_2^*. In the *CM*-step,

$$b^{*(v+1)} = \mathbf{q}_2^{(v+1)\prime}(\mathbf{Y} - \mathbf{XB}^{(v)}) \times (\mathbf{V}^{(v)})^{-1}\mathbf{1}/[2c^{(v)}\mathbf{q}_2^{(v+1)\prime}\mathbf{1}], \quad (34)$$

$$d^{*(v+1)} = [\mathbf{q}_1^{(v+1)\prime}/(c^{(v)}\mathbf{q}_1^{(v+1)\prime}\mathbf{1}) - \mathbf{q}_2^{(v+1)}/(2c^{(v)}\mathbf{q}_2^{(v+1)\prime}\mathbf{1})](\mathbf{Y} - \mathbf{XB}^{(v)})(\mathbf{V}^{(v)})^{-1}\mathbf{1}, \quad (35)$$

with $c^{(v)} = \mathbf{1}'(\mathbf{V}^{(v)})^{-1}\mathbf{1} = (\sigma_1^{2(v)} - 2\rho^{(v)}\sigma_1^{(v)}\sigma_2^{(v)} +$

$\sigma_2^{2(v)})/[\sigma_1^{2(v)}\sigma_2^{2(v)}(1-\rho^{2(v)})]$. Here, $b^{*(v+1)}$ and $d^{*(v+1)}$ are just the weighted averages of (6) and (7), respectively, weighted by the residual variance-covariance matrix $\mathbf{V}^{(v)}$. $\mathbf{B}^{(v+1)}$ and $\mathbf{V}^{(v+1)}$ are given by (8) and (9) with again b^* substituted for b_1^* and b_2^* and d^* for d_1^* and d_2^*. The log-likelihood is calculated from

$$\begin{aligned}\ln(L_3) = K &- (n/2)\ln(|\hat{\mathbf{V}}|) \\ &- (1/2)\sum_{j=1}^{n}(\mathbf{y}_j - \mathbf{x}_j\hat{\mathbf{B}})\hat{\mathbf{V}}^{-1}(\mathbf{y}_j - \mathbf{x}_j\hat{\mathbf{B}})' \\ &+ \sum_{j=1}^{n}\ln\{p_{2j}\exp[2\hat{b}^*\mathbf{1}'\hat{\mathbf{V}}^{-1}(\mathbf{y}_j - \mathbf{1}'\hat{b}^* \\ &- \mathbf{x}_j\hat{\mathbf{B}})'] + p_{1j}\exp[(\hat{b}^* + \hat{d}^*)\mathbf{1}'\hat{\mathbf{V}}^{-1} \\ &\times (\mathbf{y}_j - \mathbf{1}'\hat{b}^*/2 - \mathbf{1}'\hat{d}^*/2 \\ &- \mathbf{x}_j\hat{\mathbf{B}})'] + p_{0j}\}. \quad (36)\end{aligned}$$

The test is performed by a likelihood ratio

$$LR_3 = -2\ln(L_3/L_1) \quad (37)$$

that is asymptotically chi-square distributed with 2 degrees of freedom under the null hypothesis.

In design II, the statistical model for analysis can be specified as

$$\begin{aligned}y_{1j} &= x_{1j}^* b_1^* + z_{1j}^* d_1^* + \mathbf{x}_{1j}\mathbf{b}_1 + e_{1j} \quad j = 1, 2, \ldots, n_1, \\ y_{2j} &= x_{2j}^* b_2^* + z_{2j}^* d_2^* + \mathbf{x}_{2j}\mathbf{b}_2 + e_{2j} \quad j = 1, 2, \ldots, n_2. \quad (38)\end{aligned}$$

This can be expressed in matrix notation as

$$\begin{aligned}\mathbf{y}_1 &= \mathbf{x}_1^* b_1^* + \mathbf{z}_1^* d_1^* + \mathbf{X}_1\mathbf{b}_1 + \mathbf{e}_1, \\ \mathbf{y}_2 &= \mathbf{x}_2^* b_2^* + \mathbf{z}_2^* d_2^* + \mathbf{X}_2\mathbf{b}_2 + \mathbf{e}_2. \quad (39)\end{aligned}$$

We will assume that e_{1j} and e_{2j} are independently normally distributed with means zero and variances σ_1^2 and σ_2^2, respectively. Under H_1 of (33), since e_{1j} and e_{2j} are independent, parameters in each environment can be estimated separately, and in each environment the analysis is on one trait. The log-likelihood is then just the sum of the log-likelihoods in each environment and can be expressed as

$$\begin{aligned}\ln(L_4) &= \sum_{j=1}^{n_1}\ln\left[\sum_{i=0}^{2}p_{1ij}f_i(y_{1j})\right] + \sum_{j=1}^{n_2}\ln\left[\sum_{i=0}^{2}p_{2ij}f_i(y_{2j})\right] \\ &= \ln(L_1)_1 + \ln(L_1)_2, \quad (40)\end{aligned}$$

where $\ln(L_1)_1$ and $\ln(L_1)_2$ are the log-likelihoods of (10) (with $m = 1$) in the first and second environments, respectively.

Under H_0 of (33), the parameters have to be estimated jointly. In each ECM iteration, the *E*-step constitutes

$$q_{kij}^{(v+1)} = p_{kij}f_i^{(v)}(y_{kj})/\sum_{i=0}^{2}(p_{kij}f_i^{(v)}(y_{kj})) \quad (41)$$

for the kth environment, the ith genotype and the jth individual. The *CM*-step constitutes

$$\begin{aligned}b^{*(v+1)} = &[\mathbf{q}_{12}^{(v+1)\prime}(\mathbf{y}_1 - \mathbf{X}_1\mathbf{b}_1^{(v)})/\sigma_1^{2(v)} \\ &+ \mathbf{q}_{22}^{(v+1)\prime}(\mathbf{y}_2 - \mathbf{X}_2\mathbf{b}_2^{(v)})/\sigma_2^{2(v)}]/ \\ &[2(\mathbf{q}_{12}^{(v+1)\prime}\mathbf{1}/\sigma_1^{2(v)} \\ &+ \mathbf{q}_{22}^{(v+1)\prime}\mathbf{1}/\sigma_2^{2(v)})], \quad (42)\end{aligned}$$

$$\begin{aligned}d^{*(v+1)} = &[\mathbf{q}_{11}^{(v+1)\prime}(\mathbf{y}_1 - \mathbf{X}_1\mathbf{b}_1^{(v)})/\sigma_1^{2(v)} \\ &+ \mathbf{q}_{21}^{(v+1)\prime}(\mathbf{y}_2 - \mathbf{X}_2\mathbf{b}_2^{(v)})/\sigma_2^{2(v)}]/ \\ &[\mathbf{q}_{11}^{(v+1)\prime}\mathbf{1}/\sigma_1^{2(v)} + \mathbf{q}_{21}^{(v+1)\prime}\mathbf{1}/\sigma_2^{2(v)}] \\ &- b^{*(v+1)}, \quad (43)\end{aligned}$$

