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Heterosis in plants

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The phenomenon of heterosis

In 1876, Charles Darwin noted that cross-pollinated (hybrid) plants have "greater height, weight, and fertility" than their self-pollinated counterparts. The observation that cross-pollinated hybrids are more vigorous than their parents is nowadays commonly referred to as heterosis. Virtually all annual crops exhibit heterosis to a certain extent. The degree of heterosis is calculated as the difference in the phenotypic performance of a trait between a hybrid and the average of its two distinct parents. Naturally cross-pollinating species such as maize or rye typically display a much higher degree of heterosis than self-pollinating plants like wheat and barley. However, hybrid varieties have also been established in self-pollinating species because hybrids often display greater yield stability than line varieties. Heterosis can be monitored for a large number of traits throughout development. In maize, significant size differences between hybrids and their parents can already be observed during embryo development. After germination, seedling root traits including lateral root density, primary root length or seminal root number display differences as early as a few days after germination (Figure 1A). Later in development, hybrid plants produce significantly more biomass and yield than their parental counterparts, as illustrated by plant height (Figure 1B) and cob size (Figure 1C). Remarkably, the degree of heterosis can differ significantly for distinct traits. For instance, the levels of heterosis for plant height and grain yield are often negatively correlated in maize. It was suggested that heterosis of traits related to plant growth is primarily conditioned by proliferation, hence an increase in cell number, but not cell size. However, there are examples of increased cell size in hybrids relative to their parents', as illustrated by maize root-cortex cells.

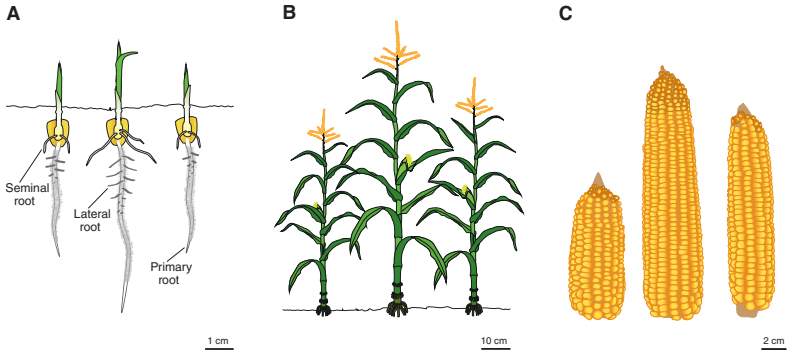


Figure 1. The manifestation of heterosis with respect to different phenotypic traits of maize.
Heterosis is observed for (A) seedling root traits, such as lateral-root density, primary-root length and seminal-root number, (B) plant height and (C) cob size of the F1-hybrid offspring (center) in comparison to both parental inbred lines (left and right).

Exploitation of heterosis in agriculture

The exploitation of heterosis in crop plants is considered to be one of the landmark innovations in modern agriculture, and is of outstanding economic importance. Maize, which provides the highest annual global kernel yield of all crop species, is exclusively hybrid based in the major corn-producing countries. In addition to maize, hybrid varieties of other crops have substantial market shares in different regions of the globe; for example, in Europe, ~100% sugar beet, >90% rapeseed, and >70% rye varieties are hybrids, as are >70% of rice in China and >80% of cotton in India. The agronomic exploitation of hybrid vigor is based on the independent rediscovery of heterosis in maize by George Shull and Edward East reported in their landmark publications in 1908. Shull and East went beyond Darwin´s experiments by generating distinct and virtually homozygous lines that were subsequently crossed to generate hybrid plants. Traditionally, homozygous lines are generated by multiple (more than seven) cycles of self-pollination to obtain homozygosity in almost all genes. The gradual reduction of heterozygosity during this process is accompanied by a reduction of vigor in these plants, a process known as inbreeding depression. In modern breeding, the so-called 'double haploid' technique allows the generation of completely homozygous inbred lines within two generations by inducing haploid embryos whose chromosomes are subsequently doubled to obtain diploid progeny.

