

THE QUANTITATIVE GENETICS OF HETEROSIS

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INTRODUCTION

Nearly 50 years have elapsed since the seminal heterosis conference was held at Iowa State College (Gowen, 1952). That conference undoubtedly grew out of the obvious importance of maize (*Zea mays* L.) hybrids in the agricultural economy of Iowa and the U.S. as well as the lack of understanding of the phenomenon of heterosis. Farmers in Iowa rapidly adopted maize hybrids. In just 15 years, Iowa went from 0 to 100% of the maize acreage being planted to hybrids. Gowen (1952) stated the following about hybrid maize "It seems likely that in no other period of like years has there been such an increase in food produced over so many acres of land. The return from hybrid corn has been phenomenal, but it is now evidently approaching an asymptotic value." If only Gowen could have looked ahead 50 years, because the best was yet to come (Fig. 1).

The term heterosis was coined by Shull (1952). He defined the heterosis concept as "...the interpretation of increased vigor, size, fruitfulness, speed of development, resistance to disease and to insect pests, or to climatic rigors of any kind, manifested by crossbred organisms as compared with corresponding inbreds, as the specific results of unlikeness in the constitutions of the uniting parental gametes." This definition is often interpreted as not implying a genetic basis for heterosis, because the definition basically describes the phenotype that results from crossing two different inbred lines.

For our purposes, we will define heterosis or hybrid vigor as the difference between the hybrid and the mean of the two parents (Falconer and Mackay, 1996). This definition is usually called midparent heterosis. Midparent heterosis is often expressed as a percentage of the midparent in the literature. It is important to note, however, that percent midparent heterosis is difficult to interpret from a quantitative genetic point of view, and statistical tests of percent midparent heterosis are nearly impossible. High parent heterosis is preferred in some circumstances, particularly in self-pollinated crops, for which the goal is to find a better hybrid than either of the parents.

To some, the terms hybrids and heterosis are synonymous. This is misleading, however, because there are hybrids that do not exhibit heterosis, but there cannot be heterosis without hybrids. In some species, hybrids are sold commercially because crossing of two varieties brings together complementary traits controlled by additive gene action. Distinguishing between hybrids and heterosis is important, because hybrids bring factors other than heterosis per se, i.e., uniformity, reproducibility, etc., to crop production. Often these factors are

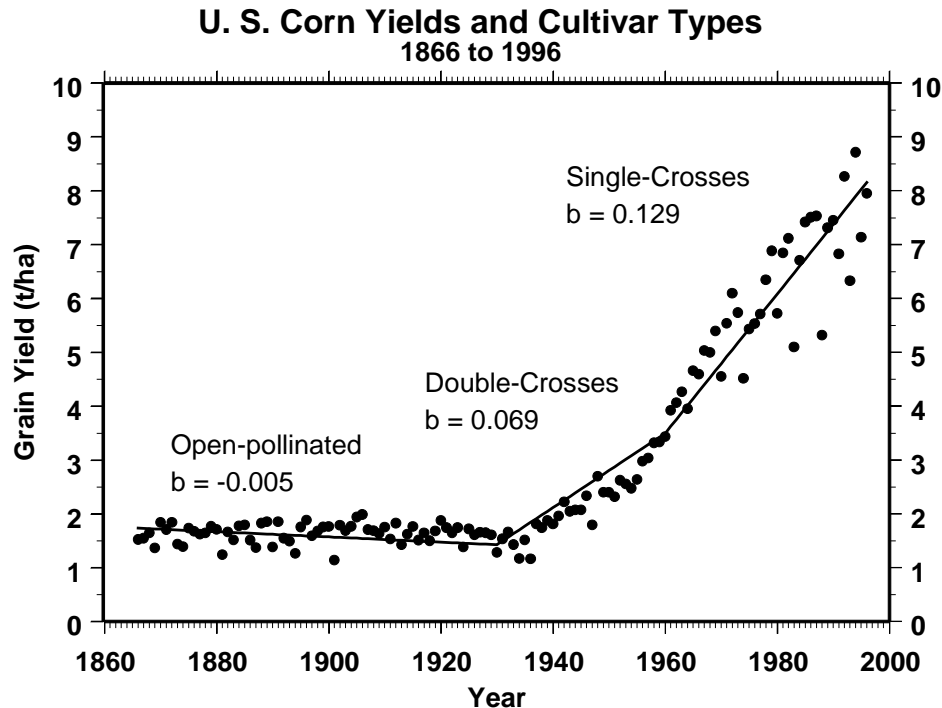


Fig. 1 Average U. S. maize yields from 1866 to 1996. Regression lines were splined together at 1930 and 1960, which corresponds roughly to when double-cross and single-cross hybrids started to become important. Regression coefficients are in $\text{t/ha}^{-1}/\text{year}$. Data are from the USDA, National Agricultural Statistics Service.

confounded and difficult to separate. For example, uniformity may result in higher yields. Is uniformity a genetic or nongenetic cause of increased yield? Is uniformity a factor in heterosis? In other species (such as wheat), hybrids are being sought as a means to prevent farmers from saving and planting their own seed and as a means of protecting research investments in transgenes.

In this manuscript we will review basic quantitative genetic concepts in heterosis, introduce the concept of baseline heterosis, review the results of over 50 years of gene action studies, and suggest needs for future research. We feel that the quantitative genetics of heterosis must be tied to plant improvement and that any theory of heterosis must explain and be consistent with the increase in yield of hybrid maize since 1930 (Fig. 1).

