Genetic Dissection of an Elite Rice Hybrid Revealed That Heterozygotes Are Not Always Advantageous for Performance

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ABSTRACT

We introduced an experimental design that produced an "immortalized F_2" population allowing for complete dissection of genetic components underlying quantitative traits. Data for yield and three component traits of the immortalized F_2 were collected from replicated field trials over 2 years. Using 231 marker loci, we resolved the genetic effects into individual components and assessed relative performance of all the genotypes at both single- and two-locus levels. Single-locus analysis detected 40 QTL for the four traits. Dominance effects for about one-half of the QTL were negative, resulting in little "net" positive dominance effect. Correlation between genotype heterozygosity and trait performance was low. Large numbers of digenic interactions, including AA, AD, and DD, were detected for all the traits, with AA as the most prevalent interaction. Complementary two-locus homozygotes frequently performed the best among the nine genotypes of many two-locus combinations. While cumulative small advantages over two-locus combinations may partly explain the genetic basis of heterosis of the hybrid as double heterozygotes frequently demonstrated marginal advantages, double heterozygotes were never the best genotypes in any of the two-locus combinations. It was concluded that heterozygotes were not necessarily advantageous for trait performance even among genotypes derived from such a highly heterotic hybrid.

THERE has been considerable interest in detection and estimation of the genetic components underlying quantitative traits. In classical quantitative genetics, such genetic components were defined as additive and dominant effects to represent linear and nonlinear effects within a locus and epistasis for deviation from additivity between loci (Falconer 1981). In a typical digenic system with two alleles per locus, the epistatic effect can be further partitioned into interactions between additive effect of the first locus and additive effect of the second locus (AA), additive effect of the first locus and dominance effect of the second locus (AD), dominance effect of the first locus and additive effect of the second locus (DA), and dominance effect of the first locus and dominance of the second locus (DD; Cockerham 1954).

A number of experimental designs were developed to decompose and estimate the genetic components (Hallauer and Miranda 1981; Mather and Jinks 1982). A statistical model based on a number of assumptions was also developed for estimating the number of loci involved in the inheritance of a quantitative trait (Wright 1934). However, the estimates obtained from an experiment using these classical methods described the characteristics of a population, from which nothing could be learned regarding the genetic effects of individual loci.

The rapid advances in the last decade in high-density molecular linkage maps and the concomitant development in mapping technology have enabled the resolution of the genetic effects of quantitative traits into individual Mendelian loci (Paterson *et al.* 1988). In plants, large efforts also have been made in constructing experimental populations for detecting and analyzing quantitative trait loci (QTL). Populations that can be permanently maintained are preferred because they can provide unlimited seed supplies for repeated experiments in multiple years and locations, thus producing accurate estimates for the QTL effects. Such efforts include the development of doubled haploid lines usually derived from culturing the pollens of F_1 plants from crosses between inbred lines and recombinant inbred lines (RILs) derived from crosses between inbred lines by single-seed descent method. Many studies have been conducted using doubled haploid and RIL populations for detecting and mapping QTL in the last decade. A shortcoming associated with both types of populations is that these populations can be used only for detecting additive types of genetic effects, including additive effect within each locus and AA between loci, and thus have limited use in quantitative genetic analyses.

It is well known that F_2 generation from a cross between two inbred lines provides theoretically the most

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complete and most informative population for most genetic analyses (Allard 1956). For a polymorphic locus, it has all three genotypes present in a proportion of 1:2:1, thus allowing for estimating both additive and dominance effects of the locus. For two unlinked polymorphic loci, nine genotypes would be present in a proportion of 1:2:1:2:4:2:1:2:1, thus allowing for the analysis of interactions between loci including AA, AD, DA, and DD. However, it is very difficult to use F_2 for genetic analysis of quantitative traits, as each distinct genotype is represented by only a single individual, which makes it difficult (if not impossible) to acquire data from replicated measurements of the same genotype. Also the population is in a transient state, and thus the experiment cannot be repeated. Efforts were also made to use F_3 families derived from individual F_2 plants, often referred to as $F_{2:3}$ populations (Edwards *et al.* 1987; Yu *et al.* 1997). Although studies using $F_{2:3}$ types of populations can produce considerable amounts of information regarding the genetic constitution of quantitative traits, such analyses suffer from several disadvantages that are inherent with this type of population. First, F_3 families are genetically heterogeneous, making it impossible to have exact replications in the field trials. Second, because an additional cycle of meiosis results in gene combinations different from those in the F_2 generation, the genotypes of $F_{2:3}$ families do not correspond exactly with those of F_2 individuals. And third, because one generation of self-fertilization theoretically reduces the level of heterozygosity by one-half, data from $F_{2:3}$ types of populations may underestimate dominant types of genetic effects, such as dominance and overdominance at the single-locus level and dominant types of interactions at the multi-locus level.

A fundamental assumption underlying any hybrid crop breeding program is that heterozygotes are advantageous over homozygotes in performance, which is also a common ground for the two long-debated hypotheses concerning the genetic basis of heterosis, *i.e.*, the dominance hypothesis (Davenport 1908) and the overdominance hypothesis (East 1908; Shull 1908). Implicit in the assumption is that the conglomerate of heterozygous advantages over various loci collectively produces what is known as hybrid vigor or heterosis. Although utilization of heterosis has greatly increased the productivity of many crops and animals (Falconer 1981; Stuber 1994; Yuan 1998), experimental data permitting critical assessments of such heterozygous advantages at the whole-genome level have been largely unavailable and especially rare for populations derived directly from breeding lines of crop species.

In the study reported in this article, we introduced an experimental design that produced an "immortalized F_2" population by intermating the RILs from a cross between Zhenshan 97 and Minghui 63, the parents of the most widely cultivated rice hybrid in China. The objectives of this study were to demonstrate the usefulness of the immortalized F_2 population in resolving the genetic effects into individual components and to assess the relative performance of all the genotypes in the entire genome, at both single- and two-locus levels.

