Understanding Reproductive Isolation Based on the Rice Model

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Annu. Rev. Plant Biol. 2013. 64:111–35

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

This article's doi:
10.1146/annurev-arplant-050312-120205

Keywords

hybrid sterility, wide compatibility, divergent evolution, Dobzhansky-Muller model, crop genetic improvement

Abstract

Reproductive isolation is both an indicator of speciation and a mechanism for maintaining species identity. Here we review the progress in studies of hybrid sterility in rice to illustrate the present understanding of the molecular and evolutionary mechanisms underlying reproductive isolation. Findings from molecular characterization of genes controlling hybrid sterility can be summarized with three evolutionary genetic models. The parallel divergence model features duplicated loci generated by genome evolution; in this model, the gametes abort when the two copies of loss-of-function mutants meet in hybrids. In the sequential divergence model, mutations of two linked loci occur sequentially in one lineage, and negative interaction between the ancestral and nascent alleles of different genes causes incompatibility. The parallel-sequential divergence model involves three tightly linked loci, exemplified by a killer–protector system formed of mutations in two steps. We discuss the significance of such findings and their implications for crop improvement.

Contents

INTRODUCTION

Reproductive isolation is both a mechanism and an indication of speciation, and thus has been among the key issues in biological studies of a range of organisms (14). Exploitation of genes from distantly related species through wide crossing has been an important strategy for crop genetic improvement, which is hindered by reproductive isolation between species. Based on the stage of occurrence, reproductive isolation can be divided into prezygotic reproductive isolation and postzygotic reproductive isolation. The classical Dobzhansky-Muller model that genetically explains reproduction isolation was based mostly on genetic studies of animal models, especially *Drosophila* species (72). With the advent of molecular biology in the past few decades, and especially genomic studies in recent years, genes for reproductive isolation have been cloned in several organisms, including fungi, animals, and plants (2–4, 9, 11, 13, 21, 43, 53, 57, 60, 77, 98, 105). Molecular characterization of these genes, also referred to as speciation genes, has begun to shed light on the biological mechanisms controlling the processes of reproductive isolation.

In plants, the best-characterized example of postzygotic reproductive isolation is perhaps hybrid sterility between the *indica* (*Oryza sativa* ssp. *indica*) and *japonica* (*O. sativa* ssp. *japonica*) subspecies of Asian cultivated rice, which has been the subject of intensive genetic studies. Several genes controlling hybrid sterility have been cloned recently. Functional analyses of these genes have uncovered interesting features that have enhanced our understanding of the biological mechanisms of reproductive isolation. Here we review recent progress in understanding postzygotic reproductive isolation in rice, and discuss the general significance of such understanding from an evolutionary perspective as well as its implications for crop genetic improvement.

REPRODUCTIVE ISOLATION IN PLANTS

Prezygotic Reproductive Isolation

Plant species are isolated by various types of reproductive barriers, which can arise at different stages during the life cycle (6, 74, 94). Prezygotic reproductive isolation prevents the formation of hybrid zygotes through mating discrimination between divergent populations.

Floral color/shape or flowering time may often differ among closely related populations, and the resulting distinct pollination patterns may prevent mating (6, 74). Variation in floral color has been studied intensively and has been shown to play an important role in prezygotic reproductive isolation through pollinator-based selection in flowering plants (6, 74). The color differences are caused either by regulation of the anthocyanin biosynthetic pathway or by functional mutations in enzymes involved in pigment biosynthesis. Such differences often result in a shift in pollinators, which may lead to assortative mating (nonrandom mating pattern), a form of prezygotic reproductive barrier (6, 74).

Physical environments such as abiotic/biotic adaptation and habitat isolation may also influence mating success (6). Plants are locally adapted to abiotic factors such as soil nutrients, metal/salt concentrations, moisture availability, and climate. Biotic adaptations, including adaptations to parasites and other organisms, may also contribute to prezygotic reproductive isolation in plants (6).

Postzygotic Reproductive Isolation

Reproductive isolation also occurs after mating owing to fitness aberration in offspring generations, including hybrid necrosis/weakness, hybrid sterility, and lethality in F_1, F_2, or backcross generations. Such postzygotic reproductive isolation restricts gene flow between populations.

