

Hybrid sterility in plant: stories from rice

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Hybrid sterility is the most common form of postzygotic reproductive isolation in plants. The best-known example is perhaps the hybrid sterility between *indica* and *japonica* subspecies of Asian cultivated rice (*Oryza sativa* L.). Major progress has been reported recently in rice in identifying and cloning hybrid sterility genes at two loci regulating female and male fertility, respectively. Genetic analyses and molecular characterization of these genes, together with the results from other model organisms especially *Drosophila*, have advanced the understanding of the processes underlying reproductive isolation and speciation. These findings also have significant implications for crop genetic improvement, by providing the feasibility and strategies for overcoming intersubspecific hybrid sterility thus allowing the development of intersubspecific hybrids.

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Introduction

Biological species are defined as populations that can actually or potentially interbreed, but are blocked from breeding with members of other species [1,2,3]. Reproductive isolation is divided into two forms depending on the stage in which it occurs: prezygotic barriers and postzygotic barriers. Prezygotic barriers act earlier in the life history preventing the formation of hybrid zygotes and consist of forces that reduce the chance of mating success. Postzygotic barriers, on the other hand, result from fitness aberration in hybrids causing weakness, inviability [4], or hybrid sterility, preventing the exchange of genes between subspecies or species [5].

In plants, hybrid sterility is the most common form of postzygotic reproductive isolation. The best-known example is perhaps the hybrid sterility between *indica* and *japonica* subspecies of Asian cultivated rice (*Oryza sativa* L.), including embryo-sac abortion and pollen sterility. Such hybrid sterility hinders the transferring of useful genes between the two subspecies, and is a major obstacle for utilization of the strong heterosis exhibited in the hybrids.

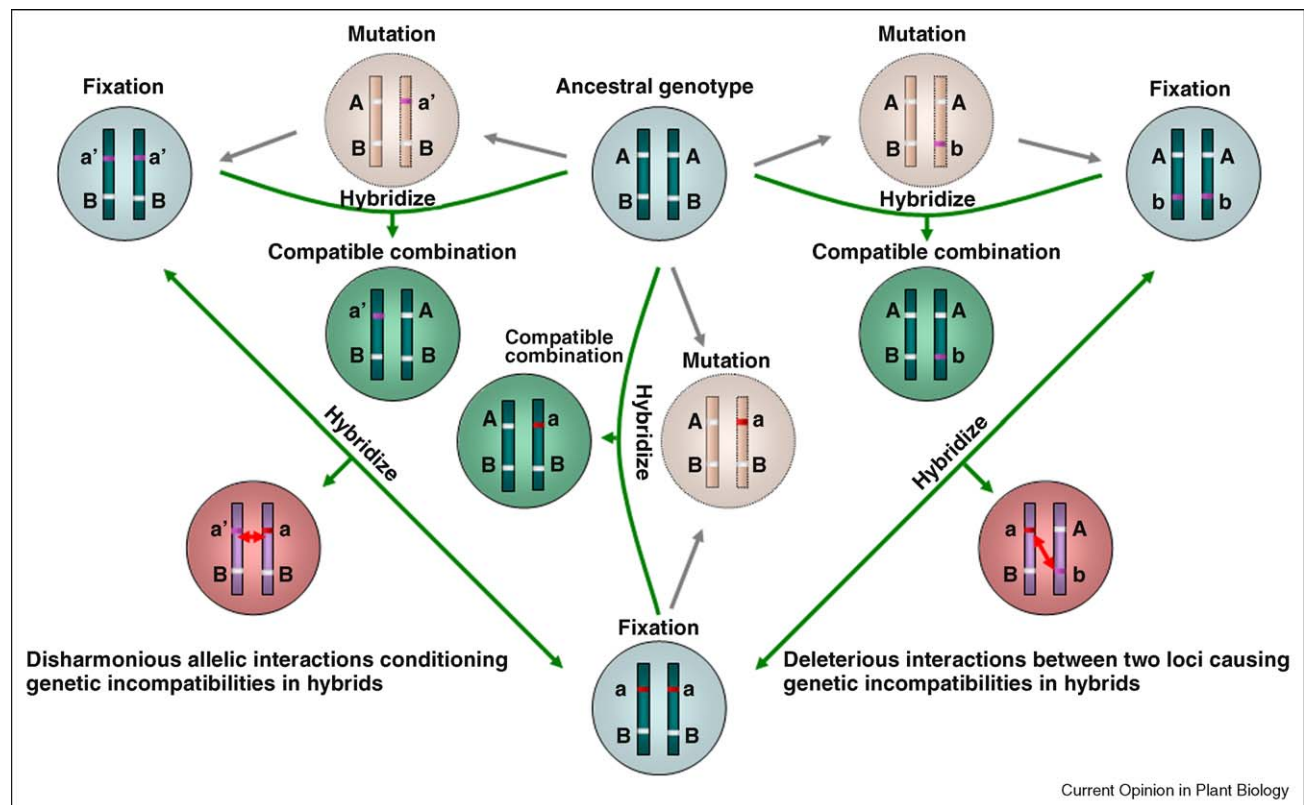
In this review we expatiate on the genetic architecture of hybrid sterility in rice based on the results of genetic analyses in the last several decades and summarize recent progress in the identification and characterization of genes involved in the intersubspecific hybrid sterility. Analyses of such unique dynamic system provided by the hybrid sterility genes and the respective neutral alleles at the corresponding loci have greatly advanced the understanding of the nature of the genes involved in reproductive isolation. These findings have shed light on the process of molecular evolution in reproductive isolation, and also have significant implications in crop genetic improvement.

