Genome-wide mapping of QTL associated with heterosis in the RIL-based NCIII design

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Heterosis represents one of the most revolutionary advancements in crop improvement. In the genetic dissection of heterosis, NCIII design is one of the most powerful and widely used mating schemes. However, the methodologies for quantitative trait loci (QTL) detection in the design were mostly based on composite interval mapping. Therefore, in this study, our purpose was to develop a statistical method for mapping epistatic QTL associated with heterosis in the RIL-based NCIII design. First, we derived the expectations of two classical linear transformations, Z_1 and Z_2 , while a quantitative trait was controlled by two QTL with digenic epistasis and arbitrary linkage under the F_∞ and F_2 metric models. Then, we constructed an epistatic genetic model that includes all markers on the whole genome simultaneously, and estimated all the parameters in the model by the empirical Bayes approach. Finally, a series of Monte Carlo simulation experiments was carried out to confirm the proposed approach. The results show that: (1) all the augmented genetic parameters for main-effect QTL could be rightly identified with satisfactory statistical power and precision; (2) the statistical powers in the detection of augmented epistatic effects were substantively affected by the signs of pure epistatic effects; (3) it is more difficult to detect epistatic QTL than to detect main-effect QTL; (4) statistical power is higher in the RIL-based NCIII design than in the F_2 -based NCIII design, especially in the detection of the augmented epistatic effect that consists of two pure epistatic effects in opposite directions.

empirical Bayes, epistasis, NCIII design, heterosis, quantitative trait locus, recombinant inbred line

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Heterosis, or hybrid vigor, results in the phenotypic superiority of a hybrid over its parents with respect to traits such as growth rate, reproductive success, and yield [1]. This phenomenon has been recognized since ancient civilization [2–4], under scientific investigation since Darwin [5] in the absence of genetics, and for over 100 years with genetic considerations [6,7]. Heterosis represents one of the most revolutionary advancements in crop improvement, i.e., maize [8] and rice [4]. In the genetic dissection of heterosis, NCIII design, proposed by Comstock and Robinson [9,10], is one of the most powerful and widely used mating schemes [11–17]. Therefore, the importance of heterosis

necessitates an in-depth study of the NCIII design.

The NCIII design is to cross the i th individual ($i=1,2,\dots,n$) of an F_2 population to both parental lines (P_1 and P_2) to produce $2n$ families (L_{i1} and L_{i2}). An analysis of variance (ANOVA) of phenotypic means and differences between the backcross progenies provides estimates of additive and dominance genetic variances with nearly equal precision when epistasis is absent, and these variances are further used to estimate the average degree of dominance. However, the conventional ANOVA approach only deals with the collective effects of all the polygenes [18]. The introduction of molecular markers has facilitated the mapping of quantitative trait loci (QTL) in numerous species, and substantial progress has been achieved in the detection of individual

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QTL and their interaction for heterosis in yield and other traits in the NCIII design.

One modification of the NCIII design is to use F_3 as the base population. The design was adopted by Stuber et al. [19] to study the genetic dissection of heterosis by mapping QTL for heterosis in grain yield and five other traits, along with single marker analysis [20] and interval mapping (IM) [21]. The results showed that overdominance (or pseudo-overdominance) plays a significant role in heterosis for the grain yield. Another modification of the NCIII design is to use recombinant inbred lines (RIL) as the base population. The results showed that the primary genetic basis of heterosis is dominance in the interval mapping by Xiao et al. [22], and epistasis and overdominance in the mixed model analysis [23] by Li et al. [24], Luo et al. [25] and Mei et al. [26]. It should be noted that repulsion linkage might lead to pseudo-overdominance. To overcome this issue, Lu et al. [12] developed the F_2 Syn3-based NCIII design and found that 24 QTL (86%) for grain yield exhibited overdominance, while most of the QTL for plant height, grain moisture, and stalk lodging showed partial to complete dominance. QTL analyses were performed separately for the two backcross populations using composite interval mapping (CIM) [27] and the additive effect, dominance effect, and the level of dominance for a QTL were indirectly estimated according to the deduction of Cockerham and Zeng [11]. Using the method of Lu et al. [12], LeDeaux et al. [28] assessed the stability of QTL involved in the expression of heterosis in the RIL-based NCIII design, and the results showed that the yield QTL showed a positive dominant gene action, whereas the ear height and DPS QTL exhibited behavior that ranged from negative to positive dominance. Among these above designs, the RIL-based NCIII design is the best. The reasons are as follows. First, RIL is a permanent segregating population, so it can be shared between research groups, and testcross progeny can be repeatedly generated and phenotyped as needed [14,15,26,29]. Second, RIL allows more recombinational segregation of tightly linked QTL than the other populations, and this offers a higher mapping resolution [15,22,29]. Finally, the use of homozygous RIL can gain the highest statistical power for QTL detection [15,17,29]. Therefore, further exploration of the RIL-based NCIII design is warranted.

To date, several theoretical studies on the genetic dissection of heterosis have been carried out. Cockerham and Zeng [11] developed the theory and statistical analysis of orthogonal contrasts for a single marker locus to characterize the effects of QTL in the F_2 - and F_3 -based NCIII designs. Four orthogonal contrasts, C_1 to C_4 , among the means of progenies from marker genotype $\times$ parental line combinations are constructed, of which C_1 and C_3 provide estimates and tests for additive and dominance effects of linked QTL, respectively, whereas C_2 and C_4 for epistatic effects. Epistatic effects contribute to the additive effects in C_1 (ad and da) and the dominance effects in C_3 (aa),

and distinct epistatic effects contribute to C_2 (aa and dd) and C_4 (ad and da). The method is used to re-analyze the maize data of Stuber et al. [19]. The results showed that dominance can explain the observed heterosis in grain yield and other traits, although epistasis can also play an important role, and overdominance at individual QTL level cannot be ruled out. In addition, QTL effects in Stuber et al. [19] are $a-d$ and $a+d$ in the two backcross populations, respectively.