Hybrid sterility is the most common form of postzygotic barrier in plants. The hybrid plants can grow viably at the vegetative stage but fail to produce fertile pollen or embryo sacs during reproductive development, thus reducing seed setting. Hybrid sterility has long been observed in a number of plants (42, 70, 71, 78, 81, 82). Hybrid lethality results in reduced fitness in zygotes or embryos of the hybrid, which then leads to the termination of development and ultimately the death of the organism. An example of hybrid lethality in *Arabidopsis* caused recessive embryo lethality in hybrids of different accessions (4). Several cases of hybrid sterility/lethality have been investigated in animals and fungi, including *Drosophila* (9, 57, 67–69), *Mus* (2, 3, 21), *Caenorhabditis* (77), and *Saccharomyces* (13, 43).

Other types of postzygotic barrier result in reduced vigor of hybrids and/or offspring in crosses between divergent plant populations; these barriers include hybrid weakness and hybrid necrosis, which have been frequently observed in plant species such as tomato, lettuce, *Arabidopsis*, and rice (1, 7, 29, 36, 100). The phenotype of hybrid necrosis is similar to the necrotic symptoms associated with environmental stresses and pathogen attack (8). Such hybrid incompatibility is associated with genes involved in immune response, and has been reviewed in the literature (5, 6, 8).

REPRODUCTIVE BARRIERS IN RICE

O. sativa was likely domesticated from its wild progenitor *O. rufipogon* and/or *O. nivara* 8,000–9,000 years ago (66, 75, 80, 86, 106). Two major rice groups in *O. sativa* had been well recognized at least 2,000 years ago in the Han dynasty of China; they were named *hsien* and *keng* and had distinct characteristics and geographical distributions (85). Based on hybridization studies, these groups were later regarded as two subspecies and were named *indica* and *japonica*, respectively (33, 52, 66). The two groups show a range of morphological and physiological distinctiveness in characteristics such as seed shape, disease resistance, cold/drought tolerance, potassium chlorate resistance, phenol reaction, plant height, and leaf color, and also have distinct patterns of adaptation to environmental conditions (66). Molecular marker analyses revealed that *indica* and *japonica* have profound genetic differentiation (18, 19, 51, 56, 63, 104, 112, 113), and recent genome sequencing analyses have clearly confirmed the distinction between them at the whole-genome level (17, 20, 22, 23, 97).

Prezygotic reproductive isolation between *indica* and *japonica* has been reported, although only a few studies have been carried out on this subject so far. One study showed that the number of pollen grains that adhere to the stigmas during intersubspecific pollination (a *japonica* variety hand-pollinated with pollen from an *indica* variety and vice versa) is much smaller than the number that do so in other pollinations (*indica* on *indica*, *japonica* on *japonica*, and a wide-compatibility variety on either *indica* or *japonica*) (96). The intersubspecific hybridization also encountered difficulties in pollen tube growth after pollination. These difficulties resulted in large differences in fertilization rate between intra- and intersubspecific hybrids, suggesting a different level of affinity between pollen and stigmas in intra- and intersubspecific pollinations. A subsequent study yielded similar results, with the affinity between pollen and stigmas much lower in intersubspecific crosses than in *indica-indica* hybridization (49). Abnormalities in pollen tube elongation after pollination were also observed in the *indica-japonica* hybrid, which had a lower fertilization rate and thus a reduced seed-setting rate (49).

Postzygotic reproductive isolation, in contrast, has been the subject of intensive investigation, including studies of hybrid sterility (70, 71) as well as hybrid weakness/breakdown and hybrid necrosis (24, 25, 31, 39, 40, 47, 58, 99, 100). Hybrid weakness/breakdown often occurs in *indica-japonica* hybrids, with progeny showing reduced viability and poor growth phenotypes. Multiple sets of gene pairs have been identified and mapped, and the results have suggested that hybrid weakness/breakdown is attributable to epistatic interaction of complementary genes from both parents (24, 25, 31, 39, 40, 47, 58, 99, 100). There has been a report that hybrid breakdown in an *indica-japonica* hybrid may be associated with the autoimmune response, which suggests a likely general mechanism involved in a wide range of plant species (5, 6, 8, 100).