BASIC QUANTITATIVE GENETIC CONCEPTS OF HETEROSIS

Much of the quantitative genetic theory of heterosis is based on single locus theory. Single locus heterosis theory assumes the absence of epistasis, which considerably simplifies the mathematics and interpretations of the theory. Single locus theory will be reviewed in detail, because basic properties of heterosis are derived from this theory. Willham and Pollak (1985) developed single locus heterosis theory for predicting the performance of the F_1 , F_2 , parents, and the backcross to the parents. They used the random mated F_1 (the F_2 generation) as the base population in which all genetic effects are defined. Willham and Pollak (1985) were interested in applying this theory to animals, for which inbreeding the parental populations is rare. Therefore, we have extended this theory to include any level of inbreeding of the F_1 , F_2 , P_1 , and P_2 generations. A pedigree showing how each

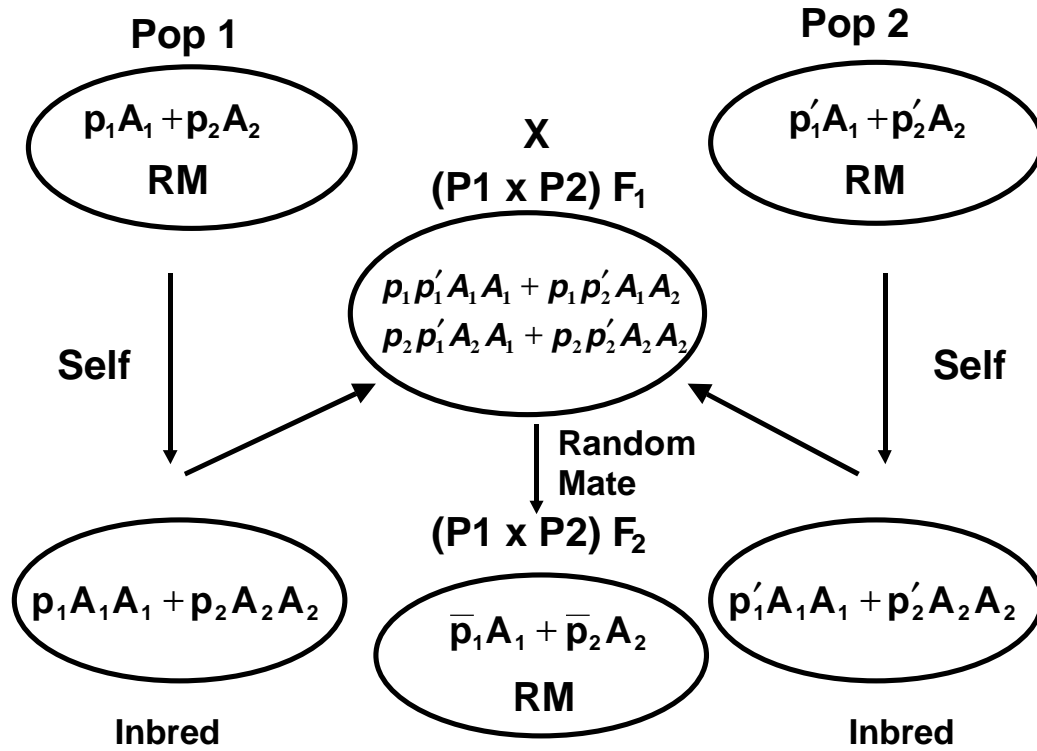


Fig. 2. Mating scheme for populations described in text. Populations 1 and 2 start with the gametic arrays shown and are in random mating equilibrium (panmixia). Crossing the panmictic populations together forms the F_1 shown. Random mating the F_1 gives rise to the F_2 generation, the population in which genetic effects are described in Willham and Pollak (1985). Inbreeding populations 1 and 2 to complete homozygosity generates the populations with the genotypic arrays shown. Crossing these two inbred populations produces the same F_1 as crossing the two panmictic populations.

generation is obtained is given in Fig. 2. Assuming two alleles per locus, the generation means are

$$\bar{F}_{1(f)} = (1-f)(\bar{F}_2 + 2\Delta^2d) + fa(\bar{p}_1 - \bar{p}_2),$$

$$\bar{F}_{2(f)} = (1-f)(\bar{F}_2) + fa(\bar{p}_1 - \bar{p}_2),$$

$$\bar{P}_{1(f)} = (1-f)(\bar{F}_2 + 2\Delta\alpha - 2\Delta^2d) + fa(\bar{p}_1 - \bar{p}_2 + 2\Delta), \text{ and}$$

$$\bar{P}_{2(f)} = (1-f)(\bar{F}_2 - 2\Delta\alpha - 2\Delta^2d) + fa(\bar{p}_1 - \bar{p}_2 - 2\Delta),$$

where

f = inbreeding coefficient of a generation;

p_i = frequency of the i th allele in population 1;

p'_i = frequency of the i th allele in population 2;

$\bar{p}_i = \frac{p_i + p'_i}{2}$ = average allele frequency in the cross of population 1 and 2; and

$$\delta_i = \frac{p_i - p'_i}{2} = \text{one half the difference in allele frequency between populations.}$$

In the two allele case,

$$\delta_1 = -\delta_2 = \Delta; \text{ and}$$

d = the deviation of the heterozygote from the homozygote midparent;

a = half of the difference between homozygotes;

$\alpha = a + d(\bar{p}_2 - \bar{p}_1)$ = average effect of an allele substitution;

$$\bar{F}_2 = a(\bar{p}_1 - \bar{p}_2) + 2\bar{p}_1\bar{p}_2d = \text{mean of } F_2 \text{ generation.}$$

Panmictic-midparent heterosis is the heterosis observed when two random mating populations are crossed to form an F_1 hybrid. The strict definition of panmictic-midparent heterosis is the difference between the mean of the F_1 and the average of the two random mated parent populations (midparent value). F_2 heterosis is defined as the difference between the mean of the F_2 generation and the midparent value. Algebraically, these heterosis values are:

$$\text{Panmictic-midparent heterosis} = 4\Delta^2d, \text{ and}$$

$$F_2 \text{ heterosis} = 2\Delta^2d.$$

Four conclusions can be drawn from these expressions: 1) heterosis is dependent on directional dominance; 2) heterosis is a function of the square of the difference in allelic frequency between two populations and therefore, heterosis is specific to a particular cross; 3) if two inbred lines are crossed, Δ can only be 0 or 1, therefore, heterosis in a cross of two inbred lines is a function of dominance at those loci that carry different alleles in the inbred lines (Falconer and Mackay, 1996); and 4) randomly mating the F_1 reduces heterosis by 50%. Although genetic divergence (difference in allelic frequency) and dominance are necessary for there to be heterosis, they are not sufficient in the case of multiple alleles. Cress (1966) showed that with multiple alleles segregating in a population the lack of heterosis cannot be used to infer a lack of genetic divergence between the parental populations. This result has important implications when breeders are screening populations to establish new heterotic groups.