Melchinger et al. [14] developed a general theoretical framework of composite interval mapping for determining the contributions of different types of genetic effects to heterosis. They defined the augmented additive and dominance effects in the RIL-based NCIII design: the former measures the net contribution of the QTL to the parental difference and the latter measures the net contribution of the QTL to midparent heterosis (MPH).

Li et al. [15] studied heterosis by QTL analysis in the RIL-based NCIII design. QTL analysis was performed separately for the RIL, MPH of two backcross populations and the SUM (L_1+L_2) and DIFF (L_1-L_2) data of two backcross populations (L_1 and L_2). In the absence of epistasis, the analysis of RIL and SUM datasets identifies QTL with additive effect, whereas the analysis of MPH and DIFF datasets detects QTL with dominance effect [30]. Main-effect QTL was detected in each mapping population by composite interval mapping [27] and digenic interaction was identified in each mapping population by the mixed linear approach [23]. The results suggested that heterosis was attributable to the orchestrated outcome of partial-to-complete dominance, overdominance, and epistasis.

However, these above methods were mostly based on composite interval mapping. Therefore, Garcia et al. [16] developed a multiple-interval mapping [31] model in the NCIII design. The method provides a platform to estimate the number, genomic positions, augmented additive and dominance effects, and epistatic interactions ($aa+dd$ and $ad+da$) of QTL simultaneously. The method was used to re-analyze the datasets in Stuber et al. [19] and Xiao et al. [22]. For the former, yield heterosis was mainly due to dominant gene action, although overdominance (or pseudo-overdominance) of individual QTL could not completely be ruled out. For the latter, the observed yield heterosis was mainly due to additive $\times$ additive epistatic effects but not dominant effects.

The objective of this study was to develop a statistical method for mapping epistatic QTL associated with heterosis in the RIL-based NCIII design. First, we calculated the genetic expectations of two classical linear transformations Z_1 and Z_2 . Then, we constructed an epistatic genetic model that included all markers on the whole genome simultaneously and estimated all the parameters in the model by empirical Bayes of Xu [32]. Finally, a series of Monte Carlo simulation experiments was carried out to confirm the proposed approach.

1 Theory and methods

1.1 Genetic design and data collection

An RIL population was derived by a single seed descent procedure from two inbred lines (P_1 and P_2) that differed significantly in the quantitative traits of interest, and possessed abundant polymorphism molecular markers. A random sample of n RIL individuals (female parents) was backcrossed to their two parental lines to produce $2n$ families (L_{1i} and L_{2i}). All of the $2n$ families, each with m replications, were planted. Molecular marker information was observed from all of the n RIL individuals, whereas quantitative traits were measured for all of the $2nm$ NCIII progeny. The phenotypic observations were denoted by y_{tij} , where $t=1$ and 2 for L_1 and L_2 , respectively; $i=1,2,\dots,n$; $j=1,2,\dots,m$. The family means were denoted by $\bar{L}_{ti} = \sum_{j=1}^m y_{tij} / m$. Following Frascaroli et al. [30] and Melchinger et al. [14], we performed two linear transformations: $Z_{1i} = \bar{L}_{1i} + \bar{L}_{2i}$ and $Z_{2i} = \bar{L}_{1i} - \bar{L}_{2i}$. The association between Z_i and the marker genotypes of the RIL lines were used to infer the genetic architecture of heterosis for the trait.

1.2 Statistical genetic models for mapping heterotic QTL in RIL-based NCIII design

The expected genetic values of Z_{1i} and Z_{2i} depended on the choice of the metric. Two main metrics were adopted for the populations derived from the cross between the two inbred lines: the F_∞ and F_2 metrics [33–35]. The derivation of the expected genetic values of Z_{1i} and Z_{2i} under both the F_∞ and the F_2 metric models is presented in Supplement Information A under the assumption that the quantitative trait was determined by two QTL with digenic epistasis and arbitrary linkage (A and B). The genetic effect symbols adopted in this research were described in Kao and Zeng [33].

(i) Statistical genetic models for mapping QTL in the RIL-based NCIII design under the F_∞ metric model. According to the expected genetic values of Z_{1i} under the F_∞ metric model (Table S2), the phenotypic value of Z_{1i} can be described as

$$Z_{1i} = 2\mu + x_{a_1i}a_1 + d_1 + x_{a_2i}a_2 + d_2 + x_{a_1a_2i}i_{a_1a_2} + x_{a_1d_2i}i_{a_1d_2} + x_{d_1a_2i}i_{d_1a_2} + x_{d_1d_2i}i_{d_1d_2} + e_{1i}, \quad (1)$$

where μ is the mean genotypic values of the four homozygotes in RIL; a_k and d_k are additive and dominance effects of the k th QTL ($k=1,2$); $i_{a_1a_2}$, $i_{a_1d_2}$, $i_{d_1a_2}$ and $i_{d_1d_2}$ are additive×additive, additive×dominance, dominance×additive and dominance×dominance epistatic effects (see Supplement Information A for details); x_{a_1i} , x_{a_2i} , $x_{a_1a_2i}$, $x_{a_1d_2i}$, $x_{d_1a_2i}$ and $x_{d_1d_2i}$ are determined by the genotype of the i th RIL line (Table S2); and e_{1i} is the residual error with an $N(0, \sigma_1^2)$ distribution. According to the results in Table S2, the coefficients of the six genetic parameters have following restrictions: $x_{a_1a_2i} = x_{d_1d_2i}$ and $x_{a_1d_2i} = -x_{d_1a_2i} = \frac{1}{2}(x_{a_1i} - x_{a_2i})$. To solve the genetic parameters, model (1) must be reduced to

$$Z_{1i} = \mu_{Z_1} + x_{a_1i}a_1^* + x_{a_2i}a_2^* + x_{i_{12}i}i_{12} + e_{1i}, \quad (2)$$

where $\mu_{Z_1} = 2\mu + d_1 + d_2$, $a_1^* = a_1 + \frac{1}{2}(i_{a_1d_2} - i_{d_1a_2})$, $a_2^* = a_2 + \frac{1}{2}(i_{d_1a_2} - i_{a_1d_2})$, $i_{12} = (i_{a_1a_2} + i_{d_1d_2})$ and $x_{i_{12}i} = x_{a_1a_2i} = x_{d_1d_2i}$.