Hybrid sterility is by far the most common form of postzygotic reproductive isolation in rice, and most *indica-japonica* hybrids show high sterility (33, 52, 70). Genetic analyses have also identified an intermediate group of rice varieties that are able to produce fertile hybrids when crossed to both *indica* and *japonica* varieties (27, 61) and thus were termed wide-compatibility varieties (WCVs) (27). Genetic analyses using mapping populations generated from various germplasms have identified approximately 50 loci controlling the fertility of *indica-japonica* hybrids, including loci with major effects and quantitative trait loci with minor effects (70). Many of the loci seem to act individually (independently) on hybrid sterility; others seem to show epistatic interactions (37, 41, 50, 79, 93). These loci were further resolved into those causing female gamete abortion (11, 27, 44, 79, 87–90, 101, 115–118), those causing pollen sterility (12, 32, 37, 38, 41, 45, 46, 53, 79, 92, 103, 119), and in a few cases those causing both. Abnormalities occur at various stages of reproductive development in *indica-japonica* hybrids (49, 79, 114), and male and female gamete abortions contribute almost equally to intersubspecific hybrid sterility (79).

HYBRID INCOMPATIBILITY GENES IDENTIFIED IN RICE

Major progress in molecular characterization of rice hybrid incompatibility genes in recent years has provided fresh data for understanding the cellular and molecular mechanisms of reproductive isolation.

The Paralogous *DPL1* and *DPL2* Causing Pollen Sterility in an *Indica-Japonica* Hybrid

A whole-genome survey of two-way interacting loci acting within the gametophyte or zygote was carried out in an F_2 population from an *indica-japonica* cross and detected one reproducible interaction on rice chromosomes 1 and 6 (**Table 1**) (60). Paralogous *DPL1* and *DPL2* genes were identified by positional cloning, which caused hybrid pollen incompatibility in the *indica-japonica* cross when the mutants of both loci occurred in the same gamete. The two *DPL* genes encode highly conserved plant-specific small proteins that are highly expressed in mature anther. The hybrid incompatibility was caused by independent disruptions of *DPL1* and *DPL2* in *indica* and *japonica*. In the *indica* rice variety Kasalath, the transcript of *DPL1-K*$^-$ had a 518-base-pair insertion of a transposable element containing diagnostic terminal inverted repeats in the predicted coding sequence; in the *japonica* variety Nipponbare, a mutation in *DPL2* resulted in a nonfunctional protein, DPL2-N$^-$. Because pollen germination requires at least one functional *DPL* copy, the pollen carrying both of the *DPL1-K*$^-$ and *DPL2-N*$^-$ loss-of-function alleles produced by the hybrid would not germinate, resulting in hybrid incompatibility (60).

Duplicated Loci *S27* and *S28* Causing Pollen Sterility in an Interspecific Hybrid

A genetic analysis revealed that an epistatic interaction between *S27* on chromosome 8 and *S28* on chromosome 4 can induce pollen sterility in the hybrid between *O. sativa* and its wild relative *O. glumaepatula* in a gametophytic manner (**Table 1**) (98). The *S27* copy is absent in *O. glumaepatula*, whereas the transcript of *S28-T65*^{s} fails to express in the rice variety of T65 in *O. sativa*. When *O. sativa* with *S27-T65*$^+$/*S28-T65*^{s} and *O. glumaepatula* with *S28-glum*$^+$/*S27-glum*^{s} hybridize, pollen carrying a set of nonfunctional *S27-glum*^{s} and *S28-T65*^{s} alleles would be sterile. However, either of the fertile alleles (*S27-T65*$^+$ or *S28-glum*$^+$) is able to rescue the sterile phenotype in hybrids. The duplicated *S27* and *S28* loci encode a mitochondrial ribosomal protein L27, which is localized in the mitochondria. It was inferred that mtRPL27 deficiency inhibits protein synthesis in mitochondria, which impairs its respiratory activity and thus induces sterile pollen (98).

