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Genetic basis of heterosis explored by simple sequence repeat markers in a random-mated maize population

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Abstract The genetic basis of heterosis in crop plants has not been completely resolved. Our objective in this study was to determine the level of dominance for quantitative trait loci (QTLs) that underlie heterosis in maize (*Zea mays* L.). An F_2 population of an elite maize single cross, LH200 × LH216, was random mated for three generations in an attempt to break up repulsion linkages that might lead to pseudo-overdominance. The population was analyzed with 160 simple-sequence repeat markers. Phenotypic data analyses indicated overdominance for grain yield and partial dominance for plant height, grain moisture and stalk lodging. A total of 28 QTLs were identified for grain yield, 16 for grain moisture, 8 for stalk lodging, and 11 for plant height. For grain yield, 24 QTLs (86%) showed overdominance. In contrast, most of the QTLs for plant height, grain moisture and stalk lodging showed partial to complete dominance. Little epistasis was detected among the QTLs for any of the traits. Our results can be interpreted in one of two ways, or a combination of both: (1) QTLs for grain yield in maize exhibit true overdominance, or (2) QTLs for grain yield show partial to complete dominance, but they are so tightly linked such that three generations of random mating failed to separate their individual effects.

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Introduction

Heterosis is the superiority of an F_1 hybrid over its parents. The high crop productivity that results from heterosis has been exploited through the development of hybrid varieties in many crop species, most notably maize. Heterosis as it applies to crop breeding was first recognized by Shull in 1908 (Shull 1908). The genetic basis of heterosis, however, has not been completely explained. The two main hypotheses that have been proposed as the genetic basis of heterosis are the dominance hypothesis (Davenport 1908) and the overdominance hypothesis (East 1908; Shull 1908).

Consider a quantitative trait locus (QTL) that affects a trait such as grain yield. The genotypic values at a locus are modelled as $\bar{P} + a$for QQ, $\bar{P} + d$for Qq, and $\bar{P} - a$for qq (Falconer and Mackay 1996). With the parents being homozygous, the mean of the QQ and qq parents is $\bar{P}$. The d/a ratio represents the level of dominance at the locus. The dominance hypothesis for heterosis suggests that favorable dominant alleles mask deleterious recessive alleles in a heterozygote. In other words, the dominance hypothesis implies that the mean of the Qq genotype in the F_1 will be superior to the mean of its parents under partial dominance (i.e., $0 < d/a < 1$) or complete dominance (i.e., $d/a = 1$). In contrast, the overdominance hypothesis suggests that the heterozygote is inherently superior to either homozygote, i.e., $d/a > 1$.

Pseudo-overdominance (or associative overdominance), however, makes it difficult to experimentally

distinguish between the dominance and overdominance hypotheses for heterosis. Pseudo-overdominance is due to the repulsion-phase linkage of two loci that exhibit partial or complete dominance (Jones 1917). Suppose two loci, Q_1 and Q_2 that control the trait are in repulsion-phase linkage. When a $Q_1Q_1q_2q_2$ parent is crossed with a $q_1q_2Q_2Q_2$ parent, the $Q_1q_1Q_2q_2$ double heterozygote will display an overdominant phenotype even though the Q_1 and Q_2 loci each exhibit only partial or complete dominance.

In contrast to pseudo-overdominance, true overdominance does not require linkage disequilibrium between loci that affect the quantitative trait. Random mating, which allows the dissipation of linkage disequilibrium, is therefore a means of distinguishing between true overdominance and pseudo-overdominance. The Design III mating scheme is a powerful experimental design for estimating the average level of dominance (i.e., across all loci affecting a trait) in the cross between two inbred parents (Comstock and Robinson 1948). In a Design III experiment for grain yield in maize (Gardner 1963), the estimate of *d*/*a* in one population decreased from 1.98 in the F_2 to 0.72 after six generations of random mating. In a second population, the estimate of *d*/*a* decreased from 1.68 in the F_2 to 1.09 after 11 generations of random mating. A summary of different studies (Bingham 1998) indicated that *d*/*a* generally decreased to less than 1.0 after three generations of random mating. These results indicated the presence of pseudo-overdominance for grain yield in maize, and that heterosis for grain yield in maize is due to partial or complete dominance at individual loci.

Molecular markers are useful for dissecting the genetic architecture of quantitative traits (Mackay 2001). Stuber et al. (1992) used 76 markers to investigate heterosis in the maize single cross B73 × Mo17. They found that for the grain yield, most of the QTL effects were larger in the heterozygote than in either homozygote, suggesting overdominance or pseudo-overdominance. Yu et al. (1997) used 150 molecular markers to study heterosis in rice (*Oryza sativa* L.). They found overdominance at most of the QTLs for yield and for a few QTLs for other yield-component traits. But in both studies, overdominance could not be distinguished from pseudo-overdominance due to the lack of random mating. A re-analysis, however, of the Stuber et al. data suggested pseudo-overdominance instead of overdominance for yield QTLs (Cockerham and Zeng 1996). Subsequent fine mapping indicated that a chromosomal region on chromosome 5, found by Stuber et al. to be overdominant for yield, comprised at least two dominant QTLs (Graham et al. 1997).