If the quantitative trait was controlled by q QTL, model (2) should be extended to

$$Z_{1i} = \mu_{Z_1} + \sum_{k=1}^q x_{a_ki}a_k^* + \sum_{k=1}^{q-1} \sum_{l=k+1}^q x_{i_{kl}i}i_{kl} + e_{1i}, \quad (3)$$

where the model mean $\mu_{Z_1} = 2\mu + \sum_{k=1}^q d_k$; $a_k^* = a_k + \frac{1}{2} \sum_{l \neq k}^q (i_{a_kd_l} - i_{d_ka_l})$ is the augmented additive effect of QTL k , which measures the net contribution of the QTL to the parental difference (PD) [14,36]; and $i_{kl} = i_{a_ka_l} + i_{d_ka_l}$ is augmented epistatic effect between QTL k and l , which estimates the compounded effect of aa and dd epistatic effects between QTL k and l . Coefficients x_{a_ki} and $x_{i_{kl}i}$ are determined by genotypes of the k th and l th QTL (marker) for the i th RIL line (Table 1).

Table 1 Coefficients of genetic parameters determined by marker genotypes of RIL line for the RIL-based NCIII Z_{1i} and Z_{2i} data under the F_∞ and F_2 metric models

Marker genotype	F_∞ metric model						F_2 metric model					
	Z_{1i}			Z_{2i}			Z_{1i}			Z_{2i}		
	x_{a_ki}	x_{a_li}	$x_{i_{kl}i}$	u_{d_ki}	u_{d_li}	$u_{i_{kl}i}$	x_{a_ki}	x_{a_li}	$x_{i_{kl}i}$	u_{d_ki}	u_{d_li}	$u_{i_{kl}i}$
$M_kM_kM_lM_l$	1	1	1	−1	−1	0	1	1	1	−1	−1	−1/2
$M_kM_lm_lm_l$	1	−1	0	−1	1	1	1	−1	0	−1	1	1/2
$m_km_kM_lM_l$	−1	1	0	1	−1	1	−1	1	0	1	−1	1/2
$m_km_lm_lm_l$	−1	−1	1	1	1	0	−1	−1	1	1	1	−1/2

In the same way, the phenotypic value of Z_{2i} can be described as

$$Z_{2i} = a_1 + u_{d_{1i}}d_1 + a_2 + u_{d_{2i}}d_2 + u_{a_1a_2i}i_{a_1a_2} + u_{a_1d_2i}i_{a_1d_2} + u_{d_1a_2i}i_{d_1a_2} + u_{d_1d_2i}i_{d_1d_2} + e_{2i}, \quad (4)$$

where $u_{d_{1i}}$, $u_{d_{2i}}$, $u_{a_1a_2i}$, $u_{a_1d_2i}$, $u_{d_1a_2i}$ and $u_{d_1d_2i}$ are determined by the genotype of the i th RIL line (Table S2), and e_{2i} is the residual error with an $N(0, \sigma^2)$ distribution. According to the results in Table S2, there are $u_{a_1d_2i} = u_{d_1a_2i}$ and $u_{a_1a_2i} = -u_{d_1d_2i} = -\frac{1}{2}(u_{d_{1i}} + u_{d_{2i}})$. To solve the genetic parameters, model (4) must be reduced to

$$Z_{2i} = \mu_{Z_2} + u_{d_{1i}}d_1^* + u_{d_{2i}}d_2^* + u_{i_{12i}}\tilde{i}_{12} + e_{2i}, \quad (5)$$

where $\mu_{Z_2} = a_1 + a_2$, $d_1^* = d_1 - \frac{1}{2}(i_{a_1a_2} - i_{d_1d_2})$, $d_2^* = d_2 - \frac{1}{2}(i_{a_1a_2} - i_{d_1d_2})$, $\tilde{i}_{12} = (i_{a_1d_2} + i_{d_1a_2})$ and $u_{i_{12i}} = u_{a_1d_2i} = u_{d_1a_2i}$.

If the quantitative trait was controlled by q QTL, model (5) should be extended to

$$Z_{2i} = \mu_{Z_2} + \sum_{k=1}^q u_{d_{ki}}d_k^* + \sum_{k=1}^{q-1} \sum_{l=k+1}^q u_{i_{kl}}\tilde{i}_{kl} + e_{2i}, \quad (6)$$

where the model mean $\mu_{Z_2} = \sum_{k=1}^q a_k$; $d_k^* = d_k - \frac{1}{2} \sum_{l \neq k}^q (i_{a_ka_l} - i_{d_kd_l})$ is the augmented dominance effect of QTL k , which measures the net contribution of the QTL to the midparent heterosis (MPH) [14,36]; and $\tilde{i}_{kl} = i_{a_kd_l} + i_{d_ka_l}$ is the augmented epistatic effect between QTL k and l , which estimates the compounded effect of ad and da epistatic effects between QTL k and l . Coefficients $u_{d_{ki}}$ and $u_{i_{kl}}$ are determined by the genotypes of the k th and l th QTL (marker) for the i th RIL line (Table 1).

Models (3) and (6) were working models for our QTL mapping approach in the RIL-based NCIII design under the F_∞ metric model. Model (3) can be used to estimate the augmented additive (a_k^*) and epistatic ($\tilde{i}_{kl}$) effects, and model (6) can be used to estimate the augmented dominance (d_k^*) and epistatic ($\tilde{i}_{kl}$) effects.

(ii) Statistical genetic models for mapping QTL in the RIL-based NCIII design under the F_2 metric model. The statistical genetic models for mapping QTL with RIL-based NCIII Z_{1i} and Z_{2i} data under the F_2 metric model have the same forms as those under the F_∞ metric model [Models (3) and (6)]. The detailed derivation is described in the Supplemental Information B. The comparisons of model parameter components under the F_∞ and F_2 metric model are given in Table 2.