Interaction of Two Adjacent Genes Leading to Hybrid Male Sterility

An incompatibility system comprising two adjacent genes in the *Sa* locus was elucidated

Table 1 Hybrid incompatibility genes causing postzygotic reproductive isolation in rice and other model organisms

Cross	Loci	Alleles	Gene and function	Hybrid phenotype	Genetics	Reference(s)
Parallel divergence model						
Oryza sativa ssp. *indica* × ssp. *japonica*	*DPL1* *DPL2*	*DPL1-N*$^{+}$/*DPL1-K*$^{-}$ *DPL2-K*$^{+}$/*DPL2-N*$^{-}$	Duplicated genes encoding plant-specific small proteins	Pollen germination failure	Loss-of-function mutations at the two loci produce abortive pollen One functional DPL is essential in pollen development	60
Oryza sativa × *Oryza glumaepatula*	*S27* *S28*	*S27-T65*$^{+}$/*S27-glum*^{s} *S28-glum*$^{+}$/*S28-T65*^{s}	Duplicated genes encoding mitochondrial ribosomal protein L27	Pollen sterility	Loss-of-function mutations at the two loci can cause pollen abortion One functional mtRPL27 is essential in pollen development	98
Arabidopsis thaliana Columbia-0 × Cape Verde Island accession Cvi-0	*HPA1* *HPA2*	*HPA1-Col/hpa1-Cvi* *hpa2-Col/HPA2-Cvi*	Duplicated genes encoding the histidinol-phosphate amino-transferase	Recessive embryo lethality	Recessive alleles at two loci in heterozygotes can cause incompatibility A transcript of either *HPA1* or *HPA2* is required for embryo development	4
Drosophila melanogaster × *Drosophila simulans*	*JYAlpha*	*JYAlpha* in chromosome 3 of *D. simulans* *JYAlpha* in chromosome 4 of *D. melanogaster*	Transposed genes encoding the catalytic subunit of an Na^{+}/K^{+} ATPase	Male sterility	Hybrids homozygous for chromosome 3 of *D. melanogaster* and chromosome 4 of *D. simulans* are male sterile One copy of *JYAlpha* is required for male fertility	57
Sequential divergence model						
Oryza sativa ssp. *indica* × ssp. *japonica*	*Sa SaF* *Sa SaM*	*SaF*$^{+}$/*SaF*$^{-}$ *SaM*$^{+}$/*SaM*$^{-}$	F-box protein Small ubiquitin-like modifier E3 ligase-like protein	Pollen sterility and preferential abortion of male gametes with *SaM*$^{-}$	*SaM*$^{+}$ and *SaF*$^{+}$ can selectively kill the male gametes with *SaM*$^{-}$	53
Drosophila simulans × *Drosophila melanogaster*	*Lhr* *Hmr*	*Lhr-sim/Lhr-mel* *Hmr-sim/Hmr-mel*	Leucine zipper–like structure MADF class of DNA-binding proteins	Hybrid male lethality	*Lhr-sim* and *Hmr-mel* can interact negatively to cause lethality in F_1 hybrid males	9

Mus musculus heterozygous *t*/+ males (males heterozygous for the *t* haplotype)	*Tcd1a*: *Tagap1* *Tcd2*: *Fgd2* *Tcr*: *Smok1*	$Tagap1^{Tcd1a}/Tagap1^{wild}$ $Fgd2^{Tcd2}/Fgd2^{wild}$ $Smok1^{Tcr}/Smok1^{wild}$	GTPase-activating protein Rho guanine nucleotide exchange factor Protein kinase Smok1	Males heterozygous for the *t* haplotype preferentially transmit the *t* chromosome to their offspring	The preferential inheritance of the *t* haplotype in heterozygous *t*/+ males is caused by interactions between *t*-complex distorters (Tcd) and responders (Tcr)	2, 3, 21
Caenorhabditis elegans Hawaii strain × Bristol strain	*zeel-1* *peel-1*	$zeel\text{-}1_{Hawaii}/zeel\text{-}1_{Bristol}$ $peel\text{-}1_{Hawaii}/peel\text{-}1_{Bristol}$	*zeel-1*: homology to the substrate-recognition subunit of a CUL-2-based E3 ubiquitin ligase complex *peel-1*: not clear	Embryonic lethality	The paternal-effect locus $peel\text{-}1_{Bristol}$ can cause lethality in the embryos homozygous for the zygotic $zeel\text{-}1_{Hawaii}$	77
Saccharomyces bayanus × *Saccharomyces cerevisiae*	*Aep2* *OLI1*	*SbAep2/ScAep2* *ScOLI1/SbOLI1*	Nuclear-encoded mitochondrial protein Mitochondrial gene encoding F0-ATP synthase subunit 9	Hybrid sterility	*SbAep2* and *ScOLI1* interact negatively, which results in hybrid sterility	43
Saccharomyces cerevisiae × *Saccharomyces bayanus* or *Saccharomyces paradoxus*	*MRS1* *COX1*	*ScMRS1/SpMRS1* *SpCOX1/ScCOX1*	Nuclear-encoded mitochondrial protein Mitochondrial gene encoding subunit I of cytochrome *c* oxidase	Hybrid lethality	Incompatibility occurs between *ScMRS1* and *SpCOX1*, which causes hybrid lethality	13
Parallel-sequential divergence model						
Oryza sativa ssp. *indica* × ssp. *japonica*	*S5 ORF3* *S5 ORF4* *S5 ORF5*	*ORF3+/ORF3−* *ORF4+/ORF4−* *ORF5+/ORF5−/ORF5n*	Heat shock protein Hsp70 Membrane protein Aspartic protease	Embryo-sac sterility and preferential abortion of female gametes with *ORF3−*	*ORF5+* in combination with *ORF4+* can selectively eliminate the female gametes without *ORF3+*	11, 105

