Theor Appl Genet (2010) 120:321–332
DOI 10.1007/s00122-009-1209-9

ORIGINAL PAPER

High congruency of QTL positions for heterosis of grain yield in three crosses of maize

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Received: 10 August 2009 / Accepted: 22 October 2009 / Published online: 13 November 2009

Abstract The genetic basis of heterosis in maize has been investigated in a number of studies but results have not been conclusive. Here, we compare quantitative trait loci (QTL) mapping results for grain yield, grain moisture, and plant height from three populations derived from crosses of the heterotic pattern Iowa Stiff Stalk Synthetic × Lancaster Sure Crop, investigated with the Design III, and analyzed with advanced statistical methods specifically developed to examine the genetic basis of mid-parent heterosis (MPH). In two populations, QTL analyses were conducted with a joint fit of linear transformations Z_1 (trait mean across pairs of backcross progenies) and Z_2 (half the trait difference between pairs of backcross progenies) to estimate augmented additive and augmented dominance effects of each QTL, as well as their ratio. QTL results for the third population were obtained from the literature. For Z_2 of grain yield, congruency of QTL positions was high across populations, and a large proportion of the genetic variance (~70%) was accounted for by QTL. This was not the case for Z_1 or the other two traits. Further, almost all congruent grain yield QTL were located in the same or an adjacent bin encompassing the centromere. We conclude that different alleles have been fixed in each heterotic pool, which in combination with allele(s) from the opposite heterotic pool lead to high MPH for grain yield. Their positive interactions very likely form the base line for the superior performance of the heterotic pattern under study.

Communicated by H. Becker.

Contribution to the special issue "Heterosis in Plants".

Electronic supplementary material The online version of this article (doi:10.1007/s00122-009-1209-9) contains supplementary material, which is available to authorized users.

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Introduction

The superiority of a hybrid over the mean of its two homozygous parents is referred to as heterosis or hybrid vigor (Shull 1908). The commercial exploitation of heterosis through the development and cultivation of hybrid cultivars was a landmark development in modern plant breeding. It started in maize (*Zea mays* L.) (Shull 1909) and has expanded over time to a large number of (partially) allogamous and even autogamous crops (for review see Coors and Pandey 1999). Despite its overwhelming importance in plant and animal breeding, the genetic basis of this biological phenomenon is still not well understood.

Three main hypotheses have been put forward to explain the genetic causes underlying heterosis. The dominance hypothesis attributes heterosis to the cumulative effect of favorable alleles with partial or complete dominance (Davenport 1908; Bruce 1910; Jones 1917). However, repulsion phase linkages of such genes may lead to pseudo-overdominance. The overdominance hypothesis assumes superiority of heterozygous genotypes over both parental homozygous genotypes (Hull 1945; Crow 1948). The epistasis hypothesis explains heterosis on the basis of interactions among genes at different loci (Powers 1944;

Jinks and Jones 1958; Williams 1959). During the twentieth century, extensive experimental data were collected on yield traits in maize and the magnitude of additive, dominance, and epistatic gene effects and variances have been estimated. Results were inconsistent, but in summary most experiments supported the hypothesis that heterosis is attributable to the cumulative action of a large number of favorable dominant genes (Hallauer and Miranda 1981).

A more profound understanding of the genetic basis of heterosis was expected from marker-aided genetic analyses, because quantitative trait loci (QTL) mapping can be employed to elucidate the genetic basis of heterosis in the same manner as molecular markers have proven useful in identifying QTL involved in the expression of complex traits. The first marker-aided experimental study on heterosis in plants was conducted by Stuber et al. (1992) on F_3 plants of the hybrid B73 × Mo17 backcrossed to each parental inbred. This corresponds to the Design III mating scheme devised by Comstock and Robinson (1952), which is to date one of the most powerful and widely used designs for the genetic analysis of heterosis. Using single-marker ANOVA and interval mapping of QTL and conducting separate analyses of each series of backcross (BC) progenies, they concluded that overdominance (or pseudo-overdominance) was the major cause of heterosis in grain yield and that there was little evidence for epistasis. Since the publication of their results, quantitative genetic theory for the analysis of heterosis with the Design III has evolved. Cockerham and Zeng (1996) developed statistical theory for simultaneous QTL mapping with both series of BC progenies in the Design III. Using single-marker analyses, they demonstrated that estimates for additive and dominance effects of a QTL are confounded with different types of epistatic effects. From a re-analysis of the data of Stuber et al. (1992), they concluded a preponderance of dominance of favorable genes, although epistasis among linked loci could also have played an important role.

Melchinger et al. (2007) developed genotypic expectations for mid-parent heterosis (MPH) and QTL mapped with the Design III progenies, which they termed augmented additive (a_i*) and augmented dominance (d_i*) effects. Linking the quantitative genetic theory for MPH with genotypic expectations of QTL effects obtained with composite interval mapping (CIM), they showed that, under digenic epistasis, MPH exactly corresponds to the sum of d_i* effects over all QTL. Using the theoretical results of Cockerham and Zeng (1996) and Melchinger et al. (2007), Garcia et al. (2008) performed multiple interval mapping (MIM) of QTL using the Design III data of Stuber et al. (1992). While their results revealed mainly d_i* effects, they found that even with the same experimental data, different statistical methods (single-marker ANOVA, CIM, and MIM, as well as the separate analysis of the two BC series) can lead to different conclusions regarding the predominant type of gene action contributing to heterosis.