1.3 Genetic parameter estimation

Models (3) and (6) can be uniformly written as

$$y_i = \mu + \sum_{k=1}^q x_{ki}g_k + \sum_{k=1}^{q-1} \sum_{l=k+1}^q x_{kli}i_{kl} + e_i = \mu + \sum_{j=1}^p z_{ji}\gamma_j + e_i, \quad (7)$$

where μ is the model mean; g_k is the augmented main effect of the k th QTL; i_{kl} is the augmented epistatic effect between the k th and l th QTL; $p = \frac{1}{2}q(q+1)$ is the total number of genetic effects, including the augmented main and epistatic effects; and $e_i \sim N(0, \sigma^2)$ is the residual error.

The true number of QTL (q) is an import parameter in model (7) and usually determined by two alternative strategies, i.e. model selection and shrinkage estimation. The latter includes the fully Bayesian [37], the penalized maximum likelihood [38,39], least absolute shrinkage and selection operator, and empirical Bayes (E-Bayes) [32,40]. The fully Bayesian method should be optimal in theory because it captures the most information from the data, but it is computationally intensive; the penalized maximum likelihood is an approximation to the fully Bayesian approach, while its main advantages over other methods are reflected by its simplicity and fast speed; least absolute shrinkage and selection operator is a good approach but the shrinkage is too weak for spurious effects and too strong for large effects; and the E-Bayes provides the optimal estimates of variance components, because shrinkage is very selective, with large effects subject to virtually no shrinkage while small effects are shrunk to zero [40]. Simulation studies have shown that the E-Bayes is the best among the above approaches in terms of small mean squares error [40]. Thus, we adopted the E-Bayes method of Xu [32] for the estimation of parameters

Table 2 Model parameter components for Z_{1i} and Z_{2i} in the RIL-based NCIII under the F_∞ and F_2 metric models

Data	Model	F_∞ metric model			F_2 metric model		
		Model mean	Main effect	Epistatic effect	Model mean	Main effect	Epistatic effect
Z_1	(3)	$\mu_{Z_1} = 2\mu + \sum_{k=1}^q d_k$	$a_k^* = a_k + \frac{1}{2} \sum_{l \neq k}^q (i_{a_kd_l} - i_{d_ka_l})$	$\tilde{i}_{kl} = i_{a_ka_l} + i_{d_ka_l}$	$\mu_{Z_1} = 2\mu - \frac{1}{2} \sum_{k=1}^{q-1} \sum_{l=k+1}^q i_{d_kd_l}$	$a_k^* = a_k - \frac{1}{2} \sum_{l \neq k}^q i_{d_ka_l}$	$\tilde{i}_{kl} = i_{a_ka_l} + i_{d_ka_l}$
Z_2	(6)	$\mu_{Z_2} = \sum_{k=1}^q a_k$	$d_k^* = d_k - \frac{1}{2} \sum_{l \neq k}^q (i_{a_ka_l} - i_{d_ka_l})$	$\tilde{i}_{kl} = i_{a_ka_l} + i_{d_ka_l}$	$\mu_{Z_2} = \sum_{k=1}^q a_k$	$d_k^* = d_k - \frac{1}{2} \sum_{l \neq k}^q i_{a_ka_l}$	$\tilde{i}_{kl} = i_{a_ka_l} + i_{d_ka_l}$

in the above model. The approach assumes that there is one QTL standing on each marker throughout the genome and shrinks the genetic effects of all “nonsignificant” QTL toward zero. For the technical details of the method, refer to the original study by Xu [32] and its application in our research [41].

1.4 Likelihood ratio test

The usual likelihood ratio test (LRT) cannot be carried out with the E-Bayes method owing to an oversaturated epistatic genetic model. We proposed the following two-stage selection process to screen the markers [38]. In the first stage, all markers with $t_j = |\hat{\gamma}_j|/\hat{\sigma}_j > 2.0$, where $\hat{\sigma}_j$ estimate of standard deviation for normal prior $\gamma_j \sim N(0, \sigma_j^2)$, are picked up. In the second stage, the epistatic genetic model is modified so that only effects past the first round of selection are included in the model. Owing to the smaller dimensionality of the reduced model, we can use the maximum likelihood method to re-analyze the data and perform the LRT [38]. The test statistic is

$$LR_j = -2[L(\theta_{-j}) - L(\theta)], \quad (8)$$

where θ is the parameters vector in the statistical genetic model in the second stage analysis of model (7); θ_{-j} is the parameters vector in θ excluding the currently tested genetic effect γ_j ; $L(\theta)$ and $L(\theta_{-j})$ are the log maximum likelihood function for θ and θ_{-j} , respectively.

2 Monte Carlo simulation studies

The purpose of the simulation experiment was: (1) to evaluate the statistical performance of the proposed approach, and (2) to compare the RIL-based NCIII design with the F₂-based NCIII design [41].

The simulated genome was 1000.0 cM in total length and covered by 210 markers (10 chromosomes, each covered with twenty-one 5.0 cM equally spaced markers). Ten QTL were assumed, of which four (QTL₁, QTL₂, QTL₃ and QTL₆) had only main effects, and the other six had pairwise epistatic effects (QTL₄ and QTL₇, QTL₅ and QTL₈, and QTL₉ and QTL₁₀ interacted each other, respectively) (Tables 3–6 and S5–S8). Two independent QTL (QTL₁ and QTL₂) and two interactive QTL (QTL₉ and QTL₁₀) were in linkage disequilibrium, respectively, and the other six QTL were located on different linkage group. The pure main and epistatic effects under both the F_∞ and the F₂ metric models were set as the same. The environmental variance was set at 6.0. Under the F_∞ (or F₂) metric model, the heritabilities for the ten main QTL varied from 0.00 to 12.69% (0.00 to 13.55%) in the L₁ population and from 0.00 to 5.44% (0.00 to 6.07%) in the L₂ population; those for the three epistatic QTL were 0.51, 7.31, and 0.00% (0.14, 4.10 and 0.00%) in