Genetic architecture of hybrid sterility: neutral evolutionary changes within populations resulting in deleterious effects in hybrid backgrounds

According to the Dobzhansky–Muller model, postzygotic isolation results from a deleterious interaction between functionally diverged genes from the hybridizing species [6]. In this model, independent mutations appeared and became fixed in different populations, and the loci interacted negatively causing genetic incompatibilities in the background of hybrids when subsequently brought together in a common genome (Figure 1). In rice, a duplicate gametic lethal model was proposed [7,8], in which two independent loci affected the gamete development and gametes carrying the recessive alleles at both loci were aborted during the development while gametes of other genotypes were normal.

Such negative interaction can also occur within a single locus as a consequence of independent evolution of the two alleles, causing significant reduction in fitness of the heterozygote compared with the two homozygotes (Figure 1). In rice, Kitamura [9] proposed that the gametes carrying the *japonica* allele would be aborted in an *indica*–*japonica* hybrid, while the gametes were fertile in hybrids having the neutral allele regardless of the combinations with either *indica* or *japonica* allele. Ikehashi and Araki [10] substantiated such one-locus

Figure 1



The genetic models of hybrid incompatibility. The left and right parts of the figure indicate the intralocus and interlocus interactions causing incompatibility in the hybrids. The genotype of the ancestral population is AA BB represented by the white bar. Independent mutations, indicated by purple or red bars, appeared and became fixed in different lineages in divergent evolution. The divergence process is indicated by the gray arrows and hybridization is indicated by the green lines. Incompatibility is indicated by the red double-headed arrow in the hybrids.

sporo-gametophytic interaction model and examined hybrid sterility caused by the *S5* locus. They found that this fertility barrier can be overcome by the utilization of a wide-compatibility variety (WCV) carrying the *S5-n* allele, such that hybrids of either *indica*/WCV or *japonica*/WCV were highly fertile. The genetic behavior of many identified hybrid sterility loci in rice in general supports this model [10–15].

Molecular divergence, by either gene transposition [16•] or divergent evolution among duplicate genes, is also a cause of genetic incompatibilities between isolated populations [17,18,19•]. Such evolutionary divergence of genomic positions exhibits neutral effects in their native genetic backgrounds, whereas negative effects occur in the heterozygotes.