In this study, an F_2 population of LH200 × LH216, an elite maize hybrid, was random-mated for three generations in an attempt to break up repulsion-phase linkages between QTLs and markers. Our objective in this study was to determine the importance of partial-to-complete dominance versus true overdominance or pseudo-overdominance for heterosis at the molecular marker level in maize.

Materials and methods

Plant materials

Maize inbreds LH200 and LH216 were developed by Holden's Foundation Seeds (MBS, Inc. 1999). The F_2 population was developed by selfing the F_1 in summer 1996 in Hawaii. The F_2 population was random-mated for three generations by chain sibbing. In chain sibbing, one plant was used to pollinate a second plant, which in turn was used to pollinate a third plant, and so on. Thus, each plant was used once as a male parent and once as a female parent. At harvest, a single kernel from each of approximately 400 ears was obtained, and the kernels were bulked to plant the next generation. This procedure generated a population denoted by F_2Syn3. The random mating was made in Hawaii in 1997–1998. Finally, the F_2Syn3 population was simultaneously selfed and backcrossed at Williamsburg, Iowa, in summer 1998. About 400 F_2Syn3 plants were selfed, and the same plant was crossed to four LH200 plants and four LH216 plants. The two resulting backcross populations, which comprised the Design III progenies, were denoted by LH200BC_1 and LH216BC_1.

Leaf tissues were harvested from LH200, LH216 and F_2Syn3 individuals that were selfed and backcrossed. Leaf tissues were stored in 2-ml microfuge tubes. All leaf tissues were lyophilized immediately after harvesting and then stored in a –70 °C freezer. The progenies of 351 F_2Syn3 plants, that were successfully selfed and crossed to both parents, were kept. DNA was extracted by the CTAB method (Saghai-Maroof et al. 1984). A part of each DNA sample (stock DNA) was diluted to a concentration of 5 $\mu g/\mu l$ with TE buffer (10 mM Tris pH 8.0, 1 mM EDTA). The stock DNA samples were stored in a –20 °C freezer, and the diluted DNA samples were stored in a 4 °C refrigerator.

Field experiments

The LH200BC_1 and LH216BC_1 populations, each with 351 backcross families, were evaluated in 1999 in a completely randomized design with a single replication in each of five locations in the USA: West Lafayette, Indiana; Evansville, Indiana; Whiteland, Indiana; Wellman, Iowa; and Monmouth, Illinois. At each location, each entry was represented by one two-row plot with 30 plants per row. The plant population density varied from 71,075 to 75,650 plants ha^{-1} among locations. The LH200BC_1 entries were planted in a separate but adjacent block from the LH216BC_1 entries. Within each block, LH200, LH216 and their F_1 were grown as checks. These checks were systematically inserted between groups of 50 BC_1 entries. (The checks were for reference purposes only and were not used in subsequent analyses.) The traits analyzed were grain yield (t ha^{-1}), grain moisture (g kg^{-1}), stalk lodging (%) and plant height (cm). All these traits were evaluated on a plot basis.

Analysis of field data

Analyses of variance, combined across the five locations, were conducted using PROC GLM in SAS. The genotypes and locations were considered as random effects, whereas the parents were considered as fixed effects. The use of a single replication at each location caused the genotype × location interaction variance and the within-location error variance to be confounded in the residual variance, V_R (Cochran and Cox 1950). Normality tests on residuals were conducted using PROC UNIVARIATE. Severe outliers were dropped and treated as missing data.

A Design III analysis was performed to estimate the average level of dominance (pooled across all loci) according to Comstock and Robinson (1948). Assuming no linkage and no epistasis among segregating loci for the trait being studied, Comstock and Robinson showed that the variance component due to individual F_2Syn3 plants estimates a quarter of the additive genetic variance, V_A. The variance component due to the interaction between the individual

Table 1 Genotypes and genotypic values of F_2 plants and their backcrosses to each parent

F_2 plants:		Backcross to LH200 (e.g., *QQ*):		Backcross to LH216 (e.g., *qq*):	
Genotype	Frequency	Genotype	Value	Genotype	Value
QQ	1/4	QQ	$\bar{P} + a$	*Qq*	$\bar{P} + d$
Qq	1/2	1/2 *QQ*, 1/2 *Qq*	$\bar{P} + (a + d)/2$	1/2 *Qq*, 1/2 *qq*	$\bar{P} + (d - a)/2$
Qq	1/4	*Qq*	$\bar{P} + d$	*qq*	$\bar{P} - a$
QQ versus *qq* contrast:			$c_1 = (a - d)/2$		$c_2 = (a + d)/2$

F_2Syn3 plants and their parents is a direct estimate of the dominance variance, V_D. Unlike in the original Comstock and Robinson design, the entries were not divided into sets in our study. The field layout was analogous to a split-plot design, where the two parents (LH200 and LH216) corresponded to the main plots and the F_2Syn3 plants corresponded to the subplots. This layout was not a limitation because the differences between main plots were confounded with the mean squares due to parents, rather than with the mean squares due to the interaction of F_2Syn3 plants and their parents, which were used to estimate V_D.