MATERIALS AND METHODS

Design and construction of the immortalized F_2 population: A population of 240 F_9 RILs, derived by single-seed descent from a cross between two rice lines, Zhenshan 97 and Minghui 63, was intermated following a design for constructing an immortalized F_2 population. These two lines were the parents of Shanyou 63, the most widely cultivated hybrid with a planting area of ~6.7 million hectares (ha)/year during its peak period in the late 1980s and early 1990s, accounting for ~25% of rice production in China.

In this design, crosses were made between the RILs chosen by random permutations of the 240 RILs. In each round of permutation, the 240 RILs were randomly divided into two groups, and the lines in the two groups were paired up at random without replacement to provide parents for 120 crosses. Each of the 240 RILs was used only once in each round of pairing and crossing. This procedure was repeated three times, resulting in a population of 360 crosses. This population resembles an F_2 population in the sense that the compositions and frequencies of single- and multi-locus genotypes are the same as those of an F_2 population. Also, as the parental seeds of the population were permanently maintained, the population can be regenerated by crossing the parental lines at any time as needed, either in exactly the same 360 combinations or by a different permutation scheme. We called this an immortalized F_2 population.

Because there was a wide range of differences in heading dates between the lines assigned as the parents for the crosses, all the RILs were planted in the nursery at intervals of 7–10 days and the seedlings were transplanted in pairs according to the mating design. The planting for crossing was carried out in four consecutive growing seasons of the summer (Wuhan, China) and winter (Hainan, China) in 1997 and 1998. At least 200 hybrid seeds per cross were produced by hand emasculation and hand pollination.

Field planting and examination: Field trials of the immortalized F_2 population were conducted in the rice growing seasons of 1998 and 1999. Adequate seeds were obtained for 324 crosses for the 1998 planting and 358 crosses for the 1999 planting. The hybrid between Zhenshan 97 and Minghui 63, called Shanyou 63, was also included in the field test. The field experiment followed a randomized complete block design with two replications. Each plot consisted of four rows with 10 hills each: two rows of the hybrid and one row for each of the respective parents. Seedlings ~35 days old for all experimental materials were transplanted to a bird-net-equipped field, with a layout of 26.5 cm between plants within a row and 33.3 cm between the rows, in the experimental farm of Huazhong Agricultural University (Wuhan, China). This planting density is lower than normal agricultural production to reduce the competition between plants in the field. The field management followed essentially normal agricultural practice.

True hybrid plants were determined by careful comparison of morphologic characters with the parents throughout the growing season. In case such field examination failed to distinguish between the hybrids and the parents, polymorphic simple sequence repeat (SSR) markers were used to determine the hybrid plants.

Each row was harvested individually at its maturity to prevent

loss from overripeness. Only the eight plants in the middle of each row were used for scoring. Traits examined included yield per plant, measured as the weight of all filled grains of the plant, which was converted to metric tons/hectare (t/ha); tillers per plant, scored as the number of seed-setting tillers per plant; grains per panicle, scored as the number of filled grains per plant divided by the number of reproductive tillers; and grain weight as the weight (in grams) of 1000 seeds.

Molecular markers and linkage maps: The molecular marker data for the RIL population were essentially as described previously (Xing *et al.* 2002), except that more SSR markers were added in certain regions to reduce the gaps. The genotype for each cross in the immortalized F_2 population was deduced on the basis of the RILs that were used as the parents for the cross. Molecular marker linkage maps were constructed using Mapmaker (Lincoln *et al.* 1992a).

Data analyses: Single-locus QTL were analyzed by composite interval mapping (Zeng 1993, 1994), using the computer program QTL Cartographer. We used a two-step process to identify significant epistatic interactions. First, the entire genome was searched at a 0.001 probability level for digenic interactions for each trait with two-way analyses of variance (ANOVA) using all possible two-locus combinations of marker genotypes. The calculation was based on unweighted cell means (Snedecor and Cochram 1980) and the sums of squares were multiplied by the harmonic means of the cell sizes to form the test criteria. For a data set with the magnitude of 20,000 possible two-locus combinations, ~20 tests could be expected to reach the preset threshold for being significant due to chance alone. Thus, as the second step, we conducted a randomization test to identify those interactions that are more likely to be "really" significant. In conducting such a test, the entry order of the trait data in the analysis was randomly permutated and the *F*-statistic for the digenic interaction was recalculated using the same marker data. This procedure was repeated 1000 times, and the resulting 1000 *F*-values were compared with the *F*-statistic from the original data. If no more than one *F*-value from the random permutations was larger than the *F*-statistic from the original data, the digenic interaction was regarded to be significant.

Each significant interaction was partitioned into four components, each specified by a single degree of freedom: AA, AD, DA, and DD. Statistical significance for each term was assessed using an orthogonal contrast test provided by the statistical package STATISTICA (StatSoft 1997).

RESULTS

The performance of the populations: The measurements of yield and the other three traits for the parents, hybrid, and the immortalized F_2 population are listed in Table 1. The yield of the F_1 in this experiment was slightly lower than that of the hybrid Shanyou 63 under normal agricultural conditions in China, due to the sparse planting. The measurements of the four traits varied widely in the immortalized F_2 population in both years. Grains per panicle showed the highest correlation with yield (Table 2). Correlations of the same traits in two years also reflected the heritabilities of the traits. It should be noted that a number of lines showed higher performance than the F_1 in both years (data not shown).

The molecular marker linkage maps: Molecular marker linkage maps consisting of 231 polymorphic loci, including 174 restriction fragment length polymorphisms (RFLP) and 57 SSR loci, were constructed for both the RIL and immortalized F_2 populations. The map constructed for the immortalized F_2 population, using the deduced marker genotypes based on RILs, spanned a total of 2646.1 cM, which was longer than the map of 2007.3 cM based on the RIL population (not shown). This is understandable because the map construction using RILs took into consideration the multiple crossovers in RILs, whereas the software for map construction of the immortalized F_2 did not consider multiple crossovers. We thus used the map based on the RIL data for QTL analyses.

