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Heterosis in elite hybrid rice: speculation on the genetic and biochemical mechanisms

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Because of the tremendous advances in functional genomics and the current availability of a large number of superior hybrids, rice is an excellent model crop system for heterosis research. Genetic dissection of yield and yield component traits of an elite rice hybrid using an ultra-high density linkage map identified overdominance as the principal genetic basis of heterosis in this hybrid. This is not an expected finding based on the reported effects of single genes. Here we propose a gene expression and protein quality control hypothesis as one possible explanation for the overdominance in hybrids bred for yield. Future studies will be directed toward the identification of the genetic and biochemical mechanisms underlying the biology of hybrid vigor.

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Introduction

Heterosis, or hybrid vigor, refers to the superior performance of hybrids relative to their parents. Utilization of heterosis has tremendously increased the global productivity of many crops. Despite the obvious importance of heterosis, however, the understanding of the underpinning biological mechanism is still only fragmentary after a century of extensive research, analysis, observation, and debate. While there have been a range of studies on various aspects of heterosis, the key to understanding the biology of heterotic performance in crop hybrids lies within the framework of genetic and biochemical mechanisms, many of which remain to be fully characterized. Three classical genetic hypotheses, that is, dominance [1–4], overdominance [5–8], and epistasis [9,10] were proposed as explanations for the genetic basis of heterosis. Although there have been a large number of genetic analyses in plants and various other species with results favoring one hypothesis or another, the full complement of genetic components pertaining to heterotic performance of crop hybrids has rarely been characterized in an experimental population for assessing the relative contributions of these genetic components to heterosis in a hybrid.

Zhou *et al.* [11••] suggested the following prerequisites for complete genetic characterization of heterosis relevant to crop production: first, the genetic materials are based on elite hybrids with demonstrated high heterotic performance and time-honored superiority in crop production; second, the targets are key traits of agronomic performance; third, the experimental population allows identification of all the genetic components concerned, including dominance, overdominance and epistasis; and fourth, a full set of markers that could detect the genetic effects of any region in the entire genome be used.

Rice provides a good model crop for heterosis studies

Rice is the staple food crop currently feeding over half of the world's population. Rice has also become an excellent model system in plant biology research for monocotyledon species because of its many advantages relative to other cereals [12]. The tremendous progress that has been achieved in rice functional genomics in the last decade, including construction and development of technological and resource platforms for high throughput functional analysis of the rice genome and cloning and molecular characterization of hundreds of genes, has greatly enhanced the understanding of a wide range of important biological processes [13]. Large scale resequencing has generated an unprecedented amount of comprehensive data for examining genetic and genomic diversity of both cultivated rice varieties and their wild relatives [14].

Tremendous efforts have been invested in the development and adoption of hybrid rice varieties in a number of countries, including China over the past half a century, and India, Bangladesh, Vietnam and other Asian countries in the past few decades. Breeding for rice hybrids has generated a large number of elite hybrid varieties including ones that have been widely used for many years. Such elite hybrids are usually highly heterotic showing greatly elevated yield potential. As much as or more than 100% mid-parent heterosis ($=F_1 - MP$, where MP is the mean of the parents) and over 40% high-parent heterosis

(=$F_1 - HP$, where HP is the higher parent value) has been frequently observed in experimental plots [15–17]. It is estimated that hybrids can out-yield conventional cultivars by 30–40% in production fields [18]. Moreover, elite hybrids often display wider adaptability due to enhanced resistance to both biotic and abiotic stresses relative to inbreds, and therefore perform more stably across locations and over time. These hybrids offer excellent genetic materials for heterosis research. Together with the available rice genomic resources and the advances made in functional genomics, rice provides an ideal model and crop system for studying the molecular mechanisms of heterosis.

Current understanding of the genetic basis of heterosis from an elite rice hybrid

Shanyou 63, a cross between the two *indica* lines Zhenshan 97 and Minghui 63, is an elite hybrid that has been widely adopted in rice production in China and other Asian countries over the past three decades. The area planted with Shanyou 63 reached 6.7 million hectares in its peak production period in the late 1980s and early 1990s. This level of production accounted for over 25% of the total rice area in China during that period. Using this hybrid cross as a model, Zhang and co-workers have conducted a series of studies in an attempt to characterize the genetic basis of heterosis [15,19,20•,21••,22••] displayed by Shanyou 63. In particular, they generated an experimental population by intercrossing recombinant inbred lines (RILs) derived from a cross between the two parents, which they referred to as an 'immortalized F_2'. Such a population possesses a number of distinct advantages for heterosis research. The genetic composition of this population is similar to an F_2, allowing estimation of all the genetic components, including dominance and overdominance at a single locus level, and epistasis involving two or more loci.