Falconer and Mackay (1996) refer to heterosis as the converse of inbreeding depression. This is sometimes an overlooked fact and represents one of the breakthroughs in the discovery of heterosis. This fact is particularly relevant to the inbred-hybrid system of breeding in which heterosis is generally calculated by using the mean of inbred parents, as opposed to the mean of random mated populations, as in Falconer and Mackay (1996). We define inbred-midparent heterosis as the difference between the mean of the F_1 and the mean of the parent populations when inbred to homozygosity. The vigor lost during inbreeding of the parent populations is restored in the F_1 . This gives rise to the concept of *baseline heterosis*. *Baseline heterosis* is simply the restoration of what was lost because of inbreeding depression. Inbred-midparent heterosis is equal to baseline heterosis plus *panmictic-midparent heterosis*. Thus, baseline heterosis is equal to inbred-midparent heterosis minus panmictic-midparent heterosis, which is equal to the difference between the panmictic midparent value and the inbred midparent value, or simply the average inbreeding depression observed in the two panmictic parent populations. Algebraically,

$$\text{Inbred-midparent heterosis} = 2\bar{p}_1\bar{p}_2d + 2\Delta^2d, \text{ and}$$

$$\text{baseline heterosis} = 2\bar{p}_1\bar{p}_2d - 2\Delta^2d.$$

Note that inbred-midparent heterosis is a function of inbreeding depression, genetic divergence, and dominance whereas panmictic-midparent heterosis is a function

only of genetic divergence and dominance.

Surprisingly little attention has been given to epistasis and heterosis. In the 1950 conference on heterosis (Gowen, 1952), epistasis as a cause of heterosis was not directly addressed. Willham and Pollak (1985) presented heterosis theory for the case of two linked loci with epistasis. Although the equations are too complicated to model, a couple of important points emerge concerning epistasis and heterosis. Panmictic-midparent heterosis is a function of dominance and unlinked additive \times additive epistasis at loci with genetic divergence. The performance of the F_1 hybrid, however, is a function of dominance and unlinked dominance \times dominance epistasis at those loci showing genetic divergence. These observations have important implications for the genetic interpretation of the midparent heterosis observed in self-pollinated crops that show little inbreeding depression. The heterosis observed may be due primarily to additive \times additive epistasis, which does not contribute to inbreeding depression.

GENE ACTION AND HETEROISIS

Theory

Single locus heterosis theory coupled with the detrimental effect of

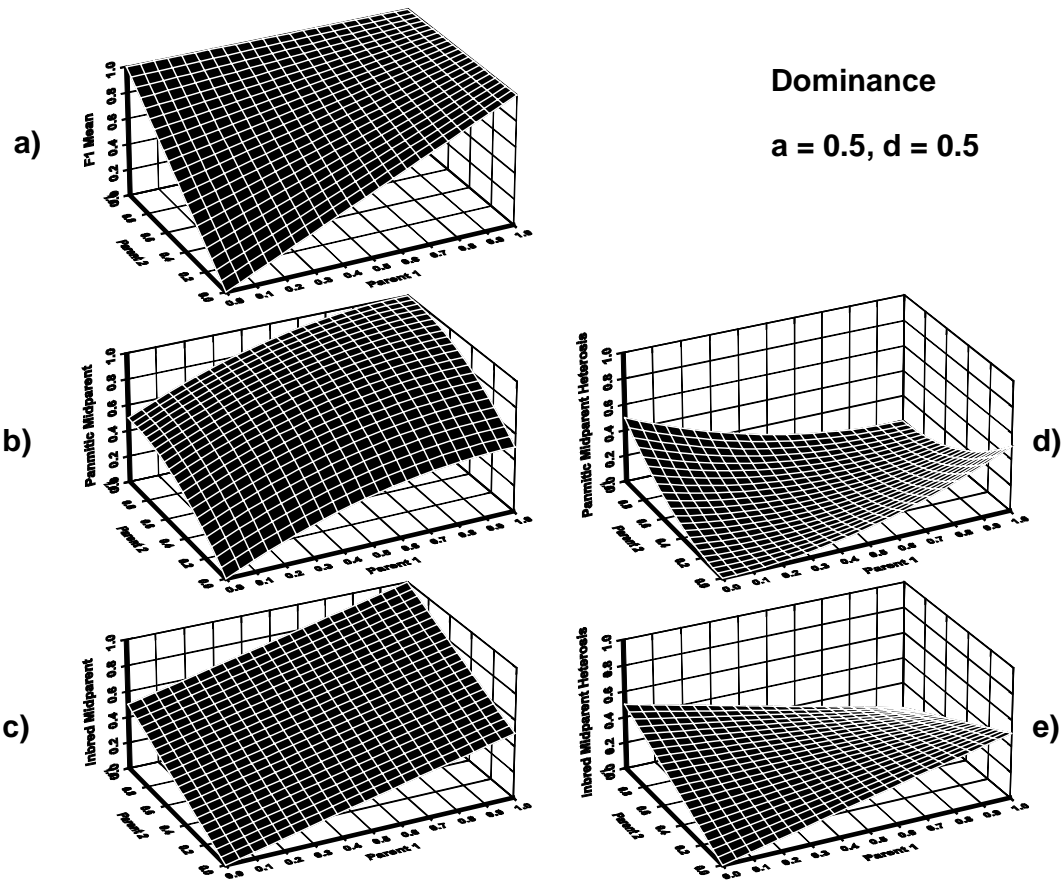


Fig. 3. Plots of mean performance and heterosis versus frequencies of the dominant allele in parent populations for the case of complete dominance. a) F_1 hybrid performance, b) midparent mean for the cross of two panmictic populations, c) midparent mean for the cross of two populations that have been inbred to $f = 1$, d) panmictic-midparent heterosis, and e) inbred-midparent heterosis.