as conditioning *indica-japonica* hybrid male sterility (**Table 1**) (53). The *Sa* locus was identified and mapped to a 30-kb region on chromosome 1 (108, 110, 120). The *Sa* locus was cloned using map-based cloning; this locus comprises two adjacent genes, *SaM* and *SaF*, encoding a small ubiquitin-like modifier E3 ligase-like protein and an F-box protein, respectively (53). Generally, the *indica* and *japonica* varieties carry the haplotype of SaM^+SaF^+ and SaM^-SaF^-, respectively. The SaM^- sequence has one nucleotide difference relative to SaM^+, which changes the 3′ splicing site of the corresponding fifth intron and results in a truncated protein. SaF^+ and SaF^- differ by only one nucleotide, which causes a Phe-to-Ser substitution in the predicted protein. Hybrid sterility is caused by selective abortion of pollen carrying SaM^- because of a selective negative interaction between SaF^+ and SaM^- in the hybrid (53). Such selective elimination of SaM^- pollen leads to semisterility of the hybrid and segregation distortion in the progeny.

A Killer–Protector System Involving Three Adjacent Genes Inducing Both Hybrid Female Sterility and Segregation Distortion

Recent studies on the *S5* locus in rice elucidated another system regulating *indica-japonica* hybrid sterility and segregation distortion in the progeny. The *S5* locus has a significant effect on embryo-sac fertility in *indica-japonica* hybrids and might be the most important locus for hybrid sterility between the *indica* and *japonica* subspecies, as demonstrated by a large number of studies using different crosses (**Table 1**) (11, 27, 30, 48, 50, 73, 91, 93, 102). This is probably due to the fact that an abortive embryo sac would set no seed in the flower, whereas abortion of a large portion of pollen may not affect seed setting.

The *S5* locus was first mapped on chromosome 6 by Ikehashi & Araki (27), using morphological markers. The presence and location of *S5* were confirmed by restriction fragment length polymorphism markers (48, 102) and quantitative trait locus analysis (50, 79, 91, 93). The *S5* region was further delimited into a 40-kb DNA fragment containing five open reading frames (*ORF1–5*) (73). Based on these results, Chen et al. (11) conducted a transformation of *ORF3*, *ORF4*, and *ORF5* from an *indica* variety individually into a *japonica* variety. The transformants carrying the *indica* allele of *ORF5* but not that of *ORF3* or *ORF4* showed reduced fertility owing to embryo-sac abortion, which identified *ORF5* encoding an aspartic protease as the candidate for the *S5* locus. Three types of *ORF5* alleles were observed. The *indica* allele (referred to as *ORF5+*) and *japonica* allele (referred to as *ORF5−*) differ by two nucleotides, both of which cause amino acid substitutions located in the central domain of the predicted protein. The allele from a WCV (referred to as *ORF5n*) has a 115-amino-acid deletion in the N terminus of the predicted protein, which contains a signal peptide plus an 87-amino-acid fragment of the central domain. The subcellular localization of the ORF5n protein was thus changed into the cytoplasm, whereas the ORF5+ and ORF5− proteins were detected in the cell wall (11).