Recently Xie *et al.* [23•] genotyped the RILs by population sequencing with a parent-independent method they developed for constructing ultra-high density linkage maps composed of high quality SNPs, based on 0.055-fold genome sequence depth per line. This enabled inference of the genotype of each cross in the immortalized F_2 population based on the parental RILs, providing data for the construction of an ultrahigh-density genetic map, which divided the genome into 1619 bins. They performed genome-wide analyses of single-locus genetic effects and digenic interactions for yield, number of tillers per plant, number of grains per panicle and grain weight to assess the relative contributions of genetic components that they considered pertinent to heterosis in the hybrid. This analysis included single-locus dominance and overdominance, and digenic dominance, which measures the advantage of the double heterozygote over the mean of the two parental genotypes, resulting from epistatic interactions that showed significant dominance by dominance interactions (Figure 1a,b). The results showed that both the overall levels of heterosis and the relative contributions of the genetic components to heterosis varied with traits. Yield showed the highest level of heterosis followed by number of grains per panicle and grain weight, while the amount of heterosis of tiller number per plant was low and inconsistent between years. Overdominance was the most important contributor to heterosis of yield, number of grains per panicle and grain weight. Digenic dominance was important for heterosis of tillers per plant, grain weight and also had a role in yield and in grain number per panicle. Single-locus dominance had a relatively small contribution in all analyzed traits. Although the results appear to be consistent with the general expectation that cumulative effects of these components may well explain the genetic basis of yield heterosis in the hybrid, the prevalence of overdominance was unexpected, and seemed to differ substantially from the perspective of previous results obtained using low-density markers [20•,21••,22••].

The cause of overdominance in hybrids is still a controversial issue in the literature. In tomato, it was reported that the flowering gene *SINGLE FLOWER TRUSS* showed overdominance for fruit number, in that the heterozygote for this gene displays higher performance than both parental lines in a near isogenic background [24]. However, there is no reported evidence of overdominance in any of the single genes cloned from rice to date, or any other published data from the comparison of rice NILs, suggesting that it is difficult to explain the overdominance observation based on specific individual genes. Thus Zhou *et al.* [11••] proposed pseudo-overdominance, resulting from genes with opposite additive effects linked in repulsion [25,26], each of which shows partial dominance but not overdominance, as a possible explanation (Figure 1c). However, testing of such a hypothesis via high-resolution genetic recombination of the experimental materials would be difficult. An alternative hypothesis and/or approach may be needed to resolve these conflicting observations. In the following section, we propose a biochemical hypothesis to explain the observed dominance and overdominance contributing to hybrid performance.

Biochemical observations and interpretations

Hybrid shellfish display increased growth and vigor over inbreds when compared in crowded or otherwise stressed conditions. Such hybrids display more efficient protein deposition per unit oxygen consumption than inbreds, and therefore grow faster and more efficiently. Gene expression analysis together with metabolic labeling studies in a number of different species suggests that hybrids have a lower basal level of protein metabolism and more efficient growth relative to their inbred parents [27–31,32•,33]. Likewise, inbreeding is known to cause increased protein turnover and slower overall growth