Variance components were estimated by equating the observed mean squares to their expectations and solving for the desired component. The average level of dominance was then calculated as $d/a = (2V_D/V_A)^{1/2}$. The *d*/*a* ratio is a weighted mean of the level of dominance over all segregating loci, and the weight was the V_A at each locus. The broad-sense heritability on a progeny mean basis, across *l* locations for each trait, was estimated as $(V_A + V_D)/(V_A + V_D + V_R/l)$.

Molecular marker analysis

Simple sequence repeat (SSR) primers were synthesized by Research Genetics Incorporated (Huntsville, Alabama, USA). Approximately 1,200 SSR primers were screened for polymorphism between LH200 and LH216. These primers were developed by the following companies or research institutions (primer codes in parentheses): Brookhaven National Laboratory (bnlg), Pioneer Hi-Bred International (phi), DuPont (dupssr), Asgrow (A), the University of Missouri-Columbia (umc) and Monsanto Company (mer).

A 3% Metaphor agarose gel was used to separate the amplified bands. Only those primers that amplified clear and distinguishable bands on 3% agarose gel were used to genotype the (LH200 × LH216) F_2Syn3 population.

PCR and electrophoresis of SSR amplification products were conducted at the Monsanto laboratory in Ankeny, Iowa. The reaction constituents were: Tris-HCl 10 mM, pH 8.3; $MgCl_2$ 2.5 mM; dATP, dCTP, dGTP, dTTP 0.2 mM each; forward- and reverse-primers 0.33 μM each; Ampli*Taq* Gold (Perkin Elmer) 0.5 units; Cresol Red 0.002% (w/v); genomic DNA 20 ng and 2.43% glycerol. ddH_2O brought the total reaction volume to 15 μl. The PCR reaction was carried out in the following fashion. The cycling profile included: (1) activation of Ampli*Taq* Gold for 10 min at 95 °C; (2) 30 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, and extension at 68 °C for 45 s; (3) a final extension at 72 °C for 5 min. After the thermocycler amplification, the SSR plates were stored in a 4 °C refrigerator until eletrophoresis. Gels were photographed by Stratagene Eagle Eye and were scored visually.

Linkage and QTL analysis

We used standard (i.e., for non-random-mated populations) software for linkage and QTL analysis, keeping in mind that the results would apply to a linkage map that is expanded by random mating. The MAPMAKER/EXP 3.0b program (Lincoln et al. 1992) was used to construct the maize genetic map. A chi-square goodness-of-fit test was performed on each locus to test segregation distortion. The Kosambi mapping function was used.

The linkage map, generated with MAPMAKER/EXP 3.0b, was subsequently used in QTL Cartographer v1.14 (Basten et al. 1998) to perform two types of QTL analyses: SRmapqtl (stepwise regression approach) and composite interval mapping (CIM). In SRmapqtl, the forward-backward stepwise regression method was used for choosing background markers to be used in CIM. The parameters used in CIM were: a window size of 15 cM (close to the average marker distance in this study); numbers of background factors of 15 for grain yield, ten for moisture content and plant height, and five for stalk lodging; and a walking speed of 1 cM. The selection and number of markers chosen as background factors (cofactors) were determined from the results of SRmapqtl. The number of cofactors was approximately the number of markers with significant effects ($P < 0.01$) in SRmapqtl. The cofactors for each trait were the markers with the highest *F*-statistic that were outside the set window size. A permutation test (Churchill and Doerge 1994) was used to define the significance threshold to declare a QTL, and 2,000 permutations were conducted for each trait. For each QTL, a one-LOD support interval was constructed according to Lander and Bostein (1989). The one-LOD support intervals are approximately equivalent to 95% confidence intervals (Lynch and Walsh 1998). If two QTLs identified from the two backcross populations were located in the same support interval, they were considered as the same QTL. It was possible, however, to detect a given QTL in one backcross population but not in the other. Suppose the alleles found in each parent are designated *Q* in LH200 and *q* in LH216 (Table 1); the $LH200BC_1$ population will have the *QQ* and *Qq* genotypes, which have identical values under complete dominance. This QTL is therefore undetectable in $LH200BC_1$. But this QTL is detectable in $LH216BC_1$ because *Qq* and *qq* differ in their genotypic values.

