Title

Theoretical maximum contribution of dominance complementation to heterosis in diploid plants

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Abstract

Although heterosis in crops has contributed to reducing hunger and malnutrition over the past 100 years, its genetic basis remains elusive. Understanding heterosis is crucial in unlocking its full potential for use in future crop production. Here we theoretically determine the maximum contribution of dominance complementation to heterosis with a focus on diploid plants. The comparisons between our theoretical results and the experimental observations imply that overdominance and/or epistasis play essential roles in heterosis exhibited by the elite maize hybrids, whereas the contribution from dominance complementation cannot be excluded. To determine the maximum contribution of dominance complementation to heterosis, based on the accumulation of superior alleles in parents, we illustrate the change of the number of heterozygous genes in the ideal hybrids where superior alleles are used to form as many heterozygous genes as possible. Like the decrease in the number of heterozygous genes occurring in the ideal hybrids, a decline in the number of heterozygous genes may eventually occur in elite

hybrids as long as newly developed inbred lines are always used as parents, and thus superior alleles continually accumulate in parents. These findings potentially open a panoramic window into a dynamic understanding of heterosis, suggesting that overdominance and/or epistasis would finally be responsible for the vast majority of heterosis to contribute to hybrid yield and the advantage of elite hybrids over concurrently developed elite inbred lines.

INTRODUCTION

Heterosis refers to the superiority of F_1 hybrid over its inbred parents [1,2]. The discovery of heterosis in maize, along with the growth of knowledge about the basis of heredity, initiated the exploitation of heterosis in plants [3-5]. The genetic circumvention of self-fertilization in female lines led to the success of hybrid seed production in some self-pollinated species such as rice [6,7]. Now over half of the production of maize and rice, two of the world's most important crop plants and two keystone model organisms for basic biology research, is from hybrid seeds [1,7-10]. Although heterosis has been widely used in the production of maize and rice, its genetic basis remains elusive [2,4,6,8,11-13]. Heterosis has been explained by three non-mutually exclusive hypotheses including dominance complementation, overdominance, and epistasis [2-4,6,8,11-13]. The current view is that dominance complementation plays a major role in heterosis, whereas it remains an open question whether elite hybrids get a boost from overdominance and/or epistasis because there is little statistical evidence for the contributions from overdominance and/or epistasis

[4]. Understanding heterosis is crucial in unlocking its full potential for use in future crop production. For example, it would be possible to combine the vast majority of superior alleles together in an inbred line [2,14], and thus elite hybrids would finally lose their advantage over inbred lines possessing the vast majority of superior alleles without the roles of overdominance and/or epistasis in heterosis [7]. A second example is that the roles of overdominance and/or epistasis in heterosis may raise the possibility of developing hybrids with super high yield. To solve the mystery, considerable experimental efforts have been made [2,4,6,8,12]; however, a detailed theoretical study, which has the potential to overcome experimental limitations to yield new insights, has not been done.

In this study, focusing on diploid plants, we theoretically determine the maximum contribution of dominance complementation to heterosis. The comparisons between our theoretical results and the experimental observations [1,15-17] imply that overdominance and/or epistasis play essential roles in heterosis exhibited by the elite maize hybrids, whereas the contribution from dominance complementation cannot be excluded. To determine the maximum contribution of dominance complementation to heterosis, based on the accumulation of superior alleles in parents, we illustrate the change of the number of heterozygous genes in the ideal hybrids where superior alleles are used to form as many heterozygous genes as possible. Like the decrease in the number of heterozygous genes occurring in the ideal hybrids, a decline in the number of heterozygous genes may eventually occur in elite hybrids as long as newly developed inbred lines are always used

as parents [1,6,17], and thus superior alleles continually accumulate in parents [2,14]. These findings suggest that overdominance and/or epistasis would finally be responsible for the vast majority of heterosis to contribute to hybrid yield and the advantage of elite hybrids over concurrently developed elite inbred lines. Thus, developing hybrids with strong overdominance and/or epistasis effects would be the challenge in future hybrid maize breeding, and the challenge has probably been faced by hybrid rice breeding.

