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PERSPECTIVE

Heterosis

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Heterosis refers to the phenomenon that progeny of diverse varieties of a species or crosses between species exhibit greater biomass, speed of development, and fertility than both parents. Various models have been posited to explain heterosis, including dominance, overdominance, and pseudo-overdominance. In this Perspective, we consider that it might be useful to the field to abandon these terms that by their nature constrain data interpretation and instead attempt a progression to a quantitative genetic framework involving interactions in hierarchical networks. While we do not provide a comprehensive model to explain the phenomenology of heterosis, we provide the details of what needs to be explained and a direction of pursuit that we feel should be fruitful.

INTRODUCTION

Heterosis refers to the phenomenon that progeny of diverse varieties of a species or crosses between species exhibit greater biomass, speed of development, and fertility than both parents. The phenomenon has apparently been recognized in one form or another for centuries by various civilizations (Chen, 2010) but has been under scientific investigation since Darwin (1876) in the absence of genetics and for over 100 years with genetic considerations (Shull, 1908; Bruce, 1910; Jones, 1917). We have borrowed the title of a paper by East (1936), who summarized his thoughts on the topic nearly 75 years ago and whose frustrations with the state of understanding of the field at that time seem eerily relevant even today.

Two terms are routinely used in discussing models of heterosis. One is the so-called "dominance" model, in which recessive alleles at different loci are complemented in the hybrid, and the second is the so-called "overdominance" model, which posits that interactions between different alleles occur in the hybrid, leading to the increase in vigor. Of the overdominance model, East (1936) stated: "Genetic knowledge, at the time, was so meagre that it seemed necessary to assume that vigor is promoted when the genes at certain loci are unlike–an assumption for which there was no proof, and which was not illuminating as a dynamic interpretation." Today, this statement is no less valid.

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Perhaps the more popular of the two is the dominance concept (Charlesworth and Willis, 2009). In the extreme version of this model, one parent contains gene copies that are missing in the opposite parent and thus the hybrid would contain more genes than either parent (Fu and Dooner, 2002). While complementation of recessive alleles and the combination of gene copies will certainly occur in hybrids, there are several arguments why this alone would not seem to account for the complete heterotic response. First, most (but not all) ecotypes or varieties that differ from each other will produce some level of heterosis, but crosses between species or genera, in which crossing barriers do not interfere with hybridization, produce some of the most spectacular cases of heterosis. East (1936) synthesized data from a large number of studies involving many species and concluded that, on average, heterosis increases as the genetic disparity of the parental stocks increases and interspecific crosses show greater heterosis than intraspecific crosses. For example, hybrids between radish (*Raphanus sativus*) and cabbage (*Brassica oleracea*) exhibit extensive biomass heterosis (Karpechenko, 1927), as do hybrids between a wild tomato species, *Solanum pennelli*, and cultivated tomato, *Solanum lycopersicum* (Eshed and Zamir, 1995).

On a simple dominance model, it would be necessary to conclude that virtually all ecotypes would carry a load of homozygous detrimental recessives and that an increasing number of homozygous detrimentals would be present with increasing phylogenetic distance. Can this be true, especially given that "better" dominant allelic alternatives are apparently so readily available in different ecotypes of the same species or between species? Although there is good evidence for a mutational load of recessive detrimental mutations that are heterozygous in

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populations (Charlesworth and Willis, 2009), different such mutations must be homozygous in opposite parents in order to produce an F1 heterotic effect. They are already complemented as heterozygotes in populations. The concept that homozygous detrimental recessives are replete in populations when better dominant alleles are so readily present runs counter to the central principle of evolution and population genetics that the most reproductively fit genotypes prevail, particularly with regard to fertility that results from biomass increase, a typically improved characteristic in heterosis. East (1936) recognized this issue from inbreeding experiments by stating: "There is similar unmasking and elimination of deleterious recessives which gradually diminishes and disappears; and there is segregation into differently characterized biotypes. But these purified biotypes exhibit as great or greater manifestations of heterosis when combined after they no longer segregate defective recessives as they did earlier. . . . How, then, are our so-called 'dominants' and recessives to be opposed to each other by crossing, since we do not use AAbb and aaBB individuals as our pure strain components? No! Heterosis must be interpreted on the basis of the behavior of non-defective allelomorphs." Of the dominance concept, East (1936) wrote: "The explanation of heterosis. . . [by complementation of detrimental recessives]. . . was so probable that it was generally accepted. . .in spite of the fact that there is no direct proof for it. This was not altogether fortunate." Today, this statement is no less valid, and we will elaborate further below.