the L₁ population, and 15.70, 7.83, and 4.98% (23.77, 2.18 and 9.47%) in the L₂ population; the total heritabilities explained by all the main and epistatic QTL were 51.28 and 42.02% (47.95 and 35.26%) in the above two populations, respectively (Table S4). Judged by pure main effects, QTL₃ and QTL₁₀ were additive; QTL₁ and QTL₄ were partially dominant; QTL₂ was completely dominant; QTL₆, QTL₇ and QTL₉ were overdominant; and QTL₅ and QTL₈ had no pure main effects (Tables 3, 5, S5 and S7). While judged by augmented main effects under the F_∞ metric model, QTL₅ and QTL₈ turned to complete dominance; the other eight QTL had the same types as the original ones, although degrees of dominance for some QTL (QTL₄ and QTL₇) changed (Tables 3 and 5). However, when judged by augmented main effects under the F₂ metric model, QTL₅ and QTL₈ became complete dominance and overdominance, respectively; QTL₄ changed from partial dominance to overdominance, and on the contrary, QTL₇ changed from overdominance to partial dominance; QTL₉ and QTL₁₀ turned to complete dominance from overdominance and additive, respectively; and the other four independent QTL had the same types as the original ones (Tables S5 and S7). The alteration of dominant mode for those interactive QTL was due to the existence of epistatic effects. The sample size (n), the family number of the RIL population, was set at two levels: 300 and 500. The replication number (m) for each NCIII family was set at 5 and 10. Each treatment was replicated 200 times. With Z_1 or Z_2 210 augmented additive or dominance effects (a_k^* or d_k^*) and $\frac{1}{2} \times 210 \times (210 - 1) = 21945$ augmented epistatic effects ($\tilde{t}_{kl}$ or $\tilde{t}_{kl}^*$) were included in the same one model and estimated simultaneously by empirical Bayes. All effects with $t_j = |\hat{b}_j|/\hat{\sigma}_j > 2.0$ were picked up. All the screened effects were re-estimated by the maximum likelihood method and tested by LRT. For each simulated QTL, we counted the samples in which the LOD statistic was greater than 3.0 and the identified QTL was within 20.0 cM of the simulated QTL. The estimate for QTL parameter was the average of the corresponding estimates in the counted samples. The ratio of the number of such samples to the total number of replicates represented the empirical power of this QTL.

To achieve the first objective of the simulation experiment, all the samples were analyzed by the proposed approach in this study. The mapping results under the F_∞ metric model were presented in Tables 3 and 4 for $n=300$ and Tables 5 and 6 for $n=500$. The results show that all the augmented main and epistatic effects were rightly and unbiasedly estimated (Tables 3–6). All of the main-effect QTL were identified with a high statistical power and precision of estimated effects and positions. The statistical powers in the detection of augmented additive and dominant effects (a_k^* and d_k^*) and precisions of its effects were comparable when they had equivalent size (Tables 3 and 5). The epi-

static QTL (QTL₄×QTL₇ and QTL₉×QTL₁₀) were also very well mapped for all simulated situations when the augmented epistatic effects consisted of two pure epistatic effects in the same sign (Tables 4 and 6). The power for uncovering epistatic QTL (QTL₅×QTL₈), of which the augmented epistatic effects consisted of two pure epistatic effects in opposite directions, were very low (25.0% for $\tilde{i}_{58}$ and 28.5% for $\tilde{i}_{58}$) when both sample size and replication number were at low levels ($n=300$, $m=5$); increased to satisfactory values (63.0% to 69.5%) when either sample size or replication number was at high level [$(n=300$ and $m=10)$ or $(n=500$ and $m=5)$]; and rose to high quantities (96.0% and 97.0%) when both sample size and replication number were at a high level ($n=500$ and $m=10$) (Tables 4 and 6). The statistical powers for detection of the two types of augmented epistatic effects ($\tilde{i}_{kl}$ and $\tilde{i}_{kl}$) and precisions of its effects were also comparable when they had equal size (Tables 4 and 6). The detection of the augmented epistatic effects ($\tilde{i}_{kl}$ and $\tilde{i}_{kl}$) was relatively more difficult than that of the augmented main effects (a_k^* and d_k^*), especially when they had small effects (Tables 3–6). The time costs were about 2.30 and 4.90 h per sample on our computer (CPU: Intel® Core™ 2 DUO 3.0G, Memory: 2.0G) for sample size 300 and 500, respectively.

The results under the F_2 metric model are listed in Tables S5 and S6 for $n=300$ and Tables S7 and S8 for $n=500$. The general conclusions were similar to those under the F_∞ metric model, although the augmented main effects (a_k^* and d_k^*) with the interactive QTL under the two models were different. In addition, the false-positive rates (FPR), the ratio of the number of false-positive effects to the total number of zero effects considered in models (3) and (6), were low (1×10^{-4} to 2×10^{-4} mostly, Table S9).

To achieve the second objective of the simulation experiment, the above results were compared with results in He and Zhang [41]. In He and Zhang [41], the parameter settings in the F_2 -based NCIII design were similar to those in this study. The results showed that the RIL-based NCIII design was superior to the F_2 -based NCIII design for mapping heterotic QTL. Although the augmented main effects (a^* and d^*) were all unbiasedly estimated with satisfactory powers and the augmented epistatic effects ($\tilde{i}$ and $\tilde{i}$) between QTL₄ and QTL₇, which consisted of two epistatic effects with the same sign, were also well mapped. The augmented epistatic effects ($\tilde{i}_{58}$ and $\tilde{i}_{58}$ between QTL₅ and QTL₈), which consisted of two epistatic effects of equal strength in opposite directions, were very poorly mapped, even though both sample size and replication number were at high levels ($n=500$ and $m=10$), i.e., the powers in the detection of $\tilde{i}_{58}$ and $\tilde{i}_{58}$ in the F_2 -based NCIII design were 0.50 and 2.00% under the F_∞ metric model, and 0.50 and

18.5% under the F_2 metric model [41].

