Genetic Expectations of Quantitative Trait Loci Main and Interaction Effects Obtained With the Triple Testcross Design and Their Relevance for the Analysis of Heterosis

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ABSTRACT

Interpretation of experimental results from quantitative trait loci (QTL) mapping studies on the predominant type of gene action can be severely affected by the choice of statistical model, experimental design, and provision of epistasis. In this study, we derive quantitative genetic expectations of (i) QTL effects obtained from one-dimensional genome scans with the triple testcross (TTC) design and (ii) pairwise interactions between marker loci using two-way analyses of variance (ANOVA) under the F_2- and the F_∞-metric model. The theoretical results show that genetic expectations of QTL effects estimated with the TTC design are complex, comprising both main and epistatic effects, and that genetic expectations of two-way marker interactions are not straightforward extensions of effects estimated in one-dimensional scans. We also demonstrate that the TTC design can partially overcome the limitations of the design III in separating QTL main effects and their epistatic interactions in the analysis of heterosis and that dominance × additive epistatic interactions of individual QTL with the genetic background can be estimated with a one-dimensional genome scan. Furthermore, we present genetic expectations of variance components for the analysis of TTC progeny tested in a split-plot design, assuming digenic epistasis and arbitrary linkage.

E STIMATION of the type of gene action at loci underlying quantitative traits has been a major research focus of quantitative genetics. When defining gene action at single loci we generally distinguish between additive gene action and deviations from additivity due to intralocus allelic interactions, such as dominance and overdominance. With more than one locus, the genotype can also be affected by interlocus interactions, *i.e.*, epistasis. New insights from molecular studies have demonstrated the importance of epistatic interactions in the inheritance of complex traits for a broad spectrum of organisms (SCHADT *et al.* 2003; BREM *et al.* 2005). However, the majority of epistatic effects are assumed to be small while available genetic models and experimental designs suffer from limited power of detection. Therefore, new strategies need to be devised for the detection and estimation of epistatic quantitative trait loci (QTL) effects.

Many models have been developed to distinguish between different types of gene action and to estimate the magnitude of genetic effects. ANDERSON and KEMPTHORNE (1954) and GAMBLE (1962) proposed the estimation of additive, dominance, and epistatic effects

from first-moment statistics, *i.e.*, generation means. These parameters reflect sums of gene effects over all loci and, consequently, positive and negative effects at individual loci may cancel each other. COCKERHAM (1954) proposed a general model for estimating the type of gene action from second-moment statistics. He developed a set of orthogonal contrasts to partition the genetic variance into additive, dominance, and epistatic components. For populations derived from a cross between two inbred lines, simpler models such as the F_2- and the F_∞-metric model have been described (see VAN DER VEEN 1959). In the presence of epistasis, the choice of metric becomes crucial and depends on the genetic material under study and the experimental design employed for estimating genetic effects. A detailed comparison of the statistical properties and genetic expectations of the F_2- and the F_∞-metric model has been given by KAO and ZENG (2002) and YANG (2004). With an F_2 population, the F_2 metric is to be preferred because genetic effects under the F_2-metric model are orthogonal and thus, in contrast to the F_∞-metric model, unbiased estimates of genetic effects can be obtained irrespective of the presence of epistasis (ALVAREZ-CASTRO and CALBORG 2007). Furthermore, the genetic variance can be partitioned into eight independent components and genetic covariances are absent, if only first-order interactions are present.

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Melchinger *et al.* (2007) gave further arguments for the superiority of the F_2 metric for the analysis of heterosis with the North Carolina Experiment III (design III), originally devised by Comstock and Robinson (1952).

In addition to different genetic models, different experimental designs have been proposed for estimating the type of gene action from second-moment statistics. However, as pointed out by Kearsey and Jinks (1968), many designs have serious limitations with respect to unbiased estimation of genetic effects. Frequently, absence of epistasis is assumed but a valid test of this assumption is not provided. Furthermore, the different components of variance are estimated with varying precision and power and linkage disequilibrium between loci is not accounted for. The design III partially overcomes these limitations. The experimental units are produced from backcross matings of F_2 plants to the two parental lines from which the F_2 was derived. Additive and dominance components of variance can be estimated with nearly equal precision under the assumption of diploidy, biallelic and equal gene frequencies, and absence of linkage and epistasis. Thus, an estimate of the average degree of dominance can be obtained from the ratio of the dominance and additive variance components. Cockerham and Zeng (1996) extended Comstock and Robinson's analysis of variance (ANOVA) to include linkage and two-locus epistasis for F_2 and F_3 progenies and developed orthogonal contrasts for marker-aided mapping of QTL using single-marker ANOVA. Melchinger *et al.* (2007) demonstrated the exceptional features of the design III for the identification of QTL contributing to heterosis. They defined a new type of heterotic gene effect, denoted as augmented dominance effect d_i^*, which equals the net contribution of QTL i to midparent heterosis (MPH). It comprises the dominance effect d minus half the sum of additive × additive (*aa*) epistatic interactions with the genetic background. The novelty of their approach is that QTL significantly contributing to MPH are identified and both dominance and epistasis are accounted for.

An elegant extension of the design III proposed by Kearsey and Jinks (1968), named the triple testcross (TTC) design, provides a test of significance for the presence of epistasis. In the TTC design, testcrosses are produced not only with the two parental lines but also with the F_1 derived from them. For every progeny from a segregating population, *e.g.*, F_2 plant or recombinant inbred line (RIL), three sets of data can be created: (i) the average parental testcross performance, (ii) the difference between the parental testcross performances, and (iii) the deviation of testcross progenies with the F_1 from the mean of the parental testcrosses. Making use of the advances of marker technology, Kearsey *et al.* (2003) and Frascaroli *et al.* (2007) presented experimental results from QTL analyses based on the TTC design with data from Arabidopsis and maize, respectively, but genetic expectations of QTL effects estimated

with their respective models were not given and the connection to the analysis of heterosis was not made.