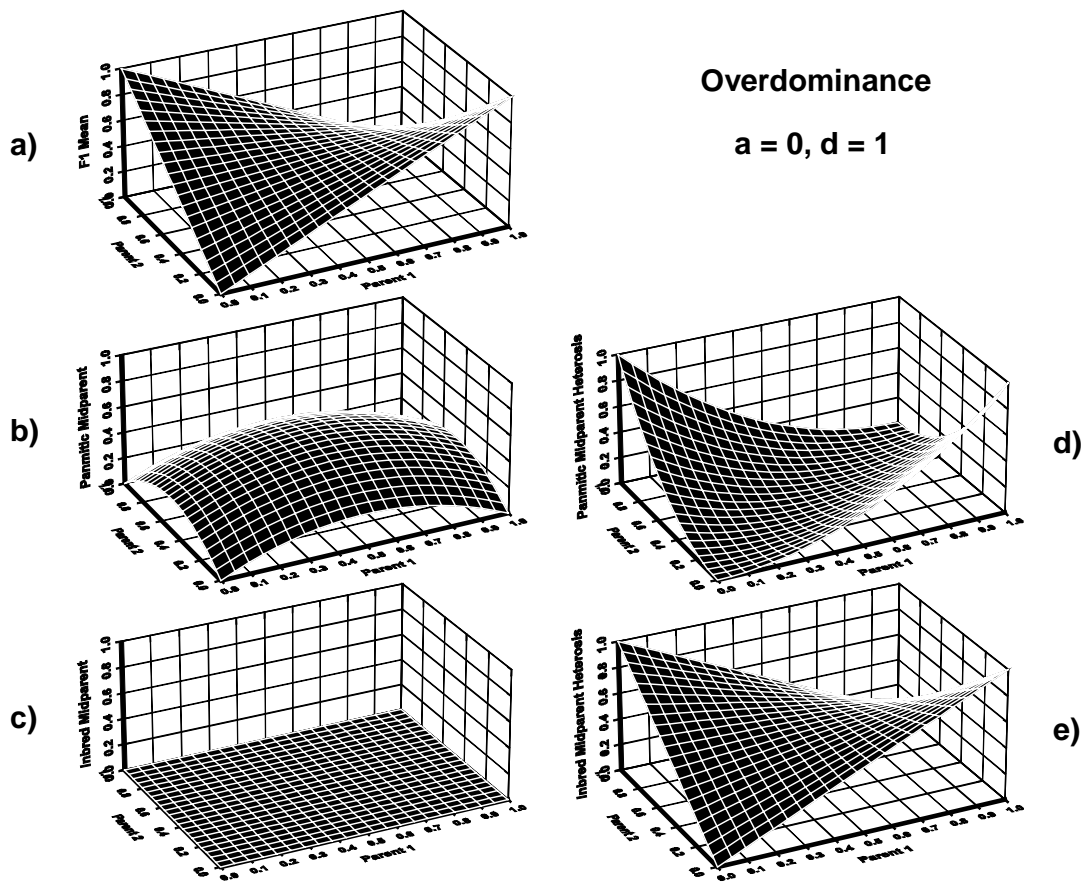


Fig. 4. Plots of mean performance and heterosis versus allele frequency in the parent populations for the case of pure over dominance. a) F_1 hybrid performance, b) midparent mean for the cross of two panmictic populations, c) midparent mean for the cross of two populations that have been inbred to $f = 1$, d) panmictic-midparent heterosis, and e) inbred-midparent heterosis.

recessiveness led to two prominent theories of heterosis called the dominance and overdominance hypotheses (Crow, 1952). Heterosis under the dominance hypothesis is produced by the masking of deleterious recessives in one strain by dominant or partially dominant alleles in the second strain. Heterosis under the overdominance hypothesis is due to heterozygote superiority and, therefore, increased vigor is proportional to the amount of heterozygosity. Supporters of the overdominance hypothesis put forth two main objections to the dominance hypothesis. First, it should be possible to accumulate by selection all the favorable dominance alleles into one homozygous strain and obtain inbreds that are as vigorous as hybrids. Second, F_2 distributions should be skewed because of the $\frac{3}{4}$ dominants to $\frac{1}{4}$ recessives segregation. Jones (1917) showed with linkage and Collins (1921) showed with large numbers of loci that the overdominance and dominance hypotheses were essentially indistinguishable. Crow (1948) using a mutation-selection equilibrium argument felt that dominance was insufficient to explain heterosis in maize. Hull (1952) presented eight reasons why he felt overdominance was the cause of heterosis in maize. The debate over the type of gene action controlling heterosis has gone on for more than 80 years. As we will see later in the section entitled gene action, this debate had a major influence on the development of breeding methodology.

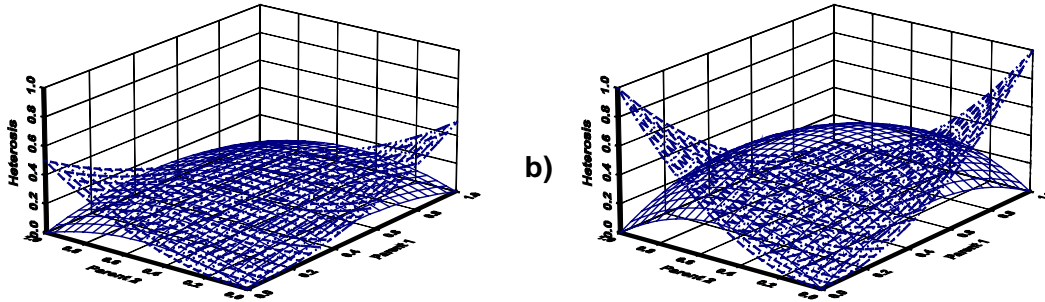


Fig. 5. Plots of baseline (solid lines) and functional heterosis (dashed lines) versus allelic frequencies in the parents. Inbred-midparent heterosis is the sum of panmictic-midparent heterosis and baseline heterosis. Fig. a) is for the case of complete dominance and b) is for the case of pure overdominance.

We have applied our extension of the theory presented by Willham and Pollak (1985) to the cases of dominance and overdominance to further illustrate some key principles. In the case of complete dominance (Fig. 3), several key points are obvious. 1) F_1 performance is maximized when the favorable allele is fixed in one of the populations. 2) With one locus the best inbred is as good as the best hybrid. 3) Panmictic-midparent heterosis is maximized as the midparent values are declining. 4) When inbred populations are crossed, heterosis exists even in the absence of genetic divergence. Pure overdominance is similar to complete dominance (Fig. 4), but the major exception is that it is not possible to obtain an inbred line as good as a hybrid with overdominance.