Figure 1

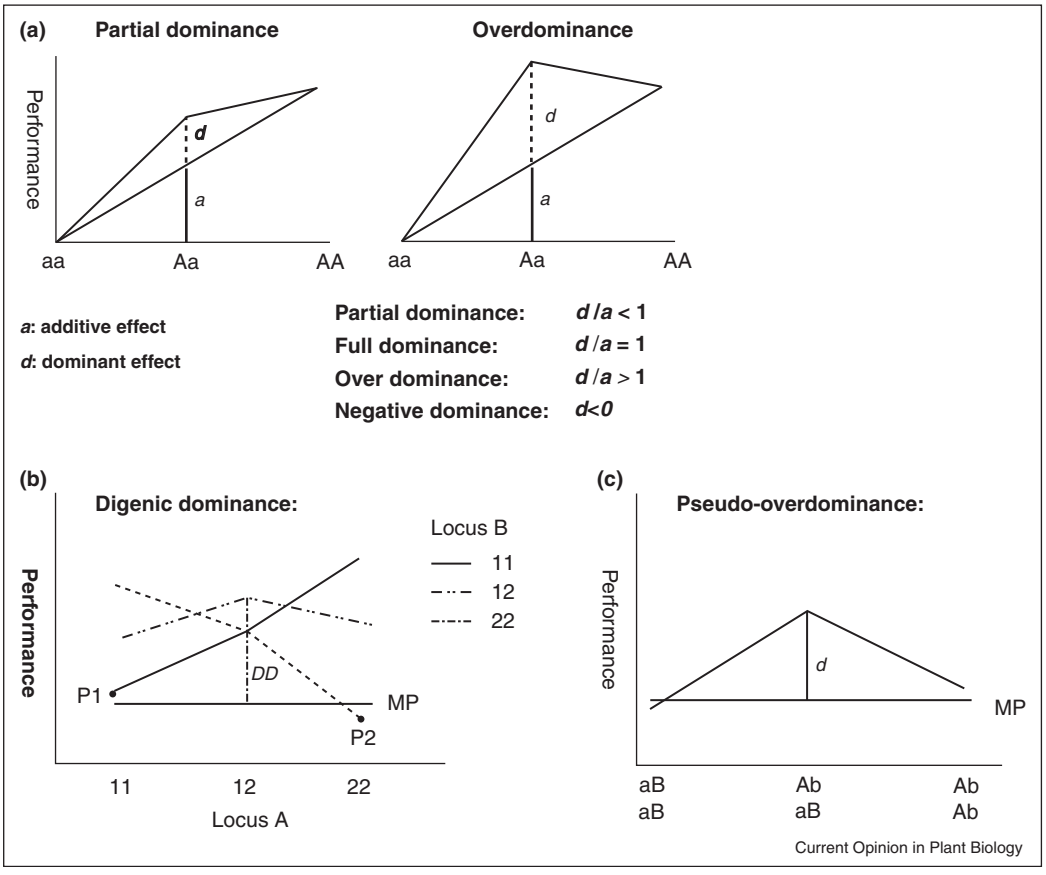


Illustration of the models for the genetic basis of heterosis. **(a)** Dominance and overdominance at the single locus level. **(b)** Digenic dominance 'DD' that measures the deviation of the performance of the double heterozygote from the mean of the two parental homozygotes (P1 and P2). '11', '12' and '22' of each locus indicate, respectively, homozygote for allele from parent 1, heterozygote and homozygote for allele from parent 2. **(c)** Pseudo-overdominance resulting from two tightly linked loci with opposite additive effects due to either reciprocal loss of functional alleles at the two loci from the two parents, or allele-specific expression. 'a' and 'b' indicate alleles that are either nonfunctional or with reduced expression in the hybrid relative to the parents.

[34]. Gene expression studies comparing hybrid to inbred oysters revealed lower expression of the genes involved in protein metabolism (both protein folding and degradation) [32•,33]. The biochemical pathways controlling nutrient sensing, protein synthesis and protein folding and catabolism are regulated by the ToR (Target of Rapamycin), myc and insulin-like regulatory factors [35,36]. These very general growth regulatory pathways stimulate coordinated changes in global gene expression. Researchers studying gene expression differences in faster growing hybrids relative to inbreds may underestimate the magnitude of expression differences when calculated per unit of mRNA or total RNA. Global regulatory changes need to be considered and may be difficult to take into account. This realization has recently brought into question tens of thousands of published studies of the impact of the myc oncoprotein [37••,38••,39], and even challenge the interpretation of routinely used gene expression techniques. Another factor that will impact the interpretation of gene expression studies when examining major metabolic systems such as protein synthesis and turnover is the high percentage of basal transcription, translation and metabolic energy devoted to these central pathways. A relatively small change in these pathways, such as less than twofold, could fall below the threshold levels in experimental analysis yet be important physiologically. It is therefore important to consider a range of experimental evidence from diverse species when studying the basic biology of heterosis.