In this study, we give genetic expectations of QTL effects estimated with the TTC design in the presence of epistasis. We show that with the TTC design dominance × additive epistatic interactions of individual QTL with the genetic background can be estimated with one-dimensional genome scans. We also demonstrate that the limitation of the design III in the analysis of heterosis to separate QTL main effects and their epistatic interactions with all other QTL can partially be overcome with the TTC design. Objectives of this study were to (1) extend the theory given by Melchinger *et al.* (2007) to derive quantitative genetic expectations of QTL effects obtained from one-dimensional genome scans with the TTC design using composite-interval mapping (CIM), (2) give quantitative genetic expectations of pairwise interactions between marker loci using two-way ANOVA, (3) derive genetic expectations of variance components of the ANOVA for the TTC progeny tested in a split-plot design for digenic epistasis and arbitrary linkage, and (4) relate our results to the analysis of MPH. Application of our theory to experimental data has been published by Kusterer *et al.* (2007).

THEORY

Experimental design: Let us assume a random population of RILs derived from the cross between two homozygous lines P1 and P2. Further, we assume that the RILs are backcrossed to their parental lines and the F_1 derived from them, yielding testcross progenies H_t of RIL p ($p = 1, \ldots, n$) with testers P1 ($t = 1$), P2 ($t = 2$), and F_1 ($t = 3$). The parental line exhibiting superior average testcross performance is denoted as P2.

To obtain maximum precision of progeny means in subsequent QTL analyses we suggest that the testcross progeny H_t are evaluated in a split-plot design with n main plots, each main plot comprising all three testcrosses of the pth RIL. The model for the phenotypic trait values Y_{tpk} can be written as

$$Y_{tpk} = \mu + r_k + g_p + (rg)_{kp} + h_t + (hg)_{tp} + e_{tpk},$$

with r_k being the effect of the kth replication ($k = 1, \ldots, r$), g_p the genetic effect of the pth RIL, $(rg)_{kp}$ the main plot error term, h_t the effect of the tth tester, $(hg)_{tp}$ the interaction between tester t and RIL p, and e_{tpk} the subplot error term. While testers are considered fixed, all other effects are assumed random. Following Kearsey and Jinks (1968), three linear transformations Z_s ($s = 1$, 2, 3) on the performance data are generated with $Z_{1pk} = (Y_{1pk} + Y_{2pk})/2$ and $Z_{2pk} = Y_{1pk} - Y_{2pk}$, and $Z_{3pk} = (Y_{1pk} + Y_{2pk} - 2Y_{3pk})$. Thus, Z_{spk} denotes the phenotypic value of transformation Z_s for RIL p grown in the kth block and Z_{sp} the mean over replicates of Z_s for RIL p. Note that Z_1 and Z_3 are not orthogonal. Thus, partitioning of the genetic variance in the ANOVA is given for the linear

transformation $Z^*_{1pk} = (Y_{1pk} + Y_{2pk} + Y_{3pk})/3$ instead of Z_{1pk}.

Following MELCHINGER *et al.* (2007) the genetic constitution of parameters in the expected mean squares of the ANOVA of the TTC design (Table 1) is derived below. Let P1 and P2 differ at loci set $Q = \{1, \ldots, q\}$ affecting the quantitative trait of interest and let v_i be an indicator variable for the genotype at locus i taking values 0 for homozygous P1 or 2 for homozygous P2. We define the additive effect a_i and the dominance effect d_i at QTL i in accordance with the definition of FALCONER and MACKAY (1996, p. 109), except that a_i is negative when the trait-increasing allele is contributed by P1. Epistatic effects between loci i and j are denoted aa_{ij} for additive $\times$ additive, ad_{ij} for additive at locus i and dominance at locus j, da_{ij} for dominance at locus i and additive at locus j, and dd_{ij} for dominance $\times$ dominance. The sum of additive $\times$ additive epistatic effects over all pairs of QTL is denoted $[aa]$ and aa epistatic interactions of QTL i with the entire genetic background are denoted $[aa_{i\bullet}]$. The same notation is followed for ad, da, and dd epistatic effects. To allow extensions to multiple loci we express linkage between loci i and j with the linkage value λ_{ij} (SCHNELL 1961), which can be calculated from the recombination frequency r_{ij} as $\lambda_{ij} = 1 - 2r_{ij}$. Linkage disequilibrium between loci i and j in the gametic array of progeny derived from cross P1 $\times$ P2 is given by the linkage disequilibrium parameter D_{ij} (FALCONER and MACKAY 1996, p. 18). For RILs derived without random mating prior to selfing, D_{ij} can be calculated as $D_{ij} = \lambda_{ij}/4(2 - \lambda_{ij})$. General derivations of expectations and variances for the TTC design based on the theory presented by MELCHINGER *et al.* (2007) are given in the APPENDIX.

Assuming digenic epistasis and with Q_i denoting the loci set Q excluding element i we obtain

$$\mathscr{E}(Z^*_1) = \mu + \frac{1}{2}\sum_{i\in Q}\sum_{j\in Q_i}\left[\left(\frac{2+\lambda_{ij}}{12} + D_{ij}\right)aa_{ij} + \frac{2+\lambda_{ij}}{3}D_{ij}dd_{ij}\right],$$

$$\mathscr{E}(Z_2) = \sum_{i\in Q}\left[-a_i + \sum_{j\in Q_i}D_{ij}(ad_{ij} + da_{ij})\right],$$

and

$$\mathscr{E}(Z_3) = \sum_{i\in Q}\sum_{j\in Q_i}(1 - \lambda_{ij})\left[\frac{1}{4}aa_{ij} + D_{ij}dd_{ij}\right].$$

As follows from the ANOVA (Table 1), the presence of epistasis can be tested with the linear transformation Z_3, the null hypothesis being H_0: $\mathscr{E}(Z_3) = 0$. In the absence of linkage ($\lambda_{ij} = D_{ij} = 0$), $\mathscr{E}(Z_3)$ simplifies to $\frac{1}{4}[aa]$, the sum of additive $\times$ additive epistatic effects over all pairs of QTL. Our results on $\mathscr{E}(Z_3)$ are in agreement with the results of KEARSEY and JINKS (1968), who developed a test of significance for the net contribution of aa effects in the TTC design performed with testcrosses of F_2

TABLE 1

Analysis of variance on linear transformations Z^*_1, Z_2, and Z_3 calculated from testcross performance of recombinant inbred lines (RIL) evaluated in a split-plot design with n main plots each comprising testcrosses of the pth RIL with testers P1 ($t = 1$) and P2 ($t = 2$) and their F_1 ($t = 3$)