$$\begin{aligned}\mathbf{b}_1^{(v+1)} = &(\mathbf{X}_1'\mathbf{X}_1)^{-1}\mathbf{X}_1'[\mathbf{y}_1 - (2\mathbf{q}_{12}^{(v+1)} \\ &+ \mathbf{q}_{11}^{(v+1)})b^{*(v+1)} - \mathbf{q}_{11}^{(v+1)}d^{*(v+1)}], \quad (44)\end{aligned}$$

$$\begin{aligned}\mathbf{b}_2^{(v+1)} = &(\mathbf{X}_2'\mathbf{X}_2)^{-1}\mathbf{X}_2'[\mathbf{y}_2 - (2\mathbf{q}_{22}^{(v+1)} \\ &+ \mathbf{q}_{21}^{(v+1)})b^{*(v+1)} - \mathbf{q}_{21}^{(v+1)}d^{*(v+1)}], \quad (45)\end{aligned}$$

$$\begin{aligned}\sigma_1^{2(v+1)} = &[(\mathbf{y}_1 - \mathbf{X}_1\mathbf{b}_1^{(v+1)})'(\mathbf{y}_1 - \mathbf{X}_1\mathbf{b}_1^{(v+1)}) \\ &- 4\mathbf{q}_{12}^{(v+1)\prime}\mathbf{1}b^{*2(v+1)} \\ &- \mathbf{q}_{11}^{(v+1)\prime}\mathbf{1}(b^{*(v+1)} + d^{*(v+1)})^2]/n_1, \quad (46)\end{aligned}$$

$$\begin{aligned}\sigma_2^{2(v+1)} = &[(\mathbf{y}_2 - \mathbf{X}_2\mathbf{b}_2^{(v+1)})'(\mathbf{y}_2 - \mathbf{X}_2\mathbf{b}_2^{(v+1)}) \\ &- 4\mathbf{q}_{22}^{(v+1)\prime}\mathbf{1}b^{*2(v+1)} \\ &- \mathbf{q}_{21}^{(v+1)\prime}\mathbf{1}(b^{*(v+1)} + d^{*(v+1)})^2]/n_2. \quad (47)\end{aligned}$$

The log-likelihood, $\ln(L_5)$, under H_0 will be similar to (40) in form with the constraint that $\hat{b}_1^* = \hat{b}_2^* = \hat{b}^*$ and $\hat{d}_1^* = \hat{d}_2^* = \hat{d}^*$. The likelihood ratio test statistic is given by

$$LR_4 = -2\ln(L_5/L_4), \quad (48)$$

which is asymptotically chi-square distributed with two degrees of freedom under the null hypothesis.

It is interesting to compare the relative efficiency of the two experimental designs on mapping QTL and testing QTL × environment interaction. When $n_1 = n_2 = n$ and n is large, the test statistic under design II can be treated approximately as a special case of design I with $\rho = 0$. With this assumption, it is shown in APPENDIX B that, with the same sample size for phenotyping in the two designs (the sample size of marker genotyping is doubled in design II), design II is likely to have more statistical power for mapping QTL, whereas design I is likely to have more statistical power for testing QTL × environment interaction.

In this analysis, we treat the QTL × environment effects as fixed effects. This may be appropriate for those environments that are distinctively different, and the inference is applied to those environments. For many experiment designs, the QTL × environment ef-

TABLE 3

Parameters and estimates of QTL positions and effects in the simulations

QTL	Position (cM)	Additive effect			Dominance effect		
		Trait 1	Trait 2	Trait 3	Trait 1	Trait 2	Trait 3
				Parameters			
1	21.0	1.00	1.00	0.30	0.43	0.43	0.13
2	84.0	−0.30	−1.00	−1.00	−0.09	−0.30	−0.30
3	142.0	−1.00	0.30	1.00	0.19	0.06	0.19
				Estimates by J-123			
1	21.0 ± 4.3	1.00 ± 0.44	1.00 ± 0.42	0.35 ± 0.36	0.43 ± 0.44	0.42 ± 0.28	0.13 ± 0.25
2	84.9 ± 4.7	−0.24 ± 0.51	−1.00 ± 0.38	−1.01 ± 0.40	−0.01 ± 0.51	−0.27 ± 0.25	−0.25 ± 0.23
3	142.4 ± 3.9	−1.03 ± 0.38	0.27 ± 0.27	1.02 ± 0.31	0.17 ± 0.38	0.05 ± 0.29	0.07 ± 0.22
				Estimates by J-12			
1	20.9 ± 5.2	1.03 ± 0.43	1.02 ± 0.41		0.44 ± 0.32	0.42 ± 0.29	
2	83.7 ± 9.6	−0.25 ± 0.56	−0.97 ± 0.43		0.03 ± 0.36	−0.24 ± 0.30	
3	141.3 ± 6.8	−1.08 ± 0.36	0.26 ± 0.34		0.17 ± 0.03	0.02 ± 0.30	
				Estimates of J-13			
1	22.4 ± 7.8	1.05 ± 0.48		0.30 ± 0.39	0.47 ± 0.32		0.14 ± 0.25
2	85.6 ± 6.0	−0.26 ± 0.52		−1.03 ± 0.40	−0.01 ± 0.33		−0.25 ± 0.24
3	143.1 ± 2.8	−1.00 ± 0.38		1.03 ± 0.30	0.18 ± 0.29		0.08 ± 0.23
				Estimates by J-23			
1	21.8 ± 6.5		1.05 ± 0.41	0.34 ± 0.40		0.41 ± 0.30	0.12 ± 0.26
2	84.9 ± 4.4		−1.01 ± 0.38	−1.04 ± 0.37		−0.27 ± 0.25	−0.25 ± 0.23
3	142.4 ± 4.5		0.28 ± 0.30	1.04 ± 0.35		0.03 ± 0.29	0.07 ± 0.23
				Estimates by S-1, S-2 and S-3[a]			
1	21.9, 21.6, 25.8 (7.7, 5.7, 13.6)	1.08 ± 0.42	1.12 ± 0.34	0.38 ± 0.49	0.48 ± 0.33	0.41 ± 0.30	0.11 ± 0.33
2	84.9, 84.3, 86.0 (17.1, 8.7, 6.7)	−0.29 ± 0.69	−1.03 ± 0.38	−1.06 ± 0.37	0.06 ± 0.42	−0.26 ± 0.29	−0.24 ± 0.26
3	141, 136, 143 (6.2, 11.7, 4.1)	−1.13 ± 0.31	0.33 ± 0.45	1.06 ± 0.30	0.17 ± 0.30	−0.01 ± 0.37	0.08 ± 0.24

Estimates are means ± SD over 100 replicates, by the joint mapping on three traits (J-123) and on two traits at a time (J-12, J-13 and J-23) and by the separate mapping on each trait (S-1, S-2 and S-3).