RESULTS

Expression for contribution of dominance complementation to heterosis

To theoretically determine the maximum contribution of dominance complementation to heterosis, it is useful to first derive a theoretical expression for the contribution of dominance complementation to heterosis. Thus, alleles at each locus are assumed to exhibit complete dominance; epistasis and environmental influence are assumed to be nonexistent. A key to deriving the expression is the understanding of the genotypic relationships between female parent, male parent, and F_1 (Fig. 1). Note that it is not exactly true that all the genes controlling a quantitative trait are heterozygous in F_1 . Combining the assumptions (see above) with the genotypic relationships (Fig. 1), the midparent value and the phenotypic value of F_1 for a quantitative trait can be given by $\sum_{h \in H} (T_h + t_h)/2 + \sum_{i \in I} T_i + \sum_{j \in J} t_j$ and $\sum_{h \in H} T_h + \sum_{i \in I} T_i + \sum_{j \in J} t_j$, where H is the set of polymorphic genes between parents and the set of heterozygous genes of F_1 (highlighted in red in Fig. 1), I is the set of homozygous superior genes possessed in common by parents and the set of homozygous superior genes of F_1 (highlighted in blue in Fig. 1), J

is the set of homozygous inferior genes possessed in common by parents and the set of homozygous inferior genes of F_1 (highlighted in green in Fig. 1), each of h , i , and j represents a locus in Set H , Set I , and Set J , respectively, each of T_h and T_i denotes the contribution of homozygous superior genotype or heterozygous genotype at a locus to a phenotypic value, and each of t_h and t_j denotes the contribution of homozygous inferior genotype at a locus to a phenotypic value. Further explanation is as follows: (i) Each of T_h , t_h , T_i , and t_j , a small effect of a minor gene on the overall phenotypic expression of a quantitative trait [6], is an actual and basic value contributing to a phenotypic value (note that each of T_h , t_h , T_i , and t_j is not based on comparisons of different mean phenotypic values, each of which is obtained from individuals with the same genotype at a locus); thus, $T_h > t_h \geq 0$, $T_i > 0$, and $t_j \geq 0$. (ii) Superior alleles from superior mutations were fixed at some loci as a result of natural and artificial selection before and during domestication [18,19]; the loci have been difficultly or impossibly identified to be relevant to a quantitative trait by experimental methods, whereas the fixed genes located at the loci have probably ensured a fundamental value of the quantitative trait [18,19]; the fixed genes are therefore included in expressing a phenotypic value; and the superior alleles from the fixed genes are used to form homozygous superior genes possessed in common by parents and thus form homozygous superior genes in F_1 (note that some, but not all, of the genes highlighted in blue in Figure 1 may be fixed genes). Finally, the contribution of dominance complementation to heterosis (D) is obtained from the ratio of difference between phenotypic value of F_1 and midparent value to midparent value to yield

$$D = \frac{\sum_{h \in H} (T_h - t_h) / 2}{\sum_{h \in H} (T_h + t_h) / 2 + \sum_{i \in I} T_i + \sum_{j \in J} t_j} \quad (1)$$

Equation 1 seems to provide a possible solution for the determination of the maximum contribution of dominance complementation to heterosis. On the basis of Eq. 1, a reduction in the contribution of dominance complementation to heterosis is conditioned by $\sum_{i \in I} T_i > 0$, and $\sum_{i \in I} T_i > 0$ depends on the existence of homozygous superior genes possessed in common by parents. In addition, superior alleles possessed in common by parents are used to form homozygous superior genes in F_1 (see the sets of genes highlighted in blue in Fig. 1). Briefly, a reduction in the contribution of dominance complementation to heterosis is linked to homozygous superior genes of F_1 . Superior alleles may be used to form both homozygous superior genes and heterozygous genes in F_1 (see the sets of genes highlighted in blue and red in F_1 in Fig. 1). Hence, the maximum contribution of dominance complementation to heterosis should be determined in the hybrids where all the superior alleles are used to form heterozygous genes.

