Understanding of Heterosis

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Abstract
Heterosis is a major reason for the commercial maize industry as well as for the success of breeding efforts in many other crops. Although some progress has been made in understanding the genetic basis of heterosis, there is relatively little information regarding the biochemical, physiological, and molecular basis of this event. In this review, we review the explanation of heterosis. Beginning in the early 1900s, scientists began designing experiments to determine the mechanism of heterosis. Over the years, the majority of the scientific community has attributed heterosis to dominance or overdominance, and recently scientists have reported that epistasis and linkage are major contributors. One common theme throughout the last century has been that no one hypothesis of heterosis holds true for every experiment or every organism.

Key Words: Heterosis, dominance, overdominance

Introduction
Heterosis can be defined as the increased performance of offspring compared with their respective parents. That is, progeny resulting from hybridization are superior to either of their two parents. Shull (1908, 1914) first described this phenomenon after observing the stimulation of heterozygosity upon cell division, growth, and other physiological characters in maize (Zea mays L.). Identification of
this phenomenon dates initially to studies conducted by Darwin in 1876, in which he observed F₁ maize hybrids were taller than either parents in young and mature stages of growth (Hallauer and Miranda 1981). With this idea of heterosis or hybrid vigor in mind, plant and animal breeders have capitalized on this occurrence to make genetic improvements.

One of the more striking examples of the utilization of heterosis has occurred in maize breeding programs over the last century (Hallauer and Miranda, 1981). Progeny from the hybridization of two inbred lines possess superior characters, such as yield and other agronomic traits. U.S. corn yields collected over 130 years illustrate the successful utilization of heterosis in maize improvement (Fig. 1).

The rate of gain in grain yield increased dramatically as breeders switched from open-pollinated varieties to hybrids. In 1918, breeders began developing double-cross hybrids based on a suggestion by Jones (1918). The principle idea was to take advantage of heterosis by using hybrid seed for commercial plantings. At this time, double-cross hybrids were needed, because parental seed production was limited using inbred lines. Breeders were able to get sufficient seed for parental lines using F₁ hybrids as parents, again utilizing heterosis. In 1960, inbred line development progressed enough so that breeders could use inbred lines as parents to produce F₁ hybrids, which increased the rate of yield advancement in maize at a tremendous rate ($b = 2.06$).
Although maize breeders have taken advantage of heterosis and used it successfully for improvement, the biological explanation for hybrid vigor remains elusive. Scientists have debated several different mechanisms of heterosis for a century, with no one argument accepted by the scientific community as a whole.

**Historical Analyses of Heterosis**

Two major explanations for heterosis have been proposed over the years. These include overdominant gene action and dominant gene action. Such hypotheses were first proposed in the early part of the 20th century. Early studies involving heterosis often used biochemical and physiological approaches in their investigations (Sprague, 1983), although geneticists and scientists have utilized biometrical approaches to investigate heterosis with the advent of quantitative genetic theory in the 1950’s (Schnell and Cockerham, 1992). Often times, studies using physiological approaches did not explain heterosis using a Mendelian approach. The earliest explanation of heterosis was presented by Shull (1908, 1914), where he reported that heterosis was caused by heterozygosity alone. East (1936) also reported that heterosis was due to multiple alleles at a locus differentiating with respect to their physiological function. These explanations gave rise to the overdominance hypothesis for heterosis.

The overdominance hypothesis for heterosis involves alleles acting in a dosage-adjusting manner in which neither homozygote is better than the heterozygote (Key 1976). Therefore, with this hypothesis, it is believed that heterozygosity alone is responsible for heterosis or hybrid vigor. In addition to the previously mentioned basis for overdominance, many physiologists thought that an increase in seed size of hybrid seed was the reason for heterosis in the hybrid plant (Key, 1976). Previously conducted studies had shown that hybrid seeds were larger than inbred seeds. Therefore, it was thought that the increase in seed size contributed to increased hybrid performance. However, Jones (1918) and East (1936) both reported that seed size did not contribute to heterosis in the mature plant, and such plants only grew faster for two weeks at the seedling stage, after which no difference in growth rate was detectable.

Hull (1945, 1946) argued for overdominance stating that additive gene action contributed to heterosis. This model assumed that additive gene action was the rule in maize and did not include the possibilities of multiplicative or epistatic gene action. A breeding experiment was designed to discriminate between dominance and overdominance using the regression of the F1 on the value of one parent when the other parent is held constant. This approach was one of the first experiments designed to discriminate between dominance and overdominance.