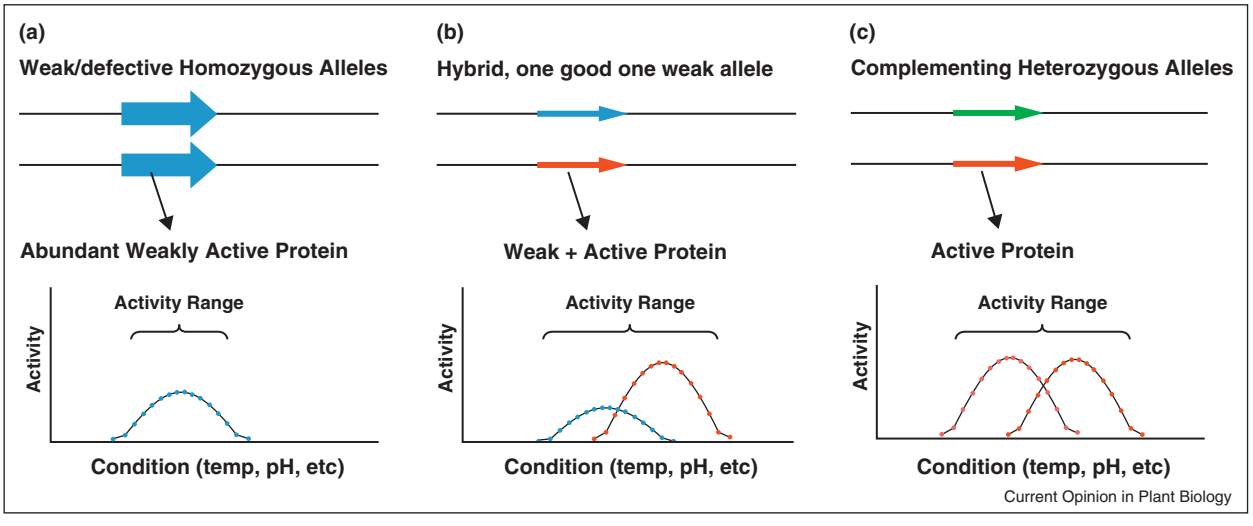
Hybrid oysters grow faster and become several times larger than inbreds, and the majority of the growth difference between hybrids and inbreds is attributed to lower protein metabolism in the hybrids [32•,33]. Protein metabolism genes (protein folding and degradation) have also been observed to be expressed at a lower level in super-hybrid rice relative to the inbred parental lines [27] suggesting a similar biochemical mechanism is

responsible for the growth differences in widely diverse species.

Observations of differences in protein metabolism raise an obvious question: if hybrids are degrading proteins at a lower basal rate than their inbred parental lines, where do the additional protein substrates come from in the inbreds? After all, the hybrid has all the same genes as both of the inbred parents. The obvious difference is that hybrids have one copy of each orthologous parental allele, and therefore cells could potentially discriminate between alleles and reduce the number of protein substrates entering the degradation pipeline if alleles encoding unstable proteins are being actively identified and differentially expressed in a quality control process. In recent years, it has become clear from studies of a large number of species that while most genes in the hybrids are expressed at the mid-parent level, certain proportions of genes in heterozygous individuals are expressed in an allele-specific fashion. Allele-specific expression (ASE) has been demonstrated in very distant species including hybrid diploids of yeast, genetic model systems such as *Drosophila*, *Caenorhabditis elegans* and *Arabidopsis*, crops such as maize and rice, invertebrates and also in humans [40••,41••,42,43,44•,45–47]. What drives ASE? Differences in noncoding regulatory regions are reported to be responsible for many of these *cis*-acting regulatory effects [48–51]. Less appreciated is the fact that some changes in apparent gene expression are correlated with changes in the protein coding sequence rather than the noncoding flanking sequences. For example, many disease-susceptibility alleles of genes have single nonsynonymous amino acid substitutions, and the protein products are not expressed in heterozygous carriers of these alleles [41••,52–56]. An excellent example of a gene displaying ASE is the human *PIT1* gene [41••]. A single amino acid substitution of PIT1 (Arg271Trp) causes the protein to become a negative inhibitor of the wild-type, but this allele is not always expressed in heterozygous carriers of the allele. When it is expressed, it blocks development of the pituitary and is fatal in newborns, but it is most often carried as a silent allele in the heterozygous form and therefore can be transmitted to subsequent generations. Approximately half of known genes that cause susceptibility to inherited diseases in humans encode proteins with single amino acid substitutions.