Although all the models discussed above seem to be simple in genetic architecture, the molecular basis of hybrid incompatibility is usually complex and often involves accumulative effects and interactions of genes at multiple loci [14,20,21•,22–24,25•,26]. In rice, approximately 50 loci controlling hybrid fertility have

been identified, including loci causing female gamete abortion and ones inducing pollen sterility (in a few cases, both) [27]. Therefore, mechanistic understanding of such reproductive isolation ultimately requires identification and characterization of genes causing hybrid sterility.

Genes causing hybrid sterility: diverse functions

There has been major progress in the identification and characterization of genes contributing to hybrid sterility. A big surprise revealed by analyzing the hybrid sterility genes is that they fall into very different functional categories (Table 1).

Two genes in rice, *S5* [28••] and *Sa* [29••], causing female and male sterility respectively in *indica-japonica* hybrids were recently cloned and characterized, providing fresh data on the molecular mechanism of hybrid sterility. Genetic analyses mapped the *S5* locus on chromosome 6 as a major locus for *indica-japonica* hybrid sterility by impairing embryo-sac fertility [10,30–36]. Chen *et al.* [28••] cloned *S5* using a map-based cloning approach.

Table 1

Hybrid sterility genes causing postzygotic reproductive isolation from rice and *Drosophila*

Species	Cross	Gene	Accession number ^a	Hybrid phenotype	Gene type	Reference
<i>Oryza sativa</i>	<i>indica</i> × <i>japonica</i>	<i>S5</i>	EU889293 (<i>S5</i> -n) EU889294 (<i>S5</i> -j) EU889295 (<i>S5</i> -i)	Embryo-sac sterility	Aspartic protease	[28**]
	<i>indica</i> × <i>japonica</i>	<i>Sa</i>	<i>SaM</i> EU337976 (<i>SaM</i> [−] , <i>japonica</i>) EU337977 (<i>SaM</i> ⁺ , <i>indica</i>)	Pollen sterility	Small ubiquitin-like modifier E3 ligase-like protein	[29**]
			<i>SaF</i> EU337974 (<i>SaF</i> [−] , <i>japonica</i>) EU337975 (<i>SaF</i> ⁺ , <i>indica</i>)	Pollen sterility	F-box protein	
<i>Drosophila</i> ssp.	<i>simulans</i> × <i>mauritiana</i>	<i>OdsH</i> (<i>Odysseus</i> -site Homeobox gene)	Dmel_CG6352	Male sterility	Transcription factor	[47*,50*]
	<i>melanogaster</i> × <i>simulans</i>	<i>JYApha</i>	Dmel_CG17923	Male sterility	The catalytic subunit of a Na ⁺ /K ⁺ ATPase	[16*]
<i>Drosophila pseudoobscura</i>	<i>pseudoobscura</i> × <i>bogotana</i>	<i>Ovd</i> (<i>Overdrive</i>)	FJ349335–FJ349342, FJ418600–FJ418631	Male sterility and female-biased sex-ratio distortion	Polypeptide with a single MADF DNA-binding domain near its C terminus end	[54*]

^a Accession numbers are those given in GenBank.

S5 encodes an aspartic protease, belonging to a protein family of at least 96 members in rice [37]. The limited evidence suggests that the function of *S5* is centered on megaspore formation or survival as the expression of *S5* is extremely low throughout the life cycle except in the ovule tissues [28**]. The *indica* (*S5*-i) and *japonica* (*S5*-j) alleles differ by two nucleotides, both of which caused amino acid substitutions located in the central domain according to the crystal structure analysis [38,39]. Chen *et al.* [28**] speculated that the conserved Phe-273 (hydrophobic and aromatic) in *S5*-i replaced by Leu (hydrophobic but nonaromatic) in *S5*-j may have reduced the stability and activity of the enzyme. However, how such likely reduced activity is related to the embryo-sac fertility only in the *S5*-i/*S5*-j heterozygote but not in the *S5*-j/*S5*-j homozygote remains to be characterized by future studies.