In general, despite numerous marker-aided studies on the genetic basis of heterosis in maize (Stuber et al. 1992; Cockerham and Zeng 1996; Graham et al. 1997; Lu et al. 2003, LeDeaux et al. 2006; Frascaroli et al. 2007, 2009; Garcia et al. 2008), results have not been conclusive. Frequently, the comparison across studies is confounded by differences in experimental design, genetic material, or statistical methods used for data analysis. In this study, we compare the results from three experimental studies on heterosis in maize (Stuber et al. 1992; Lu et al. 2003; Frascaroli et al. 2007). In each of the three studies, a single cross between two elite inbreds was investigated using the Design III. Moreover, the heterotic pattern of the crosses was the same in all three studies, i.e., Iowa Stiff Stalk Synthetic × Lancaster Sure Crop (BSSS × LSC). To characterize the mode of gene action contributing to the manifestation of MPH and assess congruency of results across studies, estimates of a_i* and d_i* effects were re-estimated from the original data of Stuber et al. (1992) and Lu et al. (2003) and compared with each other and with the results of Frascaroli et al. (2007). The specific objectives of this study were to (i) identify QTL contributing to MPH for three agronomically important traits in two single crosses of maize by applying the methods of Melchinger et al. (2007), (ii) assess the congruency of the results across three single crosses belonging to the same heterotic pattern, and (iii) draw conclusions about the number and genome-wide distribution of QTL contributing to MPH in maize.

Materials and methods

Plant materials and experimental designs

Our study is based on phenotypic data for grain yield (GY), grain moisture (GM), and plant height (PH) as well as genotypic data generated in three earlier studies in maize by Stuber et al. (1992), Lu et al. (2003), and Frascaroli et al. (2007), who each investigated an elite single cross between inbreds of the major heterotic pattern BSSS × LSC. Experimental details of these studies are summarized in Supplementary Table S1. Stuber et al. (1992) selfed F_3 plants of hybrid B73 × Mo17 (Pop1) and simultaneously crossed each plant with both parent lines according to a Design III mating scheme to develop 264 pairs of BC progenies. The BC progenies were field evaluated at six locations in the USA with one replication at each location. The genotype of each F_3 plant was determined from its F_4 line for 76 markers. Stuber et al. (1992) may be seen for details.

In the Lu et al. (2003) study, a Design III experiment was performed with hybrid LH200 × LH216 (Pop2). The F_2 generation was intermated three times by chain sibbing to produce the F_2Syn3 population. Individual plants from F_2Syn3 were crossed to the two parental inbreds. The 351 pairs of BC progenies were field evaluated at five locations in USA with one replication per location. For genotyping, the 351 F_2Syn3 parental plants were assayed with 160 markers. For further details see Lu et al. (2003).

Frascaroli et al. (2007) based their study on hybrid B73 × H99 (Pop3). A total of 142 recombinant inbred lines (RILs), derived from this hybrid through 12 selfing generations, were crossed with both parents and the F_1 hybrid following a triple test cross design (Kearsey and Jinks 1968), which is an extension of the Design III. The RILs were genotyped and 158 loci were mapped in the genetic linkage map. Phenotyping was conducted at three locations in Italy with two replications per location. Further details may be seen in Frascaroli et al. (2007).

Statistical analysis of the phenotypic data

Taking H_1 and H_2 to be the phenotypic observations on BC progenies of each F_3 or F_2Syn3 plant with the parental inbreds P1 and P2, respectively, at each location, two linear transformations Z_s ($s = 1, 2$) were computed as $Z_1 = (H_1 + H_2)/2$ (trait mean across each pair of BC progenies) and $Z_2 = (H_1 - H_2)/2$ (half the trait difference between each pair of BC progenies) in Pop1 and Pop2 as described by Melchinger et al. (2007). Backcross progenies of B73 and LH216 were considered as H_2 because their population means were greater than for BC progenies with Mo17 and LH200, respectively, for all traits resulting in a positive effect of the dominant allele in the parent with higher trait expression (B73 and LH216) except for PH in Pop2, in which case the opposite was true. All subsequent biometric and quantitative genetic analyses were performed with values of Z_1 and Z_2 for each parental F_3 or F_2Syn3 plant at each location. For the RILs from the cross B73 × H99 in Pop3, linear transformation Z_1 is given as ½ "SUM" and linear transformation Z_2 as ½ "DIFF" in Frascaroli et al. (2007). Analyses of variance and covariance over locations were performed assuming a random effects model. Estimates of the genotypic (σ_g^2) and residual error (σ_e^2) variances for Z_1 and Z_2, and their corresponding standard errors as well as phenotypic (r_p), and genotypic (r_g) correlation coefficients between Z_1 and Z_2 were computed for each trait following standard procedures (Mode and Robinson 1959; Searle 1971). Expectations, variances and covariances of Z_1 and Z_2 in terms of genetic effects of the F_2 metric are given by Melchinger et al. (2007) and Kusterer et al. (2007).

As there was only one replication at each location in the experiments with Pop1 and Pop2, the genotype × location interaction variance and the plot error variance are confounded in σ_e^2. Estimates of heritability (h^2) on an entry-mean basis together with their 95% confidence intervals were computed for Z_1 and Z_2 following Knapp and Bridges (1987). Based on $\hat{\sigma}_g^2(Z_1)$ and $\hat{\sigma}_g^2(Z_2)$, we estimated the augmented degree of dominance $\overline{D}^* = \left[\hat{\sigma}_g^2(Z_2) \Big/ \hat{\sigma}_g^2(Z_1)\right]^{0.5}$. Testing the significance of genotypic covariance (H_1, H_2) yielded a test for the null hypothesis $H_0 : \overline{D}^* = 1.0$, because the null hypothesis holds true if and only if the genotypic covariance is not significantly different from zero.

Parental genome proportion

For each F_3 or F_2Syn3 plant used as a parent for producing BC progenies (Stuber et al. 1992; Lu et al. 2003) we determined the genome proportion inherited from parent P2 as GP2 = $\sum_j m_j/n$, where $j = 1, \ldots, n$ is the number of marker loci assayed in each study. In each population and for each trait, parent P2 was defined as the parent with the higher population mean in analogy to the definition of H_2. The value of $m_j = 1$, 0.5, and 0, if the genotype at marker locus j was homozygous for the allele from P2, heterozygous, or homozygous for the allele from P1, respectively, and summation is over all n marker loci. To examine the relationship between GP2 and Z_2, phenotypic (r_p) and genotypic (r_g) correlation coefficients were calculated.