A more plausible explanation of overdominance at the molecular level can be explained in terms of a gene coding for a particular enzyme actively participating in a metabolic pathway involved in increased growth or yield. If a gene consists of alleles, or alternate forms of a gene, then one can infer that the enzyme products coded for by alleles are called allozymes. These allozymes are likely to have different properties associated with them in regard to enzymatic activity or other factors. Thus, a heterozygous individual may have an advantage due to the combination of both allozymes (Falconer and Mackay, 1996). It is possible that two
allozymes create a higher level of catalysis that is preferable to the plant, or it might be that the two allozymes work together in some different way to give the heterozygote an advantage over either homozygote.

Difficulties in identifying true overdominance are well documented. Jones (1917) first pointed out that linkage could cause considerable problems when attempting to identify overdominance, which gives rise to pseudo-overdominance. Pseudo-overdominance is a condition that gives the false presumption of overdominance. For example, if a beneficial dominant allele were tightly linked to a deleterious recessive allele of another gene, one would have difficulty in producing the recombinant individual to identify such gene action. In that case, the pair of linked loci would mimic a single, overdominant locus, thus skewing a measure of true overdominance.

The other most prevalent explanation for heterosis is the dominance hypothesis. This hypothesis is based on the idea that recessive alleles in one parent are nullified by the contribution of dominant alleles from the other parent (Xiao et al., 1995). Therefore, the F1 produced from such a cross possesses superior characters because of the contribution of dominant alleles from one parent. Bruce (1910) and Jones (1918, 1945, 1958) were the first to present the idea of dominance causing heterosis, with their respective arguments based on the accumulation of dominant, favorable alleles at different loci.

It is clear, based on the dominance hypothesis, breeders should be able to fix inbred lines with the favorable, dominant alleles through various inbreeding strategies. Therefore, it should be possible to produce inbred lines equivalent to the best hybrids. However, superior inbred lines have been difficult to identify, likely due to the large number of loci differing between two parents (Tsaftaris, 1995), an idea initially reported by East (1936). Opponents of the dominance hypothesis suggest that this occurrence is evidence against such an explanation of heterosis. Such opponents argue that several other factors provide evidence to dispute dominance. These include the lack of a binomial type distribution in an F2 population, and the presumption that selection for superior inbred lines equivalent to the F1 hybrid should be attainable (Crow, 1999). However, Collins (1921) disputed these presumptions when he reported that a trait controlled by a large number of genes showed a normal distribution regardless of gene action, and that selecting for favorable alleles in one line was extremely improbable if the number of factors (genes) controlling such a trait were large.

**Recent Evidence for Dominance or Overdominance?**

With the advent of recombinant DNA technologies and molecular markers, investigations of heterosis are becoming more advanced and informative than those of the past and are based on more precise scientific evidence (Tsaftaris, 1995). Most recent investigations of heterosis utilize molecular markers and QTL (quantitative trait loci) analyses. An abundance of DNA-based molecular markers present in many plant species allows for the identification of relationships between those markers and phenotypic traits, such as heterosis, that are segregating in a given population (Stuber et al., 1992).
Co-dominant molecular markers such as RFLPs (restriction length polymorphisms) and microsatellites are particularly advantageous when studying heterosis, because the amount of heterozygosity present in an individual or family can be estimated. Stuber et al. (1992) utilized this approach using two BC1 populations of maize derived from the F3 of a cross between two inbred lines (B73 and Mo17). One of the two BC populations was formed using B73 as the backcross parent and the other BC population was formed using Mo17 as the parent. Using this type of population, the relationship between the amount of heterozygosity present in an individual and that individual’s phenotypic value can be studied. The degree of that relationship can provide evidence in favor of overdominance or dominance, since the hypothesis of overdominance results from the presumption that heterozygosity alone contributes to heterosis. Gene action can also be estimated according to additive effects measured as a percentage deviation from the parental control (Tanksley and Monforte, 2000). This expands to the following:

\[ a = 100 \left( \frac{P_1P_1 - P_2P_2}{2P_2P_2} \right) \]

\[ d/a = \left( \frac{P_1P_2 - (P_1P_1 + P_2P_2)/2}{(P_1P_1 - P_2P_2)/2} \right) \]

where \( P_1P_1 \) = phenotypic mean of individuals homozygous for \( P_1 \) alleles for the marker locus, \( P_2P_2 \) = phenotypic mean of \( P_2 \), and \( P_1P_2 \) = phenotypic mean of individuals heterozygous for the alleles at the same marker locus.