The *Sa* locus conditioning *indica*–*japonica* hybrid male sterility was mapped on chromosome 1 within a region of 30 kb [40–43], and was subsequently cloned by Long *et al.* [29**]. The *Sa* locus consists of two adjacent genes, *SaM* and *SaF*, both of which are expressed constitutively. *SaM* encodes a small ubiquitin-like modifier E3 ligase-like protein, which is unique in rice. *SaF* encodes a protein of 476 amino acids with an F-box and a plant-specific F-box protein domain, which mediates its interaction with *SaM* of the *japonica* allele, a C-terminal-truncated product. The *indica* and *japonica* varieties contain haplotypes *SaM*⁺*SaF*⁺ and *SaM*[−]*SaF*[−], respectively. The male semi-sterility of the hybrids is caused by selective abortion of pollen carrying *SaM*[−] by a ‘two gene/three component

interaction’ model. This model proposes that three of the factors, *SaF*⁺, *SaM*⁺, and *SaM*[−], are required for the sterility process by their direct and indirect interactions, on the basis of selective transport of the *SaF*⁺, *SaM*⁺ proteins from their own microspores (in tetrads) into those carrying *SaM*[−]. Thus, in this case, the hybrid male semi-sterility is the result of selective abortion of gametes carrying a given allele, thus resulting in poor transmission of this allele into the progeny and segregation distortion of the alleles. The variations of the *SaF* and *SaM* orthologs among and within the *Oryza* species suggest that the variant alleles might have their own primary functions [29**]. Therefore, hybrid sterility genes have normal functions within populations and diverged likely due to selection for new functions, causing hybrid sterility as a by-product of these changes.

Considerable progress has been made in hybrid male sterility in *Drosophila*. An *Odysseus*-Homeobox gene (*OdsH*) was identified to cause hybrid male sterility because of the misexpression in hybrid testes [44–46,47*]. This gene has experienced accelerated evolution in the *simulans* clade even though its homologs in other species are extremely conserved [48,49,50*,51], suggesting that such hybrid sterility might result from the divergence of underlying genes that have different primary functions [51]. Another hybrid male sterility gene *JYApha* encodes the catalytic subunit of a Na–K-ATPase [16*]. Genomic and molecular analyses showed that *JYApha* transposed during the evolution in *Drosophila*, thus a fraction of hybrids completely lack *JYApha* and are sterile, representing reproductive isolation which is

different from a traditional Dobzhansky–Muller model of incompatibility. Recently, a single gene *Overdrive* (*Ovd*) in *Drosophila* was found to cause both hybrid male sterility and female-biased sex-ratio distortion in F_1 hybrids [52,53,54*].

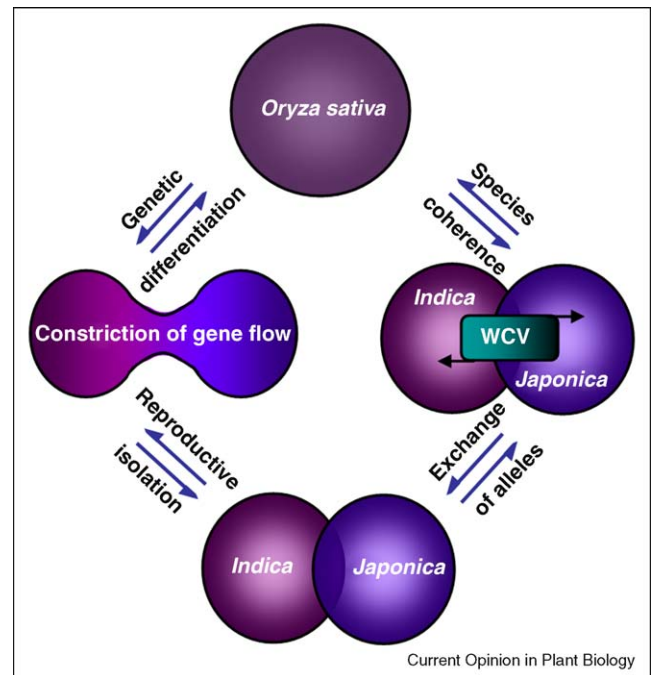
Two features thus emerge from the above analyses. First, the genes involved in hybrid sterility have distinct cellular and/or biochemical functions ranging from protease, protein degradation, to catabolism and transcription regulation. Second, the primary functions of the genes involved in hybrid sterility or postzygotic reproductive isolation may or may not be in reproduction. These features suggest complexity for studying hybrid sterility genes.