For a Design III mating scheme, the estimates of *a* and *d* at each QTL are not directly given by QTL Cartographer. The *QQ* versus *qq* contrast, estimated in $LH200BC_1$, has the expectation of $c_1 = (a - d)/2$. The *QQ* versus *qq* contrast, estimated in $LH216BC_1$, has the expectation of $c_2 = (d + a)/2$. The value of *a* was then estimated as $c_1 + c_2$, and the value of *d* was estimated as $c_2 - c_1$. The level of dominance for a QTL was therefore estimated as $d/a = (c_1 + c_2)/(c_2 - c_1)$.

Bootstrapping was used to determine the precision of the estimates of *d*/*a*. For each of 1,000 bootstrap samples, CIM analysis was used to estimate the mean and the variance of c_1 and c_2 at each testing site. The variance of *d*/*a* was subsequently estimated as the variance of a ratio (Lynch and Walsh 1998). A normal distribution was assumed for *d*/*a*, and a 95% confidence interval for *d*/*a* was calculated as $1.96\ [V(d/a)]^{1/2}$. Because bootstrapping followed by CIM analysis for each bootstrap sample was computationally demanding, confidence intervals on the *d*/*a* ratios were obtained only for grain yield.

After putative QTLs were identified by CIM, the markers nearest to each QTL were chosen for inclusion in an analysis of two-locus epistasis. Specifically, epistatic effects were obtained by fitting a two-locus linear regression model that included the main effects for each locus and an effect for the interaction between loci.

Table 2 Estimates of additive variance (V_A), dominance variance (V_D), broad-sense heritability (H) and average level of dominance (d/a) for four quantitative traits in maize

Parameter	Grain yield (t ha^{-1})	Grain moisture (g kg^{-1})	Stalk lodging (%)	Plant height (cm)
V_A	0.51	182.04	2.32	149.06
V_D	0.82	34.93	0.42	48.41
H	0.89	0.91	0.44	0.76
d/a	1.79	0.62	0.60	0.81

Results

Heritability and average level of dominance based on phenotypic data

The estimated broad-sense heritability ranged from 0.44 for stalk lodging to 0.91 for grain moisture (Table 2). For grain yield, V_D was 60% larger than V_A. In contrast, V_A was the predominant type of genetic variance for grain moisture, stalk lodging and plant height.

The estimates of the average level of dominance (d/a) were larger than zero for all traits, indicating dominance effects for at least some of the loci controlling the traits in this study (Table 2). The estimate of d/a was 1.79 for grain yield, 0.62 for grain moisture, 0.60 for stalk lodging and 0.81 for plant height. These results suggested that overdominance was involved in most loci controlling grain yield, but partial dominance was involved in most loci controlling plant height, grain moisture and stalk lodging. From a summary of several empirical studies, Bingham (1998) found that the average level of dominance indicated overdominance (i.e., $d/a > 1$) in F_2 populations that were random-mated from zero to two times, and complete to partial dominance (i.e., $d/a < 1$) in F_2 populations that were random-mated three or more times. The (LH200 × LH216)F_2 population used in this study was random-mated three times, but the d/a for grain yield remained high at 1.79.

Linkage map

A total of 160 SSR markers formed ten linkage groups that corresponded to the ten maize chromosomes. This map is published as Electronic Supplementary Material; furthermore, Lu et al. (2002) compared this map with three published maps. The total length of the ten chromosomes was 2,581 centiMorgans (cM) with an average distance of 16 cM between adjacent markers. Random mating prior to linkage mapping captures more recombination events between adjacent markers. An F_2 population samples the recombination events from meiosis in the F_1 parents. A random-mated F_2 population retains the recombination events from the F_1 parents and captures new recombination events from the F_2 intermatings. Each subsequent generation of random-mating captures additional recombination events. Random-mating therefore greatly expands the linkage map while breaking repulsion linkages that cause pseudo-overdominance. An interval of 53 cM, approximately the largest gap found on chromosomes 1, 2, 3, 4 and 5 in this study, was equivalent to 28 cM in a non-random-mated F_2 population. Similarly, an interval of 45 cM, approximately the largest gap found on chromosomes 6, 7, 8 and 9 in this study, was equivalent to 23 cM in a non-random-mated F_2 population. An internal Monsanto map indicated that markers A1518 and A1547 were both on chromosome 10. These markers were 90-cM apart in the LH200 × LH216 map, but given the prior linkage information, we joined them to form chromosome 10.