Source of variation	d.f.[a]	Expected mean square[b]
Main plots		
Replication	$r - 1$	
Z^*_1	$n - 1$	$\sigma_e^2 + 3\sigma_b^2 + 3r\sigma_{Z^*_1}^2$
Main plot error	$(n-1)(r-1)$	$\sigma_e^2 + 3\sigma_b^2$
Subplots		
Z_2	1	$\sigma_e^2 + \frac{1}{2}r\sigma_{Z_2}^2 + \frac{1}{2}rn[\mathscr{E}(Z_2)]^2$
RIL $\times$ Z_2	$n - 1$	$\sigma_e^2 + \frac{1}{2}r\sigma_{Z_2}^2$
Z_3	1	$\sigma_e^2 + \frac{1}{6}r\sigma_{Z_3}^2 + \frac{1}{6}rn[\mathscr{E}(Z_3)]^2$
RIL $\times$ Z_3	$n - 1$	$\sigma_e^2 + \frac{1}{6}r\sigma_{Z_3}^2$
Subplot error	$2n(r-1)$	σ_e^2

[a] r, number of replicates; n, number of RILs.
[b] $\mathscr{E}(Z_2)$ and $\mathscr{E}(Z_3)$ refer to expectations of Z_2 and Z_3, respectively; $\sigma_{Z^*_1}^2$, $\sigma_{Z_2}^2$, and $\sigma_{Z_3}^2$ refer to the progeny variance arising from interactions with testers; and σ_b^2 and σ_e^2 are error variances of the whole-plot and subplot errors, respectively.

plants. As mentioned by these authors, if aa_{ij} effects have different signs and cancel in the composite effect $\frac{1}{4}[aa]$, then the null hypothesis will be accepted even though strong epistasis may be present.

Assuming digenic epistasis but arbitrary linkage, genetic expectations of the variance components for the TTC are as follows:

$$\sigma_{Z^*_1}^2 = \frac{1}{4}\sum_{i\in Q}\left[a_i - \frac{1}{2}\sum_{j\in Q_i}da_{ij}\left(\frac{2+\lambda_{ij}}{3}\right)\right]^2$$

$$+ \sum_{i\in Q}\sum_{j\in Q_i}D_{ij}\left[a_i - \frac{1}{2}\sum_{k\in Q_i}da_{ik}\left(\frac{2+\lambda_{ik}}{3}\right)\right]$$

$$\times \left[a_j - \frac{1}{2}\sum_{k\in Q_j}da_{jk}\left(\frac{2+\lambda_{jk}}{3}\right)\right]$$

$$+ \frac{1}{32}\sum_{i\in Q}\sum_{j\in Q_i}(1 - 16D_{ij}^2)\left[aa_{ij} + \left(\frac{2+\lambda_{ij}}{3}\right)dd_{ij}\right]^2$$

$$\sigma_{Z_2}^2 = \sum_{i\in Q}\left(d_i - \frac{1}{2}\sum_{j\in Q_i}aa_{ij}\right)^2 + 4\sum_{i\in Q}\sum_{j\in Q_i}D_{ij}\left(d_i - \frac{1}{2}\sum_{k\in Q_i}aa_{ik}\right)$$

$$\times \left(d_j - \frac{1}{2}\sum_{k\in Q_j}aa_{jk}\right) + \frac{1}{8}\sum_{i\in Q}\sum_{j\in Q_i}(1 - 16D_{ij}^2)\left[ad_{ij} + da_{ij}\right]^2$$

$$\sigma_{Z_3}^2 = \frac{1}{8}\sum_{i\in Q}\sum_{j\in Q_i}(1 - \lambda_{ij})$$

$$\times \left[ad_{ij}^2 + da_{ij}^2 + 8D_{ij}ad_{ij}da_{ij} + (1 - 16D_{ij}^2)dd_{ij}^2\right].$$

We note that with the TTC design partitioning of the genetic variance on the basis of linear transformations Z_1^*, Z_2, and Z_3 differs from the design III. The progeny variance among RILs in the TTC design is reduced compared with the design III ($\sigma_{Z_{1^*}}^2 < \sigma_{Z_1}^2$). Both variance components comprise the same genetic effects, but the influence of da and dd epistatic effects is decreased by the factor $\left((2 + \lambda_{ij})/3\right)$, i.e., by two-thirds for unlinked loci. The variance component from interaction of RILs with the two parental testers ($\sigma_{Z_2}^2$) is identical for the TTC design and the design III. The genetic variance component arising from Z_3 is a complex function of different epistatic effects. In the absence of linkage, rejection of the null hypothesis H_0: $\sigma_{Z_3}^2 = 0$ provides evidence for epistasis of type ad and/or da.

As can be seen from Table 1, the proposed split-plot design in which each of n main plots comprises three subplots, i.e., the three testcrosses of the pth RIL, is advantageous compared with a randomized complete block design (RCB). The standard error of progeny means Z_2 and Z_3 is calculated from the subplot error, which is expected to be smaller than the error variance of the RCB. Competition effects between the three different testcross progenies are not expected due to equal inbreeding coefficients ($F = 0.5$). Compared with the design employed by FRASCAROLI et al. (2007), who assigned testers to t main plots and progenies from the same tester to subplots, this design has the advantage that the test for epistasis summed over all loci ($\mathcal{E}(Z_3)$) becomes more powerful due to more degrees of freedom of the main plot error ($(n-1)(r-1) > (t-1)(r-1)$). In addition, the subplot error variance should be decreased due to the small number of subplots per main plot resulting in higher precision of progeny means Z_2 and Z_3.