QTL for yield and yield-component traits: The QTL identified using composite interval mapping for the four traits are given in Table 3. For yield, three QTL, located on chromosomes 6 and 9, were detected in 1998, and four QTL, located on chromosomes 1, 3, 5, and 11 were resolved in 1999. The QTL *yd7*, detected in 1999, appeared to have the largest effect, followed by *yd6a*, detected in 1998. None of the QTL was observed in both years.

For tillers per plant, 6 and 5 QTL were detected in 1998 and 1999, respectively. One of them (*tp1*) was observed in both years. Thus, in total 10 QTL were detected in the 2 years. The effect accounted for by each of the QTL was small as evaluated by the amount of variance explained.

Of the four and six QTL detected for grains per panicle in 1998 and 1999, three (*gp1a*, *gp3*, and *gp7a*) were observed in both years, giving a total of seven QTL for this trait. One of the QTL (*gp7*) detected in both years on chromosome 7 appeared to have a large effect on the trait.

A total of 12 and 13 QTL were detected for grain weight in 1998 and 1999, respectively, with 9 of them resolved in both years, giving a total of 16 QTL for this trait. One QTL (*gw3a*) showed a very large effect in both years.

Taken together, a total of 40 distinct QTL were identified: 13 of them were detected in both years, and the remaining 27 were observed in only 1 year.

Dominance and overdominance: A locus is regarded as exhibiting overdominance if the ratio of the estimated dominance to the absolute value of additive effect is larger than unity and it is regarded as exhibiting partial dominance if the ratio is between 0 and 1. Thus, two QTL (*yd6b* and *yd9*) in 1998 and another two in 1999 showed overdominance for yield. Two QTL (*tp2b* and *tp3b*) for tillers per plant in 1999 showed overdominance. While overdominance was detected for none of the QTL for grains per panicle, one QTL (*gw5b*) in 1998 and another (*gw9*) in 1999 showed overdominance for grain weight. Thus, overdominance occurred more frequently in yield, but less frequently in the component traits, which is similar to the results of Yu *et al.* (1997).

Almost half of the QTL listed in Table 3 showed various degrees of negative dominance. Even more strik-

TABLE 1

Measurements of yield and yield-component traits in the parents, hybrid, and the immortalized F_2 population

Trait	Zhenshan 97	Minghui 63	F_1	Immortalized F_2 population	
				Mean	Range
1998					
Yield (t/ha)	1.80	3.97	6.53	5.15	1.06–7.76
Tillers/plant	12.7	13.2	15.3	17.6	7.8–26.2
Grains/panicle	55.7	99.6	141.3	103.1	45.2–152.9
Grain weight (g/1000)	22.6	27.3	27.1	25.8	16.2–30.9
1999					
Yield (t/ha)	0.88	3.93	5.31	4.67	2.15–7.50
Tillers/plant	6.0	11.5	13.2	14.0	8.1–19.6
Grains/panicle	64.8	107.9	132.8	123.0	73.1–188.8
Grain weight (g/1000)	20.3	27.5	26.7	24.5	19.6–29.7

ing, the "net effect" of the dominance, as evaluated by summing up the dominance effects of the QTL, appeared to be negative for tillers per plant and grains per panicle in 1998. Such negative effects of dominance indicated that heterozygosity was not necessarily favorable for the expression of the trait.

Relationship between marker heterozygosity and performance: The correlation coefficients between heterozygosity of the marker genotypes and trait measurements were small for all four traits (data not shown). They were significant only for yield (correlation coefficient 0.17, significant at $P \leq 0.01$) and grains per panicle (correlation coefficient 0.13, significant at $P \leq 0.05$) in 1998. One possibility for the low correlation is that only a portion of the 231 marker loci is related to the performance of the trait, and the correlation may be "diluted" when calculated using the 231 marker loci. To examine such a possibility, we identified the markers that detected significant effects on the trait by ANOVA, and correlation was recalculated on the basis of such positive markers. However, no significant increase in correlation was observed. This again indicates that overall heterozygosity made little contribution to the expression of the traits. This result was similar to a previous analysis of the $F_{2:3}$ population by Yu *et al.* (1997), who suggested that cancellation between positive and negative dominance among the QTL might account for the nonsignificant correlation between heterozygosity and performance, but different from the results of a diallel cross by Zhang *et al.* (1994, 1995).

Digenic interactions: The numbers of digenic interactions identified by two-way ANOVA for the four traits are listed in Table 4. The total number of tests was 23,791 for the data of 1998 and 24,259 for 1999, because only data sets formed of cells containing five or more crosses were included in the calculation. For individual tests at the 0.001 probability level, the expected number of spurious interactions would be 23.79 in 1998 and 24.26 in 1999. The number of significant interactions was greatly reduced after the randomization tests (Table 4), and the reductions were much more than the expected numbers based on chance events in all the cases, indicating that the randomization test is highly stringent in identifying the significant interactions. The interactions that survived the randomization test may therefore be regarded as the minimum number of significant interactions for each trait at the 0.001 probability level.

The randomization tests confirmed large numbers of significant digenic interactions in both years for all four traits (Table 4). The results were consistent for both years in that the number of significant interactions was the largest for grain weight and the smallest for tillers

TABLE 2

Correlations between yield and yield-component traits in the immortalized F_2 population

Trait	1	2	3	4
Yield (1)	0.53[a]			
Tillers/plant (2)	0.28/0.33	0.39[a]		
Grains/panicle (3)	0.65/0.72	−0.41/−0.27	0.71[a]	
Grain weight (4)	0.16/0.32	−0.21/−0.15	−0.10/0.01	0.83[a]

Critical values for correlation coefficients at probabilities of 0.05 and 0.01 are 0.11 and 0.15, respectively. Results in each cell are presented as 1998/1999.

[a] Correlation between the measurements of the same trait in 2 years.