[a] Values in parentheses are respective SD values.

fects may be modeled as random effects and the method of variance components may have to be used.

SIMULATION STUDIES

Joint mapping *vs.* separate mapping: To further explore some properties of joint mapping, simulation experiments were performed. For simplicity, one chromosome with 16 uniformly distributed markers was simulated for an F_2 population. Each marker interval is 10 cM in length with a total length of 150 cM. Three QTL were simulated to affect three traits with effects and positions listed in Table 3 (with no epistasis). Heritabilities of the three traits are all assumed to be 0.3. The sample size is 150. The trait values of an individual on three traits are determined by the sum of effects of QTL sampled, plus a random vector of environmental effects that are sampled from a trivariate normal distribution with means zero, and variances and covariances given in Table 4. Simulation and analyses were performed on 100 replicates.

Given the positions and effects of QTL, the genetic correlations between traits are expected to be 0.54, −0.22, 0.68 between traits 1 and 2, 1 and 3, 2 and 3, respectively. [See, for example, Appendix C of ZENG (1992) for the calculation of the expected genetic variance in an F_2 population. The genetic covariance can be calculated similarly.] However, despite the substantial genetic correlations among traits, phenotypic correlations are low after adding environmental effects (Table

TABLE 4

Summary parameters and mean statistics of traits for the simulation example in Table 3

Trait	Genetic variance	Phenotypic variance	Environmental variance	Genetic correlation		Phenotypic correlation		Environmental correlation	
				Trait 2	Trait 3	Trait 2	Trait 3	Trait 2	Trait 3
				Parameters					
1	1.02	3.40	2.38	0.54	−0.22	0.30	−0.07	0.20	0.00
2	0.76	2.53	1.77		0.68		0.06		−0.20
3	0.71	2.35	1.64						

Trait	Sample variance	Residual variance	Sample correlation		Residual correlation	
			Trait 2	Trait 3	Trait 2	Trait 3
		Mean estimates				
1	3.47	2.25	0.30	−0.07	0.21	−0.01
2	2.51	1.67		0.07		−0.14
3	2.28	1.51				

4). Sample means, variances and correlation coefficients averaged over 100 replicates are also listed in Table 4. It is interesting to observe that the observed (averaged) residual variances and correlations are very close to the expected environmental variances and correlations, as in the analysis most of the genetic variation is absorbed by markers fitted in the model.

Seven methods of QTL mapping were performed on each simulated data set at every 1 cM on the chromosome. These include the following: joint mapping for three traits (J-123), joint mapping for each pair of traits (J-12, J-13 and J-23), and separate mapping for each trait (S-1, S-2 and S-3). Simply for the convenience of discussion, in mapping except for the flanking markers, all other markers are fitted in the model to control the genetic background because markers are evenly distributed and widely separated. We used $\chi^2_{0.05/15,7} = 21.4$, $\chi^2_{0.05/15,5} = 17.7$, and $\chi^2_{0.05/15,3} = 13.6$ as the critical values of the test for the three levels of mapping.

Summary estimates of QTL positions and effects by the seven mapping methods are given in Table 3, and the observed power of detection of QTL are given in Table 5. The statistics given in Tables 3 and 5 are summarized from 100 replicates for three QTL regions separately. Although three QTL are assumed to be located on the same chromosome, they are widely separated.

TABLE 5

Observed statistical power (proportion of significant replicates over all replicates) of seven methods of QTL mapping from 100 replicates of simulations

QTL	J-123	J-12	J-13	J-23	S-1	S-2	S-3	S-123
1	0.80	0.78	0.51	0.64	0.46	0.64	0.04	0.78
2	0.79	0.37	0.36	0.84	0.00	0.39	0.41	0.64
3	0.89	0.51	0.84	0.64	0.42	0.00	0.64	0.79

Also because of the composite interval mapping used in analysis, tests in different regions are statistically independent (ZENG 1993), so that the statistical power and sampling variance of estimates can be calculated separately for three QTL at and around the intervals surrounding the QTL. In Table 5, S-123 denotes the overall performance of the three separate mappings, and its power was calculated as the frequency of the detection of the QTL by at least one of the three separate mappings. It is seen that the power of J-123 in this case is higher than that of S-123 for all three QTL. This shows that some QTL with relatively small effects may be missed by separate mappings on different traits but detected by joint mapping that combined information from different traits. The power of J-12 is very close to that of J-123 on QTL 1. This is because QTL 1 has effects mainly on traits 1 and 2, and just a small effect on trait 3. The exclusion of trait 3 in J-12 only slightly affects the power of detection of the QTL. This also shows that small pleiotropic effects on additional traits included in the joint mapping may be large enough to compensate the lose of power due to the increase of the critical value. The powers of QTL detection by J-12, J-13 and J-23 are generally comparable to the sizes of pleiotropic QTL effects involved. J-23, however, tends to have relatively higher power. This is because the pleiotropic effects of three QTL on traits 2 and 3 are all in the same direction and the environment correlation is negative (see APPENDIX A).

Means and SDs of estimates (over all replicates) of QTL positions and effects by different methods are also given in Table 3. All estimates seem to be relatively unbiased. In general, the precision of estimation of QTL positions and effects by J-123, as indicated by SDs, is better than other methods. Particularly, the joint analysis has a significant effect on improving the sampling

TABLE 6

Parameters of QTL positions and effects and their estimates in a single replicate of simulation by the joint mapping on two traits (J-12) and the separate mapping on each trait (S-1 and S-2)

QTL	Position (cM)	Additive effect		Dominance effect		Likelihood ratio
		Trait 1	Trait 2	Trait 1	Trait 2	
			Parameters			
1	54	−1.36	−1.44	1.28	1.35	
2	114	−1.16		0.75		
3	128		1.30		0.49	
			Estimates by J-12			
1	57	−1.03	−1.34	1.36	1.42	66.73
2/3	125	−0.82	1.31	−0.35	0.52	37.49
			Estimates by S-1			
1	55	−1.05		1.42		28.07
2	110	−0.75		1.16		15.01
			Estimates by S-2			
1	61		−1.22		1.24	51.95
3	127		1.34		0.45	24.24

variance of estimates of QTL positions. Sampling variances of estimates of QTL positions by J-123 are consistently smaller than those by other analyses, except of that for QTL 3 by J-13 and for QTL 2 by J-23 in which cases two trait analyses have some particular advantages as noticed above.