Segregation distortion has been observed in progenies from *indica-japonica* crosses (35, 53, 91), which is difficult to explain by *ORF5* alone. Genetic analysis combined with sequence determination suggested that two additional genes, *ORF3* and *ORF4*, which were tightly linked with *ORF5*, were required for the *S5*-induced hybrid sterility and segregation distortion (105). The hybrid sterility and preferential abortion of female gametes are controlled by the complex interaction between a killer (composed of ORF5 and ORF4) and a protector (ORF3). *ORF3* and *ORF4* are predicted to encode a heat shock protein (Hsp70) and a membrane protein, respectively. The *ORF3* sequence in typical *japonica* varieties (referred to as *ORF3−*) has a 13-base-pair deletion relative to that in typical *indica* varieties (referred to as *ORF3+*), which results in a frameshift in the C terminus of the predicted protein. The *indica* allele of *ORF4* (referred to as *ORF4−*) has an 11-base-pair deletion compared with that in typical *japonica* varieties (referred to as *ORF4+*), which causes premature termination of the predicted protein and a loss of the putative transmembrane domain. Thus, the typical *indica*-like and *japonica*-like haplotypes contain the combination of *ORF3+ORF4−ORF5+* and *ORF3−ORF4+ORF5−*, respectively. The functional ORF5+ in combination with ORF4+ acts as a killer, which selectively kills the female gametes without the protector ORF3+. ORF3+ rescues the fertility of the *indica-japonica* hybrids by preventing the gametes from being killed, which leads to preferential transmission of female gametes with *ORF3+* and thus segregation distortion in the offspring (105). It is interesting that the typical *indica*-like and *japonica*-like haplotypes at the *S5* locus already existed in wild rice, which suggests predifferentiation of *indica* and *japonica* before domestication (16, 105).

Yang et al. (105) proposed that the extracellular ORF5+ produces a molecule that is sensed by the plasma membrane–localized ORF4+, which triggers endoplasmic reticulum (ER) stress in ovaries. The ER stress would subsequently induce premature programmed cell death (PCD) in the developing megaspores without *ORF3+*, resulting in embryo-sac abortion. ORF3+ would resolve the ER stress and prevent the premature PCD. This system may provide an extreme example of egoism in the context of selfish DNA (105).

EVOLUTIONARY GENETIC MODELS FOR HYBRID INCOMPATIBILITY SUGGESTED BY THE RICE DATA

Based on the results from rice studies summarized above, we suggest three evolutionary genetic models to depict the processes for installing the hybrid incompatibility systems, two of them involving two loci and one involving three loci.

Parallel Divergence Model

The essence of postzygotic reproductive isolation might be better understood from an evolutionary genetic perspective. In the parallel divergence model, we propose that an ancestral gene *A* would be duplicated at some point during genome evolution and evolve into *A′*. An ancestral population with a genotype of *AAA′A′* would split into two allopatric populations, in which the two loci *AA* and *A′A′* functionally evolve into *aa* and *a′a′* in their respective species (**Figure 1**). Although the two mutations may be neutral or beneficial in their own genetic backgrounds, deleterious interaction would occur when the parallelly diverged *a* and *a′* alleles are combined in the same genetic background, causing incompatibility and reduced fitness (**Figure 1**).

Sequential Divergence Model

In this model, the genotype of the ancestral species is retained in one lineage, and both of the causative mutations occur in another lineage (**Figure 2**). The mutations occur sequentially such that the process of reproductive isolation comprises two steps: First, *AA* mutates into *aa* in the genetic background of *BB*; after the emergence of *aa*, *BB* then changes into *bb*. Thus, when the ancestral species and diverged species hybridize, the *A* and *b* alleles might interact negatively and cause incompatibility in the hybrids (**Figure 2**).