By 1930, breeders successfully introduced the first commercial hybrid varieties in the United States, and by the early 1940s, every commercial operator in the country was planting hybrid maize. Whereas maize yield had remained essentially unchanged for decades, a constant increase has been observed since the introduction and improvement of hybrids, accompanied by more efficient agricultural practices (Figure 2). To systematically exploit the effect of heterosis in maize, the classification of genotypes into genetically divergent heterotic groups was suggested. The concept of heterotic groups has more recently been adapted to other plant species. Generally, inter-group hybrids generated from genotypes of different heterotic groups are more vigorous than intra-group hybrids, which result from crosses within a heterotic group. Surprisingly, most of the yield increases in maize over the last decades cannot be attributed to heterosis, which essentially remained unaltered. Instead these increases are based on the better performance of newly introduced parental inbred lines, which became more vigorous likely because of their better adaptation to local climatic conditions.

In maize, large quantities of hybrid seed can be produced by physically removing the anther-containing tassel (detasseling) from plants of one inbred

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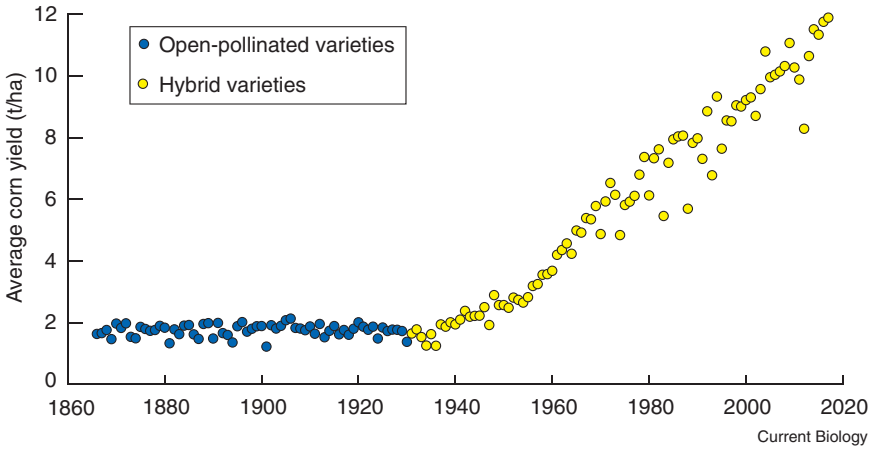


Figure 2. Average grain yield in tons per hectare for maize varieties in the United States. Yield of open-pollinated varieties (blue) from 1866 to 1930 and hybrid varieties (yellow) from 1931 to 2017. Data source: United States Department of Agriculture, National Agricultural Statistics Service (www.nass.usda.gov).

line and then pollinating the ears with a second inbred line to produce hybrid seeds. The spatial separation of male (tassel) and female (ear) reproductive organs makes maize unique among all cereals and ideally suited for hybrid production. Manual removal of stamen in crops where male and female flowers are not spatially separated, such as sunflower, barley, wheat, rice or sorghum, is tedious. Therefore, in these species, male sterility is induced by a variety of genetic systems (cytoplasmic, nuclear-encoded or photoperiod/thermo-sensitive) or by chemical agents. Such male sterile plants are unable to produce functional pollen grains. Therefore, hybrid progeny are generated by planting in close proximity to another genotype that is shedding functional pollen.

Classical genetic concepts and the basis of heterosis

Shull coined the term heterosis in 1914 with the intention to provide a denomination free from any underlying theoretical concept. By doing so, he accounted for the controversy on the genetic principle of hybrid vigor. At that time, the dominance and overdominance models had been suggested as alternatives to explain the genetic basis of hybrid vigor. The debate on these models is still ongoing to this day.