Pleiotropy *vs.* close linkage with one replicate: We also performed a simulation to test close linkage of two nonpleiotropic QTL against pleiotropy of a common QTL. In this example, one chromosome with 11 markers in 10 marker intervals, each with 15 cM, was simulated. Three QTL, one pleiotropic and two nonpleiotropic, were assumed to affect two traits with parameters given in Table 6. The heritability for each trait is 0.4. (The effects of QTL are undoubtedly very large.) The sample genetic, environmental and phenotypic correlations are 0.42, 0.2 and 0.29, respectively. The sample size is 300.

The results of joint mapping (J-12) and separate mappings (S-1 and S-2) in one replicate are presented in Table 6 and Figure 1. At least two major QTL are indicated by the analyses. There is some evidence from separate mappings that there might be two nonpleiotropic QTL in the region between 105 and 135 cM with each showing a significant effect on one trait only. To test this hypothesis, the test of pleiotropy *vs.* close linkage was performed in the two major regions that show significant effects on the traits for comparison: one between 45 and 75 cM and one between 105 and 135 cM. The results are plotted in Figure 2.

In Figure 2, the two-dimensional log-likelihood surface (as deviations from the maximum of the log-likeli-

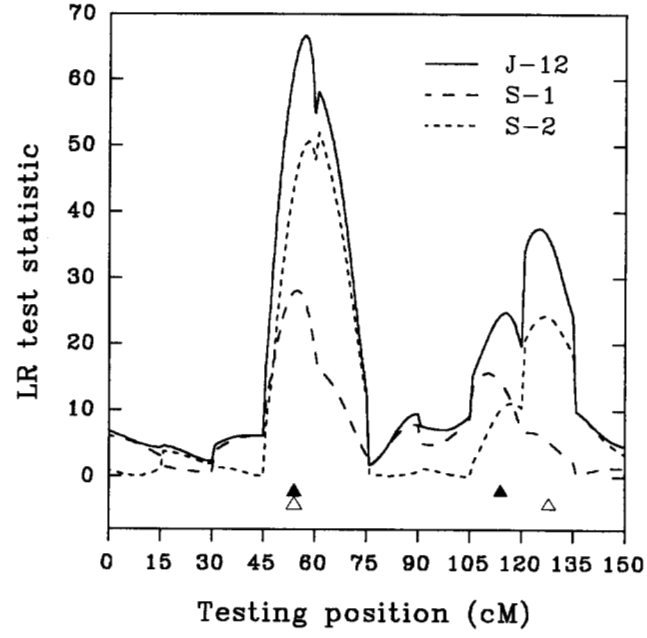

FIGURE 1.—A simulation example of QTL mapping on two traits from an F_2 population. Likelihood ratio test statistics are calculated and plotted at every 1 cM position of a chromosome for three mapping methods. J-12 is the joint mapping on two traits. S-1 is the separate mapping on trait 1 and S-2 is the separate mapping on trait 2. The genetic length of the chromosome is 150 cM with markers at every 15 cM. Three QTL, one pleiotropic and two nonpleiotropic, were simulated with positions and effects given in Table 6 and indicated by the triangles. ▲, QTL effects on trait 1; △, QTL effects on trait 2.

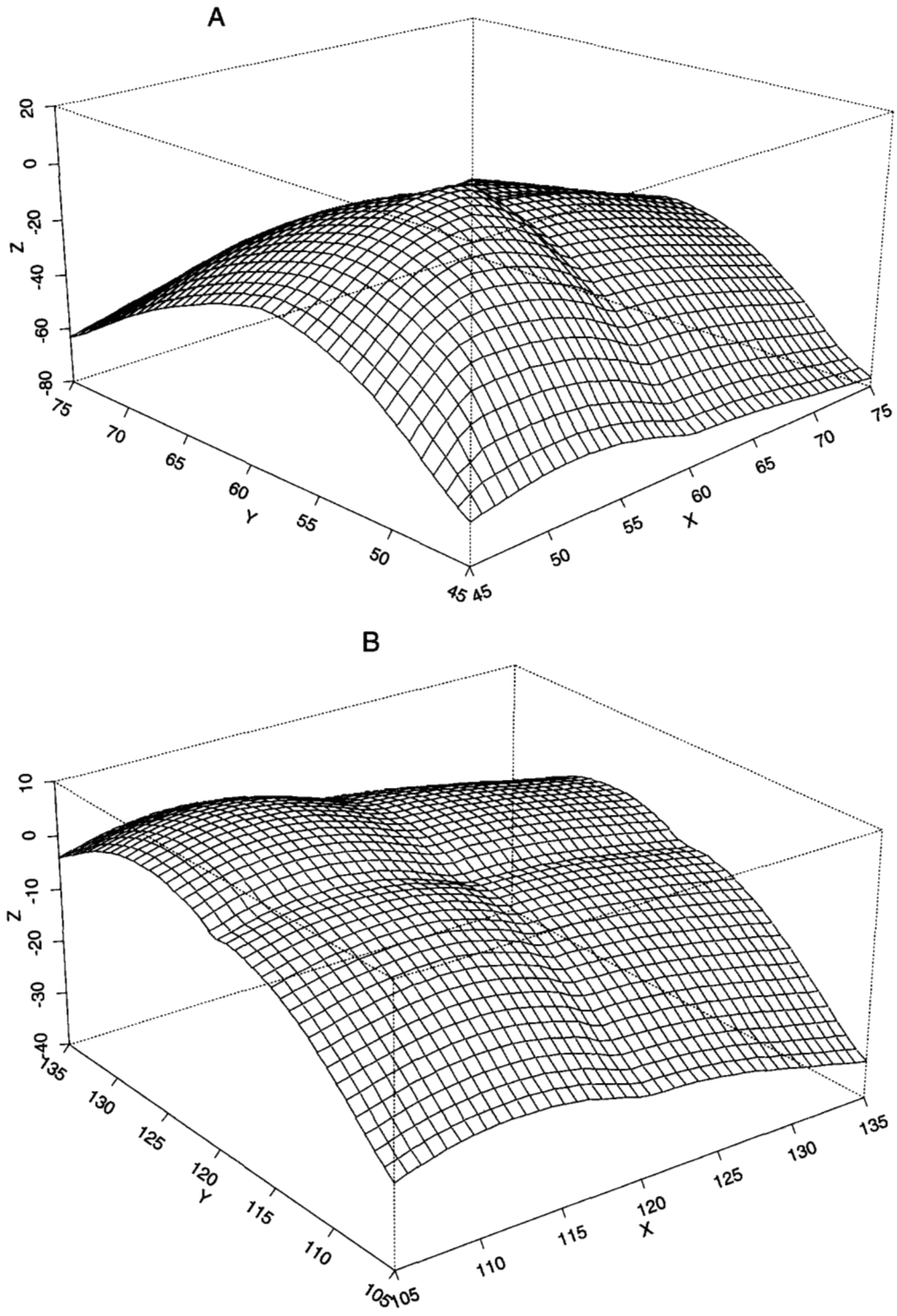

FIGURE 2.—Two-dimensional log-likelihood surfaces (expressed as deviations from the maximum of the log-likelihoods on the diagonal) for the test of pleiotropy *vs.* close linkage are presented for two regions: the region between 45 and 75 cM of Figure 1 (A) and the region between 105 and 135 cM (B). *X* is the testing position for a QTL affecting trait 1 and *Y* is the testing position for a QTL affecting trait 2. On the diagonal of *X*-*Y* plane, two QTL are located in the same position and statistically are treated as one pleiotropic QTL. *Z* is the likelihood ratio test statistic scaled to zero at the maximum point of the diagonal.