Baseline and panmictic heterosis for dominance and overdominance are plotted in Fig. 5. The graphs are very similar for the two types of gene action with the major difference being the magnitude; more heterosis is observed with overdominance than with dominance. The second and most important point is that panmictic-midparent heterosis only exceeds baseline heterosis when allelic frequencies are at the extremes. This is an important point to keep in mind when studying heterosis among inbred lines. A significant portion of the heterosis among inbred lines is due simply to recovery of what was lost during inbreeding and in some instances little of the observed heterosis may actually be due to genetic divergence.

Empirical Studies

Gene action and gene effects have been extensively studied in many crop species. Gene action is important in determining cultivar type (hybrid, pure line, synthetic, etc.), breeding methodology used to develop cultivars, and in the interpretation of quantitative genetic experiments. The study of gene action has been approached in two ways (Sprague, 1966). One characterizes the predominant types of genetic variance (additive vs. dominant) in populations, an activity that lead to development and analysis of mating designs, including the North Carolina mating designs (see Hallauer and Miranda, 1988 for a review). Because of the difficulties in artificial hybridization, the variance component approach is not used frequently in self-pollinated crops; instead generation mean analysis has been the most prominent approach to determining gene action in these species. The results of these studies lead to the proposal of many breeding methods that capitalize on different types of gene action, including recurrent selection for general combining

ability and inbred per se selection (additive effects), recurrent selection for specific combining ability (dominance effects), and reciprocal recurrent selection (both additive and dominance effects).

Of the major crop species, gene action has been most extensively studied in maize. A review of gene action studies in maize is therefore appropriate. Four lines of evidence will be reviewed: variance component estimation, generation means analysis, recurrent selection, inbreeding depression, and measured genotypes studies. Sprague and Eberhart (1977), Gardner (1963), and Hallauer and Miranda (1988) have excellent reviews of gene action studies in maize. Our review will be confined to grain yield.

Variance Component and Generation Means Studies

Numerous variance component estimation studies have been conducted in maize. Hallauer and Miranda (1988) reviewed and summarized variance component studies in maize conducted through the mid 1980s. The general conclusion from these studies is that in most maize populations, additive genetic variance for grain yield is usually 2 to 4 times larger than dominance variance. Dominance variance is important in maize populations and often is significant, but it is usually much smaller than additive variance. These results are often interpreted as implying that additive effects are of primary importance for grain yield of maize and that grain yield is controlled by genes with partial to complete dominance. The Design III mating design was developed specifically to estimate the degree of dominance (Comstock and Robinson, 1952) by utilizing F_2 populations, in which the allele frequency is 0.5. Several Design III experiments have been conducted. Estimates of degree of dominance from F_2 populations were usually in the overdominant range. These scientists realized from the outset that repulsion phase linkage would bias degree of dominance upward, so experiments were developed to reduce the linkage bias by random mating the F_2 populations. Estimates of average degree of dominance estimated from random mated F_2 populations were always smaller than the estimates from nonrandom mated F_2 populations and usually in the partial to complete dominance range. These results convinced all but the most adamant overdominance supporters that much of the observed overdominance was probably due to linkage bias.

The early variance component studies assumed that epistasis was unimportant for grain yield of maize. This assumption was required because the number of covariances of relatives were not available to estimate epistasis and because epistatic models are difficult to handle mathematically.

From both a breeding methodology and statistical point of view, epistasis is difficult to estimate. Studies estimating epistasis in maize are too numerous for comprehensive review (see Hallauer and Miranda, 1988 for an excellent review), but a few interesting conclusions can be drawn. Studies estimating epistasis by generation means analysis generally have reported significant epistatic effects. Estimates made by the analysis of variance (covariance of relatives) approach generally have reported nonsignificant epistatic effects. Studies with open-pollinated varieties generally have shown additive effects to be more important than dominance or epistatic effects, and studies with elite inbred lines generally have reported dominance and epistatic effects to be more important than additive effects.

These results are interesting and ambiguous at best. The lack of detection of epistasis with variance component studies suggests either a lack of statistical power or that epistasis is relatively unimportant. The ability to detect epistasis with generation means studies is indicative of the greater statistical power of using means, but these studies usually have a narrow inference base. Generally, it has been accepted that epistasis is relatively unimportant, but that there may be specific hybrid combinations in which epistasis is important. These conclusions are

interesting considering the findings from molecular biology during the past 15 years. It is well known now that genes at the molecular level interact with each other or exhibit epistasis (Coe et al., 1988). The question is: why have we been relatively unsuccessful at detecting epistasis at the phenotypic level? The difficulty in detecting epistasis in populations at the phenotypic level, despite its ubiquitous presence at the molecular level, may be related mostly to an inadequate understanding of epistasis at the population level. Geneticists have long known about epistasis, but their concept of epistasis (physiological epistasis) is different from a quantitative geneticist's statistical or population epistasis. Physiological epistasis occurs when phenotypic differences among individuals with various genotypes at one locus depends on their genotypes at another locus (Cheverud and Routman, 1995). Statistical epistasis is a deviation of multilocus genotypic values from the additive combination of their single locus components (Cheverud and Routman, 1995). The main distinction between these two definitions is that statistical epistasis is a population phenomenon **dependent** on allelic frequencies in a specific population, whereas physiological epistasis is a genotypic phenomenon **independent** of allelic frequencies at the loci in question (Cheverud and Routman, 1995),

Cheverud and Routman (1995) demonstrated that additivity (*a*), dominance (*d*), and epistasis (*e*) all contribute to the average effects of alleles and the additive genetic variance. Only dominance and epistasis contribute to dominance deviations and variance, and epistasis alone contributes to the epistatic interaction deviations and variance. This means that physiological epistasis makes important contributions to additive and dominance variance and only the remainder contributes to statistical epistasis. It is also important to note that this concept is different from the confounding of statistical epistasis with additive and dominance genetic variances as often happens in one and two factor mating designs.