TABLE 3

QTL for yield and yield-component traits in the immortalized F_2 population identified using composite interval mapping

Trait	QTL	Flanking markers	LOD[a]	A[b]	D[c]	Variance %[d]
Yield (t/ha) (1998)	*yd6a*	Y4073L-C751A	5.8	−0.3	−0.2	12.4
	yd6b	RG653-G342	2.5	0.1	0.2	3.4
	yd9	RG570-RG667	3.6	−0.1	0.3	4.5
Yield (t/ha) (1999)	*yd1*	C2340-C86	4.0	0.3	−0.1	4.9
	yd5	RM26-C1447	2.4	0.0	0.3	3.2
	yd7	C1023-R1440	12.7	0.3	0.4	17.2
	yd11	RG118-C1237	3.3	0.2	−0.4	3.6
Tillers/plant (1998)	*tp1*	RG236-C112	3.8	1.0	−0.9	5.2
	tp3a	C316-C63	3.5	−0.8	0.2	4.1
	tp4	G102-RM255	2.9	−1.2	0.0	3.8
	tp5a	RM42-C734b	3.3	1.0	−0.9	5.3
	tp5b	RM26-C1447	5.7	−1.4	0.2	8.7
	tp6	P-G200	2.7	0.7	−0.9	3.0
Tillers/plant (1999)	*tp1*	RG236-C112	2.9	0.4	0.0	3.6
	tp2a	R2510-RM211	3.4	0.1	0.6	5.1
	tp2b	RM208-RM207	3.8	0.6	−0.2	4.3
	tp3b	C1087-R19	2.7	−0.3	0.8	5.2
	tp5c	RZ649-C624	2.8	0.4	0.1	3.3
Grains/panicle (1998)	*gp1a*	RG532-RM259	2.8	6.0	−3.7	3.5
	gp3	RZ403-C1087	5.7	−7.9	−0.5	7.4
	gp6	RZ667-RG424	6.7	−4.7	−3.4	7.9
	gp7a	C1023-R1440	3.9	5.3	1.7	5.3
Grains/panicle (1999)	*gp1a*	G359-RG532	8.2	11.2	−3.2	9.7
	gp1b	C922-RG101	3.7	−6.6	2.8	3.7
	gp1c	C86-RG236	3.1	7.6	−2.5	4.7
	gp3	C1087-R19	5.4	−7.0	−1.9	6.2
	gp7a	C1023-R1440	13.0	7.9	6.4	16.3
	gp7b	R1789-RM18	3.0	5.1	3.4	3.4
Grain weight (1998)	*gw1a*	G359-RG532	6.3	−0.9	0.3	6.7
	gw1b	C2340-C86	4.6	0.4	0.2	4.4
	gw3a	RZ403-C1087	15.5	1.5	−0.5	16.5
	gw3b	RM55-RM203	4.6	0.5	0.3	4.4
	gw5a	R3166-RG360	9.0	−0.9	−0.2	8.9
	gw5b	C624-C246	5.2	0.5	0.8	7.8
	gw6a	C751A-RZ667	2.6	0.5	0.0	2.5
	gw9	RG667-RM201	4.1	−0.7	0.3	5.0
	gw10a	C1633-C677	3.5	−0.7	0.3	4.0
	gw10b	R2625-C371	3.7	−0.5	0.1	3.2
	gw11	G257-RM209	2.7	0.3	0.0	2.6
	gw12	G1128a-R887	3.0	−0.6	0.1	2.6
Grain weight (1999)	*gw1a*	G359-RG532	9.4	−1.0	0.3	9.3
	gw1b	C2340-C86	6.8	0.4	0.2	6.7
	gw1c	G1128b-C904	2.8	−0.5	0.4	2.3
	gw3a	RZ403-C1087	22.6	1.5	−0.2	24.0
	gw5c	RM42-C734b	6.0	−0.9	0.6	6.8
	gw6a	C751A-RZ667	3.7	0.5	−0.1	3.1
	gw6b	R2549-C962	3.4	0.6	0.1	4.5
	gw7	RG128-C1023	4.8	0.6	0.2	6.5
	gw9	RG667-RM201	2.4	−0.5	0.7	3.5
	gw10a	C1633-C677	2.9	−0.6	0.3	3.0
	gw10b	R2625-C371	2.6	−0.4	−0.1	2.4
	gw11	G44-G257	3.9	0.5	−0.2	3.5
	gw12	G1128a-R887	2.6	−0.5	0.0	2.2

[a] The threshold for logarithm of odd is set at 2.4.
[b] Additive effect.
[c] Dominance effect.
[d] Amount of variance explained.

TABLE 4

Number of significant interactions detected for yield and yield-component traits identified at 0.001 probability by searching all possible two-locus combinations and confirmed by randomization tests

	Whole-genome searching			Confirmed by randomization test		
Trait	1998	1999	Common	1998	1999	Common
Yield	130	91	3	79	63	3
Tiller/plant	98	95	4	58	54	1
Grains/panicle	111	86	23	82	59	20
Grain weight	267	187	53	175	123	50
Number of tests [a]	23,791	24,259				

[a] Number of possible two-locus combinations tested.

per plant, although more interactions were detected in 1998 than in 1999.

A number of interactions, referred to as common interactions, were simultaneously detected in both years for each trait. Again, the largest number of common interactions was detected for grain weight followed by grains per panicle, and much fewer common interactions were revealed for yield and tillers per plant.

Types of interactions: The types of interactions partitioned using orthogonal contrasts for the significant interactions that were confirmed by randomization tests are listed in Table 5. For all four traits, AA occurred at predominantly high frequencies ranging from 56% for grains per panicle in 1998 to 78% for the same trait in 1999. In contrast, DD occurred least frequently in both years for all four traits, with frequencies ranging from 6% for yield and grains per panicle in 1998 to 11% for grains per panicle in 1999. And AD/DA occurred with intermediate frequencies.