QTL analysis with the TTC design: MELCHINGER et al. (2007) derived quantitative genetic expectations of QTL effects obtained with the design III and RILs. Using CIM and assuming digenic epistasis they demonstrated that in one-dimensional genome scans on Z_1 and Z_2 the contrast of the two (unobservable) homozygous genotype classes at QTL i equals the augmented additive (a_i^*) and dominance (d_i^*) effects:

$$\mathcal{E}(Z_1(i)) = a_i - \frac{1}{2}\sum_{j \in Q_i} da_{ij} = a_i^*$$

$$\mathcal{E}(Z_2(i)) = 2d_i - \sum_{j \in Q_i} aa_{ij} = 2d_i^*.$$

They concluded that the sum of QTL effects equals genotypic expectations for the parental difference (PD) and MPH. Thus,

$$\text{PD} = \sum_{i \in Q} 2a_i^*$$

and

$$\text{MPH} = \sum_{i \in Q} d_i^*.$$

For the identification of QTL affecting the PD or MPH it is favorable that in a_i^* and d_i^* main effects and epistatic interactions of QTL i with the genetic background are confounded. However, the dissection of augmented QTL effects into their components is desirable when the relative contribution of the individual effects is of interest, i.e., the additive effect a_i at QTL i and $[da_{i\bullet}]$ epistasis contributing to the PD and the dominance effect d_i at QTL i and $[aa_{i\bullet}]$ epistasis contributing to MPH.

The contribution of the additive effect a_i at QTL i and its $[da_{i\bullet}]$ epistatic interactions can be estimated from one-dimensional genome scans with H_3 and the linear transformation Z_3. Following MELCHINGER et al. (2007), with CIM, i.e., estimating the QTL position and including cofactors in the model, we obtain the following quantitative genetic expectations of QTL effects at QTL i:

$$\mathcal{E}(Z_3(i)) = -\sum_{j \in Q_i}(1 - \lambda_{ij})da_{ij}$$

$$\mathcal{E}(H_3(i)) = a_i - \frac{1}{2}\sum_{j \in Q_i}\lambda_{ij}da_{ij}.$$

Thus, with the TTC design, genome scans on H_3 and Z_3 can be adopted for estimating to what extent the individual effects a_i and $[da_{i\bullet}]$ contribute to the augmented additive effect a_i^* in the absence of linkage.

On the basis of one-dimensional genome scans, the TTC design does not provide a solution to the dissection of the augmented dominance effect d_i^* into its components, i.e., the dominance effect of QTL i and $[aa_{i\bullet}]$. However, as commonly practiced in QTL analyses, digenic epistasis can be estimated by two-way ANOVA on the basis of interactions of two-locus combinations of marker genotypes. In the following, we derive quantitative genetic expectations of contrasts for two-locus marker genotypes with linear transformations Z_s of the TTC design. We assume two QTL i and j and two marker loci m_1 and m_2, each with genotype classes v ($v_i = 0, 2$; $v_j = 0, 2$; $v_{m_1} = 0, 2$; $v_{m_2} = 0, 2$), and define the vector $\mathbf{f'}_{m_1 m_2} = \left(f_{22|m_1 m_2}, f_{20|m_1 m_2}, f_{02|m_1 m_2}, f_{00|m_1 m_2}\right)$ with $f_{ij|m_1 m_2}$ referring to the conditional probability of the QTL genotype $v_i v_j$ ($v_i v_j = 22, 20, 02, 00$) given marker genotype $v_{m_1} v_{m_2}$ ($v_{m_1} v_{m_2} = 22, 20, 02, 00$). Following the parameterization of gamete frequencies given by SCHNELL (1961), the four-locus genotype frequencies of RILs $f_{ijm_1 m_2}$ can be expressed by using six two-locus $\left(D_{ij}, D_{im_1}, D_{im_2}, D_{jm_1}, D_{jm_2}, D_{m_1 m_2}\right)$ and one four-locus linkage disequilibrium parameter $\left(D_{ijm_1 m_2}\right)$ as

TABLE 2

Frequencies ($f_{ij|m_1m_2}$) of the four possible QTL genotypes of recombinant inbred line (RIL) parents at QTL i and j conditional on the marker genotype v ($v = 0, 2$) at marker loci m_1 and m_2 calculated using the two-locus linkage disequilibrium parameters $D_{m_1 i}$ and $D_{m_2 j}$

Genotype at QTL		Genotype $v_{m_1} v_{m_2}$			
i	j	22	20	02	00
2	2	$\frac{1}{4} + D_{m_1 i} + D_{m_2 j} + 4D_{m_1 i}D_{m_2 j}$	$\frac{1}{4} + D_{m_1 i} - D_{m_2 j} - 4D_{m_1 i}D_{m_2 j}$	$\frac{1}{4} - D_{m_1 i} + D_{m_2 j} - 4D_{m_1 i}D_{m_2 j}$	$\frac{1}{4} - D_{m_1 i} - D_{m_2 j} + 4D_{m_1 i}D_{m_2 j}$
2	0	$\frac{1}{4} + D_{m_1 i} - D_{m_2 j} - 4D_{m_1 i}D_{m_2 j}$	$\frac{1}{4} + D_{m_1 i} + D_{m_2 j} + 4D_{m_1 i}D_{m_2 j}$	$\frac{1}{4} - D_{m_1 i} - D_{m_2 j} + 4D_{m_1 i}D_{m_2 j}$	$\frac{1}{4} - D_{m_1 i} + D_{m_2 j} - 4D_{m_1 i}D_{m_2 j}$
0	2	$\frac{1}{4} - D_{m_1 i} + D_{m_2 j} - 4D_{m_1 i}D_{m_2 j}$	$\frac{1}{4} - D_{m_1 i} - D_{m_2 j} + 4D_{m_1 i}D_{m_2 j}$	$\frac{1}{4} + D_{m_1 i} + D_{m_2 j} + 4D_{m_1 i}D_{m_2 j}$	$\frac{1}{4} + D_{m_1 i} - D_{m_2 j} - 4D_{m_1 i}D_{m_2 j}$
0	0	$\frac{1}{4} - D_{m_1 i} - D_{m_2 j} + 4D_{m_1 i}D_{m_2 j}$	$\frac{1}{4} - D_{m_1 i} + D_{m_2 j} - 4D_{m_1 i}D_{m_2 j}$	$\frac{1}{4} + D_{m_1 i} - D_{m_2 j} - 4D_{m_1 i}D_{m_2 j}$	$\frac{1}{4} + D_{m_1 i} + D_{m_2 j} + 4D_{m_1 i}D_{m_2 j}$

$$f_{ijm_1m_2} = \frac{1}{16} + \frac{1}{4} \sum_{\{a,b\} \in \{i,j,m_1,m_2\}} (-1)^{(v_a + v_b)/2} D_{ab} + (-1)^{(v_i + v_j + v_{m_1} + v_{m_2})/2} D_{ijm_1m_2}.$$