A variant of the dominance model is called "pseudo-overdominance," which posits that complementation occurs for different recessive alleles that are present in close linkage but on opposite members of a pair of homologs such that overdominance appears to be operating. While these terms and ideas have dominated the literature for the past 100 years, there is no consensus because of attempts to forcibly pigeonhole results that do not fit into one or the other of these categories. For this reason, it might be useful to the field to abandon these terms that by their nature constrain data interpretation and instead attempt a progression to a quantitative genetic framework involving interactions in hierarchical networks. Below, we elaborate on aspects of heterosis that need to be explained by a useful model.

WHAT IS HETEROSIS ON THE CELLULAR LEVEL?

One must keep in mind that the changes that occur in heterosis concerning plant growth are basically differences in cell number with regard to most plant characteristics. Cell size does not usually change in a survey of a wide variety of species examined (East, 1936). The developmental program of hybrids is not dramatically altered, so a specific type of quantitative trait is involved, namely, greater cell proliferation. Heterosis can vary in different crosses in different tissues. Flowering time often changes in hybrids, but depending on the species, the heterotic phenotype can involve either faster or slower progression to flowering. It has been argued that slowing the time to flowering will prolong vegetative growth. Whether this is a valid principle for heterosis remains unknown but certainly breaks down in crosses in which flowering time is sped up in hybrids together with an increase in biomass and fertility, such as in maize (*Zea mays*). Also, alterations in the control of circadian rhythms in allotetraploid *Arabidopsis thaliana* will promote more vigorous growth typical of heterosis (Ni et al., 2009). Moreover, evidence for changes in metabolic profiles has been documented in hybrids (Gartner et al., 2009; Fievet et al., 2010).

With regard to the control of cell number, an interesting recent study might provide important clues to the basis of heterotic plant growth. The first cloned quantitative trait locus was *fruit weight 2.2* (*fw2.2*) in tomato (Frary et al., 2000). It exhibits a negative dosage effect on tomato size (Liu et al., 2003). Related genes form a substantial gene family in various plants (Guo et al., 2010; Libault et al., 2010). This family was characterized in detail from maize and consists of at least 13 members (Guo et al., 2010), which were referred to as *Zea mays* Cell Number Regulators (Zm *CNR*). Zm *CNR1* and *CNR2* are more closely related to tomato *fw2.2* than other members of the family. The expression of endogenous Zm *CNR2* was negatively correlated with plant vigor. In this study, hybrid combinations that exhibit heterosis showed reduced expression while combinations with no heterotic response at the seedling stage did not. When the Zm *CNR1* and *CNR2* genes were transformed into plants, multiple insertion events with overexpression of the former but not the latter produced reduced vigor for many aspects of plant growth. The strength of expression of Zm *CNR1* transgenes was negatively correlated with vigor of the transformed plants. Interestingly, one example that produced cosuppression of the transgene and the endogenous copy produced a more vigorous growth habit via an increase in cell number. Direct silencing of the endogenous gene produced the same result. The various other members of the gene family are expressed developmentally and might play a role in the control of cell number in different organs. Variation in the expression of Zm *CNR2* was noted, which suggests that manipulation of plant size could be performed by modulating this gene. If this gene family does indeed play a role in heterosis, then any modulation of its expression in the relevant cells would affect the response because of the dosage sensitivity involved. Furthermore, any dosage-sensitive gene in the regulatory hierarchy or network controlling this gene family might then also play a role in heterosis (see below).

STUDIES OF GLOBAL GENE EXPRESSION

With the advent of genomic methods to assay global patterns of gene expression, parents and hybrids have been studied in several species. A null hypothesis is that gene expression will be additive in the hybrid compared with the expression in the parents. For many genes, this is the result found, but depending on the particular study, there are varying numbers of genes that exhibit a nonadditive behavior (Sun et al., 2004;