TABLE 5

Summary of interaction types for yield and yield-component traits based on significant interactions identified by randomization tests

Trait	Interaction	1998	1999	Common
Yield	Positive pairs	79	63	3
	AA	68	56	3
	AD (DA)	43	21	1
	DD	7	8	0
Tillers/plant	Positive pairs	58	54	1
	AA	45	49	0
	AD (DA)	28	16	2
	DD	6	4	0
Grains/panicle	Positive pairs	82	59	20
	AA	67	57	20
	AD (DA)	45	8	4
	DD	7	8	1
Grain weight	Positive pairs	175	123	50
	AA	154	110	48
	AD (DA)	60	33	10
	DD	17	10	3

The interactions partitioned for grains per panicle for the two-locus pairs that were simultaneously identified by the randomization tests in both years are given in Tables 6–8. Three features are demonstrated in these tables. First, the types of interactions and the amounts of effects were highly consistent in the two years. Second, more than one interaction type occurred in a sizable proportion of the two-locus pairs. Third, each of the interactions accounted for only a small proportion of the phenotypic variation.

Effects of epistatic interactions: According to the coefficients used in the orthogonal contrasts (Steel and Torrie 1980), the test for an AA provided a comparison for the four homozygotes of the two loci involved. The test for an AD compared the relative performance of the heterozygote against the two homozygotes at one locus under the backgrounds of the two homozygotes of the other locus. The test for a DD provided a measurement for the performance of the heterozygote relative to the two homozygotes at one locus against the performance of the heterozygote relative to the two homozygotes at the other locus.

Several points can be made, as exemplified using grains per panicle, on the relative performance of the genotypes among the various two-locus combinations that showed significant interactions in both years. For the two-locus combinations showing significant AA (Table 6), the best-performing genotypes were double homozygotes (homozygous at both loci) in all the two-locus combinations in 1998 and almost all the two-locus combinations in 1999. Complementary two-locus homozygotes (11/22 or 22/11) were frequently the best genotypes and had large effects on the trait as evaluated by the large deviations from the means of both the two parental genotypes and the Minghui 63 genotypes. The parental two-locus genotypes (11/11 or 22/22) in some cases also performed better than others.

For those two-locus pairs showing significant AD/DA in both years (Table 7), single heterozygotes (11/12, 22/12, 12/11, or 12/22) appeared to have advantages over the means of the parental genotypes as well as the Minghui 63 genotypes. However, none of the single

TABLE 6

Comparative advantage of the best double homozygote in each of the two-locus combinations showing significant AA interactions for grains per panicle

			Best homozygote: 1998						Best homozygote: 1999				
Locus 1	Locus 2	Variance %	Genotype[a]	Over midparent[b]	Over Minghui 63[c]	Over 12/12[a]	Best genotype[a]	Variance %	Genotype[a]	Over midparent[b]	Over Minghui 63[c]	Over 12/12[a]	Best genotype[a]
R712 (2)	TEL3 (11)	4.46	11/22	15.87**	10.78*	5.82	11/22	4.63	22/11	18.80**	13.87*	10.82*	22/11
C1176 (3)	R496 (12)	3.78	22/11	17.44**	10.40*	13.26**	22/11	3.45	22/11	19.20**	14.93*	17.42**	22/11
C1176 (3)	RM17 (12)	5.04	22/11	22.34**	16.37**	22.56**	22/11	4.49	22/11	22.79**	17.92**	22.04**	22/11
C316 (3)	RM17 (12)	5.41	22/11	25.07**	18.88**	24.88**	22/11	3.17	22/11	22.17**	15.42**	23.55**	22/11
C63 (3)	RM200 (3)	3.63	22/11	21.72**	18.87**	18.57**	22/11	4.60	22/11	29.94**	27.80**	28.66**	22/11
C63 (3)	RM227 (3)	4.70	22/11	24.49**	21.02**	17.59**	22/11	2.71	22/11	25.45**	21.48**	17.18**	22/11
C63 (3)	R496 (12)	4.17	22/11	17.60**	11.80*	11.74*	22/11	3.54	22/11	19.36**	14.34*	14.82**	22/11
C63 (3)[d]	RM17 (12)	3.74	22/11	18.91**	12.79*	18.51**	22/11	3.44	22/11	20.59**	15.26**	19.53**	22/11
RG393 (3)	RM257 (9)	4.71	22/11	23.19**	24.85**	7.66	22/11	3.78	22/11	27.96**	25.09**	9.42	22/11
RG393 (3)	RM242 (9)	4.81	22/11	23.95**	21.83**	7.62	22/11	5.25	22/11	29.03**	20.83**	9.33	22/11
G144 (3)[d]	C226 (6)	2.08	11/22	20.13**	21.75**	13.26**	11/22	2.79	11/22	20.15**	15.87**	8.35	11/22
G144 (3)[d]	RZ398 (6)	2.11	11/22	20.28**	22.62**	13.42**	11/22	2.98	11/22	20.96**	18.58**	8.05	11/22
R19 (3)[d]	RM18 (7)	2.91	22/11	21.29**	27.87**	13.94**	22/11	3.09	22/11	24.55**	32.15**	13.43*	22/11
RM227 (3)	C405b (11)	5.89	22/22	1.80	3.59	0.34	22/22	6.95	11/11	1.09	0.00	4.06	12/22
C56 (4)	C952 (6)	5.26	22/22	5.96	11.92*	13.89**	22/22	3.53	11/11	0.74	0.00	8.19	22/11
C56 (4)	C688 (6)	6.09	22/22	7.24	14.47**	17.63**	22/22	3.62	22/22	1.65	3.30	8.55	22/22
C56 (4)	R1952a (6)	5.69	22/22	7.24	14.47**	17.63**	22/22	3.74	22/22	1.65	3.30	8.55	22/22
C56 (4)	C153B (9)	4.40	22/22	4.50	9.00	14.95**	22/22	3.53	22/22	1.81	3.61	14.69**	22/22
RZ467 (4)	C734 (9)	3.97	22/22	2.29	4.58	6.66	22/22	3.27	22/22	0.22	0.44	8.69	22/22
C2807 (4)	C734 (9)	3.90	22/22	2.29	4.58	6.48	22/22	3.52	22/22	0.22	0.44	8.42	22/22
R1789 (7)[d]	Y6854L (11)	4.39	11/22	28.20**	29.62**	16.27**	11/22	3.05	11/22	31.46*	29.21**	23.72**	11/22
R1687 (9)	Y6854L (11)	3.79	22/22	5.28	10.55*	9.72	22/22	3.85	22/22	5.39	10.78	20.36**	22/22

The two-locus interactions were identified by the randomization tests and the cutoff for AA was $P \leq 0.01$. **, *Significantly different from 0 at probabilities of 0.01 and 0.05, respectively.