The dominance model of heterosis is based on the hypothesis that in many genes slightly deleterious recessive alleles are complemented by superior dominant alleles in hybrids. According to this model, heterozygosity per se is not a prerequisite for heterosis. Instead, hybrid vigor is based on the combination of superior alleles at as many loci as possible. The dominance model explains inbreeding depression as the accumulation of homozygous slightly deleterious recessive alleles by subsequent cycles of self-pollination.

In this scenario, if deleterious alleles are purged over time and replaced by favorable alleles, as recently demonstrated for modern inbred lines compared to traditional landraces, a slight decrease of relative heterosis would be expected. However, the degree of heterosis (in maize, for instance) remained constant over the last decades despite the significant improvement of inbred line vigor. An explanation for this, one that is not necessarily in conflict with the dominance model, is that linkage of loci representing deleterious and beneficial alleles might substantially hamper the enrichment of favorable alleles. Moreover, in modern inbred lines of maize it has been demonstrated that deleterious alleles are fixed in regions with low recombination rates, such as the centromere. However, the situation is further complicated when the analysis of heterosis is extended to polyploids, which exhibit progressive heterosis that cannot be explained by the dominance model. Progressive heterosis describes the scenario in which hybrid vigor generally increases with the number of distinct genomes present in allopolyploids. A similar observation has been made in wheat, where tetraploids and hexaploids display progressive increases in heterosis, even though the ratio of positive and negative alleles has not been altered in polypoid versus diploid plants. Moreover, autotetraploids in maize and the corresponding diploids display a similar rate of inbreeding depression over several generations, although homozygosity is reached much faster in the diploids than in the tetraploids. It has been recently demonstrated in maize that most deleterious alleles display incomplete dominance. Although the effects observed in polyploids cannot be explained by complementation of purely recessive alleles, the action of incompletely dominant alleles and gene-dosage effects described below can contribute to the understanding of the genetic patterns observed in polyploids.

The overdominance model attributes the superiority of hybrids to heterozygosity itself and hence allelic interactions at multiple loci. Several examples of overdominance have been reported in *Arabidopsis* species (*erecta* and *augustifolia*), maize (*pl*) and tomato (*SFT*) where a heterotic trait was conditioned by a single gene. In these examples, the trait was superior in its heterozygous over its homozygous state. However, heterozygosity cannot solely explain heterosis. For instance, in some species highly homozygous, modern inbred lines perform better than highly heterozygous hybrids developed some decades ago.

Neither the dominance nor the overdominance model can exclusively explain heterosis. However, they also do not exclude each other. Furthermore, epistatic interactions between different genes are likely to contribute to heterosis. It is important to keep in mind that these genetic hypotheses were suggested before the era of molecular biology and cannot be directly associated with molecular principles.

Molecular observations related to heterosis

It has been shown in maize that hybrid vigor correlates with the genetic distance between the parental inbred lines. The genetic diversity of maize genotypes is based on intensive

structural diversity between different genotypes including copy number variation and presence/absence variation. For instance, it has been demonstrated that ~10% of genes present in either of the maize inbred lines B73 and Mo17 are absent in the other genotype. The activity of genes absent in one parental genotype can be complemented in the hybrid by the second genotype. Recently, it has been shown that complementation occurs not only on the genomic level but also on the level of gene expression. In a panel of distinct inbred lines and their hybrids, ~1,000 genes that were active in the hybrid were only expressed in one of the two parental inbred lines. This phenomenon was designated as 'single parent expression complementation'. Remarkably, only about 10% of these genes are accounted for by genomic presence/absence variations. Likely, other classes of genomic variations — such as single-nucleotide polymorphisms and insertion/deletion polymorphisms in the regulatory sequences of these genes — might account for this observation. As a consequence of single parent expression complementation, it was demonstrated that hybrids expressed, on average, ~600 additional genes compared to the average of their parental inbred lines in the surveyed tissue. Intriguingly, the class of evolutionarily younger non-syntenic genes (that is, genes that emerged after the evolutionary separation of the lineages that led to maize and sorghum) were significantly overrepresented (~60–65%) among the genes displaying single parent expression patterns relative to their genomic prevalence (38%) and prevalence among all expressed genes (~18–20%). It was hypothesized that the activity of these additionally active genes could be beneficial for hybrids, for instance in the adaptation to changing environmental conditions, because it has been demonstrated before that non-syntenic genes are often involved in such processes. These extreme instances of gene expression complementation of hundreds of genes fit into the dominance model of heterosis. However, one needs to keep in mind that these global gene expression patterns have not yet been linked to the manifestation of phenotypic traits.