Hybrids where all superior alleles are used to form heterozygous genes

The following work outlines how the hybrids where all the superior alleles are used to form heterozygous genes are theoretically generated. Eighty is assigned to n (the number of loci related to a quantitative trait). Superior alleles from fixed genes are used to form homozygous superior genes in F_1 (see above). Hence, in the work with the aim of generating the hybrids where all the superior alleles are used to form heterozygous genes, the fixed genes are assumed to be nonexistent. Improvements in inbred lines are accompanied by an increase of z (the number of homozygous superior genes in an inbred

line) [2,14]. For each value of z , we define and use four types of hybrid combinations that express the difference between x (the number of homozygous superior genes in one parent) and y (the number of homozygous superior genes in the other parent) (Table 1; Supplementary Data 1). It is assumed that, without loss of generality, $z = x$ and $x \geq y$. For each value of z , in each type of hybrid combination, we generate hybrids with all possible values of l (the number of homozygous superior genes in F_1) and m (the number of heterozygous genes in F_1) and identify a hybrid with m_{max} (the highest value among all possible values of m) (Supplementary Data 1). It should be noted that l_{min} (the lowest value among all possible values of l) is also in the hybrid with m_{max} (Supplementary Data 1).

We examine the hybrids with m_{max} to ascertain the hybrids where all the superior alleles are used to form heterozygous genes. For $x + y > n$, $m_{max} > 0$ and $l_{min} > 0$ (Fig. 2a-d) mean that superior alleles are used to form both heterozygous genes and homozygous superior genes in the hybrids. An exception is that, in hybrid combinations with $z = x = y$, for $x + y = 2n$, $m_{max} = 0$ and $l_{min} = n$ (Fig. 2a) mean that superior alleles are totally used to form homozygous superior genes in the hybrids. Importantly, for $x + y \leq n$, $m_{max} > 0$ and $l_{min} = 0$ (Fig. 2a-d) mean that all the superior alleles are used to form heterozygous genes in the hybrids.

Maximum contribution of dominance complementation to heterosis

Equation 1, combined with the hybrids where all the superior alleles are used to form heterozygous genes (see the hybrid combinations with $x + y \leq n$ in Fig. 2a-d), enables the determination of the maximum contribution of dominance complementation to heterosis. In a hybrid combination with $x + y \leq n$, $l_{min} = 0$ (Fig. 2a-d) indicates that homozygous superior genes are absent in F_1 , and homozygous superior genes possessed in common by parents are also absent in each parent; thus, $\sum_{i \in I} T_i = 0$ for F_1 and each parent. Hence, substituting $\sum_{i \in I} T_i = 0$ in Eq. 1 yields

$$D = \frac{\sum_{h \in H} (T_h - t_h)/2}{\sum_{h \in H} (T_h + t_h)/2 + \sum_{j \in J} t_j} \quad (2)$$

for the hybrids with $l_{min} = 0$. In a hybrid combination with $x + y = n$, $m_{max} = n$ (Fig. 2a-d) indicates that only heterozygous genes are present in F_1 , and only polymorphic genes between parents are present in each parent; thus, $\sum_{i \in I} T_i = 0$ and $\sum_{j \in J} t_j = 0$ for F_1 and each parent. Hence, Eq. 1 along $\sum_{i \in I} T_i = 0$ and $\sum_{j \in J} t_j = 0$ becomes

$$D = \frac{\sum_{h \in H} (T_h - t_h)}{\sum_{h \in H} (T_h + t_h)} \quad (3)$$

for the hybrids with $m_{max} = n$. Equation 2 combined with $t_h = 0$ and $t_j = 0$ and Eq. 3 combined with $t_h = 0$ reach their highest value, and the highest value is 1, which is definitely the maximum contribution of dominance complementation to heterosis.