hoods on the diagonal) is presented. In this analysis, unlike joint mapping and the separate mappings that used all but flanking markers for background control, only markers 1–3 and 7–11 are used in the model for background control in Figure 2A, and only markers 1–7 and 11 are used for background control in Figure 2B. On this two-dimensional surface, the diagonal elements represents null hypotheses of one pleiotropic QTL, and the off-diagonal elements represent alternative hypotheses of two nonpleiotropic QTL. The likelihood ratio test is performed at the maximum of the surface against the maximum of the diagonals. This likelihood ratio is 0.53 for Figure 2A at the position 56 cM for trait 1 and 57 cM for trait 2 (the maximum diagonal is at 57 cM), which is clearly not significant, and 7.26 for Figure 2B at the position 111 cM for trait 1 and 126 cM for trait 2 (the maximum diagonal is at 125 cM), which is significant at 0.01 level (for chi-square distribution with one degree of freedom). There is clear evidence to support the presence of two QTL with one located

TABLE 7

Parameters of QTL positions and effects and their estimates over 100 replicates of simulation under the close linkage model

	QTL *1*	QTL *2*	QTL *3*	QTL *4*	QTL *5*
			Parameters		
Position (cM)	23	36	81	89	127
Effect					
b_1^*	1.00		−1.00		1.00
d_1^*	0.40		0.40		0.35
b_2^*		−1.00		1.00	−1.00
d_2^*		−0.30		−0.30	0.35
			Estimates[a]		
Position (cM)	23.4 ± 4.03	36.2 ± 4.60	81.1 ± 1.90	89.0 ± 2.20	127.2 ± 3.20[b] 127.4 ± 2.40[b]
Effect					
b_1^*	1.00 ± 0.19		−1.00 ± 0.17		1.05 ± 0.19
d_1^*	0.46 ± 0.15		0.49 ± 0.16		0.38 ± 0.16
b_2^*		−0.96 ± 0.16		1.02 ± 0.18	−1.04 ± 0.17
d_2^*		−0.32 ± 0.15		−0.27 ± 0.14	0.25 ± 0.14
Power					
JMP	1.00		1.00		1.00
TLK	0.81		0.89		0.10

[a] Values are means ± SD.

[b] Here the estimates of position of QTL *5* are given for each trait separately (under the nonpleiotropic model) to indicate the closeness of the estimates.

around 111 cM showing a significant effect on trait 1 and one located around 126 cM showing a significant effect on trait 2.

The maximum positions of diagonals in Figure 2 are the same maximum positions under the joint mapping in Figure 1 and Table 6. The maximum positions of off-diagonals in Figure 2 are also very close to the maximum positions indicated by the separate mappings (Table 6). As discussed above, in practice this two-dimensional search for the best fit of two nonpleiotropic QTL is unnecessary, although the two-dimensional surface is more illuminating. The test can be constructed at the peak suggested by joint mapping for the null hypothesis and at or around the peak suggested by separate mappings for the alternative hypothesis. This is clearly supported by the results of Figure 2. These are, however, the results of simulation in one replicate.

Pleiotropy *vs.* close linkage with multiple replicates: To show the behavior of the test statistic, we also performed simulations and tests with multiple replicates. The parameters of this simulation are, however, different from the above example and are shown in Table 7. In this simulation, one chromosome with 16 uniformly distributed markers in 15 10-cM marker intervals was simulated. Five QTL, one pleiotropic and four nonpleiotropic, are assumed to affect two traits with effects and positions listed in the table. The magnitudes of QTL effects are assumed to be the same for additive effects and slightly different for dominant effects. In this way, the statistical power of the test is comparable for different QTL. Heritabilities of the two traits are all assumed to be 0.6 and the environmental correlation coefficient between the traits is 0.2. Sample size is 150 and the number of replicates of simulation is 100.

For each replicate, the joint mapping is first performed using the procedure stated above. In the joint mapping, QTL 1 and 2 can be detected only as one QTL and so are QTL 3 and 4. Therefore, only three QTL are indicated by the joint analysis, and they are significant in all the replicates (with the estimated power of 1 in Table 7). Again, by using the result of ZENG (1994), the critical value of the joint mapping is set to be 17.7, which corresponds to the chi-square value with 5 degrees of freedom at the significance level of (0.05/15=) 0.0033. The separate mappings, however, failed to detect some QTL in a few replicates (results not shown). The corresponding critical value used in the separate mappings is 13.6.

Statistical tests for the hypotheses of pleiotropy *vs.* close linkage are then performed in the three regions indicated by the joint mapping. The observed powers of the test are given in Table 7. The power of the test for QTL 1 and 2 is 0.81, which is slightly lower than that for QTL 3 and 4. This may be because QTL 1 and 2 are relatively more distant from nearby markers than QTL 3 and 4 that are only 1 cM away from the respective closest markers. Similar results were also observed in

other simulations. This implies that dense marker coverage can help the test and separation of multiple QTL, as expected. QTL 5 is a pleiotropic locus. In the simulation, however, it can still be mapped to different positions (and even to different intervals in some replicates), due to sampling effects, by separate mapping on different traits. The observed power of the test in this case is 0.1, which is slightly higher than, but not significantly different from, the nominal level 0.05 (the difference is below the SE of the estimated power.) The critical value of the test used is 3.84 here.

Estimates of QTL positions and effects averaged over 100 replicates under the hypothesis of close linkage are also listed in Table 7. It is seen that, under the correct model, estimates tend to be *asymptotically* unbiased. Although it is seen that the mean estimates of d_1^* for QTL 1 and 3 (0.46 and 0.49, respectively) are statistically significantly different from the expected value of 0.40, these slightly biases in estimation are due to finite sample size ($n = 150$). It is known that the convergence of maximum likelihood estimates of dominance effects to their expected values is slower than those of additive effects. On the other hand, the estimates under the pleiotropic model are biased for QTL 1, 2, 3 and 4 (results not shown) as expected. For QTL 5, both models give very similar mean estimates, but the estimates under the pleiotropic model have less sampling variation (results not shown).

Complications can occur when two or more pleiotropic QTL are closely linked. In this case, use of incorrect (nonpleiotropic linkage) model can bias the test and estimation. The extent of the bias depends on the relative magnitudes of pleiotropic QTL effects on different traits and the extent of linkage. When each QTL has a predominant effect on one (and different) trait only, the bias can be small and the test for linked nonpleiotropic QTL may still be appropriate.