Linkage map

For localization of QTL for Z_1 and Z_2 on the genetic map, we used linkage maps constructed in the original publications based on the Kosambi mapping function. In the study of Stuber et al. (1992), the total map distance spanned 1,149 cM with an average interval length of 18 cM. Lu et al. (2003) reported a total map distance of 2,581 cM with an average distance of 16 cM between markers, not accounting for the effect of three generations of intermating of the F_2 population. In the study by Frascaroli et al. (2007), the total map length calculated on RILs was 2,260 cM with an average distance of 14 cM between markers (Frova et al. 1999). Due to a limited number of common markers, joint map construction for the three populations was not possible. Marker positions from individual studies were projected on the IBM2 2008 neighbors reference map obtained from MaizeGDB (http://www.maizegdb.org). To facilitate the assignment of markers to bins, calculation of map distances must take into account the effect of selfings or intermatings as described by Falke et al. (2006). For the map of Pop2 (Lu et al. 2003) the appropriate transformation resulted in a total map distance

of 1,376 cM which is similar to the length of the map of Stuber et al. (1992) but with an average interval length of 10 cM.

QTL analyses

For Pop1 and Pop2, entry means of Z_1 and Z_2 across locations as well as the genotyping data of the parental F_3 or F_2Syn3 plants and the corresponding linkage maps were used for QTL analyses. Composite interval mapping of Jansen and Stam (1994) and Zeng (1994) was employed to detect, map, and characterize QTL. Marker cofactors were selected by stepwise regression using the Bayes information criterion (BIC) computed according to the method of Burnham and Anderson (2004). Initially, the QTL scans for Z_1 and Z_2 were based on a genetic model with two effects, comparable to the linear and the quadratic effects in QTL mapping for per se performance. At the marker positions, this corresponds for Z_1 to the contrasts C_1 (comprising additive a_i and dominance × additive da_{ij} epistatic effects) and C_2 (comprising additive × additive aa_{ij} and dominance × dominance dd_{ij} epistatic effects) and for Z_2 to the contrasts C_3 (comprising dominance d_i and aa_{ij} epistatic effects) and C_4 (comprising da_{ij} and additive × dominance ad_{ij} epistatic effects) defined by Cockerham and Zeng (1996). As the QTL contrasts corresponding to C_2 and C_4 were not significant for Z_1 and Z_2, respectively, we applied for QTL mapping with Z_1 and Z_2 a genetic model with only one main (gene dosage) effect corresponding to the contrasts C_1 and C_3 of Cockerham and Zeng (1996). Besides separate QTL scans for each Z_s, we also carried out a joint mapping of Z_1 and Z_2 for each trait, following Jiang and Zeng (1995) and Melchinger et al. (2007). Significance thresholds of LOD scores for declaring presence of a QTL were determined by permutation tests (Churchill and Doerge 1994) using 6,000 permutations. For an experiment-wise error rate of 30% (corresponding to a comparison-wise error rate of about 5%), the mean LOD threshold for Z_1 and Z_2 across all three traits was 2.84 in Pop1 and 3.27 in Pop2 with minor deviations for individual traits. Using the same error rate, the mean LOD threshold across traits for joint mapping of Z_1 and Z_2 was 3.68 in Pop1 and 4.10 in Pop2.

Under the assumptions of the F_2-metric model, arbitrary linkage and digenic epistasis, Melchinger et al. (2007) defined the augmented additive effect of QTL i as $a_i^* = a_i - 0.5\,[da_i]$, where a_i is the additive effect of QTL i and $[da_i]$ is the sum of dominance × additive effects of QTL i with all other QTL in the genetic background irrespective of linkage. Likewise, they defined the augmented dominance effect of QTL i as $d_i^* = d_i - 0.5\,[aa_i]$, where d_i is the dominance effect of QTL i, and $[aa_i]$ is the sum of additive × additive effects of QTL i with all other QTL in the genetic background irrespective of linkage.

Estimates of QTL positions were obtained from joint mapping with Z_1 and Z_2 at the point where the LOD score of the joint analysis reached its maximum in the chromosomal region under consideration. At this position the QTL effects a_i^* and d_i^* were estimated for Z_1 and Z_2, respectively. The augmented dominance ratio (ADR) for QTL i, defined as $d_i^*/|a_i^*|$, was computed from estimates of a_i^* and d_i^*. The proportion of total genotypic variance for Z_1 or Z_2 explained by all detected QTL in the model was obtained as $p = R^2_{adj}/h^2$, where R^2_{adj} is the adjusted multiple correlation coefficient in the simultaneous fit (Utz et al. 2000). For each putative QTL i, the normed partial correlation coefficient p_i was calculated according to Zhu et al. (2004), which adds up over all QTL to p. Unbiased proportions of genotypic variance explained by QTL (p_{TS}) were estimated with fivefold cross validation using 2,000 splits for each trait according to the procedure described by Utz et al. (2000).

For a given trait and linear transformation (Z_1 or Z_2) if QTL were found in the same or adjacent bins across Pop1 and Pop2, the respective genomic regions were examined for presence of QTL in Pop3. In these genomic regions, map distances for Pop1 and Pop2 were re-scaled based on genetic distances calculated from the IBM2 2008 Neighbors map (http://www.maizegdb.org) and QTL positions were compared based on the re-scaled maps. QTL were declared as congruent across Pop1 and Pop2 when the re-scaling resulted in localization of LOD peaks within 20 cM distance from each other according to the concept of overlapping bins suggested by Tuberosa et al. (2002).

Following the EPISTACY software of Holland (1998), all possible marker × marker interactions among homozygous marker genotypes were calculated separately for Z_1 and Z_2 to test the presence of different types of epistatic effects as described in detail by Melchinger et al. (2008). To eliminate the effect of other QTL on these interactions, the same set of cofactors was used in the comparison of the models with and without interactions as in the genome-wide scan for QTL. We employed mBIC significance criterion (Baierl et al. 2006) to account for the multiple test situation.

Software package PLABQTL (Utz and Melchinger 1996) was used for all QTL analyses. The package was extended to carry out the joint analysis of Z_1 and Z_2 as well as the calculation of the BIC and mBIC.