There are several examples in which single-gene overdominance has been observed. For instance, heterozygosity of loss-of-function alleles of the *SINGLE FLOWER TRUSS* (*SFT*) gene in tomato (mentioned above) increases yield by up to 60%. The observed effects are a consequence of the suppression of growth termination mediated by the *SFT* antagonist *SELF PRUNING* (*SP*). Moreover, the expression of the maize cell number regulator genes *ZmCNR1* and *ZmCNR2* is negatively correlated with plant vigor for many traits related to plant growth. Hence, these genes display significantly higher expression levels in homozygous inbred lines compared to their heterozygous parents. If it could be demonstrated that these dosage-dependent genes — and the molecular networks in which they are interwoven — play a role in heterosis, they could be considered as examples for negative overdominance.

Several observations — including the previously mentioned experiments with polyploids and the identification of dosage-dependent regulators of plant growth — suggest that gene dosage likely plays a role in heterosis manifestation. The theoretical framework underlying these observations is known as the gene-balance hypothesis and was developed by James Birchler and Reiner Veitia. This concept does not oppose the dominance and overdominance hypotheses, but it does provide an additional molecular explanation for observations that are not covered by these classical genetic concepts. In brief, the gene-balance hypothesis suggests that regulatory genes, such as transcription factors, often function as part of complexes that are sensitive to changes in the abundance, and thus dosage, of their different subunits. Non-additive gene expression as well as protein and metabolite patterns related to regulatory genes are frequently observed in hybrids and might be associated with dosage-sensitive molecular complexes. Since these complexes are often involved in multi-level regulatory hierarchical cascades, alteration of expression of such regulatory genes can result in the altered expression of a large number of downstream genes. A similar concept proposed by Stephen Goff suggests that hybrids achieve greater cellular energy efficiency by selectively regulating transcription and translation of the more stable, energy-efficient alleles in hybrids.

Despite the large number of heterosis-related gene expression studies that have revealed non-additive, differential and allele-specific gene-activity patterns, no consensus set or enriched functional categories related to heterosis have been identified. This can be attributed in part to the different experimental platforms used, as well as to tissue- and genotype-specific gene expression differences. Nevertheless, even if there are a limited number of regulatory genes that control heterosis manifestation, it could be very difficult to identify them because their activities might be masked by the plethora of downstream genes affected by them. Moreover, the interpretation of heterosis-related transcriptome, proteome and metabolome datasets is hampered by the fact that hybrids and their parental inbred lines are often compared at developmental stages when the hybrids are substantially more vigorous than their parents. Hence, a substantial number of the expression differences observed might be the result of growth differences rather than directly related to processes involved in the manifestation of heterosis. In such analyses, it is therefore virtually impossible to separate heterosis-related and growth-difference conditioned effects.

Finally, genotype-specific epigenetic variation and unique epigenetic constitutions of hybrids have been observed and merit further experiments to explore their possible role in heterosis manifestation.

Conclusion

In summary, the introduction of hybrids was one of the most important innovations in modern agriculture. The exploitation of heterosis by constant improvement of hybrid yield and environmental stability is one pillar of global food security. Despite the agronomic — and thus economic — success of hybrids, to date no unifying genetic or molecular theory

can explain hybrid vigor completely. One explanation for this is simply that there is no single principle underlying this phenomenon. Alternatively, this principle might be masked by the genetic diversity between and within plant species or by trait-specific networks of gene expression, proteome accumulation and metabolome activity. Today, heterosis is extensively exploited without detailed knowledge of the underlying molecular principles. The identification of the genetic and epigenetic framework underlying heterosis would be a landmark discovery revolutionizing modern plant breeding by allowing the optimal exploitation of crop productivity and stability.