We have also performed the simulation studies on the performance of the statistical tests of QTL × environment interaction, particularly on comparing the powers of designs I and II in detecting QTL and testing QTL × environment interaction. The results agree with the analyses of APPENDIX B. To save the space, these simulation results are not presented.

DISCUSSION

Many data in QTL studies contain multiple traits. These traits are often correlated genetically and nongenetically (or environmentally). One way to analyze these data is to map QTL on each trait separately. Alternatively and preferably, different traits are analyzed together to map QTL affecting one or more traits by taking the correlated structure of data into account. There are generally three advantages for this joint analysis. First, the joint analysis may increase statistical power of detecting QTL. Second, the joint analysis can improve the precision of parameter estimation. Third and probably most importantly, the joint analysis provides appropriate procedures to test a number of biologically interesting hypotheses involving multiple traits. We examined, in detail, various advantages and disadvantages of the joint analysis as compared to separate analyses for mapping QTL and also for testing a number of hypotheses involving genetic correlations among multiple traits. Among the test procedures considered are those for joint mapping, pleiotropy, QTL × environment interaction, and pleiotropy *vs.* close linkage.

As the number of traits in an analysis increases, the number of relevant significant QTL involved may increase. So it is very important to have procedures to test whether the significant association on different traits in certain genome regions is due to possibly a pleiotropic QTL or multiple (more or less) nonpleiotropic QTL. Separation of these two hypotheses undoubtedly has important implications to our understanding of the nature of genetic correlations between the traits involved and also to practical breeding of genetic materials because one of the main purposes in animal and plant breeding is to break unfavorable linkage of some genes involved.

We also analyzed two commonly used experimental designs for studying QTL by environment interaction. As expected, design I tends to has relatively more power to detect QTL by environment interaction, whereas design II, which would require more genotyping on markers, tends to have relatively more power to detect QTL.

In this paper, we formulate our analysis in terms of some specific experimental designs for mapping QTL, namely F_2 populations (and backcross populations as well with some modifications). When F_1 [F_2 or F_3, *e.g.,* STUBER *et al.* (1992)] individuals are backcrossed to both parental lines, two backcrosses can be analyzed together by taking respective maker and QTL genotypes into account. There are, however, several other specifications for this design that need also to be taken into account in analysis and are not discussed here. Many experiments for mapping QTL are subdivided into a number of groups or blocks. These groups or blocks sometimes have significant effects on phenotypes observed. Eliminating these experimental effects when mapping QTL can further increase the precision and efficiency of mapping. Some of these effects may be random and others may be fixed. So it is very desirable to combine the composite interval mapping with a mixed model. Epistatic effects of QTL are also ignored here and should also be taken into account for a mature package of methods and programs for mapping QTL.

We sincerely thank BRUCE WEIR for the encouragement on the project and the constructive comments on the manuscript. We also thank reviewers for many constructive comments on the manuscript. This study was supported in part by grants GM-45344 from the National Institutes of Health, DEB-9220856 from the National Science Founda-

tion, and a grant from the Natural Science Foundation of China. Computer programs implementing methods and procedures of the analyses described in ZENG (1994) and in this paper have been written by Drs. CHRISTOPHER BASTEN and ZHAO-BANG ZENG in C language and are available by sending email to basten@essspg.stat.ncsu.edu or by anonymous ftp to esssjp.stat.ncsu.edu (in the directory /pub/qtlcart). The program package is called QTL CARTOGRAPHER. Send comments to basten@essspg.stat.ncsu.edu or zeng@stat.ncsu.edu. The development of the programs was also supported in part by U.S. Department of Agriculture Plant Genome Program 9400746 and a Pioneer Hi-Bred International Inc. Competitive Research Award.

LITERATURE CITED

CHURCHILL, G. A., and R. W. DOERGE, 1994 Empirical threshold values for quantitative trait mapping. Genetics **138:** 963–971.

DONGARRA, J. J., C. B. MOLER, J. R. BUNCH and G. W. STEWART, 1979 *LINPACK Users' Guide.* SIAM, Philadelphia.

FALCONER, D. S., 1952 The problem of environment and selection. Amer. Nat. **86:** 293–298.

HALEY, C. S., and S. A. KNOTT, 1992 A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity **69:** 315–324.

JANSEN, R. C., 1992 A general mixture model for mapping quantitative trait loci by using molecular markers. Theor. Appl. Genet. **85:** 252–260.

JANSEN, R. C., and P. STAM, 1994 High resolution of quantitative traits into multiple loci via interval mapping. Genetics **136:** 1447–1455.

LANDER, E. S., and D. BOTSTEIN, 1989 Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics **121:** 185–199.

MENG, X.-L., and D. B. RUBIN, 1993 Maximum likelihood estimation via the ECM algorithm: a general framework. Biometrika **80:** 267–278.

PATERSON, A. H., E. S. LANDER, J. D. HEWITT, S. PETERSON, S. E. LINCOLN *et al.*, 1988 Resolution of quantitative traits into Mendelian factors by using a complete RFLP linkage map. Nature **335:** 721–726.

PATERSON, A. H., S. DAMON, J. D. HEWITT, D. ZAMIR, H. D. RABINOWITCH *et al.*, 1991 Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. Genetics **127:** 181–197.

STUBER, C. W., S. E. LINCOLN, D. W. WOLFF, T. HELENTJARIS and E. S. LANDER, 1992 Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics **132:** 823–839.

ZENG, Z.-B., 1992 Correcting the bias of Wright's estimates of the number of genes affecting a quantitative trait: a further improved method. Genetics **131:** 987–1001.

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

ZENG, Z.-B., 1994 Precision mapping of quantitative trait loci. Genetics **136:** 1457–1468.

Communicating editor: P. D. KEIGHTLEY

APPENDIX A: COMPARING THE JOINT AND SEPARATE ANALYSES FOR MAPPING QTL

Here we discuss the relationship between the joint (for multiple traits) and separate (for one trait) analyses for mapping QTL. To simplify the argument, we will consider the situation where the testing position for a putative QTL is right on a marker, say marker k, so that the comparison can be made based on the regression analysis. Then, by induction, the general conclusion on the comparison can be extended to mapping for a QTL within a marker interval.