Evolutionary significance of the triallelic systems at the hybrid sterility loci

Speciation is a gradual process and represents the emergence of barriers to gene flow between populations. In the early stages of speciation, extensive exchange of alleles is still possible, while the gene flow essentially ceases when the speciation process is completed, and the genomes of the nascent species will diverge independently [55]. Hybrid sterility is recognized as one of the most common postzygotic genetic barriers in plants [56–58]. A very interesting outcome of such evolution is that there is a neutral allele at the hybrid sterility locus, which does not cause sterility when present in a heterozygous genetic background with either of the incompatible alleles (Figure 2).

In rice, for example, extensive *indica*–*japonica* hybridization identified a group of rice varieties, named WCVs, that produce highly fertile hybrids when crossed with both *indica* and *japonica* varieties [59]. Subsequent studies showed that WCVs carry a neutral allele at the *S5* locus [10], such that there is a triallelic system: an *indica* allele (*S5-i*), a *japonica* allele (*S5-j*), and a neutral allele (*S5-n*) also referred to as the wide-compatibility gene (WCG) [10]. Sequence analysis of *S5-n* in comparison with the other two alleles *S5-i* and *S5-j* [28**] detected a 136-bp deletion, causing a large deletion in the N-terminus of the predicted *S5* protein, which contains the signal peptide and the N-terminal segment of the central domain. The deletion of the signal peptide results in subcellular mislocalization of *S5-n* protein in the cytoplasm, instead of secretion into its normal destination in the cell wall as in the case of *S5-i* and *S5-j*. In addition, the loss of the N-terminal fragment in the central domain may greatly affect the stability and activity of the enzyme. Similar compatible haplotype *SaM⁺SaF⁻* is also found at the *Sa* locus [29**], which is proposed to be an intermediate product during the evolution from *SaM⁺SaF⁺* to *SaM⁻SaF⁻* in wild rice. This haplotype is compatible with *SaM⁻SaF⁻* as well as with *SaM⁺SaF⁺* due to the absence of *SaF⁺* or *SaM⁻*, both of which are necessary for the male sterility process in the hybrids. Thus this is an

Figure 2



Evolutionary dynamics of the three varietal groups. Differentiation caused by geographical adaptation gradually builds up the constriction of gene flow eventually leading to the development of reproductive barriers between the isolated groups, referred to as two subspecies *indica* and *japonica* (left). Widely compatible varieties (WCVs) enable gene flow between the subspecies by producing fertile hybrids with both subspecies, thus providing genetic coherence at the species level (right).

analogy of the triallelic system of *S5*, *Sa-i* (*SaM⁺SaF⁺*), *Sa-j* (*SaM⁻SaF⁻*), and *Sa-n* (*SaM⁺SaF⁻*). Sequence analysis of rice germplasm shows that the triallelic systems at both *S5* and *Sa* loci are widespread in the species *O. sativa*.

The coexistence of *indica*, *japonica*, and WCVs in rice provides a highly interesting system for studying the evolutionary consequence of reproductive isolation and gene flow. During the course of evolution, hybrid sterility genes have acted as an important promoting factor for the genetic differentiation between *indica* and *japonica*, which constitute a major form and source of genetic diversity in the cultivated rice gene pool [60–63]. Such genetic differentiation has enabled the dispersion and wide adaptation of cultivated rice from the tropics and subtropics, where the cultivated rice originated, to more temperate regions, as well as a diverse range of environmental conditions, thus making rice a major staple food crop throughout the world. Such process of adaptation has greatly promoted genetic diversification of the species resulting in a large range of ecotypes, which has tremendously enriched the gene pool. Conversely, the WCGs that enable hybridization can serve as a bridge for gene flow and exchanges between the two subspecies, thus providing an opposing force that

suppresses differentiation. Such triallelic system suggests a counter-acting dynamic relationship between adaptive differentiation and genetic coherence of the cultivated rice species during evolution and artificial selection (Figure 2). It is highly interesting that the WCGs, as shown in the analyses both of *S5* and *Sa*, may not be essential for growth, development, or reproduction, as loss-of-function mutations do not have obvious phenotypic effect at the whole plant level. At the species level, however, WCGs play important roles for holding the differentiated groups together.