On the basis of gene expression and metabolic studies from phylogenetically diverse species, the following model was proposed to explain the growth and vigor differences observed between hybrids and their inbred parents: highly inbred individuals have identical alleles at most orthologous genes, and therefore cannot take advantage of allele-specific differences in protein stability and/or gene expression. In other words, inbreds express both alleles of each homozygous gene. Hybrid individuals have two different alleles of many or most genes, providing the opportunity to take advantage of the best allele for

Figure 2



A biochemical model explaining the hybrid advantage. **(a)** A homozygote where one weak allele is present in two copies and must be expressed at a relatively high level (thick arrows) to create the enzyme activity required by the cell. This assumes that the specific enzymatic activity is under feedback regulatory control (induced by low activity). The enzyme encoded by this allele could have a shorter half-life or not fold efficiently, so more of the protein needs to be made per unit activity of enzyme. This will be an energetically unfavorable condition. **(b)** A heterozygote of the weak allele with an allele encoding a more active protein with a broadened range of activity. Less of the weak allele needs to be transcribed and translated, since the stronger allele provides more activity per unit protein. This again assumes a feedback regulation of the enzymatic activity. This would provide higher enzymatic activity than the homozygous condition in (a) under most conditions with less energy. **(c)** A heterozygote consisting of two stronger alleles complementing each other across a broad range of conditions creates the most favorable situation. Less of these proteins need to be made to generate activity over any given point in the activity range.

a given environment [57••]. A protein quality control system exists within the cell that monitors proper protein-folding during translation. If the protein is not folding efficiently, the nascent polypeptide is degraded for this weakly folding protein and the transcript for that protein is also eliminated efficiently before bulk translation. The proposed protein quality control step would be most effective if it takes place during the pioneer round of translation [58–61], with appropriate feedback mechanisms to lower gene expression, as does the feedback that reduces transcription in the unfolded protein response of the endoplasmic reticulum. Feedback mechanisms that decrease transcription of alleles encoding proteins that do not fold efficiently would reduce the number of mRNAs used for bulk translation. Such a quality control mechanism would save a considerable amount of energy otherwise wasted on generating and degrading faulty proteins. Figure 2 describes the biochemical interpretation from a protein quality and enzyme activity perspective, which illustrates the low efficiency in an inbred (Figure 2a) and higher efficiency in the hybrids, underpinning the dominance and overdominance genetic hypotheses (Figure 2b and c).

A protein quality control mechanism would be expected to result in non-additive gene expression differences. Guo *et al.* [44•] reported that yield in different inbred-hybrid lines was proportional to the level of additive gene expression differences between the inbreds and hybrids, or inversely proportional to the non-additive expression differences. This would be consistent with the protein quality control mechanism if fewer defective alleles were present in the inbred parents of high yielding hybrids. Alleles encoding defective proteins would display non-additive gene expression differences in the absence of dosage compensation.

Similar quality control mechanisms such as nonsense-mediated decay and nonstop-mediated decay also appear to require close coupling of transcription and translation. The ability of cells to discriminate between alleles based on the folding and stability of the encoded protein would explain why many disease-susceptibility alleles are not expressed in heterozygous carriers of these alleles even though they frequently result from only single nucleotide and single amino acid substitutions. A protein quality control system would also explain why allopolyploids display higher levels of heterosis, and are more vigorous than autopolyploids or diploid hybrids [62–67]. Allopolyploids have more available discrete alleles for cells to use under different environmental conditions.

Conclusion and perspective

Like any trait displayed by an individual, heterosis is the phenotypic manifestation of a genetic and biochemical program originating from DNA sequence information being transcribed into ncRNA or mRNA, functional as ncRNA or translated into proteins via mRNA, then creating specific regulatory or biochemical activities as unique gene products. Unlike an ordinary trait, however, heterosis occurs in heterozygous genetic backgrounds such that any non-additive activity that occurs in the hybrid relative to the parents may result in heterosis (positive or negative). It is now clear that the classical genetic hypotheses, for example, dominance, overdominance and epistasis, and hence the genetic analyses, are based on conceptually oversimplified hypothetical direct relationships between the genotype and phenotype. The prevalence of overdominance as detected in the immortalized F_2 population is not expected, based on the presently available information from analyses of the cloned individual genes and NILs. Transcriptome analysis of the Shanyou 63 triad also showed that large numbers of genes are differentially expressed at various stages of the rice plant development, and more genes are downregulated in the hybrid relative to the parents than ones that are upregulated [68]. Recent studies of a rice intersubspecific hybrid revealed substantial epigenetic changes in the hybrid compared with the parents [69,70]. This indicates that much more is happening in the hybrid, due to hybridization and genome heterozygosity, than would be expected based on the DNA sequence polymorphisms. However, whether such epigenetic modifications of the hybrid genome and differential expression of the genes have any role in the agronomic performance of the hybrids remains to be investigated. It poses a tremendous challenge to relate the differential epigenetic modifications of the hybrid genome and the non-additive gene expression of the genes with performance of the hybrids.

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