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Lateral eyes direct principal eyes as jumping spiders track objects

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One way of circumventing the functional tradeoffs on eye design [1,2] is to have different eyes for different tasks. For example, jumping spiders (Salticidae), known for elaborate, visually guided courtship and predatory behavior [3], view the same object simultaneously with two of their four pairs of eyes: the antero-lateral eyes (ALEs) and the principal eyes (reviewed in [2]; Figure 1A). The ALEs, with immobile lenses and retinas, wide fields of view, and hyperacute sensitivity to moving stimuli [4], are structurally distinct from the principal eyes, which have the best spatial acuity known for terrestrial invertebrates and can discern fine details of stationary objects [5]. Behind the immobile corneal lenses of the principal eyes are miniature, boomerang-shaped retinas with correspondingly small fields of view (Figure 1B). The principal-eye visual fields are greatly expanded and overlap because of eye movements: these retinas are at the proximal ends of long, moveable tubes within the spider's cephalothorax [6]. By designing and using a specialized eyetracker, we tested whether principal-eye gaze direction is influenced by what the ALEs see. The principal eyes scanned stationary objects regardless of whether the ALEs were masked, but only when the ALEs were unmasked did the principal eyes smoothly track moving disks. The principal eyes, with high acuity but a narrow field of view, can thus precisely target moving stimuli, but only with the guidance of the secondary eyes.

'Tracking' and 'scanning' refer to distinct principal-eye retinal movement patterns that Land [2,6] identified in 1969 by using a modified ophthalmoscope that became the basis for our salticid-specific eyetracker [7]. Our eyetracker allows for accurately mapping the shifting salticid gaze on images it sees, similar to what is done in human-eyetracker research. By presenting stimuli from video recordings to tethered salticids (adult *Phidippus audax* females), we elicited two distinct types of principal-eye behavior. To ascertain proficiency at tracking moving objects, we varied the size (3, 4 or 5° of a spider's field of view subtended) and speed (2.77, 7.60, or 15.13 °/s) of the moving disks shown to spiders. Each disk appeared first in the center of the screen, and after 10 s traversed back and forth across the screen for 1 min. In random order, the spider viewed all size and speed combinations while the ALEs were unmasked. Then, after we masked its ALEs, the same spider viewed the same stimuli in a new random order. To ascertain how salticids scan stationary visual objects situated in a standardized position in front of the principal-eye retinas, we let tethered spiders view a cricket silhouette and a black oval for 1 min while the ALEs were unmasked and then while masked. Pilot data showed that freely moving spiders readily attacked the cricket silhouette, but not the oval stimulus.

We video-recorded principal-eye retinal movement, then overlaid and aligned the retinal and stimulus videos and measured three dependent variables (Figure 1C) frame-by-frame. Each spider generated one mean value per stimulus for each masking condition. For the moving-disk experiment, we analyzed data for the first complete pass of the stimulus across the spider's field of view. We performed a MANOVA with all dependent variables included, then dissected the results with separate ANOVAs for each dependent variable. The independent variables were masking treatment, stimulus shape, stimulus order, and masking x stimulus shape interaction. For the still-image experiment, we included an additional dependent variable: time spent examining each stimulus. Multiple presentations to the same spider were accounted for by using spider identity as a random variable. We applied Bonferroni corrections for multiple tests.

The salticid's principal-eye retinas closely tracked moving disks when the ALEs were not masked (Video S1, Figure 1D, Figure S1, Figure S2); irrespective of disk size and speed, they generally kept both 'elbows' (high-acuity regions) of the