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Swanson-Wagner et al., 2006, 2009; Wang et al., 2006; Meyer et al., 2007; Uzarowska et al., 2007; Zhuang and Adams, 2007; Chen et al., 2008; Guo et al., 2008; Hoecker et al., 2008; Pea et al., 2008; Stupar et al., 2008; Zhang et al., 2008; Tirosh et al., 2009; Wei et al., 2009; Andorf et al., 2010; He et al., 2010; Jahnke et al., 2010; Paschold et al., 2010; Riddle et al., 2010). The various studies have utilized diverse species, different inbred lines within species, distinct tissues, and a variety of microarray platforms, which might account for the differences found to some degree. Over the range of studies, there has been no obvious consensus about genes that are differentially expressed in hybrids. Nevertheless, there does appear to be a correlation between the size of the fraction of genes that show nonadditive expression and the magnitude of the heterotic response (Li et al., 2009; Riddle et al., 2010), but it is not clear if this effect is causative (Stupar et al., 2008). Heterotic plants will probably have slightly different distributions of cell types in the assayed tissues, and such a correlation might simply reflect this possibility. However, nonadditive expression in hybrid *Drosophila* produced from divergent parents occurs on the individual cell level (Hammerle and Ferrus, 2003). Experiments to determine definitively whether this is routinely the case in plants have not been reported. In a broad sense, gene expression becomes more divergent in hybrids from parents of increasing genetic divergence (Birchler and Veitia, 2010). Whether global discordant regulation due to diverged alleles and regulatory elements at multiple loci contributes to heterosis is unknown. This concept does not on the surface account for why hybrids typically show positive heterosis rather than random positive or negative effects. Thus, the studies of gene expression to date on the whole are ambiguous as to whether any observed changes are correlative, causative, or predictive of heterosis. However, some attempts to correlate parental expression with hybrid performance show promise (Frisch et al., 2010; Thiemann et al., 2010).

OBSERVATIONS ABOUT HETEROSIS THAT ARE OFTEN OVERLOOKED BUT MUST BE EXPLAINED BY A VALID MODEL

Most discussions of heterosis focus on standard analysis of crosses of diploid parents with examination of the resulting hybrid for several characteristics. In parallel, inbreeding experiments have been conducted as a corollary with an interpretation as being the opposing effect of heterosis. While these results are important, there are a few neglected observations about heterosis that have accumulated over the decades that should be revived into current thought about the genetic and molecular basis of heterosis. The major observations follow.

Despite the Multigenic Nature of Heterosis, Single Genes or Small Genomic Segments Can Produce a Heterotic Effect

The typical thinking about heterosis is that it involves many genes, and this is certainly true in most cases. This concept probably arises from the fact that inbreeding depression is cumulative over generations until it stabilizes but will be reversed immediately upon outcrossing to a different strain (Darwin, 1876). However, through the decades, examples of single gene heterosis have been documented, most notably by cases in *Arabidopsis* (Redei, 1962), cereals (Gustafson, 1946; Dollinger, 1985), and yeast (Delneri et al., 2008). Analyses of introgression lines of portions of the genome of a wild tomato species into domesticated tomato are most readily interpreted as due to the action of single genes (Semel et al., 2006). Recently, a single gene in tomato has been demonstrated to produce an effect that exhibits heterosis for yield in tomato (Krieger et al., 2010). While these cases might be interpreted as examples of overdominance, it is possible that they involve dosage effects on regulatory networks that are not incompatible with the concept of multigenic control. If alterations to regulatory networks contribute to heterosis, then variation in single genes or multiple genes that are not necessarily the same in different varieties could also contribute.

There Is No Decrease in Heterosis after Purging of Obvious Detrimental Alleles

In an intriguing experiment, Duvick (1999) analyzed heterosis using inbred lines that had been selected for improvement over many decades at Pioneer Hi-Bred seed company. Whereas there was a consistent improvement in yield for the inbreds, the magnitude of heterosis (as measured by yield) from these lines selected over decades was not changed in a major way. The thinking was that the improvement of the inbred lines was successful in purging them of detrimental mutations. On the concept that heterosis results from the complementation of recessive detrimental mutations in the hybrid, one might expect that the magnitude of heterosis would decline with the continuing accumulation of superior alleles in elite inbred lines. This, however, is not the observed result. On the other hand, as noted above, East (1936) described how the purging of obvious detrimental mutations during inbreeding did not seem to affect the heterotic response, which observation is consistent with the results of Duvick (1999).

Polyploids Exhibit Progressive Heterosis

The behavior of heterosis in polyploids is almost never raised in discussions of the mechanism, perhaps because this behavior defies categorization into the classical models of dominance and overdominance. Given that all plants have a history of multiple polyploidization and diploidization cycles (Comai, 2005; Van de Peer et al., 2009) and many are fairly recently formed polyploids (Wood et al., 2009), this is an unfortunate circumstance. Progressive heterosis is a phenomenon in polyploid plants that is critical to developing a viable model of heterosis. This phenomenon involves the fact that maximizing diverse genomes in a polyploid results in increasingly greater magnitudes of heterosis as a general rule. Perhaps one of the first observations of this

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phenomenon is that crosses between different allotetraploids in the same genus will produce exceptional heterosis. East (1936) noted: "I have crossed *Nicotiana Tabacum* [sic] and *N. rustica*, obtaining plants showing more heterosis than any other crosses I have ever observed." Both tobacco (*N. tabacum*) and *N. rustica* are allotetraploids of fairly recent origin (<200,000 years ago) that originated from different pairs of parental species (Leitch et al., 2008). Thus, this cross brings together four different genomes that have diverged from each other. The vigor of this hybrid is superior to that of either *N. tabacum* or *N. rustica*, both of which exhibit good vigor because they are allotetraploids containing different sets of genomes.