Using the previously mentioned BC populations, Stuber et al. (1992) found a significant correlation between grain yield and amount of heterozygosity (\( r = 0.68 \)), but did not for the remaining six traits measured, indicating the superiority of the heterozygote for grain yield. Several QTL were detected for yield on nine different chromosomes, and QTL present on 6 of the 9 chromosomes mapped to the same location in both BC populations. Accordingly, Stuber et al. (1992) concluded that overdominance was the cause of heterosis since both BC populations contained the majority of the QTLs identified for yield, although it was noted that pseudo-overdominance could not be ruled out as a contributor to heterosis because of their inability to detect linked loci with favorable alleles in repulsion phase linkage.

Xiao et al. (1995) conducted a similar experiment in rice studying two F7 derived BC1 populations resulting from a cross between two elite rice lines representing the indica and japonica subspecies of rice that have contributed to the foundation of the success of hybrid rice in China. Contrasting the results of Stuber et al. (1992), Xiao et al. (1995) reported that only 10% of the QTLs identified were present in both BC populations. Only 2 of the 12 traits measured showed a significant correlation with genome heterozygosity and inbred lines were identified which outperformed the F1 hybrid. These findings all indicate that dominance, not overdominance, contributes to the heterosis observed in rice.

Other studies, including Tanksley and Monforte (2000) and Yu et al. (1997), have reported that heterosis is not controlled by overdominance or dominance alone, but is influenced by linkage and/or heterosis. Tanksley and Monforte (2000) studied a subNIL population consisting of recombinant inbred lines developed from a cross between a near isogenic line (NIL) containing a favorable introgression (from a wild species) and an inbred line. This experimental design allowed for their analysis to be concentrated on genes present in a 40 cM introgression that contained QTL for agronomic traits in tomato. Using methods outlined by Stuber et al. (1992), it was determined that the predominant form of gene action acting on several traits was
additive. However, heterosis was not attributed to a single, overdominant locus or a two-locus dominance complementation model. Hence, Tanksley and Monforte (2000) attributed heterosis to a model consisting of two or more closely linked loci.

Graham et al. (1998) conducted a similar study in maize utilizing an introgression from the population used by Stuber et al. (1992). In this study, the conclusion of overdominance reported by Stuber et al. (1992) was found to be false. Graham et al. (1998) reported that the heterosis present in the Stuber et al. (1992) population resulted from the expression of two dominant genes in repulsion phase linkage, a possibility that was reported to possibly explain the initial results of the first mapping population.

Other evidence disputing dominance and overdominance hypotheses of heterosis was reported by Yu et al. (1997) in rice. Analysis of an F3 population indicated that epistasis was an important factor contributing to heterosis. Overdominance and dominance of individual loci were found, but significant D x D, D x A, A x D, and A x A interactions were identified in regard to each of these loci. In addition, higher order interactions between more than two loci were identified, but could not be analyzed thoroughly because of the need for a greater population size to do the test.

**Concluding Thoughts**

The true mechanism of heterosis has remained elusive to scientists over the last century. A large contingent of scientists, particularly those working in maize, have utilized this phenomenon extensively in genetic improvement, but have failed to solve the puzzle that is heterosis. Beginning in the early 1900s, scientists began designing experiments to determine the mechanism of heterosis. Over the years, the majority of the scientific community has attributed heterosis to dominance or overdominance, and recently scientists have reported that epistasis and linkage are major contributors. One common theme throughout the last century has been that no one hypothesis of heterosis holds true for every experiment or every organism. Heterosis is very likely organism dependent and population dependent. This would begin to explain the conflicting reports involving experiments designed to study the mechanism of heterosis. For example, heterosis observed in self-pollinated species like rice may be very different from heterosis observed in a naturally cross-pollinating species like maize. The recent reports by Yu et al. (1997), Tanksley and Monforte (2000), and Graham et al. (1998) provide substantial evidence that heterosis is not controlled by a single locus alone, whether that locus behaves in a dominant or overdominant fashion. Linkage and epistasis among multiple loci must play a large role in the phenomenon of hybrid vigor.

Undoubtedly, future experiments will approach the phenomenon of heterosis utilizing new technologically advanced tools. Future experiments must be carefully designed to provide greater evidence for a mechanism of heterosis. Hopefully, these experiments will build upon the body of knowledge collected thus far concerning heterosis. For example, Pioneer Hi-Bred International, Inc. is approaching the dissection of heterosis in maize using a “Gene Calling” technology (Howie Smith, personal communication). This approach utilizes the molecular biology toolbox and bioinformatics to dissect expressed DNA sequences responsible for hybrid vigor.
Literatures


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