Cheverud and Routman (1995) were able to show that physiological epistasis can either suppress or enhance additive and dominance genetic variance. In some instances, depending on allelic frequencies, genetic variances and in particular dominance variance, can be suppressed or enhanced up to 50%. Using this same two locus approach and varying allelic frequencies at the two loci, Cheverud and Routman (1996) set up models with only additive-by-additive, additive-by-dominance, and dominance-by-dominance epistasis. Additive (*a*) and dominance (*d*) genotypic values for these models were zero. They were able to show that under certain allele frequencies that additive and dominance genetic variance exists in these populations. In essence, they along with others have shown that with finite populations, epistasis can contribute to the additive genetic variance.

Results from generation means analyses have been more ambiguous. Hallauer and Miranda (1988) reviewed the advantages and disadvantages of generation means models. Generally, two types of generation means studies have been conducted. One type involves a diallel among a group of inbred lines or populations. For diallel studies, models of Griffing (1956), Eberhart and Gardner (1966), and Gardner and Eberhart (1966) are often used. With these studies, the reference or inference population is restricted to the set of lines or populations included in the study. Typically, only general and specific combining ability effects can be estimated, although in the more advanced models of Eberhart and Gardner (1966) epistatic effects can be estimated as well. The second type of generation means analysis involves the cross between two inbred lines and generations derived from this cross (e.g., F_2 , backcrosses to the parents). These studies are even more restricted in their inference base and have been used mostly for studying the inheritance of specific traits. Several methods are available for analyzing these types of studies (See Hallauer and Miranda, 1988 for a review).

Classical generation means studies involving inbred lines and derived generations typically have the F_2 as the inference population. This is a

disadvantage in crops exhibiting heterosis, because the inference population is not reflective of elite maize hybrids. Melchinger (1987) proposed a generation means model to analyze testcrosses of generations derived from two inbred lines. The reference population for this model is the F₂ testcross population in gametic phase equilibrium, which is more directly applicable to elite maize hybrids. He developed models for means and variances that included linkage and epistasis.

The typical genetic design for Melchinger's model involves choosing two inbreds from the same heterotic group (P1 and P2) and an inbred from the opposite heterotic group (PT). P1 and P2 are used to generate F₁, F₂, BCP1, and BCP2 generations. In addition, to enhance the power of the model, an F_∞ generation can be developed by selfing the F₂ to homozygosity or the F₂ generation can be random mated for several generations (See Melchinger, 1987 and Lamkey et al., 1995). Each of these generations is testcrossed to PT and generation means analysis is calculated by using the testcross generation means. Variances of each of the segregating generations can also be analyzed by crossing individual plants from each of the generations onto the tester, PT. Because linkage has no effect on the means in the absence of epistasis, only two models need to be fit to the data. Model 1 allows for linkage, but not epistasis:

$$Y = m^T + x(d^T),$$

where

Y = generation testcross mean;

M^T = testcross mean of the F₂ population in gametic equilibrium,

$$(d^T) = \sum_j \theta_j d_j^T,$$

θ_j = + 1 if P1 carries the favorable allele at locus j and - 1 otherwise,

d_j^T = half the average effect of a gene substitution at locus j in the F₂ testcross population, and

x = coefficient that is generation dependent.

Superscript T denotes parameters that are intrinsic to the tester used in the study. Model 2 allows for epistasis, but not linkage:

$$Y = m^T + x(d^T) + x^2(i^T),$$

where

$$(i^T) = \sum_{j < k} \theta_j \theta_k i_{jk}^T \text{ and}$$

i_{jk}^T = additive x additive epistatic effect between loci j and k .

Lamkey et al. (1995) fit Melchinger's model to the P1, P2, F₂, F₂-Syn 8, BCP1, and BCP2 generations derived from the inbreds B73 (P1) and B84 (P2). B73 and B84 are from the same heterotic group and are related to the extent that they were both developed from Iowa Stiff Stalk Synthetic (BSSS). Inbred Mo17 from the Lancaster Sure Crop heterotic group was used as the tester. The results of fitting Models 1 and 2 to the testcross means are shown in Fig. 6. Model 1, which allows linkage, but not epistasis, explained 48% of the variation among testcross means, but had a highly significant lack of fit. Model 2, which allows epistasis, but not linkage, explained 69% of the variation among testcross means and detected significant epistatic effects. These results indicated that unlinked epistatic effects accounted for 21% of the variation among generation means. In addition, the lack

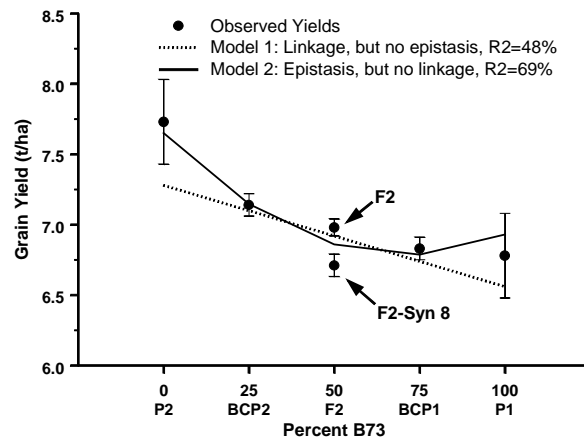


Fig. 6. The results of fitting Models 1 and 2 to the six testcross generation means. Testcross performance is plotted against the percentage of B73 germplasm in the population. From Lamkey et al. (1995).

of fit for Model 2 was significant as well, indicating that other epistatic effects, both linked and unlinked, are important in this population. Favorable epistatic gene combinations have been accumulated in B73 and B84. Lamkey et al. (1995) found that the genetic variance among BCP2 progenies was not significant. Melchinger et al. (1988) also reported that backcrossing to the higher yielding parent resulted in a nonsignificant genetic variance component. These results are further evidence of the importance of epistasis and suggest that it may not be possible to accumulate favorable alleles for grain yield into one parent in an additive fashion as predicted by the dominance theory of heterosis (Lamkey et al., 1995). This result has important implications for marker assisted selection and backcrossing programs, which rely on the additive accumulation of favorable alleles into a parent.