Following MELCHINGER *et al.* (2007), conditional expectations of linear functions Z_s ($Z_s = Z_1$, Z_1^*, Z_2, Z_3) and testcross progenies H_t ($t = 1, 2, 3$) are obtained by

$$\mathcal{E}(Z_{s|m_1m_2}) = \mathbf{f'_{m_1m_2}} \mathbf{K_s} \mathbf{E}$$

and

$$\mathcal{E}(H_{t|m_1m_2}) = \mathbf{f'_{m_1m_2}} \mathbf{H_t} \mathbf{E}$$

with the matrices $\mathbf{H_t}$ and $\mathbf{K_s}$ denoting the coefficients of genetic effects given the genotype of the parental RIL, the tester t, and for $\mathbf{K_s}$ the linear transformation s (for details see the APPENDIX). $\mathbf{E}$ denotes the vector of genetic effects defined according to the F_2 metric. From this, we obtain the expectations of the interaction between markers m_1 and m_2 for linear functions Z_s and testcross progenies H_t as

$$\mathcal{E}(Z_s(m_1 \times m_2)) = (\mathbf{f'_{22}} - \mathbf{f'_{20}} - \mathbf{f'_{02}} + \mathbf{f'_{00}}) \mathbf{K_s} \mathbf{E}$$

and

$$\mathcal{E}(H_t(m_1 \times m_2)) = (\mathbf{f'_{22}} - \mathbf{f'_{20}} - \mathbf{f'_{02}} + \mathbf{f'_{00}}) \mathbf{H_t} \mathbf{E}.$$

For arbitrary linkage between all four loci (i, j, m_1, m_2) calculations of conditional QTL genotype frequencies become rather unwieldy. Therefore, we exemplify our derivations for the special case in which marker loci m_1 and m_2 are unlinked ($\lambda_{m_1m_2} = 0$) and QTL i is linked to marker m_1 and QTL j to marker m_2 ($\lambda_{ij} = \lambda_{m_1 j} = \lambda_{m_2 i} = 0$; $\lambda_{m_1 i} \geq 0$; $\lambda_{m_2 j} \geq 0$). Conditional probabilities of QTL genotypes at loci i and j are given in Table 2. Summation over QTL i and j yields genotypic expectations for interactions between marker pairs with linear transformations Z_s calculated from the TTC design,

$$\mathcal{E}(Z_1(m_1 \times m_2)) = \sum_{i \in Q(m_1)} \sum_{j \in Q(m_2)} 4D_{m_1 i} 4D_{m_2 j}(aa_{ij} + dd_{ij})$$

$$\mathcal{E}(Z_1^*(m_1 \times m_2)) = \sum_{i \in Q(m_1)} \sum_{j \in Q(m_2)} 4D_{m_1 i} 4D_{m_2 j}\left(aa_{ij} + \frac{2}{3} dd_{ij}\right)$$

$$\mathcal{E}(Z_2(m_1 \times m_2)) = \sum_{i \in Q(m_1)} \sum_{j \in Q(m_2)} 4D_{m_1 i} 4D_{m_2 j}(ad_{ij} + da_{ij})$$

$$\mathcal{E}(Z_3(m_1 \times m_2)) = 2 \sum_{i \in Q(m_1)} \sum_{j \in Q(m_2)} 4D_{m_1 i} 4D_{m_2 j} dd_{ij},$$

where $Q(m_1)$ denotes all loci in set Q in linkage disequilibrium with marker m_1.

For the dissection of heterotic-effect d_i^* into its components, estimates of aa epistatic interactions of QTL i with other QTL in the genome are of particular interest and can be obtained from two-way ANOVAs of marker interactions with H_3. The genotypic expectation for interactions between marker pairs with testcross progenies H_3 is given by

$$\mathcal{E}(H_3(m_1 \times m_2)) = \sum_{i \in Q(m_1)} \sum_{j \in Q(m_2)} 4D_{m_1 i} 4D_{m_2 j} aa_{ij}.$$

With cofactors in the model and assuming (i) that QTL linked to markers m_1 and m_2 interact only with each other and not with other QTL and (ii) $\lambda_{m_1 i} = \lambda_{m_2 j} = 1$, then the genotypic expectations of interactions between markers simplify to

$$\mathcal{E}(Z_1(m_1 \times m_2)) = aa_{ij} + dd_{ij}$$

$$\mathcal{E}(Z_1^*(m_1 \times m_2)) = aa_{ij} + \frac{2}{3} dd_{ij}$$

$$\mathcal{E}(Z_2(m_1 \times m_2)) = 2(ad_{ij} + da_{ij})$$

$$\mathcal{E}(Z_3(m_1 \times m_2)) = 2dd_{ij}$$

$$\mathcal{E}(H_3(m_1 \times m_2)) = aa_{ij}.$$

TABLE 3

Genetic expectations of QTL effects obtained with the triple testcross (TTC) design and estimated from one-dimensional genome scans ($\mathcal{E}(X(i))$) and two-way ANOVAs of marker interactions ($\mathcal{E}(X(m_1 \times m_2))$) between unlinked marker loci m_1 and m_2 ($\lambda_{m_1 m_2} = 0$) on the three possible testcrosses (H_1, H_2, H_3), the RIL lines (H_4), and the linear transformations Z_s under the F$_2$- and F$_\infty$-metric models