Comparisons between our theoretical results and experimental observations

The maximum contribution of dominance complementation to heterosis, a 1: 1 ratio in difference between phenotypic value of F_1 and midparent value: midparent value, offers the possibility of making comparisons with experimental results. After a literature review,

the results from the two maize experiments [1,15-17], Experiment A and Experiment B (Fig. 3), are suitable for the comparisons on the basis of our selection criteria: (i) grain yield, the most complex trait and the key determinant of food security, is evaluated; (ii) elite hybrids, the most successful examples of hybrid breeding, are used as experimental materials; and (iii) hybrids and their parents are tested together in bordered plots over multiple replications, densities, locations, and years to minimize environmental influence [10,12,20-22], which ensures comparability with the maximum contribution of dominance complementation to heterosis determined under the assumption of the nonexistence of environmental influence (see above). Heterosis for grain yield (shown in Fig. 3) is calculated as the ratio of difference between phenotypic value of F_1 and midparent value to midparent value. The experimental data provided by the published literature can be only used to calculate the ratios, each of which is obtained from a set of hybrids and their parents but not obtained from a hybrid and its parents (Fig. 3) [1,15]. Statistical analysis is performed using one-tailed one-sample Student's t test ($P = 0.0098$, $t = 2.1318$, and $df = 4$ for Experiment A and $P = 0.0109$, $t = 2.0150$, and $df = 5$ for Experiment B), and thus the mean ratio obtained from each of the two maize experiments (the actual mean) is statistically significantly higher than the maximum contribution of dominance complementation to heterosis (the hypothetical value). Hence, the maximum contribution of dominance complementation is inadequate to explain heterosis exhibited by the elite maize hybrids.

Even if alleles at each locus exhibit complete dominance, the contribution of dominance complementation to heterosis, even in elite hybrids, cannot reach the maximum contribution of dominance complementation. The reasons can be summarized as follows:

(i) The maximum contribution of dominance complementation to heterosis is determined in the hybrids where all the superior alleles are used to form heterozygous genes (see the hybrid combinations with $x + y \leq n$ in Fig. 2a-d). However, homozygous superior genes are formed by fixed superior alleles in hybrids (see above). In addition, unfixed superior alleles can also be used to form homozygous superior genes in hybrids because of an adequate number of superior alleles in parents (refer to the hybrid combinations with $x + y > n$ in Fig. 2a-d) and inadequate breeding capacity. (ii) A key factor for determining the maximum contribution of dominance complementation to heterosis is $t_h = 0$ and $t_j = 0$ in Eq. 2 and $t_h = 0$ in Eq. 3; however, it is possible that $t_h > 0$ and/or $t_j > 0$ because each of t_h and t_j is an actual and basic value contributing to a phenotypic value (see above).

DISCUSSION

Roles of overdominance and/or epistasis in heterosis

Our results may be summarized as follows: (i) combining the assumptions that environmental influence, fixed genes, overdominance, and epistasis are nonexistent with $t_h = 0$ and $t_j = 0$, the maximum contribution of dominance complementation to heterosis is determined in the hybrids where all the superior alleles are used to form heterozygous genes; (ii) the maximum contribution of dominance complementation is inadequate to explain heterosis exhibited by the elite maize hybrids from the experiments with