QTLs for grain yield

A total of 28 QTLs for grain yield were identified in either or in both backcross populations (i.e., to each of the two parents; Table 3). The QTLs for grain yield were detected on all ten chromosomes. The 15 QTLs identified in LH200BC_1 accounted for 85% of the phenotypic variance for grain yield in that population. The 18 QTLs found in LH216BC_1 accounted for 90% of the phenotypic variance for grain yield in that population. A QTL located in the region A1792–A1808 on chromosome 7 was identified as a major QTL (LOD score > 7.0) in both of the backcross populations.

Five of the QTLs were detected in both backcross populations (Table 3). The QTL on chromosome 5 was detected at the same location in each backcross population. The four QTLs located between the following pairs of markers were regarded as having the same locations in the two backcross populations, in accordance with a one-LOD support interval for QTLs location: A1452–phi053 on chromosome 3; A1792–A1808 on chromosome 7; A1808–bnlg339 on chromosome 7; and A2181–asg105 on chromosome 8.

On the basis of the absolute value of the d/a ratio ($|d/a|$), 24 out of 28 (86%) QTLs for grain yield showed overdominance, four (14%) showed dominance, and none showed the absence of dominance (Table 3). The level of overdominance ranged from 1.1 for the QTL in A1329–A1109 on chromosome 2 to 143.0 for the QTL in A1067–asg025 on chromosome 8. On the basis of the 95% confidence intervals on d/a, 13 out of the 28 QTLs for grain yield had $|d/a|$ values that were significantly greater than 1, indicating overdominance (Table 3). The very high level of dominance (with $|d/a|$ larger than 12.0) at six QTLs was due to the small value of a (less than 0.05) at these QTLs.

Table 3 Map locations, genetic effects and levels of dominance of QTLs for maize grain yield

QTL	Chromosome	Detected in:	Flanking markers	Nearest distance (cM)[a]	Position (cM)[b]	LOD score	R^2 (%)	a[c]	d[d]	$\|d/a\|$	95% Confidence interval on d/a
1	1	$LH200BC_1$	A1556–A2057*	9.9	129.7	11.1	10.1	–0.27	0.52	1.94	(0.44, 3.45)
2	1	$LH216BC_1$	dupssr26–A2238*	6.0	198.4	4.7	4.1	0.18	0.28	1.52	(0.64, 2.41)
3	1	$LH216BC_1$	A1016*–A1866	1.0	206.8	4.8	3.3	0.13	0.28	2.12	(1.50, 2.75)
4	1	$LH200BC_1$	A1953*–A1429	12.0	288.7	4.5	2.2	–0.12	0.32	2.74	(–2.64, 8.12)
5	2	$LH200BC_1$	A1537*–A2248	13.0	59.9	8.2	9.9	–0.25	0.45	1.82	(1.03, 2.62)
6	2	$LH216BC_1$	A1018–phi092*	8.1	127.5	12.0	9.8	0.37	0.34	0.91	(0.27, 1.55)
7	2	$LH216BC_1$	nc003*–A1329	2.0	145.6	7.9	6.1	0.24	0.29	1.19	(0.34, 2.04)
8	2	$LH216BC_1$	A1329*–A1109	20.0	172.9	7.5	9.8	0.36	0.40	1.11	(–0.79, 3.01)
9	2	$LH216BC_1$	phi090–A1746*	6.7	246.6	4.2	3.8	0.29	0.09	0.30	(–0.27, 0.88)
10	2	$LH216BC_1$	A1746*–bnlg469	11.0	264.3	3.8	4.0	0.26	0.13	0.50	(–0.32, 1.32)
11	3	$LH216BC_1$	phi029–A1638*	6.9	69.7	4.5	2.7	0.07	0.35	4.76	(1.12, 8.39)
12	3	$LH216BC_1$	A1452–phi053*	6.5	85.1	6.1	3.7	–0.00	0.50	125.50	(99.75, 151.25)
		$LH200BC_1$	A1452–phi053*	4.5	87.1	5.2	4.9				
13	3	$LH216BC_1$	A1951*–A1160	1.0	165.5	15.1	7.1	0.33	0.40	1.21	(0.63, 1.78)
14	4	$LH216BC_1$	A1621*–A2291	3.0	189.5	5.6	4.3	0.16	0.28	1.79	(1.01, 2.58)
15	5	$LH200BC_1$	A1208–umc1019*	19.4	115.4	5.4	7.0	0.05	0.68	13.64	(2.80, 24.28)
		$LH216BC_1$	A1208–umc1019*	19.4	115.4	9.4	11.7				
16	6	$LH200BC_1$	A1600*–A2191	11.0	11.0	3.9	2.8	–0.29	0.17	0.59	(–0.11, 1.29)
17	6	$LH200BC_1$	umc1006*–A1188	2.0	43.2	5.6	3.0	–0.10	0.37	3.85	(–0.11, 7.82)
18	6	$LH216BC_1$	A1867*–bnlg426	0.0	59.0	4.7	1.8	0.03	0.36	12.24	(10.94, 13.54)
19	7	$LH216BC_1$	A1792*–A1808	6.0	11.4	24.2	16.8	0.25	0.78	3.13	(2.01, 4.25)
		$LH200BC_1$	A1792–A1808*	8.5	15.4	7.1	3.7				
20	7	$LH200BC_1$	A1808*–bnlg339	7.0	30.8	6.9	4.9	–0.09	0.46	5.33	(2.59, 8.06)
		$LH216BC_1$	A1808–bnlg339*	0.6	38.8	4.4	3.7				
21	7	$LH216BC_1$	bnlg339*–umc1029	18.0	57.4	5.8	13.3	–0.03	0.56	17.63	(16.25, 19.01)
22	8	$LH216BC_1$	A2181–asg105*	14.4	126.8	9.6	17.6	0.23	0.60	2.57	(–0.17, 5.31)
		$LH200BC_1$	A2181–asg105*	8.4	132.8	3.9	1.4				
23	8	$LH200BC_1$	A2082*–A1067	4.0	146.8	4.1	0.5	0.05	0.34	6.61	(–5.43, 18.65)
24	8	$LH200BC_1$	A1067–asg025*	11.3	168.2	4.2	0.6	–0.00	0.29	143.00	(105.78, 180.22)
25	9	$LH200BC_1$	asg093*–A1714	0.0	167.0	4.0	1.1	–0.15	0.23	1.52	(0.05, 2.99)
26	9	$LH200BC_1$	phi065*–bnlg244	0.0	182.7	4.1	1.2	–0.13	0.26	1.91	(–0.37, 4.19)
27	10	$LH216BC_1$	asg010*–phi059	27.0	250.8	3.7	3.1	–0.04	0.57	16.20	(11.38, 21.02)
28	10	$LH200BC_1$	asg010–phi059*	24.4	270.8	4.7	3.6	–0.24	0.69	2.94	(1.37, 4.51)