Progeny	$\mathcal{E}(X(i))$		$\mathcal{E}(X(m_1 \times m_2))$
	F$_2$ metric	F$_\infty$ metric	
H_1	$a_i + d_i - \frac{1}{2}\sum_{j\in Q_i} aa_{ij} - \frac{1}{2}\sum_{j\in Q_i} da_{ij}$	$a_i + d_i - \frac{1}{2}\sum_{j\in Q_i} aa_{ij} + \frac{1}{2}\sum_{j\in Q_i} ad_{ij} + \frac{1}{2}\sum_{j\in Q_i} dd_{ij}$	$aa_{ij} + ad_{ij} + da_{ij} + dd_{ij}$
H_2	$a_i - d_i + \frac{1}{2}\sum_{j\in Q_i} aa_{ij} - \frac{1}{2}\sum_{j\in Q_i} da_{ij}$	$a_i - d_i + \frac{1}{2}\sum_{j\in Q_i} aa_{ij} + \frac{1}{2}\sum_{j\in Q_i} ad_{ij} - \frac{1}{2}\sum_{j\in Q_i} dd_{ij}$	$aa_{ij} - ad_{ij} - da_{ij} + dd_{ij}$
H_3	$a_i - \frac{1}{2}\sum_{j\in Q_i} \lambda_{ij} da_{ij}$	$a_i + \frac{1}{2}\sum_{j\in Q_i} ad_{ij} - \frac{1}{2}\sum_{j\in Q_i} \lambda_{ij} da_{ij}$	aa_{ij}
H_4	$2a_i - \sum_{j\in Q_i} ad_{ij}$	$2a_i$	$4aa_{ij}$
Z_1	$a_i - \frac{1}{2}\sum_{j\in Q_i} da_{ij}$	$a_i + \frac{1}{2}\sum_{j\in Q_i} ad_{ij} - \frac{1}{2}\sum_{j\in Q_i} da_{ij}$	$aa_{ij} + dd_{ij}$
Z_1^*	$a_i - \frac{1}{6}\sum_{j\in Q_i} \left(2 + \lambda_{ij}\right) da_{ij}$	$a_i + \frac{1}{2}\sum_{j\in Q_i} ad_{ij} - \frac{1}{6}\sum_{j\in Q_i} \left(2 + \lambda_{ij}\right) da_{ij}$	$aa_{ij} + \frac{2}{3} dd_{ij}$
Z_2	$2d_i - \sum_{j\in Q_i} aa_{ij}$	$2d_i - \sum_{j\in Q_i} aa_{ij} + \sum_{j\in Q_i} dd_{ij}$	$2ad_{ij} + 2da_{ij}$
Z_3	$-\sum_{j\in Q_i} \left(1 - \lambda_{ij}\right) da_{ij}$	$-\sum_{j\in Q_i} \left(1 - \lambda_{ij}\right) da_{ij}$	$2dd_{ij}$
Z_4	$\sum_{j\in Q_i} ad_{ij} - \sum_{j\in Q_i} \lambda_{ij} da_{ij}$	$\sum_{j\in Q_i} ad_{ij} - \sum_{j\in Q_i} \lambda_{ij} da_{ij}$	$-2aa_{ij}$

While augmented additive and dominance effects are estimated in one-dimensional scans of Z_1 and Z_2, respectively, estimates of two-way marker interactions on Z_1 and Z_1^* yield a confounded estimate of aa and dd interactions, and Z_2 captures ad and da epistasis. A first estimate of genetic background interactions contributing to d_i^* at QTL i can be obtained with two-way marker interactions on H_3. Unbiased estimates of dd epistasis can be obtained with two-way ANOVAs on Z_3.

It becomes obvious that genetic expectations of two-way marker interactions obtained with the TTC design are not straightforward extensions of effects estimated with the same linear transformation in one-dimensional scans. Note that this is also true under the F$_\infty$ model employed by Kearsey *et al.* (2003) and Frascaroli *et al.* (2007) and for the separate analysis of backcross progenies as performed by Stuber *et al.* (1992). A summary of genetic expectations of QTL effects estimated from one-dimensional genome scans and two-way ANOVAs of marker interactions on (i) the three possible testcrosses (H_1, H_2, H_3), (ii) the RIL lines (H_4), and (iii) the linear transformations Z_s are given in Table 3 for both the F$_2$- and the F$_\infty$-metric models. Genetic expectations of QTL effects comprising epistatic effects but no main effects are identical for the two models. Genetic expectations of QTL effects obtained with one-dimensional genome scans on Z_1 and Z_2 differ, because under the F$_\infty$ metric estimates of QTL main effects are confounded with epistasis. Following Yang (2004), genetic expectations for the F$_\infty$-metric model are obtained by substituting a_i and d_i of the F$_2$ model with

$a_i + \frac{1}{2}\sum_{j\in Q_i} ad_{ij}$ and $d_i + \frac{1}{2}\sum_{j\in Q_i} dd_{ij}$, respectively. As evident from Table 3, additional complexity is introduced by the use of the F$_\infty$ metric. In the general case of populations with arbitrary gene frequencies the NOIA model devised by Alvarez-Castro and Calborg (2007) could be used for transforming the QTL effects determined in such a population to the genetic effects defined under the F$_2$ or the F$_\infty$ metric.

DISCUSSION

Genetic expectations of QTL effects: In this study, genetic expectations of QTL effects are estimated with the TTC design. Accounting for all types of digenic epistasis and arbitrary linkage, genetic expectations are given for one-dimensional genome scans and two-way marker ANOVAs under both the F$_2$- and the F$_\infty$-metric models. These theoretical results contribute significantly to the interpretation of QTL mapping experiments estimating the type of gene action with the TTC design and the design III. The advantages of these two designs have been widely recognized but, to date, genetic expectations of QTL effects have been given for the design III using only single-marker ANOVA (Cockerham and Zeng 1996) and one-dimensional genome scans with CIM (Melchinger *et al.* 2007).

When making inferences on the predominant type of gene action, profound knowledge on the genetic expectations of QTL effects is crucial, as was demonstrated by Cockerham and Zeng (1996) with a reanalysis of data from Stuber *et al.* (1992). Stuber *et al.* (1992) estimated

the type of gene action in a marker-aided design III experiment with F_3 lines of maize. They performed the QTL analysis separately for the testcrosses with each parent (*i.e.*, on H_1 and H_2) and detected that overdominance but not epistasis plays a major role in the inheritance of grain yield. Accounting for epistasis and performing a joint analysis of both testcrosses, COCKERHAM and ZENG (1996) found mostly QTL with dominant and epistatic gene action. Similarly divergent results were found for experimental studies on rice grain yield. XIAO *et al.* (1995) found dominance to be the most important type of gene action. When accounting for epistasis in the genetic model, LI *et al.* (2001) and LUO *et al.* (2001) detected strong evidence for overdominance and epistasis. Thus, interpretation of experimental results from QTL mapping studies on the predominant type of gene action can be severely affected by the choice of statistical model and the provision of epistasis. As evident from Table 3, genetic expectations of QTL effects estimated with the TTC design and the design III are complex and comprise both main and epistatic effects. This fact has been widely neglected in the literature. The theoretical results presented here assist in the interpretation of experimental results obtained with two of the major designs employed in the analysis of gene action and heterosis. We therefore believe that reanalysis of previously collected data sets with the statistical methods presented here as well as the joint analysis of similar data sets with a special focus on epistasis will be rewarding. The general quantitative genetic theory given in the APPENDIX allows derivation of genetic expectations of QTL effects for experiments where production of testcross progenies was performed with double-haploid lines or F_2 or F_3 populations.