minimized environmental influence; and (iii) even if alleles at each locus exhibit complete dominance, the contribution of dominance complementation to heterosis, even in elite hybrids, cannot reach the maximum contribution of dominance complementation because of the existence of fixed genes, an adequate number of superior alleles in parents, inadequate breeding capacity, and/or the possibilities of $t_h > 0$ and/or $t_j > 0$. Briefly, the maximum contribution of dominance complementation is inadequate to explain heterosis exhibited by the elite maize hybrids and cannot be reached by the contribution of dominance complementation in elite hybrids even if alleles at each locus exhibit complete dominance. Thus, our results imply that overdominance and/or epistasis play essential roles in heterosis exhibited by the elite maize hybrids, whereas the contribution from dominance complementation cannot be excluded. On one hand, the roles of overdominance and/or epistasis in heterosis not only ensure that the advantage of elite hybrids over concurrently developed elite inbred lines can be maintained forever [7] but also raise the possibility of developing hybrids with super high yield. On the other hand, the roles of overdominance and/or epistasis in heterosis complicate genomic prediction for performance of hybrid yield [7,12,20].

Dynamic understanding of heterosis

Different species and different parents within species may result from different degrees of evolution and improvement. Hybrids may include ordinary hybrids and elite hybrids. Focusing on elite hybrids, covering all the degrees of evolution and improvement, a dynamic view should be vital for understanding heterosis. To determine the maximum

contribution of dominance complementation to heterosis, based on the accumulation of superior alleles in parents, we illustrate the change of the number of heterozygous genes in the ideal hybrids where superior alleles are used to form as many heterozygous genes as possible (Fig. 2a-d). The ideal hybrids cannot reflect the actual complexity; even if heterozygous genes are important for heterosis, because breeding capacity is inadequate, it is practically unachievable to obtain the ideal hybrids. However, like the decrease in the number of heterozygous genes occurring in the ideal hybrids (see the hybrid combinations with $x + y > n$ in Fig. 2a-d), a decline in the number of heterozygous genes may eventually occur in elite hybrids as long as newly developed inbred lines are always used as parents [1,6,17], and thus superior alleles continually accumulate in parents [2,14]. These findings potentially open a panoramic window into a dynamic understanding of heterosis.

The ultimate goal of a hybrid breeding program is to release new hybrid varieties with higher yield than existing hybrid varieties [1,17]. Hybrid yield depends on both heterosis and yields of parents [1,17]. The first elite maize hybrids, in 1920s, exhibited strong heterosis for grain yield [1,3,4], which might be attributed to dominance complementation, overdominance, and/or epistasis (as demonstrated above) and was inseparable from a large number of heterozygous genes [7,8,20]. The number of heterozygous genes in F_1 equals the number of homozygous inferior genes included in polymorphic genes between parents (see the sets of genes highlighted in red in Fig. 1). The inferior alleles included in polymorphic genes between parents, together with inferior

alleles possessed in common by parents (see the sets of genes highlighted in red and green in each parent in Fig. 1), existed in the maize inbred parents derived from open-pollinated maize at the beginning of the exploitation of heterosis in breeding and caused the poor seed production of the maize inbred parents [3,4]. Because newly developed inbred lines have always been used as parents [1,6,17], improvements in inbred lines have enhanced both yields of parents and contributions of parents to hybrid yield [1,17] and have been accompanied by the accumulation of superior alleles in parents [2,14]. The continual accumulation of superior alleles in parents would finally lead to a shortage of heterozygous genes in hybrids (refer to the hybrid combinations with $x + y > n$ in Fig. 2a-d). Consequently, dominance complementation relying largely on the number of heterozygous genes would play a negligible role in heterosis, whereas overdominance and/or epistasis would finally be responsible for the vast majority of heterosis to contribute to hybrid yield and the advantage of elite hybrids over concurrently developed elite inbred lines. Thus, developing hybrids with strong overdominance and/or epistasis effects would be the challenge in future hybrid maize breeding. The challenge has probably been faced by hybrid rice breeding [23] because parents of hybrids in rice are armed with high yield resulting from intentional and unintentional selection through millennia of traditional farming [19,24] and thus possess the vast majority of superior alleles [2,14].