Assuming that a quantitative trait was controlled by only two QTL with digenic epistasis and arbitrary linkage, the genetic variances of Z_{1i} and Z_{2i} among the RIL-based NCIII families have the same expression under the F_∞ and F_2 metric models when the symbols of augmented effects are used (Supplement Information A),

$$\sigma_{Z_1}^2(\text{RIL}) = (a_1^*)^2 + (a_2^*)^2 + R(1-R)\tilde{i}_{12}^2 \\ + 2(1-2R)a_1^*a_2^* \stackrel{r=R=0.5}{=} (a_1^*)^2 + (a_2^*)^2 + \frac{1}{4}\tilde{i}_{12}^2,$$

$$\sigma_{Z_2}^2(\text{RIL}) = (d_1^*)^2 + (d_2^*)^2 + R(1-R)\tilde{i}_{12}^2 \\ + 2(1-2R)d_1^*d_2^* \stackrel{r=R=0.5}{=} (d_1^*)^2 + (d_2^*)^2 + \frac{1}{4}\tilde{i}_{12}^2,$$

where $R=2r/(1+2r)$ and r is the recombination fraction between the two loci. Similarly, the genetic variances of Z_{1i} and Z_{2i} among the F_2 -based NCIII families also have the same expression under the F_∞ and F_2 metric models,

$$\sigma_{Z_1}^2(F_2) = \frac{1}{2}(a_1^*)^2 + \frac{1}{2}(a_2^*)^2 + \frac{1}{4}r(2-5r+4r^2)\tilde{i}_{12}^2 \\ + (1-2r)a_1^*a_2^* \stackrel{r=0.5}{=} \frac{1}{2}(a_1^*)^2 + \frac{1}{2}(a_2^*)^2 + \frac{1}{16}\tilde{i}_{12}^2,$$

$$\sigma_{Z_2}^2(F_2) = \frac{1}{2}(d_1^*)^2 + \frac{1}{2}(d_2^*)^2 + \frac{1}{4}r(2-5r+4r^2)\tilde{i}_{12}^2 \\ + (1-2r)d_1^*d_2^* \stackrel{r=0.5}{=} \frac{1}{2}(d_1^*)^2 + \frac{1}{2}(d_2^*)^2 + \frac{1}{16}\tilde{i}_{12}^2.$$

These variance formulae were helpful for explaining the above results.

3 Discussion

NCIII design is widely used to infer the genetic basis of heterosis for many economically important traits. However, previous studies have shown that a conclusion on the genetic basis of heterosis depends on a statistical approach adopted by [8,16,17,36]. Therefore, the development and choice of appropriate methodology for NCIII design are critical. In Stuber et al. [19] and Xiao et al. [22], the separate analysis on each backcross population actually estimated d^*+a^* or d^*-a^* [11,14,16]. To overcome this issue, Cockerham and Zeng [11] separated the additive and dominance effects in their combined analysis. However, their single marker analysis method could not separate multiple linked gene effects and did not provide exact location of the QTL [11,16]. Based on this situation, Lu et al. [12] and Ledeaux et al. [28] developed the use of composite interval mapping [27] on each backcross separately and, after QTL are mapped in one or both backcrosses, a^* and d^* effects were estimated by a linear combination of the contrasts for each backcross according to the derivations of Cockerham and Zeng [11]. Although a^* and d^* effects can be indirectly estimated, the results of QTL mapping are still based on the analysis of each backcross separately in a similar way to

that of Stuber et al. [19]. The extended CIM analysis on progeny mean values for pair means and pair differences, which correspond to contrasts C_1 and C_3 of Cockerham and Zeng [11], suggested by Melchinger et al. [14], not only can estimate a^* and d^* directly, but also can eliminate the influence of other linked QTL. However, the augmented epistatic effects ($\tilde{i} = i_{aa} + i_{dd}$ and $\tilde{j} = i_{ad} + i_{da}$) are not included in the model of Melchinger et al. [14]. This avoidance would result in a larger residual variance, further a lower power of QTL detection and poorer precision (little larger in standard deviation) in estimated QTL effects and positions [41]. The two dimensional scanning methods, which consider both main and epistatic effects of pair QTL simultaneously with genetic background controlling, such as the mixed model approach of Wang et al. [23] and inclusive composite interval mapping (ICIM) of Li et al. [42], are alternative methods. However, the genetic design matrix should be reconstructed (Table 1) and the genetic parameter should be correctly explained [14–16,24,30,41]. Although the multiple interval mapping in Garcia et al. [16] can estimate the augmented main and epistatic effects simultaneously, it differs from our method in two aspects. First, Garcia et al. [16] adopt a model selection strategy, such as AIC criteria, to search the final model, whereas we fit all markers on the whole genome simultaneously. As a result, the latter may capture the best model more easily than the former. Second, the former uses orthogonal contrasts and the original observations, and we take advantage of linear regression and the two classical transformations.

The proposed approach is similar to He and Zhang's [41] method in spirit, but we extend He and Zhang's [41] method from an F_2 -based triple testcross (TTC) design to a RIL-based NCIII design. Models (3) and (6) can also be used for the NCIII design derived from other base populations, such as DH, F_2 , BC and F_2 Syn3, after the genetic design matrix (Table 1) was appropriately reconstructed. For a quantitative trait determined by two unlinked QTL, the genetic variances among NCIII families on Z_{1i} [Z_{2i}] are $(a_1^*)^2 + (a_2^*)^2 + \frac{1}{4}\tilde{i}_{12}^2$ [$(a_1^*)^2 + (a_2^*)^2 + \frac{1}{4}\tilde{i}_{12}^2$] and $\frac{1}{2}(a_1^*)^2 + \frac{1}{2}(a_2^*)^2 + \frac{1}{16}\tilde{i}_{12}^2$ [$\frac{1}{2}(a_1^*)^2 + \frac{1}{2}(a_2^*)^2 + \frac{1}{16}\tilde{i}_{12}^2$] for RIL and F_2 , respectively. The coefficients for genetic variance components of the augmented main effects are two times larger for RIL than for F_2 ; and it becomes four times when for augmented epistatic effects. Therefore, the RIL-based NCIII design is much more powerful in the detection of main, and especially epistatic QTL, than in the F_2 -based NCIII design. The conclusion is similar to that in Schön et al. [17] and is also confirmed by our simulated experiments. For example, in the detection of $QTL_5 \times QTL_8$ interaction that consists of two epistatic effects in opposite directions, the power is satisfactory when either sample size or replication number is at a high level [($n=300$ and $m=10$) or ($n=500$ and $m=5$)] in the RIL-based NCIII design (Tables 4 and 6), and is ex-

tremely low even when both sample size and replication number are at high levels ($n=500$ and $m=10$) in the F_2 -based NCIII design. The result helps us to choose an optimal base population in their NCIII design.