A related intraspecific phenomenon has been reported in alfalfa (*Medicago sativa*; Groose et al., 1989), potato (*Solanum tuberosum*; Mok and Peloquin, 1975), and maize (Randolph, 1942; Levings et al., 1967; Chase, 1980; Sockness and Dudley, 1989a, 1989b; Riddle and Birchler, 2008). In autotetraploids, crosses between homozygous tetraploid lines will produce single cross hybrids (AABB and CCDD) that exhibit heterosis. However, if different single cross hybrids are mated that have originated from different parents (for a total of four grandparental lines) to produce a double cross hybrid (ABCD), the heterotic response is almost always superior to the single cross tetraploid hybrids. These results with intraspecific tetraploid crosses are analogous to the tobacco interspecific crosses of different allotetraploid species and form a coherent concept that needs to be accommodated in heterosis models. This principle is also upheld in considering the vigor of increasing ploidy and diversity of genomes from diploid to tetraploid to hexaploid wheat (*Triticum aestivum*; Briggle, 1963) and further on with the addition of a rye (*Secale cereale*) genome to produce triticale as an octoploid with four diverse genomes (Goral et al., 2005). Despite the considerable spectrum of alleles present in hexaploid wheat and octoploid triticale, it is still possible to obtain further vigor increase by crossing together diverse derivatives within each species (Briggle, 1963; Sun et al., 2004; Goral et al., 2005).

There Is a Dosage Component to Heterosis

An underappreciated aspect of heterosis is that it has a dosage component. East (1936) noted this in terms of crosses of tobacco species. In multiple instances of crossing an allotetraploid species back to a diploid species that was one of the progenitor contributors to the polyploid, there was often less heterosis than in the allotetraploid itself. In other words, plants with AABB genomic constitutions were more vigorous than those with AAB. A similar relationship has been noted within intraspecific maize tetraploids (Chase, 1980). Tetraploids of constitution AAAB or ABBB are less vigorous that AABB, and as noted above, AABB is less vigorous than ABCD. Such a dosage component is consistent with the dosage effects described for the *fw2.2* gene family of cell number regulators mentioned above. The fact that allelic dosage impacts the magnitude of heterosis is an additional argument why complementation of recessive detrimental alleles is an inadequate model for heterosis. Such complementation would occur regardless of allelic dosage.

In a study using *Mimulus guttatus*, inbreeding depression was attributed to slightly deleterious alleles whereas recessive lethals and strongly deleterious alleles were discounted (Willis, 1999). Interestingly, the slightly deleterious alleles were judged to be only partially recessive whereas the more deleterious effects were judged to be completely recessive. Thus, the "slightly deleterious" alleles might be in fact dosage sensitive and produce responses in hybrids that are not exactly the same as envisioned for the molecular basis of complementation of fully recessive mutations, in which a normal allele produces the gene function that is missing in a mutant allele. This dichotomy might simply reflect the possibility that new null mutations in metabolic functions are tolerated in the heterozygous state whereas only weak loss-of-function dosage-sensitive genes can survive negative selection as heterozygotes (Birchler and Veitia, 2010). These slightly deleterious alleles are likely to be in quantitative trait loci (QTLs) that are typically dosage sensitive to some degree. While they will contribute to the decline of plants following inbreeding, it is unclear if they contribute to the heterotic response of surpassing the better parent in the hybrids. Indeed, with the complementation model, the principle still applies that for heterosis to occur between strains, the parents must be homozygous at different such loci affecting any one trait, which is unlikely to be the case.

Inbreeding Depression Is Not Appreciatively Different between the Diploid and Autotetraploid Levels, Despite Extremely Different Rates of Homozygosis of Alleles

Self-pollination of a diploid hybrid will produce half of the progeny that is homozygous at any one locus. By contrast, self-pollination of an autotetraploid hybrid will only produce homozygosis of a locus at ~1/18th of the progeny if the locus in question is near the centromere. Loci with increasing distal locations will have slightly different segregation ratios but will never approach that of the diploid. Interestingly, studies in both tetraploid alfalfa (Busbice and Wilsie, 1966; Bingham et al., 1994) and maize (Alexander and Sonnemaker, 1961; Levings et al., 1967; Rice and Dudley, 1974) indicate that despite this large difference in segregation at the two ploidy levels, the decline in vigor is quite similar. These observations deserve further theoretical and experimental consideration in terms of the size of recombinational blocks that are made homozygous at the two ploidy levels. In light of the dosage component to heterosis, the impact that the change of allelic dosage might have during inbreeding in tetraploids, which shifts more quickly than complete homozygosity, also deserves further exploration.