[a] Genotype of the first locus/second locus: 11, homozygous for the Minghui 63 allele; 22, homozygous for the Zhenshan 97 allele; 12, heterozygote.

[b] Midparent is the mean of the two parental genotypes of the respective locus pair.

[c] The Minghui 63 genotype of the respective locus pair.

[d] Another type of interaction was also detected for this two-locus combination.

TABLE 7

Comparative advantage of the best single heterozygote in each of the two-locus combinations showing significant AD/DA interactions for grains per panicle

			Best single heterozygote: 1998						Best single heterozygote: 1999					
Locus 1	Locus 2	Variance %	Genotype[a]	Over midparent[b]	Over Minghui 63[c]	Over 12/12[a]	Best genotype[a]	Variance %	Genotype[a]	Over midparent[b]	Over Minghui 63[c]	Over 12/12[a]	Best genotype[a]
G144 (3)[d]	C226 (6)	2.63	22/12	11.99**	13.60**	5.12	11/22	2.06	22/12	16.58**	12.30**	4.78	11/22
G144 (3)[d]	RZ398 (6)	2.57	22/12	12.11**	14.46**	5.25	11/22	2.98	22/12	17.28**	14.89**	4.37	11/22
G144 (3)	Y6855RA (11)	5.24	11/12	9.96**	17.96**	3.90	11/12	2.90	11/12	2.90	4.26	0.89	22/11
R19 (3)[d]	RM18 (7)	1.30	22/12	13.46**	20.03**	6.11	22/11	1.53	22/12	14.26**	21.86**	3.15	22/11
C63 (3)	RM227 (3)	1.34	12/22	6.38	2.91	−0.52	22/11	1.69	12/22	5.39	1.42	−2.88	22/11
R1789 (7)[d]	Y6854L (11)	1.92	12/11	7.96*	9.38**	−3.97	11/22	1.12	12/11	6.41	4.17	−1.33	11/22

The two-locus interactions were identified by the randomization tests and the cutoff for AD/DA was $P \leq 0.01$. The first four two-locus pairs showed significant AD interactions and the last two two-locus pairs showed significant DA interactions. **, *Significantly different from 0 at probabilities of 0.01 and 0.05, respectively.

[a] Genotype of the first locus/second locus: 11, homozygous for the Minghui 63 allele; 22, homozygous for the Zhenshan 97 allele; 12, heterozygote.

[b] Midparent is the mean of the two parental genotypes of the respective locus pair.

[c] The Minghui 63 genotype of the respective locus pair.

[d] Another type of interaction was also detected for this two-locus combination.

heterozygotes was the best genotype of the respective two-locus combinations, except in one case in 1998, whereas the complementary two-locus homozygotes performed the best in almost all the cases.

In the only two-locus combination that showed significant DD in both years (Table 8), the double heterozygote (12/12) was not the best genotype. However, in a total of the 24 two-locus combinations (Tables 6 and 7), double heterozygotes performed better than the midparental genotypes in 16 cases, indicating that double heterozygotes may have advantages over the means of the two parental genotypes.

We also examined the data from two-locus combinations with significant interactions identified in only 1 year by the randomization tests (data not shown). The trend was the same: namely, the complementary two-locus homozygotes were frequently the best-performing genotypes and had large effects on the trait. Parental homozygotes were better than others in a considerable proportion of the two-locus combinations. Single heterozygotes often had advantages over parental means and, in some cases, were the best genotypes of the respective two-locus combinations. Double heterozygotes sometimes had marginal advantages over the means of the two parental genotypes, but were never the best-performing genotypes.

DISCUSSION

Usefulness of the immortalized F_2 population: This study demonstrated the use of the immortalized F_2 population for complete dissection of the genetic components underlying yield and yield-component traits at both single- and two-locus levels. As illustrated in the study, this population possesses several distinct advantages for QTL analyses. First, the genotypes and their proportions are similar to those in an F_2 population. Thus such a population is genetically as informative as an F_2 population. Second, instead of only one individual per genotype represented in an F_2 population, each genotype in this population is represented by as many plants as the researcher desires, thus permitting replicated trials. The whole population can be recreated when needed, either in exactly the same way or by different permutation schemes, thus allowing for trials in multiple years and locations. Third, the molecular marker data need to be collected from only the 240 RILs, no matter how many crosses are included in the population. It is also obvious that the immortalized F_2 population can be created using any segregating homozygous populations, such as RILs and doubled haploid lines, and thus may have general applications for complete resolutions of genetic components of quantitative traits.

Detection of digenic interactions: The approach that we followed for the confirmation of the two-locus interactions may also be worth noting. A common problem

TABLE 8

Comparative advantage of the double heterozygote in the two-locus combination consistently showing significant DD interaction for grains per panicle

				Double heterozygote			
Locus 1	Locus 2	Year	Variance %	Over midparent[a]	Over Minghui 63[b]	Over best genotype	Best genotype[c]
C63 (3)[d]	RM17 (12)	1998	1.33	0.39	−5.73	−18.51**	22/11
		1999	2.41	1.06	−4.27	−19.53**	22/11

The two-locus interaction was identified by the randomization test and the cutoff for DD was $P \leq 0.01$.
**Significantly different from 0 at probability of 0.01.
[a] Midparent is the mean of the two parental genotypes of the respective locus pair.
[b] The Minghui 63 genotype of the respective locus pair.
[c] Genotype of the first locus/second locus: 11, homozygous for the Minghui 63 allele; 22, homozygous for the Zhenshan 97 allele.
[d] Another type of interaction was also detected for this two-locus combination.

associated with detection of two-locus interactions using the whole-genome search approach is the possible false-positive interactions that occur as chance events. Although this problem has been generally recognized in the literature (Edwards *et al.* 1987; Xiao *et al.* 1995; Yu *et al.* 1997), statistically sound method has not been adopted to distinguish between the interactions that are more likely to be real and those that are less likely to be real. In this study, we devised a randomization test to identify the interactions by comparing the observed *F*-statistic with the results from 1000 random permutations. Statistically, this test is nonparametric and free from all the assumptions about the statistical properties of the data imposed by the two-way ANOVA and thus can provide a nearly exact probability for the *F*-value calculated from each of the two-way ANOVA. Our results indicate that this test is highly stringent for determining significant interactions with the given threshold and may offer a useful method for eliminating possible false-positive digenic interactions, supplementary to the whole-genome search approach.