Implications of the triallelic systems for crop genetic improvement

The hybrid sterility and wide-compatibility system also has significant implications in crop genetic improvement. Rice is a main staple crop providing food for a large segment of the world population. The genetic differentiation between *indica* and *japonica* leads to strong hybrid vigor in F_1 hybrids, utilization of the intersubspecific heterosis has been regarded as a promising strategy for increasing rice productivity. Large efforts have been invested in the last several decades in breeding for *indica-japonica* hybrids. However, such efforts have been hindered by hybrid sterility that frequently occurs in intersubspecific crosses. Discovery of the WCVs has brought hope for breaking the fertility barrier between *indica* and *japonica* subspecies and provided a possibility for exploiting the strong heterosis between them [30,64–68].

At least three strategies have been identified for overcoming the hybrid sterility in *indica-japonica* crosses. First, the neutral alleles (WCGs) can be introgressed from the WCVs into the parents whose hybrids exhibit strong yield heterosis. Thus, crossing of the parents would produce highly heterotic hybrids with normal fertility. The second strategy is to breed '*indica-compatible japonica* lines' by introgressing *indica* alleles of several hybrid sterility loci into *japonica* lines by backcrossing [69]. Crossing of such '*indica-compatible japonica* lines' with targeted *indica* lines would produce desired hybrids. The third is to produce artificial neutral alleles by suppressing expression of the genes causing hybrid sterility with RNAi or microRNA technology, if such gene silencing does not affect the plant growth or development. However, the effectiveness of these strategies may be affected by the fact that hybrid sterility in a cross frequently involves multiple loci, most of which have not been characterized. Thus genetic manipulation, either through introgression or transgenics, should target multiple loci simultaneously in order to achieve a desired level of fertility. This suggests the need for further identification and molecular characterization of more hybrid sterility loci to find new wide-compatibility alleles and to understand the molecular mechanism. Such finding would facilitate the development of intersubspecific rice hybrids for efficient utilization of the heterosis, which may open a new horizon in rice breeding.

Concluding remarks and future directions

Identification and molecular analysis of the hybrid sterility genes have refocused attention to the genetic basis of reproductive isolation. Although the present understanding is still rudimentary and tentative, several conclusions can be drawn from the existing results. First, essentially neutral evolutionary changes within populations can produce deleterious interactions which cause sterility in hybrids between the populations. Second, genes involved in hybrid sterility may change their primary functions or genomic locations during divergent evolution. And third, the factors that cause hybrid sterility are ordinary genes which have diverse functions without preference of special functional classes. Neutral alleles (WCGs) may arise as loss-of-function mutants at these loci that may provide bridges for gene flow between differentiating populations serving as a cohesive force at the species level.

The coexistence of *indica* and *japonica* subspecies and the triallelic systems governing hybrid sterility in cultivated rice provide an excellent model system for studying the evolutionary processes underlying reproductive isolation and speciation. For complete understanding of these processes, it is essential to clone and functionally characterize the genes at the loci identified as conditioning hybrid sterility. It is also imperative, although highly challenging, to characterize the mechanisms at molecular, cellular, and organ levels of how the gene products function to induce hybrid sterility. Efforts should also be made to investigate the sequence diversity and geographical distribution of the alleles of various loci at the species level, including wild relatives. With these data together it would be possible to provide a full elucidation on the origin of the genes for hybrid sterility and the evolutionary processes for the establishment of the subspecies and species. In turn, such knowledge would facilitate the formulation and development of strategies for rice improvement, which may also have implications for the improvement of other crop species.

Conflicts of interest

The authors declare that there are no conflicts of interest related to this publication.

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