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Heterosis-What art thou?

James A Birchler

Division of Biological Sciences, University of Missouri, Columbia, MO 65211 USA
E-mail: birchlerj@missouri.edu

Heterosis is the phenomenon that hybrids between two inbred lines or varieties exhibit biomass, fertility and flowering time outside the upper limits of the parents. It has been recognized by several civilizations for practical use millennia ago but came under scientific investigation with Darwin (Darwin, 1876) and then genetic analysis approximately a century ago (Shull, 1908; Bruce, 1910; Jones, 1917). In this, the genomic era, there has been a proliferation of hypotheses to explain the phenomenon that vary from complementation of generalized slightly deleterious recessive mutations (Charlesworth and Willis, 2009) to optimization of metabolites (Frievert et al, 2010; Lisec et al, 2011) to protein quality control (Goff, 2011) to circadian rhythm modulation (Ni et al, 2009) to cell number modulation (Guo et al, 2010) to si and miRNA interactions (Groszmann et al, 2011; Chen et al, 2010) to DNA and histone methylation (Greaves et al, 2012; He et al, 2010; Shen et al, 2012) among others. Of course, any of these suggested bases of heterosis are not necessarily mutually exclusive of others. The diversity of ideas about the underlying basis of heterosis illustrates the lack of consensus.

The diversity of ideas to explain heterosis and their scientific study are indeed of value to characterize further the parameters surrounding heterosis. In terms of establishing the underlying basis, the standard scientific difficulty of assigning correlation versus causation will always be present. When any biochemical parameter is found to differ in the hybrid relative to the two parents or to deviate from the midparent value, there is a tendency to view this particular parameter as a contributor to heterosis as opposed to a consequence. Indeed with the publication philosophy in which we operate if one claims that a parameter is a consequence of heterosis as opposed to a contributor, the observations might not gain residence in the literature. And given the need to establish the nature of heterosis in its every detail, this scenario would be unfortunate.

How then might the scientific community go about narrowing or consolidating the panoply of ideas about heterosis? Perhaps a discussion of the genetic knowledge of heterosis might allow the framework into which features of hypotheses must fit. In this regard, a popular idea for some time has been that slightly deleterious recessive alleles at different loci from the two parents are complemented in the hybrid to condition the superior performance (Jones, 1917; Charlesworth and Willis, 2009). One can imagine the concept involved because there is an abundance of recessive mutations that compromise the growth of mutant plants. And clearly there will be complementation of the respective recessives from different parents. But is this concept consistent with the phenomenology of heterosis?

Some of the first skepticism about this model comes from the finding that improvement of inbred lines that continues over the years does not seem to diminish the ability of two inbreds to exhibit a strong heterotic response. This realization was noted long ago (East, 1936). An experiment widely cited in this realm is that of Duvick (Duvick, 1999). He analyzed inbred lines developed at Pioneer Hi-Bred over several decades. When they were crossed to a common inbred line and characterized for heterosis, the yield of the hybrids increased as did the yield of the improved inbreds but the magnitude of heterosis seemed quite constant. If the improvement of the inbreds had purged detrimental recessives, the prediction would be that the component of yield contributed by heterosis might diminish. That this is not the case draws into question the complementation concept in its simplest form.

Another observation about heterosis that seems counter to the complementation idea is that wide hybrids produce some of the most spectacular examples of heterotic biomass. A classical example is a cross between radish and cabbage (Gravatt, 1914; Karpechenko, 1927). Such hybrids have been photographically depicted as encircling the ceiling in greenhouse situations. With exception, there does seem to be an increasing degree of heterosis with increasing phylogenetic distance. For the complementation model to be consistent with this generalization, it would be necessary for the continued accumulation of homozygous slightly deleterious alleles as evolution proceeds. The mutations would have to be homozygous for complementation to produce heterosis in the hybrids. If present as genetic load in a heterozygous condition, they would already be complemented and produce no detrimental effect. However, given that the complementation idea would require superior alleles to be common in populations

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to produce heterosis so readily in crosses even within species, which is obviously the case, the concept, when followed through strictly, would lead to the nonsensical conclusion that there is an increasing accumulation of homozygous deleterious alleles over evolutionary time.

Indeed, a study of visible recessive mutations in maize suggested several cases of single gene heterosis (Dollinger, 1985). This finding indicated that even though the phenotypic effect was not manifested in hybrids, an impact on heterosis was not in fact recessive. This finding suggested a semi-dominant effect in heterozygotes.