However, the genetic effects estimated for the various two-locus genotypes may not be independent of each other due to linkages of the markers (Zeng 1994). Such interdependence may sometimes cause bias in the estimated effects of digenic genotypes. However, for a specific two-locus combination, these estimates may still provide direct comparison of the relative performance of the digenic genotypes in the population.

QTL detected in the immortalized F_2 population: A comparison of the results from analyzing the immortalized F_2 population with the QTL that we detected for the RIL population (data not shown) in the same field experiments showed that these two populations were quite consistent; 16 of the 28 QTL detected in the RILs were also resolved in the immortalized F_2 population. However, as expected, the immortalized F_2 is much more informative than the RIL population as shown by the detection of 12 (~40%) more QTL than were detected in the RIL population. This is partly due to the ability to detect dominance effects of the QTL. However, there were also cases in which QTL with no prominent dominance effects were detected in the immortalized F_2 but not in the RILs, indicating that the immortalized F_2 population seems to be more powerful even for the detection of additive genetic effects.

We also compared the immortalized F_2 with the $F_{2:3}$ population (Yu *et al.* 1997) and a vegetatively propagated F_2 population by ratooning (Li *et al.* 2000) from the same cross; in both cases the data were analyzed by the interval mapping method using Mapmaker/QTL (Lincoln *et al.* 1992b). To compare the results directly, the data from the immortalized F_2 population were also analyzed using Mapmaker/QTL. A total of 37 QTL were resolved in the immortalized F_2, as compared to 32 detected in the $F_{2:3}$ population and 20 in the vegetatively propagated F_2. Ten of the QTL were observed in both the immortalized F_2 and $F_{2:3}$ populations, and 8 in both the immortalized F_2 and vegetatively propagated F_2 populations.

A number of QTL have been observed in all the populations derived from the cross between Zhenshan 97 and Minghui 63 that we have analyzed so far. Examples of such QTL include *yd7* (located in the interval of R1440-R1023 or nearby region) for yield; *gp1b* (G359-RG532 or nearby region), *gp3* (RZ403-C1087 or nearby region), and *gp7* (C1023-R1440) for grains per panicle; and *gw3* (RZ403-C1087 or nearby region), *gw5a* (RG360-C734b or nearby region), and *gw7a* (RG128-C1023 or nearby region) for grain weight. Some of the QTL showed consistently large effects in all the populations, despite the widespread occurrence of epistatic interactions. Comparison of the QTL detected for different traits in the various populations also revealed that some of the QTL had pleiotropic effects. An example of such pleiotropic QTL is the one in the region marked by C1023 and R1440 on chromosome 7, which has significant effect on yield, grains per panicle, and grain weight.

In contrast, many of the QTL were detected in only one experiment but not in others. For example, in the

present study, only 27 of the 40 QTL for the four traits were detected in only 1 year and none of the QTL for yield were detected in both years. Furthermore, even for the QTL detected in both years, there were also considerable differences in the estimated genetic effects. Such results clearly indicate that genotype-by-environment interactions had large influences on the expression of the QTL in this population.

Epistasis: Cheverud and Routman (1995) discussed the differences between physiological and statistical genetic definitions of epistasis and also proposed an analysis for what they referred to as physiological epistasis. Epistasis, as dealt with by most statistical genetic models, is a population genetic phenomenon, in which the occurrence and effects of epistasis are dependent on the frequencies of population genotypes, in addition to the effects of the genotypes (Crow and Kimura 1970). In contrast, the epistatic effects identified by the two-way ANOVA and partitioned by orthogonal contrasts employed in this analysis did not depend on the genotypic frequencies in the population and thus are properties of the genotypes. Such epistasis may reflect physiological interactions, although many studies are needed to identify the underlying physiological processes.

The analysis of the immortalized F_2 population has clearly revealed the prevalence of epistatic interactions in the rice genome conditioning the expression of yield and yield-component traits. The highly frequent occurrence of AA clearly indicates that AA is by far the most important component in the genetic bases of these traits. The large numbers of AD/DA and the detection of DD indicate that interactions involving dominant types of genetic effects also have important roles to play. This feature is also similar to the results of Yu *et al.* (1997).

Heterozygosity and performance: We used the number of grains per panicle to demonstrate the genetic effects resolved in the analyses. Number of grains per panicle is probably the most appropriate trait for such a purpose for a number of reasons. This trait is much less complex than yield *per se*, yet highly correlated with yield; in both years the correlations of this trait with yield were higher than the correlation between yield in two years. The heritability of grains per panicle is high as demonstrated by the correlation of this trait in 2 years. Also, number of grains is the best indicator of the fitness of a genotype. In addition, this trait has consistently demonstrated a high level of heterosis (Zhang *et al.* 1994, 1995; Yu *et al.* 1997).