* Marker nearest to the putative QTL

[a] Map distance (cM) from the putative QTL to the nearest marker

[b] Map position (cM) of the putative QTL on the chromosome; QTLs found in both $LH200BC_1$ and $LH216BC_1$ were considered the same QTL if they were within the same one-LOD support interval

[c] Estimate of half the difference between homozygotes

[d] Estimate of dominance effect

Table 4 Map locations, genetic effects, and levels of dominance of QTLs for maize grain moisture, stalk lodging and plant height

QTL	Chromosome	Detected in:	Flanking markers	Nearest distance (cM)[a]	Position (cM)[b]	LOD score	R^2 (%)	a[c]	d[d]	$\|d/a\|$
Grain moisture (kg ha^{-1})										
1	1	LH200BC$_1$	A2238*–A1016	0.0	204.9	4.5	4.9	–4.15	1.67	0.4
2	3	LH200BC$_1$	A1904–phi029*	5.3	54.4	6.0	5.8	–4.59	2.61	0.6
3	3	LH200BC$_1$	phi029–A1638*	6.9	69.7	7.0	8.4	–3.58	4.03	1.1
4	3	LH200BC$_1$	A1452*–phi053	5.0	82.1	6.5	8.0	–2.58	4.50	1.7
5	3	LH200BC$_1$	A1601*–A1117	5.0	100.6	4.5	3.9	–1.56	4.46	2.9
6	4	LH216BC$_1$	phi079*–phi026	0.0	152.3	4.1	6.8	3.57	1.94	0.5
7	4	LH216BC$_1$	phi026–A1755*	2.1	164.8	3.9	6.7	3.56	2.20	0.6
8	5	LH200BC$_1$	phi128–A1118*	0.9	233.5	4.9	5.3	4.19	–1.68	0.4
9	5	LH200BC$_1$	A1118*–A1885	15.0	249.3	5.0	10.8	6.86	–1.18	0.2
10	5	LH216BC$_1$	A1118–A1885*	25.2	264.3	3.8	14.0	7.64	0.22	0.0
11	7	LH216BC$_1$	A1792*–A1808	5.0	10.4	5.3	2.7	2.69	3.16	1.2
12	8	LH200BC$_1$	A2181–asg105*	14.4	126.8	5.2	9.3	5.51	–2.65	0.5
13	8	LH200BC$_1$	A2082–A1067*	1.4	152.8	5.1	5.0	3.44	–2.78	0.8
14	9	LH200BC$_1$	A1129–A1375*	9.6	40.0	4.1	3.8	4.88	–1.36	0.3
15	9	LH200BC$_1$	A1375*–A1525	10.0	59.7	4.2	3.6	4.97	–1.15	0.2
16	9	LH216BC$_1$	A1375–A1525*	0.8	89.7	5.1	7.9	3.27	0.62	0.2
Stalk lodging (%)										
1	1	LH216BC$_1$	phi011*–dupssr12	15.0	15.0	22.8	10.2	–3.0	–2.3	0.8
2	4	LH216BC$_1$	A2162*–A1565	35.0	284.5	12.7	8.2	3.8	0.9	0.2
		LH200BC$_1$	A2162*–A1565	35.0	284.5	4.9	7.0			
3	5	LH216BC$_1$	A1346–A1306*	0.1	217.3	4.7	7.5	0.5	0.4	0.8
4	7	LH200BC$_1$	A1094*–A1792	0.0	3.2	4.5	7.9	0.6	–0.4	0.6
5	7	LH200BC$_1$	A1792*–A1808	5.0	10.4	4.0	8.4	0.6	–0.4	0.7
6	10	LH216BC$_1$	A1518*–A1547	40.0	121.6	8.0	2.4	–3.6	–0.8	0.2
7	10	LH200BC$_1$	A1518–A1547*	5.0	166.6	6.7	2.0	–1.4	1.7	1.2
8	10	LH200BC$_1$	A1547–asg010*	7.4	216.6	8.0	5.6	1.6	–1.6	1.0
Plant height (cm)										
1	1	LH200BC$_1$	phi011–dupssr12*	15.0	25.0	7.5	15.8	–10.0	4.3	0.4
2	1	LH200BC$_1$	dupssr12*–phi039	5.0	45.0	6.0	9.2	–7.5	–3.5	0.5
3	1	LH200BC$_1$	phi037*–A1564	0.0	56.4	5.6	7.4	–6.7	3.5	0.5
4	1	LH200BC$_1$	A2057*–asg057	5.0	144.6	4.4	5.5	–4.0	4.2	1.0
5	1	LH200BC$_1$	asg057*–A2295	4.9	163.7	3.5	4.9	–3.9	3.9	1.0
6	3	LH216BC$_1$	A1452–phi053*	4.5	87.1	4.8	5.2	2.3	4.4	1.9