We first consider the case of testing only the marker additive effects in a simple bivariate regression setting. Let the array of the regression coefficients of y_1 and y_2 on x_k be denoted as $\mathbf{b}_k = (b_{k1}, b_{k2})$. For the joint analysis, the hypotheses to be tested are

$$H_0: \mathbf{b}_k = \mathbf{0} \quad \text{and} \quad H_1: \mathbf{b}_k \neq \mathbf{0}. \tag{A1}$$

Under H_1 of (A1), the expected maximum likelihood is

$$\begin{aligned} L_1 &\propto |\mathbf{V}|^{-n/2}, \\ &= \begin{vmatrix} \sigma_1^2 & \rho\sigma_1\sigma_2 \\ \rho\sigma_1\sigma_2 & \sigma_2^2 \end{vmatrix}^{-n/2}, \\ &= [\sigma_1^2\sigma_2^2(1-\rho^2)]^{-n/2}, \end{aligned} \tag{A2}$$

where σ_1^2 and σ_2^2 are residual variances and ρ is the residual correlation coefficient. Under H_0,

$$\begin{aligned} L_0 &\propto |\mathbf{V}_0|^{-n/2}, \\ &= |\mathbf{V} + \mathbf{b}_k'\sigma_{x_k}^2\mathbf{b}_k|^{-n/2}, \\ &= \left|\begin{pmatrix} \sigma_1^2 & \rho\sigma_1\sigma_2 \\ \rho\sigma_1\sigma_2 & \sigma_2^2 \end{pmatrix} + \begin{pmatrix} \sigma_{x_k}^2 b_{k1}^2 & \sigma_{x_k}^2 b_{k1}b_{k2} \\ \sigma_{x_k}^2 b_{k1}b_{k2} & \sigma_{x_k}^2 b_{k2}^2 \end{pmatrix}\right|^{-n/2}, \\ &= [(\sigma_1^2 + \sigma_{x_k}^2 b_{k1}^2)(\sigma_2^2 + \sigma_{x_k}^2 b_{k2}^2) \\ &\qquad - (\rho\sigma_1\sigma_2 + \sigma_{x_k}^2 b_{k1}b_{k2})^2]^{-n/2}, \end{aligned} \tag{A3}$$

where $\sigma_{x_k}^2$ is the variance of x_k that is $^1/_2$ for an F_2 population. The likelihood ratio test statistic for the joint analysis is then expected to be

$$\begin{aligned} LR_J &= -2\ln(L_0/L_1), \\ &= n\ln\left[1 + \frac{\beta_1^2 + \beta_2^2 - 2\rho\beta_1\beta_2}{1-\rho^2}\right], \end{aligned} \tag{A4}$$

where $\beta_1 = b_{k1}\sigma_{x_k}/\sigma_1$ and $\beta_2 = b_{k2}\sigma_{x_k}/\sigma_2$. If $\beta_1^2 \ll 1$ and $\beta_2^2 \ll 1$,

$$LR_J \simeq n\frac{\beta_1^2 + \beta_2^2 - 2\rho\beta_1\beta_2}{1-\rho^2}. \tag{A5}$$

Similarly, it can be shown that for the separate analyses

$$LR_{S1} \simeq n\beta_1^2, \tag{A6}$$

$$LR_{S2} \simeq n\beta_2^2. \tag{A7}$$

Several observations can be made from (A5), (A6) and (A7):

1. If $\rho = 0$, $LR_J \simeq LR_{S1} + LR_{S2}$. That is, the joint test statistic is approximately the sum of separate test statistics for independent traits.
2. If $\beta_2 = 0$, $LR_J \simeq LR_{S1}/(1-\rho^2) \geq LR_{S1}$. This shows that even if a QTL has an effect only on one trait, say trait 1, using the joint analysis on two traits to test $b_{k1} = 0$ can still have some advantage as compared to

the separate analysis on trait 1 only, if the two traits are correlated.

3. $LR_J \geq$ maximum $[LR_{S1}, LR_{S2}]$ always. This can be shown, for example, for two extreme cases. Case 1: $\beta_2 = 0$ and $\beta_1 \neq 0$, $LR_J \simeq LR_{S1}/(1 - \rho^2) \geq LR_{S1}$, the observation 2. Case 2: $\beta_1 = \beta_2 = \beta$, $LR_J \simeq [2/(1 + \rho)]\,\beta^2 \geq LR_{S1}$ or LR_{S2}.
4. If $\rho\beta_1\beta_2 < 0$, *i.e.*, ρ and $\beta_1\beta_2$ are in different signs, $LR_J > LR_{S1} + LR_{S2}$. In this case, the power of the joint analysis would be higher than that of any separate analysis for mapping QTL. This is the most favorable situation for the joint analysis.

The same argument and comparisons can also apply to the multiple regression analysis. If we test the additive effects of marker k conditional on fitting other markers, $\mathbf{b}_k$ of (A3) become the partial regression coefficients of y_1 and y_2 on x_k conditional on other markers, and $\sigma^2_{x_k}$ of (A3) now becomes the variance of x_k conditional on other markers, which can be denoted as $\sigma^2_{x_k \cdot s_k}$ (see below). Then by simply redefining $\beta_1 = b_{k1}\sigma_{x_k \cdot s_k}/\sigma_1$ and $\beta_2 = b_{k2}\sigma_{x_k \cdot s_k}/\sigma_2$ in (A4) – (A7), the above comparisons still apply.

When we take dominance effects into account in mapping, the analysis becomes a little complicated. Now let the array of the partial regression coefficients of y_1 and y_2 on x_k and z_k be denoted as

$$\mathbf{B}_k = \begin{pmatrix} b_{k1} & b_{k2} \\ d_{k1} & d_{k2} \end{pmatrix}.$$

When we test the hypotheses H_0: $\mathbf{B}_k = \mathbf{0}$ and H_1: $\mathbf{B}_k \neq \mathbf{0}$, the expected maximum likelihood under H_1 can still be expressed as (A2), and under H_0 it becomes

$$L_0 \propto |\mathbf{V} + \mathbf{B}_k' \mathbf{A}_k \mathbf{B}_k|^{-n/2}, \qquad \text{(A8)}$$

where $\mathbf{A}_k$ is the covariance matrix of x_k and z_k conditional on other markers fitted in the model. Using the same notation of ZENG (1993), this is denoted as

$$\mathbf{A}_k = \begin{pmatrix} \sigma^2_{x_k \cdot s_k} & \sigma_{x_k z_k \cdot s_k} \\ \sigma_{x_k z_k \cdot s_k} & \sigma^2_{z_k \cdot s_k} \end{pmatrix},$$

where s_k denotes a set of selected markers that does not contain marker k. For F_2, these conditional variances and covariance are

$$\sigma^2_{x_k \cdot s_k} = \frac{2r_{k-1,k}(1 - r_{k-1,k})\, r_{k,k+1}(1 - r_{k,k+1})}{r_{k-1,k+1}(1 - r_{k-1,k+1})},$$

$$\sigma^2_{z_k \cdot s_k} = \frac{[1 - (1 - 2r_{k-1,k})^4][1 - (1 - 2r_{k,k+1})^4]}{4[1 - (1 - 2r_{k-1,k+1})^4]},$$

$$\sigma_{x_k z_k \cdot s_k} = 0,$$

where r denotes the corresponding recombination frequency between markers. The conditional and unconditional covariances between x and z within and between markers are all zero. This shows that asymptotically the additive and dominance effects can be estimated and tested independently in an F_2 population, because they are orthogonal.