Analysis of heterosis with the TTC design: MELCHINGER *et al.* (2007) demonstrated that in the analysis of the genetic causes of heterosis we need to identify genomic regions that harbor augmented dominance effects d_i^* instead of identifying QTL with maximum dominance d_i and dd interactions that control F_1 performance. With the design III, the confounding of QTL effects with epistatic background variation is desirable for the identification of heterotic QTL. However, it is also a limitation because partitioning of augmented QTL effects a_i^* and d_i^* into their main and epistatic components is not possible. By adding the F_1 as a third tester, this limitation can partially be overcome. The contribution of $[da_i.]$ epistatic interactions of QTL i with the genetic background to the augmented additive effect a_i^* can be estimated with genome scans on Z_3 and two-way marker interactions with H_3 can be used to estimate aa interactions of individual QTL with the genetic background. Because the search for interactions may be restricted to those QTL with significant augmented dominance effects (d_i^*), the problem of multiple testing is alleviated. However, two-way marker interactions will provide only a rough estimate of the contributions of $[aa_i.]$ epistasis

to d_i^* due to limited power of detection. Furthermore, QTL with a significant positive dominance effect d_i and positive $[aa_i.]$ epistasis may remain undetected in a genomewide scan with Z_2 if the two effects cancel each other, resulting in nonsignificant d_i^* effects. We are currently in the process of developing new experimental designs that allow separate estimation of d_i and $[aa_i.]$ components contributing to d_i^* and, consequently, MPH in one-dimensional genome scans.

Detection of epistatic interactions: The development of statistical tools and powerful experimental designs for an efficient identification of genetic interactions is a major challenge in the analysis of quantitative traits. As a result of limited statistical power, detection of significant epistatic QTL interactions has proved difficult in marker-aided studies on complex traits, such as yield, even with dense marker coverage and large populations (*e.g.*, SCHÖN *et al.* 2004; MIHALJEVIC *et al.* 2005). On the contrary, the presence of significant epistasis has been demonstrated when clearly defined genes were investigated and efficient molecular tools were at hand. DOEBLEY *et al.* (1995) demonstrated dependency of QTL effects on genetic background for plant and inflorescence architecture in maize and teosinte. Epistatic interactions of QTL and expression QTL (eQTL) involved in regulation of flowering in Arabidopsis were reported by KEURENTJES *et al.* (2007). KROYMANN and MITCHELL-OLDS (2005) cloned two QTL for growth rate in Arabidopsis exhibiting significant epistasis with the genetic background. The authors pointed out that the two QTL would not have been detected with classical QTL analysis approaches and that we are likely to introduce an ascertainment bias because QTL with significant epistatic interaction effects might not be representative of the majority of QTL with small effects contributing to gene networks.

In this study, we have developed a one-dimensional genome scan for epistatic interactions of type dominance × additive. QTL detected with CIM on the linear transformation Z_3 exhibit significant $[da_i.]$ epistasis with the genetic background. With this method, statistical power of detection is increased compared with statistical tests for epistasis based on interactions of all possible marker pairs, because the number of significance tests is greatly reduced and thus safeguarding against a high false discovery rate becomes less rigorous. Employing diallel crosses of three homozygous parents, JANNINK and JANSEN (2001) proposed a one-dimensional search for significant background interactions of QTL. With simulated data, they reported a twofold increase in power with the proposed one-dimensional search compared with standard two-dimensional searches. BLANC *et al.* (2006) employed a similar method in an experimental study with multiparental crosses of maize and found substantial evidence for QTL × genetic-background interactions, especially for grain yield. With both methods the partitioning of epistasis into its components is

not feasible. Nevertheless, with the increasing availability of QTL mapping populations derived from multiline crosses these methods are a valuable contribution to the identification of QTL with significant interactions with the genetic background.

In addition to genetic interactions of type dominance $\times$ additive $[da_{i\bullet}]$, interactions of type additive $\times$ dominance $[ad_{i\bullet}]$ can be detected in the absence of linkage with a one-dimensional genome scan on the linear transformation $Z_4 = 2H_3 - H_4$ if, in addition to the testcrosses of the RILs, their line *per se* performance (H_4) is tested, as was done by FRASCAROLI *et al.* (2007) and KUSTERER *et al.* (2007) (see the APPENDIX and Table 3). Epistatic interactions of type $[ad_{i\bullet}]$ or $[da_{i\bullet}]$ of QTL i may not play a major role in elite breeding material, because when summed over the entire genetic background, they are likely to cancel each other due to opposite signs of individual interactions. However, so far we have not been able to verify this hypothesis in QTL analyses. Many experimental studies demonstrated only minor importance of significant *ad* or *da* epistasis on the basis of two-way ANOVAs of marker interactions (*e.g.*, HUA *et al.* 2003) but it has been difficult to distinguish between true and false negatives due to the limitations of statistical tests. With the variance component $\sigma^2_{Z_3}$, the TTC design provides a significance test for the presence of *ad* and *da* epistasis. With the one-dimensional genome scan on Z_3 and Z_4 (if data on *per se* performance of RILs are available) and reasonable sample sizes we can achieve sufficient power to estimate the magnitude of $[ad_{i\bullet}]$ or $[da_{i\bullet}]$ interactions of individual QTL as well as the hypothesis $[ad_{i\bullet}] = [da_{i\bullet}]$. Once QTL are identified to interact with the genetic background, two-way marker ANOVAs on Z_2 can be performed to obtain individual estimates of QTL interactions between these QTL and other QTL in the genome $(\mathcal{E}(Z_2(m_1 \times m_2)) = ad_{ij} + da_{ij})$.