HETEROSIS AS A QUANTITATIVE TRAIT

East (1936) wrote that, "the problem of heterosis is the problem of the inheritance of quantitative characters." Today, this

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statement is no less valid and must be the way forward in thinking about the problem in light of new understanding of the control of quantitative traits (Frascaroli et al., 2007; Flint-Garcia et al., 2009; Meyer et al., 2010; Schon et al., 2010). To the extent that there is an understanding of the genetic basis of quantitative characters, some principles seem to be emerging.

Quantitative traits are typically affected by multiple genes (Buckler et al., 2009; McMullen et al., 2009), and crosses between extreme phenotypic types tend to show intermediate or dosage-dependent effects (Tanksley, 1993). Indeed, of the various QTLs that have been identified on the molecular level, most have involved some type of regulatory molecule that exhibits a dosage effect (Birchler and Veitia, 2010). These observations parallel the classical observations from aneuploid studies that changing multiple chromosomal segments can alter quantitative characteristics in a dosage-dependent manner (Guo and Birchler, 1994; Lee et al., 1996). A long-term study to identify dosage-dependent modifiers of a single phenotype in *Drosophila* identified a collection of diverse genes, but all those of known molecular basis have transcriptional or signal transduction functions (Birchler et al., 2001). Stoichiometric interactions of members of multisubunit complexes will impact phenotypic characteristics (Veitia, 2002; Papp et al., 2003; Rosado and Raikhel, 2010) and are candidates for QTL.

Our bias in terms of the genetic control of heterosis is that quantitative traits are affected in large measure by the kinetics and mode of assembly of multisubunit complexes of proteins that include various types of regulatory components that act in hierarchies (Birchler et al., 2001; Veitia, 2002, 2010). This idea is based on evidence from QTL behavior (Tanksley, 1993), aneuploidy syndromes and their gene expression modulations (Birchler, 1979; Birchler and Newton, 1981; Guo and Birchler, 1994), phenotypic manifestations of transcription factor null heterozygotes (Veitia, 2002), biophysical properties of protein–protein interactions and their impact on the prevalence of gene duplications (Liang et al., 2008; Schuster-Bockler et al., 2010), and the complementary pattern of preferential retention of duplicated regulatory genes and other members of macromolecular complexes from whole genome duplications (polyploidization) versus the fact that they are depleted in copy number variants (Freeling and Thomas, 2006; Freeling et al., 2008; Van de Peer et al., 2009; Schuster-Bockler et al., 2010). This concept is referred to as the gene balance hypothesis and is elaborated upon in more detail elsewhere (Birchler et al., 2005; Birchler and Veitia, 2007, 2010; Veitia et al., 2008). The consequence of dosage balance is that many determinants of quantitative characters will exhibit some degree of dosage sensitivity, and for any one characteristic, multiple genes and their different variants will have an impact on the trait in question in different ecotypes. This quantitative trait framework can accommodate the observations that single genes (Gustafson, 1946; Redei, 1962; Dollinger, 1985; Krieger et al., 2010) or small genomic segments (Semel et al., 2006) have been documented to exhibit effects that are essentially heterotic, but at the same time the relevant characters are under multigenic control. By contrast, the concept of the cumulative effects of complementation of slightly deleterious alleles as the basis of heterosis is not consistent with single gene effects of recognizable magnitude. Moreover, if the regulatory network is more apt to be reconfigured with increasing divergence, the observed generalized (but not absolute) positive correlation between greater heterosis and greater phylogenetic distance is satisfied as well as the phenomenon of progressive heterosis in polyploid intraspecific and interspecific crosses. An examination of the impact of dosage effects of regulatory alleles and network interactions of dissimilar alleles might be a direction for investigation in this field. Clearly, however, this concept in the context of heterosis needs much further experimental exploration, and the targets of such dosage network modulation as well as how they operate are unknown. Further integration of aspects of development, circadian rhythms, and metabolic characteristics with the genetic determinants of heterosis is needed. While we cannot provide a comprehensive model to explain the phenomenology of heterosis, we provide the details of what needs to be explained and a direction of pursuit that we feel should be fruitful.

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