Heterozygote advantage has been the fundamental assumption for hybrid breeding programs and is also a common ground for the two long-debated hypotheses concerning the genetic basis of heterosis (Allard 1960; Stuber *et al.* 1992; Xiao *et al.* 1995; Yu *et al.* 1997; Li *et al.* 2001; Luo *et al.* 2001). Because the analysis is based on the molecular marker polymorphisms that are detectable between the parents of Shanyou 63, only the heterozygotes are pertinent to the F_1 hybrid, and the superior performance of the hybrid, or heterosis, of necessity would be the results of heterozygosity. However, the analyses clearly showed that the level of heterozygote advantage is low as revealed by dominance effects at the single-locus level, DD effects (double heterozygotes) at the two-locus level, as well as correlation between heterozygosity and performance at the whole-genome level. In contrast, the most advantageous genotypes in many of the two-locus combinations are the complementary two-locus homozygotes, which frequently showed significant superiority over other genotypes. This also corroborates the prevalence of AA effects detected in the analyses.

The results also suggest that accumulation of the small advantages over individual loci and two-locus combinations may partly explain the genetic basis of heterosis of grains per panicle in the F_1 hybrid. Although the challenge still remains for a full characterization of the genetic basis of heterosis, the implication of such results is clear. Despite the fact that Shanyou 63 has been the best hybrid widely used for decades, it has not realized the genetic potential set by the genotypes of the two parents, and alternative approaches for exploiting the complementary genotypes may lead to better attainment.

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LITERATURE CITED

Allard, R. W., 1956 Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia **24:** 235–278.

Allard, R. W., 1960 *Principles of Plant Breeding*. John Wiley & Sons, New York.

Cheverud, J. M., and E. J. Routman, 1995 Epistasis and its contribution to genetic variance components. Genetics **139:** 1455–1461.

Cockerham, C. C., 1954 An extension of the concept of partitioning hereditary variance for analysis of covariances among relatives when epistasis is present. Genetics **39:** 859–882.

Crow, J., and M. Kimura, 1970 *An Introduction to Population Genetic Theory*. Burgess Publishing, Minneapolis.

Davenport, C. B., 1908 Degeneration, albinism and inbreeding. Science **28:** 454–455.

East, E. M., 1908 *Report of the Connecticut Agricultural Experimental Station for Years 1907–1908,* pp. 419–428. New Haven, CT.

Edwards, M. D., C. W. Stuber and J. F. Wendel, 1987 Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. Genetics **116:** 113–125.

Falconer, D. D., 1981 *Introduction to Quantitative Genetics*. Longman, London/New York.

Hallauer, A. R., and J. B. Miranda, 1981 *Quantitative Genetics in Maize Breeding*. Iowa State University Press, Ames, IA.

Li, J. X., S. B. Yu, C. G. Xu, Y. F. Tan, Y. J. Gao *et al.*, 2000 Analyzing quantitative trait loci for yield using a vegetatively replicated F_2 population from a cross between the parents of an elite rice hybrid. Theor. Appl. Genet. **101:** 248–254.

Li, Z. K., L. J. Luo, H. W. Mei, D. L. Wang, Q. Y. Shu *et al.*, 2001

Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. Genetics **158:** 1737–1753.

Lincoln, S., M. Daly and E. Lander, 1992a *Constructing Genetics Maps with MAPMAKER/EXP3.0*, Ed. 3. Whitehead Institute Technical Report, Whitehead Institute, Cambridge, MA.

Lincoln, S., M. Daly and E. Lander, 1992b *Mapping Genes Controlling Quantitative Traits with MAPMAKER/QTL1.1*. Whitehead Institute Technical Report, Whitehead Institute, Cambridge, MA.

Luo, L. J., Z. K. Li, H. W. Mei, Q. Y. Shu, R. Tabien *et al.*, 2001 Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. II. Grain yield components. Genetics **158:** 1755–1771.

Mather, K., and J. L. Jinks, 1982 *Biometrical Genetics*, Ed. 3. Chapman & Hall, London/New York.

Paterson, A. H., E. S. Lander, J. D. Hewitt, S. Peterson, S. E. Lincoln *et al.*, 1988 Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction length polymorphisms. Nature **335:** 721–726.

Shull, G. H., 1908 The composition of a field of maize. Am. Breed. Assn. **4:** 296–301.

Snedecor, G. W, and W. G. Cochram, 1980 *Statistical Methods*. Iowa State University Press, Ames, IA.

StatSoft, 1997 *Statistica*. StatSoft, Tulsa, OK.

Steel, R. G. D., and J. H. Torrie, 1980 *Principles and Procedures of Statistics*, Ed. 2. McGraw-Hill, New York.

Stuber, C. W., 1994 Heterosis in plant breeding. Plant Breed. Rev. **12:** 227–251.

Stuber, C. W., S. W. Lincoln, D. W. Wolff, T. Helentjaris and E. S. Lander, 1992 Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics **132:** 823–839.

Wright, S., 1934 The results of crosses between inbred strains of guinea pigs, differing in number of digits. Genetics **19:** 537–551.

Xiao, J. H., J. M. Li, L. P. Yuan and S. D. Tanksley, 1995 Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. Genetics **140:** 745–754.

Xing, Y. Z., Y. F. Tan, J. P. Hua, X. L. Sun, C. G. Xu *et al.*, 2002 Characterization of the main effects, epistatic effects and their environmental interactions of QTLs in the genetic basis of yield traits in rice. Theor. Appl. Genet. **105:** 248–257.

Yu, S. B., J. X. Li, C. G. Xu, Y. F. Tan, Y. J. Gao *et al.*, 1997 Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc. Natl. Acad. Sci. USA **94:** 9226–9231.

Yuan, L. P., 1998 Hybrid rice breeding in China, pp. 27–33 in *Advances in Hybrid Rice Technology*, edited by S. S. Virmani, E. A. Siddiq, and K. Muralidharan. International Rice Research Institute, Los Baños, Philippines.

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

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

Zhang, Q., Y. J. Gao, S. Yang, R. Ragab, M. A. Saghai Maroof *et al.*, 1994 A diallel analysis of heterosis in elite hybrid rice based on RFLPs and microsatellites. Theor. Appl. Genet. **89:** 185–192.

Zhang, Q., Y. J. Gao, M. A. Saghai Maroof, S. H. Yang and J. X. Li, 1995 Molecular divergence and hybrid performance in rice. Mol. Breed. **1:** 133–142.

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