Results

Linear transformations Z_1 and Z_2

Results of the analysis of GY, GM, and PH are presented in Table 1 for Pop1 and Pop2 and by Frascaroli et al. (2007)

Table 1 Summary statistics (mean $\bar{X}$, genotypic variance σ_g^2, error variance σ_e^2, heritability h^2, phenotypic correlation r_p, genotypic correlation r_g, augmented degree of dominance $\overline{D}^*$) with associated standard errors for linear transformations Z_1 and Z_2 of three traits in Pop1 and Pop2

Statistic[b]		Grain yield (q/ha)	Grain moisture (%)	Plant height (cm)
Pop1				
Z_1	$\bar{X}$	88.5 ± 0.4**	16.72 ± 0.04**	257.1 ± 0.7**
	σ_g^2	24.4 ± 3.9**	0.24 ± 0.04**	65.2 ± 6.9**
	σ_e^2	111.0 ± 4.3**	0.99 ± 0.04**	81.7 ± 3.2**
	h^2	56.1	53.5	82.7
	CI (h^2)	(46.6, 63.5)	(43.1, 61.5)	(79.0, 85.6)
Z_2	$\bar{X}$	2.24 ± 0.25**	0.10 ± 0.02**	4.98 ± 0.36**
	σ_g^2	96.8 ± 9.6**	0.03 ± 0.01**	27.6 ± 3.0**
	σ_e^2	79.4 ± 3.1**	0.33 ± 0.01**	43.2 ± 1.7**
	h^2	87.9	30.5	79.3
	CI (h^2)	(85.3, 89.9)	(15.4, 42.3)	(74.8, 82.7)
r_p (Z_1, Z_2)		−0.16**	−0.03	0.10
r_g (Z_1, Z_2)		−0.21[a]	−0.02	0.11
$\overline{D}^*$		1.99†	0.35†	0.65†
Pop2				
Z_1	$\bar{X}$	85.5 ± 0.3**	15.1 ± 0.04**	241.6 ± 0.4**
	σ_g^2	13.2 ± 1.8**	0.45 ± 0.04**	34.4 ± 5.1**
	σ_e^2	45.3 ± 1.8**	0.43 ± 0.02**	53.5 ± 4.0**
	h^2	57.2	80.1	55.9
	CI (h^2)	(47.4, 65.2)	(76.1, 83.3)	(45.3, 64.5)
Z_2	$\bar{X}$	0.37 ± 0.40	0.52 ± 0.03**	−2.84 ± 0.35**
	σ_g^2	46.2 ± 4.1**	0.17 ± 0.02**	20.3 ± 3.9**
	σ_e^2	39.4 ± 1.5**	0.37 ± 0.02**	50.6 ± 3.8**
	h^2	85.3	65.3	44.4
	CI (h^2)	(82.6, 87.5)	(58.6, 70.6)	(31.4, 55.5)
r_p (Z_1, Z_2)		0.02	0.09	0.05
r_g (Z_1, Z_2)		0.02	0.11	0.02
$\overline{D}^*$		1.87†	0.62†	0.77†

* Significant at $P < 0.05$, ** significant at $P < 0.01$

† Significantly different from 1

[a] Genotypic correlation coefficient exceeded twice its standard error

[b] For the description of linear transformations Z_1 and Z_2 see materials and methods

[c] 95% confidence interval

for Pop3. (Estimates of h^2 were kindly provided by E. Frascaroli, personal communication). Considering that BC progenies have an expected inbreeding coefficient of $F = 0.5$, means of Z_1 for GY reflected a high performance level in all three populations ranging from 6.3 to 8.9 Mg ha^{-1}. Estimates of σ_g^2 for Z_1 and Z_2 were significantly greater than zero ($P < 0.01$) for all traits in all populations. For all three populations, heritability estimates for Z_2 were high $\left(\hat{h}^2 > 0.8\right)$ for GY and medium to high for GM and PH, except for the low value for GM in Pop1 $\left(\hat{h}^2 = 0.3\right)$. Compared to Z_2, h^2 estimates for Z_1 were significantly lower for GY, not significantly different for PH, and significantly higher for GM. Correlations (r_p, r_g) between Z_1 and Z_2 were small and in most cases not significantly different from zero. Estimates of $\overline{D}^*$ were high for GY (1.18–1.99), medium for PH (0.61–0.77) and low for GM (0.35–0.62). Based on correlations between H_1 and H_2, estimates of $\overline{D}^*$ in Pop1 and Pop2 were significantly greater than 1.0 for GY, but less than 1.0 for the other traits (data not shown).

QTL analyses for Z_1 and Z_2

For GY, the genome scan with Z_2 identified 10 (Pop1) and 13 (Pop2 and Pop3) QTL affecting MPH (Fig. 1 and Supplementary Table S2 and S3). Three genomic regions on chromosomes 1, 8, and 10 harbored significant QTL for Z_2 of GY in all three populations (same or adjacent bins) (Fig. 1). In two additional genomic regions on chromosomes 5 and 7, congruent QTL positions were found in Pop1 and Pop2, each exhibiting a high level of significance and explaining approximately 10% of the genetic variance. With only a few exceptions, genomic regions carrying QTL for Z_2 of GY had d_i* larger than a_i* in each of the three populations (ADR > 1). In the genomic regions where congruent QTL were detected for Pop1 and Pop2

