Unraveling the genetic basis of hybrid vigor

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The study of hybrid vigor and inbreeding depression traces back to Charles Darwin, who was the first scientist to examine the phenomenon in a systematic manner (1). Hybrid vigor, or heterosis, is the increase in stature, biomass, and fertility that characterizes the progeny of crosses between diverse parents such that the F_1 is superior to the better of the two parents. In plants, this is basically achieved by a greater proliferation of cells in some but not all tissues (2). Inbreeding depression refers to the decline in the quantitative measure of these characters upon self-fertilization or other forms of homozygosis of alleles (inbreeding). The genetic basis of heterosis has been debated for nearly a hundred years without an emerging consensus (3–5) (Fig. 1). An early view was that the combination of different alleles in an organism resulted in a superior state for growth and vigor compared with the presence of identical alleles (3). As genetic knowledge increased, the concept that inferior alleles of different genes in the two parents were complemented in the hybrid (6), thus leading to the superior characteristics, gained favor. Although the latter explanation is simple and easily envisioned, results that seemed to favor interactions of diverse alleles have been repeatedly found. In this issue of PNAS, the work of Semel *et al.* (7) examined an extensive set of quantitative traits in partial hybrids of domesticated tomato and a wild relative. They conclude that most traits that exhibit heterosis do so as a result of heterozygosity of the controlling genomic regions to produce traits superior to the better parent. They also suggest that heterosis was selected over evolutionary time for characteristics that impact reproductive success.

The complementation explanation for heterosis has been classically referred to as the dominance model (Fig. 1). It postulates that different inbred parents are limited by slightly deleterious alleles at several loci that differ between the parents. In the hybrid, the superior allele from the respective parent will complement the inferior allele from the other parent. Based on modern knowledge of the molecular lesions responsible for mutations, this concept is reasonable. Although such complementation will certainly occur in hybrids, the unresolved question is whether the complementation at different loci is cumulative across these genes. In other words, would the complementation only result in characteristics being equal to the better of the two parents, or could it result in superior performance to the better parent?

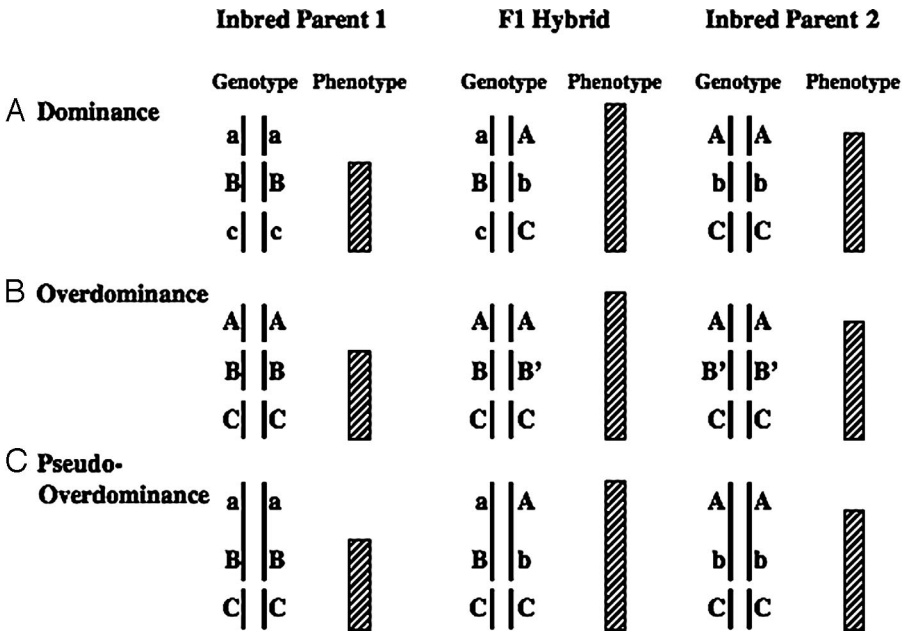

Fig. 1. Genetic models for heterosis. It is hypothesized in the diagrammed models that a phenotype or trait is controlled by multiple linked or unlinked loci (e.g., a, b, and c). (*A*) The dominance model. Inbred parents 1 and 2 carry slightly deleterious homozygous alleles (a and c in parent 1; b in parent 2). In the F_1 hybrid, at each locus, the superior allele A, B, or C will complement the inferior alleles a, b, or c. This complementation could cause the F_1 hybrid to exhibit a superior phenotype than the better of its parents. (*B*) The overdominance model. The homozygous alleles at the b locus are different between the inbred parent 1 (BB) and 2 (B′B′). When brought together in the F_1 hybrid, allele B and B′ can interact to cause a superior phenotype compared with both the parental BB and B′B′ homozygous states. (*C*) The pseudooverdominance model. The superior phenotype in the F_1 hybrid can be attributed to a small chromosomal region, which contains two or more different loci (e.g., a and b) that are linked in repulsion phase. The presence of superior alleles A and B in the hybrid leads to a better phenotype due to complementation, giving the impression of overdominance.

The allelic interaction explanation for heterosis has been classically referred to as the overdominance model (Fig. 1). It postulates that diverse alleles interact so as to create a superior function than that which could happen with homozygous alleles. Although various molecular mechanisms might be imagined for such interactions, there are none that present themselves as obvious. The persistence of this explanation owes to the fact that observations are repeatedly made that heterozygosity for small regions of a genome will produce a heterotic response.