7	4	LH216BC$_1$	A1621*–A2291	0.0	186.5	5.7	8.2	2.6	3.7	1.4
8	7	LH216BC$_1$	A1792*–A1808	5.0	10.4	5.0	5.3	4.6	2.4	0.5
9	8	LH216BC$_1$	bnlg240–A2181*	2.3	104.5	5.1	6.4	4.5	2.3	0.5
10	9	LH200BC$_1$	A1209*–asg124	0.0	155.9	3.9	1.5	–0.5	2.9	5.5
11	9	LH200BC$_1$	asg093–A1714*	1.7	172.0	4.2	1.7	–0.9	3.7	4.2

* Marker nearest to the putative QTL

[a] Map distance (cM) from the putative QTL to the nearest marker

[b] Map position (cM) of the putative QTL on the chromosome; QTL found in both LH200BC$_1$ and LH216BC$_1$ were considered the same QTL if they were within the same one-LOD support interval

[c] Estimate of half the difference between homozygotes

[d] Estimate of dominance effect

The results from the two backcross populations suggested that epistasis was not important for grain yield in this study: no two-locus interactions among all the major putative QTLs for grain yield in either population were significant. Stuber et al. (1992) and Xiao et al. (1995) also did not find evidence of two-locus epistasis contributing to heterosis. Therefore, additive and dominance genetic effects (allelic interactions within a gene) were the main types of gene action at QTLs for grain yield. Our analysis did not permit the detection of epistatic loci that did not have significant effects by themselves. The high R^2 values for grain yield (i.e., 85 to 90%), however, indicated that any epistatic loci with non-significant values of a and d could have only minor effects.

QTLs for other traits

For grain moisture, one of 16 (6.3%) QTLs showed no dominance; 13 (81.3%) showed partial dominance, with $|d/a|$ estimates ranging from 0.2 to 0.9; and two (12.5%) showed overdominance, with $|d/a|$ estimates ranging from 1.1 to 2.9 (Table 4). The majority of the QTLs for grain moisture therefore exhibited partial to complete dominance. The average $|d/a|$ ratio across all the 16 QTLs was 0.58. This result agreed with the results from the phenotypic data analysis, where the average level of dominance across all loci controlling grain moisture was 0.62 (Table 2).

For stalk lodging, six of eight (75%) QTLs showed partial dominance, one (12.5%) QTL showed complete dominance, and one (12.5%) QTL showed overdominance (Table 4). The majority (87.5%) of the QTLs for stalk lodging therefore showed partial to complete dominance. The QTL located in the A2162–A1565 region on chromosome 4 was detected in both backcross populations. The average level of dominance across the eight QTLs for stalk lodging was 0.48. This result was consistent with that from phenotypic data analysis, where the average level of dominance was 0.60 (Table 4).