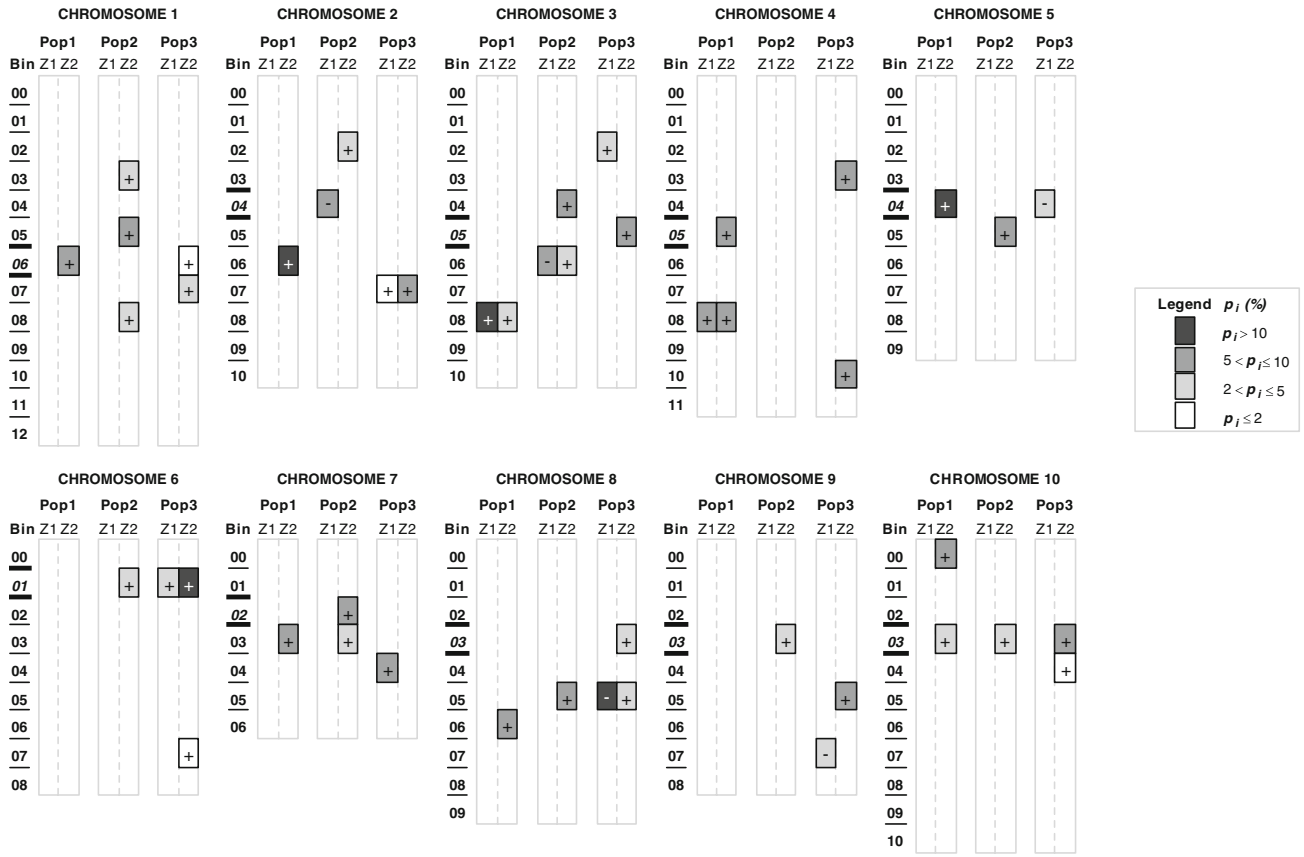

Fig. 1 Chromosomal positions and magnitude of effects of QTL detected for linear transformations Z_1 and Z_2 of grain yield in Pop1, Pop2, and Pop3. Bin assignment of QTL was performed based on bin assignment of flanking markers in the IBM2 2008 neighbors reference map obtained from MaizeGDB. The bin harboring centromere is shown in italics in between bold lines. The *plus or minus sign* indicates the sign of a_i^* (Z_1) and d_i^* (Z_2) effects

(chromosomes 1, 5, 7, 8, and 10), estimates of the ADR were substantially greater for F_3 plants (Pop1) than for the intermated F_2Syn3 population (Pop2), as expected for pseudo-overdominance. With the exception of chromosome 8, all congruent QTL were located in the same or an adjacent bin harboring the centromere.

In a simultaneous fit of all putative GY QTL for Z_2, 63–78% of the genotypic variance was explained in the three populations. In the re-analyses of Pop1 and Pop2, cross-validated estimates for the genetic variance explained for Z_2 of GY were high, i.e., 66 and 58%, respectively.

The genome scan with Z_1 identified two (Pop1 and Pop2) and seven (Pop3) genomic regions significantly affecting GY with no apparent overlap across populations. In all three populations, the estimated genotypic variance explained by QTL for Z_1 ($24\% < p < 40\%$) was smaller than for Z_2. In Pop1 and Pop2, QTL explained only 11–15% of the genetic variance after cross validation.

As compared to GY, fewer QTL were detected for GM in the genome scan with Z_2 (3, 6, and 0 in Pop1, 2, and 3, respectively). This was expected for a trait generally not exhibiting significant heterosis. QTL congruency for Z_2 of GM across Pop1 and Pop2 was detected on Chromosomes 3 and 8. The ADR in Pop1 was less than 1.0 for the majority of QTL, whereas an equal number of QTL had an ADR higher or smaller than 1.0 in Pop2. In the simultaneous fit of all putative QTL for Z_2 of GM, 46% (Pop1) and 38% (Pop2) of the genetic variance were explained; cross validation reduced these estimates to 20 and 12%, respectively.

The genome scan for GM with Z_1 identified two (Pop1), four (Pop2), and six (Pop3) genomic regions significantly affecting this trait. On chromosomes 1, 8, and 9, QTL were identified in the same or adjacent bins for at least two of the three populations (data not shown). In Pop1 and Pop2, genetic variance explained by all QTL for Z_1 was comparable to Z_2, with 25% for Pop1 and 13% for Pop2 after cross validation.

For Z_2 of PH, nine QTL were detected in Pop1, three in Pop2, and 11 in Pop3. Congruent QTL were detected on seven chromosomes for at least two of the three populations (data not shown). Considering all detected QTL, there was a prevalence of QTL with ADR greater than 1.0 in Pop1 and Pop3, whereas an equal number of QTL had ADR larger or smaller than one in Pop2. More than 50% of

the genotypic variance was explained by QTL in each of three populations. The proportion of the genotypic variance explained by QTL in Pop1 and Pop2 after cross validation was 58 and 30%, respectively.

Results from the genome scan with Z_1 of PH were similar to those obtained for GM. Four (Pop1), one (Pop2), and seven (Pop3) genomic regions significantly affected PH; however, the genetic variance explained by QTL was low when cross validation was applied (25% in Pop1 and 13% in Pop2). Three genomic regions carried QTL for Z_1 of PH in more than one population.