With $\sigma_{x_k z_k \cdot s_k} = 0$,

$$L_0 \propto [(\sigma_1^2 + \sigma^2_{x_k \cdot s_k} b_{k1}^2 + \sigma^2_{z_k \cdot s_k} d_{k1}^2) \times (\sigma_2^2 + \sigma^2_{x_k \cdot s_k} b_{k2}^2 + \sigma^2_{z_k \cdot s_k} d_{k2}^2) - (\rho\sigma_1\sigma_2 + \sigma^2_{x_k \cdot s_k} b_{k1} b_{k2} + \sigma^2_{z_k \cdot s_k} d_{k1} d_{k2})^2]^{-n/2}.$$

Thus the likelihood ratio under the joint analysis can be expressed as

$$LR_J = n \ln\left[1 + \frac{\beta_1^2 + \beta_2^2 - 2\rho\beta_1\beta_2 + \gamma_1^2 + \gamma_2^2 - 2\rho\gamma_1\gamma_2 + (\beta_1\gamma_2 - \beta_2\gamma_1)^2}{1 - \rho^2}\right]$$

$$\simeq n\,\frac{\beta_1^2 + \beta_2^2 - 2\rho\beta_1\beta_2 + \gamma_1^2 + \gamma_2^2 - 2\rho\gamma_1\gamma_2 + (\beta_1\gamma_2 - \beta_2\gamma_1)^2}{1 - \rho^2}$$

under the similar conditions of (A5) where $\beta_1 = b_{k1}\sigma_{x_k \cdot s_k}/\sigma_1$, $\beta_2 = b_{k2}\sigma_{x_k \cdot s_k}/\sigma_2$, $\gamma_1 = d_{k1}\sigma_{z_k \cdot s_k}/\sigma_1$ and $\gamma_2 = d_{k2}\sigma_{z_k \cdot s_k}/\sigma_2$. Similarly, for the separate analyses

$$LR_{S1} \simeq n(\beta_1^2 + \gamma_1^2),$$

$$LR_{S2} \simeq n(\beta_2^2 + \gamma_2^2).$$

The comparison of the joint analysis *vs.* separate analyses is basically the same as above. However, since $(\beta_1\gamma_2 - \beta_2\gamma_1)^2 \geq 0$, the joint analysis in this case has some extra advantage.

APPENDIX B: COMPARING TWO EXPERIMENTAL DESIGNS FOR MAPPING QTL AND FOR TESTING QTL × ENVIRONMENT INTERACTION

On testing QTL: In this paper, we regard a trait expressed in multiple environments as multiple traits in analysis. Then, the likelihood ratio test statistic under design I for testing a QTL is just (A5) under the same assumptions and approximations. Under design II, since different individuals are independent, the likelihood ratio test statistic for the joint analysis is just the sum of those for separate analyses, a special situation of design I with $\rho = 0$ (observation 1 in APPENDIX A). Thus under the same conditions (*e.g.*, sample size), the expected difference on the test between the two designs in the analysis is reflected on the value of ρ in (A5). Depending on ρ, β_1 and β_2, the test statistic for design I may be smaller or larger than that for design II. However, in a special case in which $\beta_1 = \beta_2$, the ratio of the test statistics for the two designs is expected to be

$$\frac{LR_{M \cdot I}}{LR_{M \cdot II}} \simeq \frac{1}{1 + \rho}.$$

Thus, unless $\rho \leq 0$, the test under design II is likely to

be more powerful for mapping QTL than that under design I in this case. Of course, the number of individuals for marker genotyping in design II is doubled.

On testing QTL × environment interaction: To test QTL × environment interaction, we test hypotheses H_0: $b_{k1} = b_{k2} = b_{k0}$ and H_1: $b_{k1} \neq b_{k2}$ under the same assumptions of APPENDIX A. Under H_0, the maximum likelihood estimate of b_{k0} can be shown to be the weighted mean of the estimates of b_{k1} and b_{k2} under H_1 with weights

$$c_1 = \frac{\sigma_1^2 - \rho\sigma_1\sigma_2}{\sigma_1^2 + \sigma_2^2 - 2\rho\sigma_1\sigma_2}, \quad c_2 = \frac{\sigma_2^2 - \rho\sigma_1\sigma_2}{\sigma_1^2 + \sigma_2^2 - 2\rho\sigma_1\sigma_2}.$$

Then the maximum likelihood under H_0 is expected to be

$$L_0 \propto |\mathbf{V}_0|^{-n/2} = \left| \begin{pmatrix} \sigma_1^2 & \rho\sigma_1\sigma_2 \\ \rho\sigma_1\sigma_2 & \sigma_2^2 \end{pmatrix} + \begin{pmatrix} \sigma_{x_k}^2 (b_{k1} - b_{k0})^2 & \sigma_{x_k}^2 (b_{k1} - b_{k0})(b_{k2} - b_{k0}) \\ \sigma_{x_k}^2 (b_{k1} - b_{k0})(b_{k2} - b_{k0}) & \sigma_{x_k}^2 (b_{k2} - b_{k0})^2 \end{pmatrix} \right|^{-n/2}.$$

The expected maximum likelihood under H_1 is the same as (A2). The likelihood ratio under design I is then expected to be

$$LR_{Q\times E\cdot I} = n \ln\left[1 + \frac{\Delta_1^2 + \Delta_2^2 - 2\rho\Delta_1\Delta_2}{1 - \rho^2}\right]$$

$$\simeq n \frac{\Delta_1^2 + \Delta_2^2 - 2\rho\Delta_1\Delta_2}{1 - \rho^2},$$

with $\Delta_1 = (b_{k1} - b_{k0})\sigma_{x_k}/\sigma_1$ and $\Delta_2 = (b_{k2} - b_{k0})\sigma_{x_k}/\sigma_2$. When $\sigma_1^2 = \sigma_2^2$, $b_{k0} = c_1 b_{k1} + c_2 b_{k2} = {}^1/_2(b_{k1} + b_{k2})$, and $\Delta_1 = -\Delta_2 = \Delta$.

$$LR_{Q\times E\cdot I} \simeq n \frac{2\Delta^2}{1 - \rho}.$$

The likelihood ratio under design II is expected to be

$$LR_{Q\times E\cdot II} \simeq 2n\Delta^2,$$

and then

$$\frac{LR_{Q\times E\cdot I}}{LR_{Q\times E\cdot II}} \simeq \frac{1}{1 - \rho} \quad \text{for} \quad \sigma_1^2 = \sigma_2^2.$$

Thus, unless $\rho \leq 0$, the power of design I for testing QTL × environment interaction will be higher than that of design II.