Recurrent Selection

Patterns of response to recurrent selection are also indicative of the type of gene action controlling a trait. Sprague and Miller (1950) proposed a selection experiment to test what type of gene action was important for a trait. The premise of their method was that selection for general combining ability is made on the assumption that dominant favorable genes are important in heterosis and selection for specific combining ability is made on the assumption that overdominance and epistasis are mainly responsible for heterosis. With selection for general combining ability, the average allele frequency for genes affecting a trait will approach 1.0 as a limit. With selection for specific combining ability, the allele frequency in the population undergoing selection would approach $1-q$ if the average allele frequency in the homozygous tester is q . The experiment involves choosing two populations A and B, in which selection will be practiced and an inbred line C as the tester parent. Standard half-sib selection is conducted in A and B for a number of cycles by using C as the tester. Improved cycles of A and B are designated as A', A'', etc. If selection has been primarily fixing dominant alleles in A and B, the crosses between A' x B', A'' x B'', etc should exhibit an increase in yield relative to A x B. Similarly, A', A'', etc. should be higher in yield than the original A. If selection has been to primarily fix recessive alleles for those loci where tester C carries dominants and dominant alleles where tester C carries recessive alleles, then the crosses A' x B', A'' x B'', etc. should exhibit a downward trend relative to the original cross A x B. Trends in A', A'', etc. relative to A would depend on allele frequency in the tester C.

Table 1. Selection response in Alph and the F₂ of (WF9 x B7) after five cycles of selection using B14 as the tester. Data from Russell et al. (1973).

Tester	Alph Cn	(WF9 x B7)Cn
Population per se	2.06 ± 0.44	1.55 ± 0.44
B14	3.09 ± 0.50	1.32 ± 0.50
BSBB	3.63 ± 0.50	1.51 ± 0.50
C0 of population per se	2.29 ± 0.44	2.38 ± 0.44
C0 of other population	2.63 ± 0.18	1.46 ± 0.18
Interpopulation cross	4.09 ± 0.18	

Russell et al. (1973) reported on an experiment to compare the importance of dominance and overdominance for yield heterosis in maize by using the procedure of Sprague and Miller (1950). They conducted five cycles of selection for specific combining ability in the open-pollinated variety 'Alph' and the F₂ of WF9 x B7. Responses for grain yield for six different testers are shown in Table 1. They found significant increases in grain yield in both the populations per se and in the interpopulation crosses suggesting that overdominance was not important for grain yield in these two populations. A significant result of this study was that selection for specific combining ability was effective for improving general combining ability as well as evidenced by the performance of the populations when crossed to BSBB (a broad based population) as well as the C0 of the other population. The implication of this study was that using a single tester would also give improvement with other testers as well. This was further evidence in support of the concept of early testing (Jenkins, 1935; Sprague, 1946) that is commonly used in maize breeding today.

The finding that additive effects were of primary importance for grain yield of maize and that overdominance was relatively unimportant increased interest in inbred progeny selection methods and the search for high yielding inbred lines. Comstock (1964) demonstrated that in the absence of overdominance S₁- or S₂-progeny selection was expected to be superior to other methods of recurrent selection for population improvement. Inbred progeny selection has had variable levels of success. Lamkey (1992) and others (see Lamkey (1992) for references) found that results from S₂-progeny selection were discouraging. Several reasons could account for the lack of response including lack of genetic variance, overdominance, and random genetic drift. Horner et al. (1989) compared S₂-progeny selection with half-sib selection by using an inbred tester in two maize populations. They found greater rates of gain for half-sib selection and concluded that nonadditive gene action in the overdominant range was important for grain yield in these populations.

Long-term reciprocal recurrent selection (RRS) studies also provide evidence on the type of gene action for heterosis. Cress (1967) conducted simulation studies of RRS by using both dominant and overdominant gene action models. With complete dominance, the mean of the interpopulation cross (hybrid), and the mean of the two populations are expected to increase, except by chance. But, with overdominance, the change in population mean depends on the equilibrium gene frequency. It is possible to get short term increases in the population per se means, but in the long-term they always decrease.

Keeratinijakal and Lamkey (1993a) evaluated response to 11 cycles of RRS in BSSS and Iowa Corn Borer Synthetic #1 (BSCB1). They reported gains of 7% per cycle in the interpopulation cross, no change in BSSS per se, and a small significant increase in BSCB1. The small genetic gains in the populations per se did not resemble the response patterns that Cress (1967) predicted for overdominance and was attributed to random genetic drift due to small effective population size (Keeratinijakal and Lamkey, 1993b). Inbreeding depression in

BSSS decreased over cycles of selection and showed no change in BSCB1, whereas inbreeding depression in the interpopulation cross doubled from C0 to C11. Heterosis of the interpopulation cross increased from 0.86 to 2.92 Mg ha⁻¹. These changes in inbreeding depression and heterosis suggest that selection has been for alleles at complementary loci in each population, such that the interpopulation cross is becoming more heterozygous with selection. More recent molecular data seem to support this conclusion (Labate et al., 1997; 1998). An analysis of genetic divergence of dominance-associated distances also indicated that overdominance was not important in these populations (Keeratinijakal and Lamkey, 1993b).

Inbreeding Depression

Inbreeding depression is the converse of heterosis. The mean of a population with inbreeding coefficient f is:

$$M_f = M_0 - 2f \sum d\bar{p}\bar{q}$$

where summation is over all loci controlling a trait and p and q are the allele frequencies in the whole population (Falconer and Mackay, 1996). Several conclusions can be drawn from this equation, like the one for heterosis. First of all, a locus will not contribute to inbreeding depression if $d = 0$ or there is no dominance. Second, the direction of the change in mean is toward the value of the recessive allele. Third, inbreeding depression is maximized when $p=q=0.5$, which is also where the number of heterozygotes are maximized. Fourth, in the absence of epistasis, inbreeding is a linear function of f . Fifth, if there is epistasis, but no dominance, there will not be any inbreeding depression (Crow and Kimura, 1970). Sixth, if there is epistasis and dominance, then inbreeding depression will be a quadratic or higher function of f (Crow and Kimura, 1970).

These basic results have several important implications regarding heterosis and hybrids in self-pollinated crops. Several studies with soybean [*Glycine max* (L.) Merr.] have reported significant heterosis (Burton, 1987). Over all the heterosis studies that Burton reviewed, 85% of the F₁ crosses showed midparent heterosis and 62% showed high parent heterosis. In soybean, there obviously is heterosis, but the genetic cause of the heterosis remains obscure. Heterosis alone, is not good evidence for dominance; however, heterosis studies conducted in conjunction with inbreeding depression studies should give a clear picture of the types of gene action involved in heterosis in soybean. For example, if there is midparent heterosis, but no inbreeding depression then there would be good evidence for the existence of additive x additive epistasis.