As expected, results for PH were intermediate between GY and GM with respect to the relative importance of d_i* and a_i*. Further, only three QTL were detected for GM in Pop1 and PH in Pop2, by the genome scan with Z_2. We therefore concentrated our analyses of the genetic basis of MPH on the most heterotic trait GY.

Epistasis

Based on the BIC significance criterion, we did not detect significant first-order marker × marker interactions for all possible combinations of markers linked to a QTL detected with Z_1 or Z_2 for all traits in Pop1 and Pop2. In the genome-wide search for first-order marker × marker interactions between genomic regions without significant main effects for Z_1 or Z_2, 20 marker pairs for Z_1 and 19 marker pairs for Z_2 in Pop1 and 74 marker pairs for Z_1 and 82 marker pairs for Z_2 in Pop2 were detected for GY. However, when correcting for multiple testing by applying the more stringent significance criterion mBIC, none of the significant interactions detected with BIC were confirmed. Likewise, no significant additional first order marker × marker interactions were detected based on the mBIC for PH and GM in Pop1 and Pop2.

Parental genome proportion and Z_2

The parental genome proportion (GP2) ranged from 21.1 to 80.2% among F_3 plants of Pop1 and from 30.9 to 69.4% among F_2Syn3 plants of Pop2, with means of 50.2 and 49.1%, respectively. Mean homozygosity was 77.5% in Pop1 and 51.0% in Pop2, close to the expected values. GP2 was highly correlated with Z_2 for GY in all three populations with $r_g(Z_2, \text{GP2}) \geq 0.77$ (Table 2). Phenotypic and genotypic correlations were medium to high for PH and relatively low for GM.

Discussion

The genetic analysis of heterosis has benefitted from advances in quantitative genetics and molecular biology.

Table 2 Phenotypic ($r_p(Z_2, \text{GP2})$) and genotypic ($r_g(Z_2, \text{GP2})$) correlation coefficients between linear transformation Z_2 and genome proportion of parent P2 (GP2) in Pop1, Pop2, and Pop3

Population	Correlation coefficient	Grain yield (q/ha)	Grain moisture (%)	Plant height (cm)
Pop1	r_p	0.80**	0.14*	0.71**
	r_g	0.85	0.25	0.80
Pop2	r_p	0.71**	0.33**	0.44**
	r_g	0.77	0.41	0.66
Pop3[a]	r_p	0.76**	0.31**	0.64**
	r_g	0.82	0.40	0.70

* Significant at $P < 0.05$, ** significant at $P < 0.01$

[a] Based on Frascaroli et al. (2007) and E. Frascaroli (personal communication)

Novel quantitative genetic theory provided a generalized mathematical derivation of genetic effects contributing to MPH (Melchinger et al. 2007) and genetic expectations of QTL effects for two major mating designs employed in heterosis research, the Design III and the triple testcross design (Melchinger et al. 2007, 2008). Experimental QTL studies generated information on the number of genomic regions contributing to GY, their localization in the genome, and the magnitude of QTL effects (Stuber et al. 1992; Beavis et al. 1994; Graham et al. 1997; Melchinger et al. 1998; Austin et al. 2000; Lu et al. 2003, Schön et al. 2004; LeDeaux et al. 2006; Frascaroli et al. 2007, 2009; Garcia et al. 2008). Studies on gene expression (Guo et al. 2006; Swanson-Wagner et al. 2006) and microcolinearity of DNA sequences (Fu and Dooner 2002) have broadened our understanding of the action of individual genes. Integrating theoretical advances and experimental results on heterosis from several studies has been the goal of this study.

Congruent QTL for MPH across populations

We compared QTL mapping in three populations of maize specifically designed for the analysis of heterosis. For QTL detection with Z_2, the information content per progeny for the linear (gene dosage) effect increases with homozygosity. Thus, with identical sample sizes, the power of QTL detection is expected to be smallest for F_2Syn3 (Pop2), highest for RIL (Pop3), and intermediate for F_3 (Pop1) populations. However, differences in power of QTL detection for d_i* or a_i* due to different population types were at least partially compensated by differences in sample size (Pop2 > Pop1 > Pop3).

The comparison across Pop1 and Pop2 yielded five genomic regions with congruent QTL for Z_2 of GY. Three of these regions were also found to significantly affect MPH for GY in Pop3. Meta-analyses of QTL positions are

complex when the number of common markers between populations is small, as was the case in our study; a consensus map could not be constructed. Confidence intervals of QTL positions are often wide, especially for QTL with small effects, and CIM does not allow straightforward calculation of confidence intervals (Visscher et al. 1996). In the present study, additional uncertainty of QTL positions was introduced by the differences in genetic structure among the populations compared (F_3, F_2Syn3, and RILs). Thus, our recourse was to compare QTL bins.

Based on these first results, an in-depth analysis of QTL positions was performed in the respective candidate regions. Map distances of the individual maps were recalculated to account for the varying degrees of selfing and intermating. In all five genomic regions, QTL across Pop1 and Pop2 were placed within 20 cM of each other. Thus, we are confident that these genomic regions warrant further study with respect to their role in the expression of MPH despite the uncertainties of QTL localization discussed above. Statistical evidence for a significant contribution to MPH was strong in all populations and the proportion of the genetic variance explained by the d_i* effects of congruent QTL was substantial ($6\% < p_i < 13\%$) in all cases except those on chromosome 10 in Pop1 and Pop2.

A logical next step is the development of near isogenic lines (NILs) that allow contrasting of genetic effects for these congruent genomic regions. Quantitative genetic theory for the analysis of MPH with one- or two-segment NILs has been developed (Melchinger et al. 2008; Reif et al. 2009). Generation mean analysis of NILs allows estimation of the relative magnitude of *aa* epistasis and, thus, will provide insight into the predominant type of gene action contributing to d_i* effects in candidate genomic regions. For the region on chromosome 5, Graham et al. (1997) created NILs from Pop1 and found two QTL linked in repulsion. In Pop3, production of NILs for genomic regions on chromosome 8 and 10 has been started (Frascaroli et al. 2007).