The augmented additive effect (a_k^*) equals to $a_k + \frac{1}{2}\sum_{l \neq k}^q (i_{a_k d_l} - i_{d_k a_l})$ [$a_k - \frac{1}{2}\sum_{l \neq k}^q i_{d_k a_l}$] and the augmented dominant effect (d_k^*) equals to $d_k - \frac{1}{2}\sum_{l \neq k}^q (i_{a_k a_l} - i_{d_k d_l})$ [$d_k - \frac{1}{2}\sum_{l \neq k}^q i_{a_k a_l}$] under the F_∞ [F_2] metric model (Table 2).

In these augmented main effects, the pure main effects (a_k and d_k) are confounded with pure epistatic interactions ($i_{a_k d_l}$, $i_{d_k a_l}$, $i_{a_k a_l}$ and $i_{d_k d_l}$). However, the augmented additive and dominant effects just precisely measure the net contribution of the k -th QTL to parental difference and midparent heterosis; this is the advantage of the NCIII design [14]. However, the relative contribution of the pure main effects cannot be assessed independently from the sum of epistatic interactions of QTL k with the genetic background, and this is a limitation of the NCIII design [14,36]. Likewise, the pure epistatic effects ($i_{a_k a_l}$ and $i_{d_k d_l}$, $i_{a_k d_l}$ and $i_{d_k a_l}$) between QTL k and l are also confounded ($\tilde{i}_{kl} = i_{a_k a_l} + i_{d_k d_l}$ and $\tilde{j}_{kl} = i_{a_k d_l} + i_{d_k a_l}$) and cannot be individually estimated. If $i_{a_k a_l}$ and $i_{d_k d_l}$ ($i_{a_k d_l}$ and $i_{d_k a_l}$) have the same sign, the augmented epistatic effect $\tilde{i}_{kl}$ ($\tilde{j}_{kl}$) will be inflated and can be more easily detected; if the two componential pure epistatic effects have like magnitude and opposite signs, the augmented epistatic effects will be weak and can be hardly detected [16](Tables 4, 6, S6 and S8 in this study). Fortunately, the RIL-based NCIII design has relatively high power for detecting $\tilde{i}_{kl}$ and $\tilde{j}_{kl}$. By increasing sample size and/or replication number, we still have considerable power to identify $\tilde{i}_{kl}$ and $\tilde{j}_{kl}$, which consisted of two epistatic effects of equal strength in opposite directions (Tables 4, 6, S6 and S8). To dissect the augmented main and epistatic effects, a special mating design, such as the TTC design, will be needed. In the TTC design, the F_1 is added as a third tester to produce the L_{3i} family and the third linear transformation $Z_{3i} = \bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i}$ is constructed. Under the F_2 metric model, Melchinger et al. [36] suggested that one-dimensional genome scans on L_{3i} and Z_{3i} can be adopted for separately estimating a_k and $\sum_{l \neq k}^q i_{d_k a_l}$ in a_k^* ; two-way ANOVA for marker interactions with L_{3i} can be used to acquire $i_{a_k a_l}$ and these $i_{a_k a_l}$ can be further used for dissection of heterotic-effect d_k^* . Under both the F_∞ and F_2 metric models, He and Zhang [41] developed a two-step approach to completely dissect pure main and epistatic effects of QTL in the F_2 -based TTC

Main effect QTL	True value				Estimate											
	Pure effects		Augmented effects		$m=5^u$				$m=10$							
	a	d	a^*	d^*	Z_1		Z_2		Z_1		Z_2					
	Posi. (cM)															
QTL ₁	30.00	1.00	-0.50	1.00	-0.50	0.956 (0.106)	29.966 (0.394)	1.000	-0.474 (0.088)	29.889 (2.988)	0.900	0.980 (0.076)	30.000 (0.000)	-0.476 (0.073)	29.867 (1.558)	0.995
QTL ₂	75.00	-1.00	1.00	-1.00	1.00	-0.969 (0.118)	75.000 (0.000)	1.000	0.966 (0.109)	74.993 (0.237)	1.000	-0.980 (0.067)	75.000 (0.000)	0.975 (0.074)	75.024 (0.228)	1.000
QTL ₃	150.00	0.80	0.00	0.80	0.00	0.778 (0.107)	150.033 (1.017)	0.990	.	.	.	0.796 (0.075)	149.996 (0.357)	.	.	.
QTL ₄	235.00	1.50	-1.00	1.75	-0.75	1.714 (0.111)	234.991 (0.238)	1.000	-0.714 (0.109)	234.978 (1.297)	1.000	1.720 (0.072)	235.005 (0.072)	-0.736 (0.070)	234.999 (0.351)	1.000
QTL ₅	465.00	0.00	0.00	0.90	-0.90	0.872 (0.104)	465.013 (0.404)	1.000	-0.873 (0.105)	465.030 (0.714)	1.000	0.892 (0.076)	464.972 (0.446)	-0.892 (0.070)	465.020 (0.270)	1.000
QTL ₆	555.00	0.50	1.00	0.50	1.00	0.480 (0.097)	554.722 (2.515)	0.940	0.977 (0.107)	555.003 (0.413)	0.995	0.478 (0.066)	555.178 (1.467)	0.975 (0.068)	554.982 (0.213)	1.000
QTL ₇	675.00	-1.00	1.50	-1.25	1.75	-1.236 (0.109)	674.959 (0.416)	1.000	1.708 (0.096)	675.006 (0.156)	1.000	-1.225 (0.069)	674.968 (0.197)	1.719 (0.072)	674.998 (0.030)	1.000
QTL ₈	740.00	0.00	0.00	-0.90	-0.90	-0.886 (0.101)	740.092 (1.317)	1.000	-0.862 (0.102)	740.128 (0.762)	0.995	-0.875 (0.074)	739.987 (0.221)	-0.885 (0.068)	739.993 (0.318)	1.000
QTL ₉	830.00	0.00	-0.75	0.00	-0.75	.	.	.	-0.722 (0.105)	829.903 (1.237)	0.990	.	.	-0.735 (0.067)	830.021 (0.376)	1.000
QTL ₁₀	870.00	-0.75	0.00	-0.75	0.00	-0.732 (0.107)	869.848 (1.931)	0.985	.	.	.	-0.745 (0.065)	869.983 (0.404)	.	.	.