The advocates of the dominance model have countered that this situation is, in fact, only a pseudooverdominance (Fig. 1). Pseudooverdominance refers to the possibility that these small regions could, in fact, contain variation at two or more different genes in repulsion (dominant and recessive alleles on opposite homologues for the two genes) that exhibit complementation and provide the appearance of an overdominant action. In some cases, continued self-pollination of plants that apparently exhibit overdominance will result in the dissolution of the heterotic effect as predicted by the pseudooverdominance postulate. This result is suggested to occur by the recombinational separation of the variation at the two loci, thus allowing homozygosis of alleles for both genes.

The difficulty in formulating the genetic basis of heterosis has at least two major contributors. First, in most cases, multiple genes contribute to the response of the F_1 hybrid. Thus, sorting through the contributions of the responsible factors is not an

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easy task. Secondly, the multiple genes interact in ways that mask the action of each other in the process of epistasis. Thus, not only do multiple genes present a complicating factor, but also the shifting states of heterozygosity or homozygosity of individual factors can influence the impact of other genes. As noted below, Semel *et al.* (7) have used an approach that minimizes the potential influence of epistasis.

Although the dominance model has maintained a "dominance" among practitioners of the field, there are several observations on heterosis that do not seem to be easily explained by this concept. First, E. M. East (2), while reflecting on nearly 30 years of research on the topic, noted that the apparent purging of detrimental alleles during an inbreeding regime did not diminish the potential for a heterotic response when the end products were again joined in a hybrid state. More recently, Duvick (8) has analyzed the heterotic response of increasingly improved inbred lines used in the hybrid corn industry over many years. Although the performance of inbred lines has been continually improved, the contribution of heterosis to yield has changed very little. It certainly has not declined as might be anticipated if detrimental alleles were purged from the inbred lines during the breeding program. Lastly, the behavior of heterosis in polyploids plants is not readily explained by the dominance concept (4).

Semel *et al.* (7) addressed the genetic basis of heterosis by first creating a series of lines of domesticated tomato (*Solanum lycopersicum*) that exchange small segments of the genome with the diverged but similar chromosomal sequences of the wild tomato species, *Solanum pennellii*. These introgression lines replace portions of the domesticated tomato genome with that of the wild relative. Collectively, they cover the entire genomic complement. Using these materials, Semel *et al.* measured an exhaustive collection of traits on the domesticated line used in the recurrent introgression process. They could then compare these results to the same measures performed on each introgressed line as homozygotes and on heterozygotes between the parental line and each introgressed line. By dividing up the genome into small parts that are present in an otherwise uniform background, the effects of epistasis on the results could be largely avoided.

The action of genes contributing to quantitative traits in hybrids can exhibit a dominant–recessive relationship, an additivity of being at the midparent value or an overdominant action in which the better parent is exceeded. Some degree of additivity is the general rule for quantitative trait behavior (9). The actions of the various segments of the tomato genome were classified into these categories for a very large number of characteristics. When correlation studies were performed, an interesting dichotomy emerged. Those characteristics that correlated with reproductive success measures, such as the number of seeds produced, showed largely overdominant action, whereas other quantitative traits fell into the range of additivity or dominant/recessive behavior. Reproductive traits in this case are defined as any that would tend to foster greater numbers of seeds, for example by generating or providing resources for their production, because they correlate with this character.

This dichotomy allowed the authors to argue against the involvement of pseudooverdominance. If linked alleles in repulsion were responsible for the overdominance of the reproductive traits, then one might also expect that overdominance would be observed for the nonreproductive characteristics as well. This circumstance was very rarely observed and suggests that the overdominance found for the reproductive traits results from true overdominance.

The implication of these results is that heterosis is mainly the result of heterozygosity of single genes or gene complexes. Semel *et al.* (7) postulate that this scenario has evolved to promote gene flow. Small, isolated populations would tend to suffer inbreeding depression were it not for mechanisms that tend to foster outcrossing and the migration of new alleles to the population. The maintenance of a heterozygous state in most individuals would prevent the homozygosis of highly detrimental completely recessive alleles because they would be masked most of the time.

Many quantitative trait loci for which a molecular basis is known are transcription factors or members of signal transduction cascades (10). These two classes of genes are also those that tend to exhibit dosage-modifying effects that mimic aneuploid conditions (10). It is likely that both phenomena are a reflection of these same classes of genes and that they exhibit a dosage balance among gene products because they comprise molecular complexes (11). Thus, if many quantitative traits are controlled by dosage-dependent genes, then the effects of new mutations might affect the phenotype of the heterozygote upon which selection could act, be that purifying or adaptive. One might then imagine that selection could foster interactions between alleles for increased reproductive success, because the new variation can be acted upon in the heterozygous condition.

These results inspire new directions. First, if heterosis is mainly due to overdominance of heterozygous alleles, then fine-scale mapping for positional identification of the responsible genes could be potentially illuminating. The system described of introgressed lines of tomato provides a good one for such pursuits because any interacting epistatic interactions from elsewhere in the genome have already been eliminated from the experiment. It will be of interest to know whether such reduction to individual genes is possible and, if so, their nature.

Interestingly, G. Redei (12) described single gene heterosis in *Arabidopsis* in 1962. The two loci studied that exhibited overdominance were *erecta* and *angustifolia*, which are now known to encode a signal transduction kinase (13) and a transcription factor (14), respectively. Thus, a second direction of research would be to ask what the nature of heteroallelic action is in those cases for which single gene heterosis can be documented.

Heterosis has played an important role in agricultural practices. Many crops are planted as hybrids to increase yield over open pollinated varieties. Also, as noted, hybrid vigor plays an important role in evolution. The new results will provide grist for the mill of further research into the genetic and molecular basis of this important biological problem.

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