Over the decades when breeding efforts enabled not only the yields of parents (the midparent value) of elite maize hybrids to have a consistent improvement but also the

magnitude of heterosis for grain yield (the difference between phenotypic value of F_1 and midparent value) to be unchanged in a major way, the ratio of difference between phenotypic value of F_1 and midparent value to midparent value decreased [1,2,15-17]. The ratio would continually decline (on the basis of the above discussion). When the ratio is equal to or less than the maximum contribution of dominance complementation to heterosis, the approach to realizing the roles of overdominance and/or epistasis in heterosis by comparing our theoretical results with the experimental observations (see above) is no longer valid. The approach is not valid in rice for the same reason.

METHODS

Defining types of hybrid combinations

Four types of hybrid combinations are defined and used. For example, for $z = 19$, the type of hybrid combination $z = x = y$ represents hybrid combinations with $x = 19$ and $y = 19$, $z = x = y + 4$ represents hybrid combinations with $x = 19$ and $y = 15$, $z = x = y + 8$ represents hybrid combinations with $x = 19$ and $y = 11$, and $z = x = y + 12$ represents hybrid combinations with $x = 19$ and $y = 7$ (Table 1; Supplementary Data 1). As another example, for $z = 21$, $z = x = y$ represents hybrid combinations with $x = 21$ and $y = 21$, $z = x = y + 4$ represents hybrid combinations with $x = 21$ and $y = 17$, $z = x = y + 8$ represents hybrid combinations with $x = 21$ and $y = 13$, and $z = x = y + 12$ represents hybrid combinations with $x = 21$ and $y = 9$ (Table 1; Supplementary Data 1).

Generating all possible values of l and m

There are numerous hybrid combinations where one parent has x homozygous superior genes, and the other parent has y homozygous superior genes. In a hybrid combination where y superior alleles from each parent are used to form homozygous superior genes of F_1 , l reaches its highest value, and the highest value of l equals y . In a hybrid combination where $x + y \leq n$, and all the superior alleles from both parents are used to form heterozygous genes of F_1 , l reaches l_{min} , and $l_{min} = 0$. Thus, $0 \leq l \leq y$ for hybrid combinations with $x + y \leq n$. To understand that l reaches l_{min} , and $l_{min} = x + y - n$ in a hybrid combination with $x + y > n$, we may imagine that superior alleles from both parents are first used to form heterozygous genes of F_1 to make m reach n , and the remaining superior alleles from both parents then give the conversion of heterozygous genes to homozygous superior genes in F_1 to contribute to l . Thus, $x + y - n \leq l \leq y$ for hybrid combinations with $x + y > n$. In each hybrid combination, $m = x + y - 2l$ because l superior alleles from each parent are used to form homozygous superior genes of F_1 , and the remaining superior alleles from both parents are used to form heterozygous genes of F_1 . For example, for $n = 80$ and $z = 19$, $z = x = y + 4$ represents hybrid combinations with $x = 19$ and $y = 15$. Because $x + y < n$, $0 \leq l \leq y$. Thus, $0 \leq l \leq 15$ ($l = 0, 1, 2, 3, \dots, 15$), and $m = x + y - 2l = 34 - 2l$ ($m = 34, 32, 30, 28, \dots, 4$) (Supplementary Data 1). As another example, for $n = 80$ and $z = 59$, $z = x = y + 8$ represents hybrid combinations with $x = 59$ and $y = 51$. Because $x + y > n$, $x + y - n \leq l \leq y$. Thus, $30 \leq l \leq 51$ ($l = 30, 31, 32, 33, \dots, 51$), and $m = x + y - 2l = 110 - 2l$ ($m = 50, 48, 46, 44, \dots, 8$) (Supplementary Data 1).

Statistical analysis

One-tailed one-sample Student's t test is used to compare the actual mean with the hypothetical value. P values lower than 0.05 are considered statistically significant.