Table 4 Simulated and estimated epistatic QTL positions and effects for imitated real data in the RIL-based NCIII design under the F_{∞} metric model (200 replications)

	True value						Estimate									
	Posi. A (cM)		Posi. B (cM)		Pure effects		Augmented effects		$m=5^{(1)}$				$m=10$			
	i_{aa}	i_{ad}	i_{da}	i_{dd}	i_{adt}	$\tilde{i}$	$\tilde{i}$	Z_1	Z_2	Z_1	Z_2	Z_1	Z_2	Z_1	Z_2	
	i_{aa}	i_{ad}	i_{da}	i_{dd}	i_{adt}	$\tilde{i}$	$\tilde{i}$	Posi. A	Posi. B	Power	$\tilde{i}$	Posi. A	Posi. B	Power	$\tilde{i}$	
Epistatic QTL																
QTL ₄ ×QTL ₇	235.00	675.00	1.00	1.50	1.00	1.50	2.50	2.432 (0.213)	675.023 (0.315)	1.000	2.453 (0.228)	234.960 (0.462)	674.985 (0.437)	1.000	2.465 (0.153)	
QTL ₅ ×QTL ₈	465.00	740.00	1.20	0.60	-1.20	-0.60	0.60	0.778 (0.122)	740.316 (7.255)	0.250	-0.758 (0.128)	464.737 (6.507)	741.579 (8.460)	0.630	-0.618 (0.111)	
QTL ₉ ×QTL ₁₀	830.00	870.00	-0.75	-0.75	-0.75	-1.50	-1.50	-1.454 (0.237)	870.378 (2.348)	1.000	-1.479 (0.238)	829.844 (1.516)	869.874 (1.225)	1.000	-1.476 (0.161)	

a) m denotes family replication number. The numbers in parentheses are standard deviation. Sample size was 300.

design. In the first step, with Z_1 and Z_2 the augmented main and epistatic effects were estimated, and with Z_3 three pure epistatic effects were obtained. In the second step, the three pure epistatic effects obtained in the first step were integrated with the augmented epistatic and main effects for the further estimation of all other pure effects.

Although the exploitation of heterosis in plant breeding is very successful, its genetic basis has been debated for more than 100 years and is still not completely resolved [12,19,30]. The genetic analysis of QTL can provide both molecular and practical understanding of this key phenomenon [8,15,19,28]. It should be noted that not all QTL detected in these studies contribute to trait heterosis [14,25,43]. In fact, two ways are tried to infer heterotic loci. The first one is based on the type of gene action. The additive QTL do not contribute to heterosis, while the non-additive QTL, including dominant, overdominant, and epistatic do contribute [12,15,19,22,24,25,28]. The second one is to use the measurements of heterosis as the data input to perform QTL analysis [13–15,24–26,30,43]. QTL effects for Z_1 and Z_2 in the RIL-based NCIII design provide estimates of the augmented effects a^* , which measures the net contribution of the QTL to the parental difference, and d^* , which measures the net contribution of the QTL to midparent heterosis [14]. Thus, QTL detected in the analysis of Z_2 data may be directly associated with trait heterosis, and the augmented dominance ratio ($ADR=d^*/|a^*|$) may measure the type of heterotic QTL.

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Supporting Information

Supplemental Information A The expected genetic values of Z_{1i} and Z_{2i} under the F_∞ and the F_2 metric models in the RIL-based NCIII design.

Table S1 Genetic constitutions of L_{1i} and L_{2i} families derived from the RIL-based NCIII design

Table S2 Expected genetic values of $Z_{1i} = \bar{L}_{1i} + \bar{L}_{2i}$ and $Z_{2i} = \bar{L}_{1i} - \bar{L}_{2i}$ under the F_∞ metric model in the RIL-based NCIII design

Table S3 Expected genetic values of $Z_{1i} = \bar{L}_{1i} + \bar{L}_{2i}$ and $Z_{2i} = \bar{L}_{1i} - \bar{L}_{2i}$ under the F_2 metric model in the RIL-based NCIII design

Supplemental Information B Statistical genetic models for mapping heterotic QTL in the RIL-based NCIII design under the F_2 metric model.

Table S4 Heritability (%) of each main and epistatic QTL for L_1 and L_2 populations in the simulated RIL-based NCIII design under both the F_∞ and F_2 metric models

Table S5 Simulated and estimated main-effect QTL positions and effects for imitated real data in the RIL-based NCIII design under the F_2 metric model (200 replications)

Table S6 Simulated and estimated epistatic QTL positions and effects for imitated real data in the RIL-based NCIII design under the F_2 metric model (200 replications)

Table S7 Simulated and estimated main-effect QTL positions and effects for imitated real data in the RIL-based NCIII design under the F_2 metric model (200 replications)

Table S8 Simulated and estimated epistatic QTL positions and effects for imitated real data in the RIL-based NCIII design under the F_2 metric model (200 replications)

Table S9 False positive rate ($\times 10^{-4}$) for the analysis of Z_1 and Z_2 datasets in the simulated RIL-based NCIII design under both the F_∞ and F_2 metric models

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