In conclusion, we are still at the beginning of understanding the complex interactions of individual genes and gene networks even with extensive genomic tools at hand. Knowledge about genetic expectations of QTL effects in the presence of epistasis will facilitate the assessment of gene action and function and will help elucidate the quantitative genetic basis of heterosis. As pointed out by JANNINK and JANSEN (2001), marker-assisted transfer of single genes affecting quantitative traits may be a fruitless endeavor if alleles show strong epistasis and fail to interact with the target genome in the same way as with the donor genome. On the other hand, with a more profound understanding of gene interactions, breeders may be empowered to utilize new alleles from nonadapted genetic resources or genetic engineering that exhibit favorable epistasis with the genetic background. In combination with newly developed statistical methods, such as Bayesian approaches (XU and JIA 2007), multiple-interval mapping (KAO *et al.* 1999), or two-stage analyses (BREM *et al.* 2005), powerful experimental designs can significantly increase the ef-

ficiency of experiments analyzing phenotypic data on agronomic traits such as yield. Furthermore, the same experimental designs can be used for molecular studies on the quantitative genetics of transcription, protein, or metabolite data. We believe that the analysis of gene interactions will be of increasing importance in future molecular and quantitative genetics research and that the theoretical results from this study provide improved analytical tools for the interpretation of a wide range of experimental data.

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APPENDIX: GENERAL DERIVATION OF EXPECTATIONS, VARIANCES, AND COVARIANCES OF LINEAR TRANSFORMATIONS Z_S FOR THE TTC DESIGN

Following MELCHINGER *et al.*'s (2007) expectations, variances and covariances of linear transformations Z_s ($Z_s = Z_1, Z_1^*, Z_2, Z_3, Z_4$) are given by

$$\mathscr{E}(Z_s) = \mathbf{f}'\mathbf{K_s}\mathbf{E},$$

$$\sigma_{Z_S}^2 = \mathbf{E}'\mathbf{K_s}'(\mathbf{F} - \mathbf{ff}')\mathbf{K_s}\mathbf{E}$$

and

$$\mathrm{cov}(Z_s, \ Z_t) = \mathbf{E}'\mathbf{K_s}'(\mathbf{F} - \mathbf{ff}')\mathbf{K_t}\mathbf{E}.$$

Assuming digenic epistasis $\mathbf{f}'$ denotes the frequencies of the four possible genotypes at two QTL i and j, $\mathbf{F}$ denotes a diagonal matrix with these frequencies on the diagonal, and $\mathbf{E}$ denotes the vector of genetic effects α_{AD}; *i.e.*, $\mathbf{E}' = \left(\mu, a_i, \ d_i, \ a_j, \ d_j, \ aa_{ij}, \ ad_{ij}, \ da_{ij}, \ dd_{ij}\right)$.

Elements of the matrix $\mathbf{H_t}$ denote the coefficients of genetic effects α_{AD} in the conditional genotypic expectation of testcross progeny H_t of a RIL with genotype $v_i v_j$ ($v_i v_j = 22, 20, 02, 00$) at QTL i and j for testcross performance ($t = 1, 2, 3$) with tester P1 ($t = 1$), P2 ($t = 2$), or F$_1$ ($t = 3$) or *per se* performance ($t = 4$). Assuming digenic epistasis, elements of $\mathbf{H_t}$ are given in Table A1. The matrices $\mathbf{K_s}$ are obtained for the TTC design by calculating

$$\mathbf{K_1} = (\mathbf{H_1} + \mathbf{H_2})/2, \quad \mathbf{K_1^*} = (\mathbf{H_1} + \mathbf{H_2} + \mathbf{H_3})/3, \quad \mathbf{K_2} = \mathbf{H_1} - \mathbf{H_2},$$

$$\mathbf{K_3} = \mathbf{H_1} + \mathbf{H_2} - 2\mathbf{H_3}, \quad \text{and} \quad \mathbf{K_4} = 2\mathbf{H_3} - \mathbf{H_4}.$$

TABLE A1

Coefficients in 4×9 matrix H_t ($t = 1, 2, 3, 4$) for the conditional expectation of the genotypic value of testcross progeny H_t of a RIL with genotype $v_i v_j$ ($v_i v_j$ = 22, 20, 02, 00) at QTL i and j for testcross performance ($t = 1, 2, 3$) with tester P1 ($t = 1$), P2 ($t = 2$), or F_1 ($t = 3$) or *per se* performance ($t = 4$)

RIL genotype $v_i v_j$	Genetic parameter								
	μ	a_i	$1/2 d_i$	a_j	$1/2 d_j$	aa_{ij}	$1/2 ad_{ij}$	$1/2 da_{ij}$	$1/4 dd_{ij}$
H_1 (testcrosses with P1)									
22	1	0	1	0	1	0	0	0	1
20	1	0	1	−1	−1	0	0	−1	−1
02	1	−1	−1	0	1	0	−1	0	−1
00	1	−1	−1	−1	−1	1	1	1	1
H_2 (testcrosses with P2)									
22	1	1	−1	1	−1	1	−1	−1	1
20	1	1	−1	0	1	0	1	0	−1
02	1	0	1	1	−1	0	0	1	−1
00	1	0	1	0	1	0	0	0	1
H_3 (testcrosses with F_1)									
22	1	1/2	0	1/2	0	$(1 + \lambda)/4$	$-\lambda/2$	$-\lambda/2$	λ
20	1	1/2	0	−1/2	0	$-(1 + \lambda)/4$	$\lambda/2$	$-\lambda/2$	$-\lambda$
02	1	−1/2	0	1/2	0	$-(1 + \lambda)/4$	$-\lambda/2$	$\lambda/2$	$-\lambda$
00	1	−1/2	0	−1/2	0	$(1 + \lambda)/4$	$\lambda/2$	$\lambda/2$	λ
H_4 (*per se* performance)									
22	1	1	−1	1	−1	1	−1	−1	1
20	1	1	−1	−1	−1	−1	−1	1	1
02	1	−1	−1	1	−1	−1	1	−1	1
00	1	−1	−1	−1	−1	1	1	1	1