Genetic architecture of MPH

The genetic architecture of quantitative traits can be characterized by the number of segregating QTL and the magnitude of their effects. In plants, animals, and humans it has been shown that quantitative traits are controlled by a large number of genes with small effects (Schön et al. 2004; Hayes and Goddard 2001; Visscher 2008). Therefore, it was surprising that in all three populations, QTL detected with Z_2 of GY explained 63–78% of the genetic variance and that major QTL explaining 10% or more of the genetic variance were detected. After cross validation, 58–66% of the genetic variance could still be accounted for in Pop1 and Pop2, which indicated that estimates of QTL effects were not strongly biased upward. On the other hand, for GM the cross-validated genetic variance explained by QTL for Z_2 was small in Pop1 and Pop2 ($p_{TS} \leq 20\%$). The same was true for results obtained with genome scans for Z_1 with all traits ($p_{TS} \leq 25\%$).

Based on these findings, we hypothesize that in each heterotic pool (BSSS and LSC), different alleles or allele clusters for GY have been fixed, which in combination with the allele(s) from the opposite heterotic pool lead to high MPH. Their positive interactions very likely form the base line for the superior performance of this heterotic pattern. Due to the fixation of favorable alleles within heterotic pools and consequent "fixation" of allele combinations in inter-pool crosses, these genomic regions will not contribute to the variance of general and specific combining ability effects in intra-pool testcrosses. Thus, in the development of new inbred lines from intra-pool recycling, these genomic regions will not contribute to genetic gain.

This hypothesis may explain the occasional quantum leap in maize hybrid performance observed in the past. When new genetic material originating from sources other than the existing heterotic pools is introduced, new allelic combinations with major effects on MPH might become effective. However, these complementary favorable allelic combinations will be rapidly fixed with careful management of heterotic pools and cannot be exploited in subsequent cycles of selection.

Pronounced positive interaction of divergently fixed alleles can also serve as an explanation why in some studies on inter-pool crosses major QTL for GY were consistently detected across populations (e.g., Abler et al. 1991), while in studies on intra-pool crosses (e.g., Melchinger et al. 1998), the QTL had small effects and congruency was limited. Thus, in the analysis of the genetic architecture of polygenic traits, the genetic structure of experimental populations plays a crucial role because the relative contributions of dominance, additive, and epistatic genetic effects to trait variation may differ in inter- versus intra-pool crosses.

Even though a few genomic regions had a major effect on MPH ($p_i > 10\%$), a substantial proportion of the variance for Z_2 remained unaccounted for after cross validation in Pop1 and Pop2. To further assess the genetic architecture of MPH, we calculated the correlation between the GP2 and Z_2. The magnitude of this correlation depends on (i) the proportion of QTL tagged by markers, (ii) the proportion of markers tagging a QTL, and (iii) the coefficient of variation of d_i* effects (Melchinger et al. 2010). For GY estimates of this correlation were high ($0.77 < r_g < 0.85$), which for the given marker densities is only expected if numerous QTL distributed across the entire genome contribute to MPH. The somewhat lower correlation detected for Pop2 when

compared to Pop1 and Pop3 was expected due to reduced linkage between QTL and markers in the F_2Syn3. Based on these results we conclude that many QTL with effects on MPH below the LOD threshold remained undetected in the genome scan with Z_2. This strongly supports the conclusion that MPH is a polygenic phenomenon.

Type of gene action

The types of gene action that have been investigated in the context of heterosis are dominance, (pseudo)-overdominance, and epistasis. The Design III provides different estimates for assessing their relative importance. From genetic variances $\hat{\sigma}^2_g(Z_1)$ and $\hat{\sigma}^2_g(Z_2)$, the augmented degree of dominance $\bar{D}^*$ can be estimated. However, $\bar{D}^*$is not a meaningful measure of the type of gene action at the level of individual loci, because it is severely biased by epistasis and linkage disequilibrium between QTL (Melchinger et al. 2007). QTL effects obtained from genome scans with Z_1 and Z_2 provide estimates of the augmented effects a_i* and d_i* which allow calculation of the ADR. Epistatic effects can be estimated from marker–marker interactions, but their genotypic expectations are complex (Melchinger et al. 2008).

In all three populations, $\bar{D}^*$ estimates varied considerably among the traits reflecting a relatively higher importance of d_i* effects for GY than for the other two traits. These results are in good agreement with findings from individual QTL, where the ratio $d_i^*/|a_i^*|$ was much greater for GY than for the other traits. Almost all congruent QTL for GY were located close to the centromere where gene density tends to be high and recombination tends to be suppressed (McMullen et al. 2009). These results point to pseudo-overdominance as a major cause for heterosis in maize. However, in the presence of epistasis, interpretation of $d_i^*/|a_i^*|$ with respect to degree of dominance is not straightforward. Different types of epistasis contribute to d_i* and a_i*: aa_{ij} epistasis mainly contributes to d_i* effects and da_{ij} epistasis mainly to a_i* effects. Thus, neither $\bar{D}^*$ nor ADR is an unbiased estimate of the degree of dominance.

Differences between Z_1 and Z_2 for the number of detected GY QTL were large, and most genomic regions with large d_i* effects did not have significant a_i* effects. These findings are most likely the result of tight repulsion phase linkage of loci with (partially) dominant gene action. With a few recombination events, linkage equilibrium cannot be attained and, as a consequence, additive genetic effects of opposite sign cancel each other, thereby, leading to subthreshold effects of QTL in genome scans with Z_1 and very small estimates of a_i*. On the other hand, directional dominance is exhibited in the same genomic regions with large significant d_i* effects. This hypothesis is also supported by the findings of Graham et al. (1997). For the genomic region on chromosome 5 they developed NILs from Pop1 and showed that the large QTL effect on chromosome 5 was the result of the joint action of two independent QTL with additive effects of opposite sign.