Numerous inbreeding depression studies have been conducted in maize. The majority of the studies have reported a linear relationship between inbreeding depression and f , and have concluded that epistasis is unimportant for grain yield (Hallauer and Miranda, 1988). It should be realized, however, that these studies all measured population bulks, and hence were looking at the average over the whole population. To our knowledge, there is no published data in maize on the variation in inbreeding depression. Pray and Goodnight (1995) reported that inbreeding depression can be genetically variable among lineages within a single population of flour beetle (*Tribolium castaneum*). Variation in inbreeding depression can be due to variation in the actual level of inbreeding, past history of inbreeding (whether inbreeding is due to the expression of deleterious recessives or overdominance), genetic drift and fixation of different alleles in different lines (Pray and Goodnight, 1995). Pray and Goodnight found evidence for nonlinearity in inbreeding depression suggesting that epistasis may be important for some traits. They concluded that the genetic variation present for inbreeding depression suggests that inbreeding depression may be a heritable trait.

Measured Genotypes

Measured genotypes refers to the situation in which a phenotype is scored on all possible genotypes of a two or three locus system in an otherwise homogeneous genetic background. These types of studies provide considerable power in estimating genetic effects. The disadvantage of these studies, however, is that only two or three loci can be studied at a time. Measured genotype studies may offer us the best opportunity for doing detailed studies of gene effects.

Conducting a measured genotype study requires two features. First, you need genes controlling traits that you are interested in and second, you need a method of creating the appropriate genotypes in an isogenic background. The only technique for doing this in plants is backcrossing. Backcrossing has the usual problem of linkage drag, but if the drag is the same for all gene combinations, then the bias may not be too severe. In *Drosophila* for example, the appropriate genotypes can be created in identical genetic backgrounds without backcrossing (Clark and Wang, 1997). More recently, in plants, data from QTL mapping studies have been used like a measured genotype study primarily to estimate epistasis.

For the sake of simplicity, only two measured genotype studies will be discussed. One from maize and one from *Drosophila*. Together, these two studies bring out the salient features of the analyses. Russell (1976) developed B14 isolines of the 27 genotypes possible for three loci with two alleles. The experiment was grown for three years at one location and data were collected for 10 traits. The standard Cockerham model was fit to the data to estimate additive, dominance, and epistatic effects. Russell (1976) found that 87, 27, 47, 15, 23, and 30% of the additive, dominance, additive x additive, additive x dominance, and dominance x dominance effects were significant at the 5% level, indicating that even at the population level, loci were interacting fairly frequently. This study also demonstrates the pleiotropic effect of loci.

Clark and Wang (1997) reported on a measured genotypes study in *Drosophila*. They constructed all possible two locus genotypes for each of 8 pairs of *P*-element insertions. They found significant epistatic effects in 27% of their comparisons. They applied the method of Cheverud and Routman (1995) and found significant physiological epistasis in 15% of the comparisons. Clark and Wang reported epistatic effects on the same order of magnitude as main effects.

These studies clearly demonstrate that genes interact. Measured genotypes using a combination of Cockerham's analysis and Cheverud and Routman's analysis, may be one of the best tools for understanding epistasis and its contribution to heterosis.

NEEDS FOR FUTURE RESEARCH

The data clearly indicate a need for future empirical and theoretical research into heterosis; however, we need to be very careful about how future experiments are designed and analyzed. Enfield (1977) was critical of empirical quantitative genetic experiments indicating that the literature was either cluttered with experiments that were meaningless because of standard errors that were too large or because standard errors were absent altogether. Despite the tremendous developments that have recently occurred in molecular biology, quantitative genetics is still the only theory linking genotype to phenotype. It is imperative that we design better experiments to test the adequacy and validity of quantitative genetic models.

We would like to propose several areas of research that are needed to better understand heterosis. We will not present experimental approaches, because we do not have all of the answers.

- 1) Gene action and effects are key to understanding the inheritance of

quantitative traits. For maize at least, it seems that from average population estimates, there is no evidence for overdominance. Although this is useful information, what would be even better is to gain insight into the distribution of gene effects and gene action for individual traits. Are there lots of loci with equal and small effects or is there some type of distribution? The conventional approach to gene action studies will not answer this question and new approaches will be needed.

- 2) Selection experiments in plants need to be better designed. Most of our current recurrent selection experiments are not adequately designed to separate the effects of selection from drift, so it becomes nearly impossible to reliably interpret the results of these experiments. It is clear that recurrent selection works, and future experiments to demonstrate the effectiveness of recurrent selection are probably not needed. But we do need well-designed experiments with adequate controls and replication. New selection experiments should either be replicated or include an unselected, replicated control population of the same effective size.
- 3) The view of random genetic drift in agricultural is the one that drift leads to a loss of heterozygosity and eventual erosion of genetic variance. Although this is true in the additive gene action case, with dominance and epistasis, drift may reduce heterozygosity with a corresponding increase in additive genetic variance. We need to incorporate this new information from evolutionary biology into the design of our breeding programs.
- 4) Epistasis has long been ignored in breeding programs and is generally assumed to be absent or unimportant. Evidence from molecular biology clearly shows that genes interact. Recent theory from evolutionary biology that distinguishes physiological epistasis from population epistasis may indicate why population level epistasis may be undetectable. More theoretical work is needed to optimally design breeding programs to select for epistatic effects.
- 5) Despite several classic studies, we know very little about inbreeding depression in plants. Because much of the observed heterosis among inbred lines may be due to the recovery of inbreeding depression, the genetics of heterosis may be best elucidated by studying the genetics of inbreeding depression. Our preoccupation with heterosis has caused us to overlook the importance of inbreeding depression.

There are of course several problems in designing and conducting good quantitative genetics experiments. First, they are often large and consume considerable physical resources, even for lab species. Second, there seems to be little funding available in agricultural species to do quantitative genetics and plant breeding related research. Third, there are few scientists being trained to do this type of research. Lack of funding and support in quantitative genetics may in the long term severely limit future genetic gains.

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