Further, the characteristics of heterosis in polyploids are seldom discussed. Indeed, this is unfortunate because the parameters of heterosis in polyploids reveals behavior that needs to be accommodated. Some might argue that these characteristics are from artificial situations but given that all plants are undergoing cyclical polyploidization over evolutionary time and that the development of inbred lines in diploids is clearly not natural, this argument is a thin rationalization.

An aspect of heterosis in polyploids of note is called progressive heterosis (Groose et al, 1989; Bingham et al, 1994). This phenomenon was first described in alfalfa. Progessive heterosis involves the fact that single cross hybrids between two inbred lines will produce a heterotic response but when a double cross hybrid is produced from two different single cross hybrids, i.e. the double cross hybrid has four different grandparents, the magnitude of heterosis is typically superior to either of the single cross hybrid parents. While defined and studied in some detail in alfalfa, the same type of result has been found in maize (Levings et al, 1967; Sockness and Dudley, 1989a; 1989b; Riddle and Birchler, 1980) and potato (Mak and Peloquin, 1975). Thus, it appears to be a generalizable aspect of heterosis.

If we return to the complementation concept and attempt to reconcile that idea with progressive heterosis, what would have to be the case is that each set of single cross hybrids would need to complement a certain set of slightly deleterious recessive alleles. Then in the double cross hybrid there must be complementation of a new set of recessives that are homozygous still in the respective single cross hybrids. In addition, in order for the second boost of vigor, the double cross hybrid could not return substantial numbers of common recessives from the two single cross hybrids to the homozygous state to offset any of the new complementations. In other words, there would always have to be more new complementations in the double cross hybrids than return to homozygotes. While the degree of homozygous alleles in various inbreds and their presence across inbreds has not been documented, it seems unlikely that by chance different combinations of inbreds would satisfy the conditions outlined above.

A second aspect of heterosis in polyploids is the finding, again first in alfalfa (Busbice and Wilsie, 1966) but recapitulated in maize (Alexander and Sonnemaker, 1961; Rice and Dudley, 1974), that the curves of inbreeding depression of matched diploid and tetraploids are quite similar despite the fact that the predicted rate of homozygosis is very different. In a diploid, homozygosis for any one gene will progress by half at each selfing generation. In an autotetraploid, the segregation will vary somewhat depending on the location of a gene on the chromosome and meiotic pairing, but for genes near the centromere it will approximate homozygosis of about 1/18th of the progeny at each selfing. The loss of vigor in an inbreeding scheme would thus be predicted to be much slower at the tetraploid level but this is not the case. Again, this does not fit with the concept of homozygosis of slightly deleterious recessive alleles as the basis of inbreeding depression.

When this phenomenon was first documented in alfalfa, the similarity of diploid and tetraploid inbreeding curves was used to question whether alfalfa was indeed an autotetraploid. However, Busbice and Wilsie (1966) conducted several tests to confirm the tetraploid nature to be the case. And, as noted above, similar results have been found in maize. One might rationalize these results that the tetraploid segregation is complicated or that tetraploids react differently for the cumulative effects of recessive mutations; however, there is no evidence to support these contentions.

Busbice and Wilsie (1966) provided a potential explanation in that they noted that there is a shifting dosage of alleles. In an autotetraploid, while the homozygosis of alleles is different from the diploid, the duplex hybrid condition (AABB) is present in half of the progeny following selfing of a hybrid similarly to a diploid in which a hybrid situation (AB) is found in half of the progeny from selfing an F_1. The manner in which the tetraploid differs is that there are shifts in allelic dosage (ABBB) or (AAAB) in other genotypes.

East (1936) also suggested an effect of dosage of alleles on heterosis from work in tobacco in which different doses of a genome in a polyploid would impact heterosis differently. Thus, from these combined insights one might postulate that heterosis is controlled by genes that exhibit an allelic dosage effect or in other words show additive allelic effects. In this regard, heterosis would exhibit genetic control that is basically similar to the majority of quantitative traits (Tanksley, 1993).

If we allow this conclusion and accept that the complementation concept is inadequate on many scores, we can advance the thinking about mechanisms of heterosis. Rather than suggesting that complementation of slightly deleterious alleles is the sole basis of heterosis, we can come to the realization that it is a quantitative trait that is controlled multigenically. While this might seem to be a small step forward, it suggests that heterosis does not result from complementation of generalized mutations but rather is a process that is modulated similarly to other quantitative traits.

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