Consistently smaller estimates of ADR of QTL with significant effects on Z_2 of GY in Pop2 than in Pop1 may be because of recombinations due to intermating. Three generations of intermating should have broken up some associations between genes linked in repulsion. Therefore, more QTL for Z_1 were expected in Pop2 than Pop1. However, it might be that three intermatings were not sufficient to offset the cancelation of a_i* effects with opposite sign in Pop2. Bingham (1998) summarized studies on the effect of random mating on $\bar{D}^*$, and concluded that about eight generations of random mating are required to obtain stable estimates of $\bar{D}^*$.

In populations with non-inbred progenies, contrasts can be constructed between heterozygous and homozygous marker classes from Z_1 and Z_2 to estimate epistatic effects for linked QTL (contrasts C_2 and C_4 of Cockerham and Zeng 1996). In addition, pair-wise marker interactions also provide estimates of epistatic effects. When testing for the presence of epistasis with both methods, the re-analysis of Pop1 and Pop2 did not yield QTL with significant results. These findings do not agree with results of Cockerham and Zeng (1996) and Garcia et al. (2008) who detected significant epistasis with their analyses of data of Pop1. The variable results are probably due to different type 1 error rates. We corrected for multiple testing by applying mBIC (Baierl et al. 2006) in the two-marker interaction analysis. Because Garcia et al. (2008) specifically focussed on detecting epistatic effects by MIM, they used the less conservative Akaike information criterion (Akaike 1974). However, in summary they concluded that detectable epistasis did not likely play an important role in the manifestation of heterosis for GY in maize.

Estimation methods

The QTL analysis for GY on Z_2 in the joint analysis of the two series of BC progenies yielded results that agreed partially with the separate analysis of each of the two series of BC progenies presented by Stuber et al. (1992) and Lu et al. (2003). In Pop1, the number of detected QTL and the estimates of QTL positions were in good agreement for the joint and separate analyses. In the genome scan with Z_2, LOD scores were mostly higher than those obtained in the separate analysis of each BC series. In Pop2, all five genomic regions identified to carry a segregating QTL in both BC series (Lu et al. 2003) were confirmed by us in the genome scan with Z_2. Eight additional QTL were identified with Z_2 and six of these were common with the separate analyses of BC series. For the analysis of MPH, estimates

derived from the genome scan with Z_2 are expected to be more precise because genetic expectations of QTL effects precisely meet the net contribution of QTL to MPH and are not confounded by additive effects as is the case in the separate analysis of each series of BC progenies (for details see Cockerham and Zeng 1996 and Melchinger et al. 2007).

In the re-analysis of data from Pop1 and Pop2, we determined the most likely QTL position for Z_1 and Z_2 by a joint genome scan with Z_1 and Z_2 (Melchinger et al. 2007), because, for estimation of ADR, determination of a common QTL position for Z_1 and Z_2 is necessary. In the absence of correlations between Z_1 and Z_2, the LOD score for their joint analysis is expected to be the sum of LOD scores obtained from their separate analyses. However, in the case of closely linked genes with significant a_i* and d_i* effects, the QTL position of the joint fit might be a compromise between QTL for Z_1 and Z_2. However, testing the hypothesis of a common QTL affecting Z_1 and Z_2 at a given position in the genome versus separate QTL for Z_1 and Z_2 requires higher marker densities and larger populations than those analyzed in the present study.

Analysis of MPH across species

Hybrid maize breeding is the most notable example of the exploitation of heterosis through long-term reciprocal recurrent selection with genetically diverse heterotic pools. If the high congruency of QTL positions and the large magnitude of some QTL effects are the result of divergent selection of alleles or allele combinations contributing to MPH for GY, it is unlikely that similar results would be observed in a species in which hybrid breeding has only had a short history, heterotic pools have not been developed, or traits other than GY played an important role in selection. Comparing results from a Design III MIM analysis on heterosis for GY in maize (Pop1) and rice (*Oryza sativa* L.), Garcia et al. (2008) found pronounced differences between the two species. In contrast to their findings in maize, which are in good agreement with our results, they found in rice relatively few QTL for genome scans with Z_2, d_i* effects were positive and negative for approximately the same number of genomic regions, and a_i* effects seemed to be of similar number and magnitude as d_i* effects. Similar results were reported by Radoev et al. (2008) from a study of a doubled haploid population backcrossed to one of the parents in rapeseed (*Brassica napus* L.). Only a few QTL for MPH were identified explaining a rather small proportion of the phenotypic variance despite a satisfactory sample size ($N = 250$). In Arabidopsis too, in a large triple testcross experiment, only a few genomic regions could be identified that contributed to heterosis for biomass yield (Kusterer et al. 2007). Taking these results into account we postulate that genomic regions having large effects on heterosis are only likely to be identified in species with a long history of hybrid breeding using divergent germplasm groups. Therefore, it would be worthwhile to perform genetic analyses on heterosis with the Design III in sunflower (*Helianthus annuus* L.), rye (*Secale cereale* L.), and sugar beet (*Beta vulgaris* L.) that have like maize, undergone selection in genetically divergent pools over long time periods.

Conclusion

In conclusion, we are still at the beginning of understanding the complex mechanisms underlying the genetic basis of heterosis. However, with novel genomic tools at hand we will be able to support or reject some of the genetic hypotheses that have been put forward by classical quantitative genetics and marker-based studies. Identification of genomic regions involved in the manifestation of heterosis across populations within established heterotic patterns is the starting point. The next step will be the generation of experimental NIL populations for comparative analyses of specific genomic segments in different heterotic pools and patterns. High-throughput genotyping of the candidate regions and their characterization in these populations will give us first ideas about their role in germplasm development. Next generation sequencing should generate information on the colinearity of the genomes between and within heterotic pools and, thus, provide additional insights into the genetic mechanisms underlying heterosis.

Acknowledgments We are grateful to R. Bernardo, C.W. Stuber, and Z.-B. Zeng for providing the raw data for Pop1 and Pop2 and to E. Frascaroli for providing heritability estimates for Pop3. We thank G. Haseneyer and D. Campell for their help with the linkage map comparisons, and an anonymous reviewer for suggestions. This research was financed by the Deutsche Forschungsgemeinschaft (DFG) within the priority program SPP 1149 "Heterosis in Plants" (Research Grant ME931/4-3).

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