For plant height, all 11 QTLs showed dominance at different levels. Five QTLs (46%) showed partial dominance with $|d/a|$ estimates of approximately 0.5. Two QTLs (18%) showed complete dominance, and four QTLs (36%) showed overdominance with $|d/a|$ estimates ranging from 1.4 to 5.5 (Table 4). Even though QTLs with overdominance were found in both backcross populations, the majority (63%) of the QTLs for plant height showed partial to complete dominance. The average level of dominance across all 11 QTLs was 0.71. This result was close to that from phenotypic data analysis, where the average level of dominance was 0.81 (Table 4).

For plant height, grain moisture and stalk lodging, there was little evidence of epistasis: less than 5% of all possible pairwise interactions among QTLs for each of these three traits were significant ($P < 0.05$).

Discussion

The overdominance hypothesis for heterosis was strongly advocated in the 1940s and 1950s (Crow 1999). Empirical evidence in maize in the 1960s, however, led to a general acceptance of the dominance hypothesis for heterosis. But the phenotypic estimates and the marker-based estimates of the level of dominance for grain yield in this study suggest overdominance. Our study therefore renews old questions on the genetic basis of heterosis. The results from our study can be interpreted in one of two ways, or a combination of both: (1) QTLs for grain yield in maize exhibit true overdominance, or (2) QTLs for grain yield show partial to complete dominance, but they are so tightly linked such that three generations of random mating failed to separate their individual effects.

With regard to the first reason, it is possible that true overdominance exists for some elite maize single crosses. Maize breeding involves the development of inbreds and the evaluation of crosses between these inbreds. Because phenotypic evaluations for grain yield are performed among hybrids (which are heterozygous) rather than inbreds, selection for heterozygote superiority (i.e., true overdominance) may have occurred in the course of maize breeding. Possible mechanisms for heterozygote superiority include selection for better metabolic balance (Mangelsdorf 1952) or metabolic control of fluxes (Kacser and Burns 1981), or selection for modifier genes that favor the heterozygote as proposed by Fisher (1928). We are aware of objections to Fisher's hypothesis for the evolution of dominance in the context of natural populations, but we speculate that these objections do not apply to maize populations in which stringent artificial selection has been practiced. The modification, by selection, of the level or direction of dominance has been shown in *Drosophila* (Helfer 1939), poultry (Dunn and Landauer 1934), cotton (*Gossypium* spp., Harland 1936) and different moth species (Ford 1940; Kettlewell 1965). On the other hand, we note that true overdominance for grain yield in maize contradicts the results of Duvick (1999). If the level of dominance is increasing, then the amount of heterosis should also increase. In contrast, Duvick found that improvements in the performance of hybrids and of their parents were largely parallel from the 1930s to the 1980s. The amount of heterosis has therefore been constant over the years. Selection for higher seed yields of inbreds (i.e., for producing hybrid seeds) may have indirectly limited the potential amount of heterosis due to true overdominance.

With regard to the second reason – persistence of pseudo-overdominance – we decided to random mate the (LH200 × LH216)F_2 population for three generations on the basis of two pieces of information. First, Dijkhuizen et al. (1996) found that after an F_2 maize population was random-mated for four generations, only 38 of the 95 original marker-QTL linkages for kernel-composition traits remained significant. Second, previous Design III experiments indicated partial to complete dominance for

maize grain yield after three generations of random-mating (Bingham 1998).

Random-mating the (LH200 × LH216)F_2 population for three generations was therefore an attempt to strike a balance between reducing pseudo-overdominance and breaking marker-QTL linkages needed for QTL detection. But the approach to linkage equilibrium is slow for closely linked loci. If the recombination frequency between two loci is 0.05, then three generations of random-mating will lead to the retention of $(1 - 0.05)^3$ = 86% of the linkage disequilibrium that was originally present in the non-random-mated F_2 population. If the recombination frequency is 0.20, then three generations of random-mating will lead to the retention of $(1 - 0.20)^3$ = 51% of the original linkage disequilibrium. Our three generations of random-mating would therefore have dissipated loose linkages between QTLs, but not tight linkages. Unfortunately, the lack of a non-random-mated (LH200 × LH216)F_2 population prevented us from estimating $|d/a|$ prior to random-mating.

Perhaps the phenomenon we have observed should be termed *functional overdominance*: each functional unit of inheritance comprises either one QTL that exhibits overdominance, or more than one QTL that each exhibits only partial or complete dominance, but are so tightly linked so that they function as one inherited unit. On the practical side, the current breeding procedures in hybrid crops – create a segregating population, self the population to develop inbreds, and test single-cross hybrids between different inbreds – exploit heterosis regardless of its genetic basis. We concur with the conclusion of Rhodes et al. (1992) that "Only when location and function have been elucidated will the question of dominance versus overdominance likely be resolved for each locus contributing to heterosis." Our study provides evidence of overdominance at the molecular marker level, but analysis at the gene level is necessary for resolving the level of dominance that causes heterosis.

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