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Tables

Table 1 Examples illustrating types of hybrid combinations

z	x	y	Types of hybrid combinations
19	19	19	$z = x = y$
19	19	15	$z = x = y + 4$
19	19	11	$z = x = y + 8$
19	19	7	$z = x = y + 12$
21	21	21	$z = x = y$
21	21	17	$z = x = y + 4$
21	21	13	$z = x = y + 8$
21	21	9	$z = x = y + 12$

Figures

Figure 1 Examples illustrating genotypic relationships between female parent, male parent, and F₁

A represents a superior allele, and a represents an inferior allele. Eighty is assigned to the number of genes controlling a quantitative trait. The set of polymorphic genes between parents and the set of heterozygous genes of F₁ are highlighted in red, the set of homozygous superior genes possessed in common by parents and the set of homozygous superior genes of F₁ are highlighted in blue, and the set of homozygous inferior genes possessed in common by parents and the set of homozygous inferior genes of F₁ are highlighted in green.

Example A	
Female parent	$A_1A_1a_2a_2a_3a_3a_4a_4A_5A_5A_6A_6a_7a_7a_8a_8A_9A_9 \dots a_{78}a_{78}A_{79}A_{79}A_{80}A_{80}$
Male parent	$a_1a_1a_2a_2A_3A_3a_4a_4A_5A_5a_6a_6A_7A_7A_8A_8A_9A_9 \dots a_{78}a_{78}a_{79}a_{79}a_{80}a_{80}$
F ₁	$A_1a_1a_2a_2A_3a_3a_4a_4A_5A_5A_6a_6A_7a_7A_8a_8A_9A_9 \dots a_{78}a_{78}A_{79}a_{79}A_{80}a_{80}$
Example B	
Female parent	$A_1A_1a_2a_2a_3A_4A_4A_5A_5A_6A_6A_7A_7a_8a_8A_9A_9 \dots a_{78}a_{78}a_{79}a_{79}a_{80}a_{80}$
Male parent	$A_1A_1A_2A_2A_3A_3a_4a_4A_5A_5A_6A_6a_7a_7a_8a_8a_9a_9 \dots a_{78}a_{78}A_{79}A_{79}a_{80}a_{80}$
F ₁	$A_1A_1A_2a_2A_3A_4A_4A_5A_5A_6A_6A_7a_7a_8a_8A_9a_9 \dots a_{78}a_{78}A_{79}a_{79}a_{80}a_{80}$

Figure 2 Changes of $x + y$, m_{max} , and l_{min} based on increase of z

The horizontal grey line indicates $x + y = n$; the vertical grey line indicates the values of m_{max} , l_{min} , and z corresponding to $x + y = n$. **a**, Changes of $x + y$, m_{max} , and l_{min} in hybrid combinations with $z = x = y$. **b**, Changes of $x + y$, m_{max} , and l_{min} in hybrid combinations with $z = x = y + 4$. **c**, Changes of $x + y$, m_{max} , and l_{min} in hybrid combinations with $z = x = y + 8$. **d**, Changes of $x + y$, m_{max} , and l_{min} in hybrid combinations with $z = x = y + 12$.

Figure 3 Heterosis for grain yield exhibited by elite maize hybrids

In Experiment A, 50 hybrids were grouped into five sets, each of which included all possible cross combinations of five unrelated inbred lines that were most widely used in one of the five decades (1930s through 1970s); the hybrids and their parents were tested together for three years, in two locations per year, at three densities per location, with two replications per density, and in bordered plots [1,15,17]. In Experiment B, 42 hybrids were grouped into six sets, each of which included seven hybrids that were made based on the pedigrees of the Era hybrids released in one of the six decades (1930s through 1980s); the hybrids and their parents were tested together for two years, in two or three locations per year, at three densities per location, and in bordered plots [1,16,17]. Heterosis for grain yield is calculated as the ratio of difference between phenotypic value of F_1 and midparent value to midparent value. The black dots denote the ratios, each of which is an average ratio obtained from a set of hybrids and their parents over multiple years, locations, densities, and replications [1,15-17]. The red lines and blue error bars represent the averages and standard deviations.