Parallel-Sequential Divergence Model

When more than two loci are involved in the system, reproductive isolation might result from the evolution of genes that have undergone both parallel and sequential divergence (**Figure 3**). Suppose that the divergence between *AA* and *BB* is insufficient to induce hybrid malfunction, and that the *CC* locus would not mutate until the divergence of *AA* or *BB*. The initial species with the *AABBCC* genotype would therefore diverge into two allopatric populations, one carrying *AAbbCC* and one carrying *aaBBCC*, which are able to interbreed with each other. The *CC* locus then evolves into *cc* after the emergence of either *aa* or *bb*. Thus, incompatible interactions arise

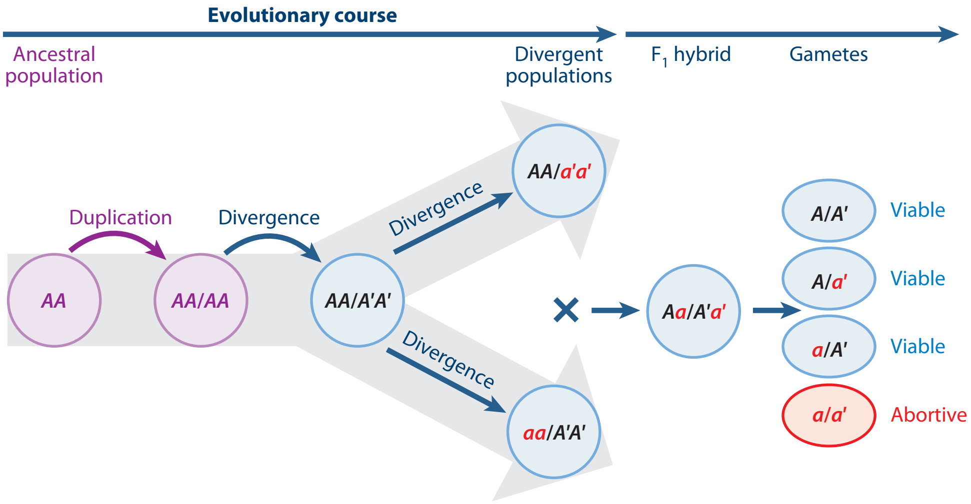

Figure 1

The parallel divergence model for the genetic architecture of the genes (duplicated loci, unlinked) involved in postzygotic reproductive isolation.

from the ancestral *A* in combination with *B* and the nascent *c*, resulting in incompatibility in the hybrids (**Figure 3**). The evolutionary genetics of reproductive isolation might be rather complex when more than two loci are involved, which generates more intermediate populations and genotypes during evolution.

The Commonalities and Differences of the Models

The three cases suggested reflect diversifying strategies in evolution. First, the mutations occur in parallel in two allopatric populations in the parallel divergence model (**Figure 1**) but emerge in only one lineage in the sequential

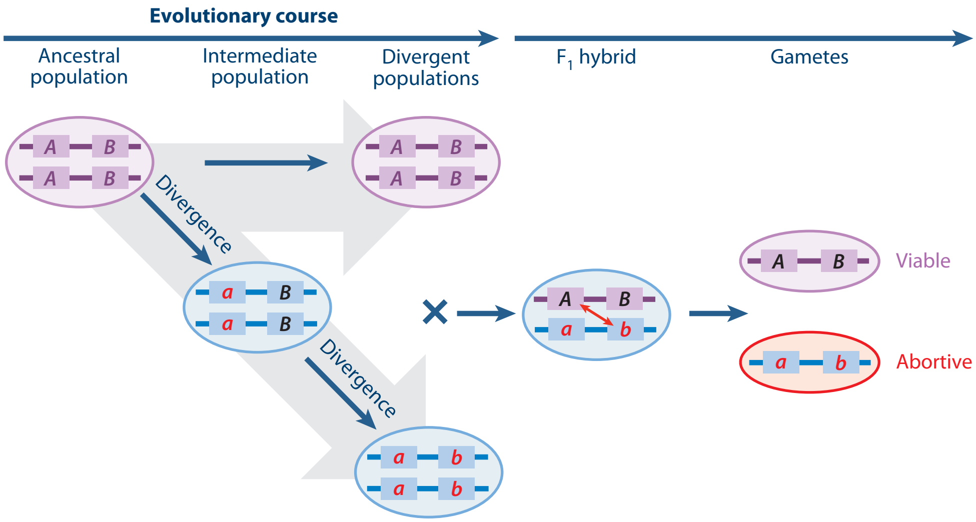

Figure 2

The sequential divergence model for the genetic architecture of the genes (tightly linked loci) involved in postzygotic reproductive isolation. The red arrow indicates the interaction between *A* and *b*.

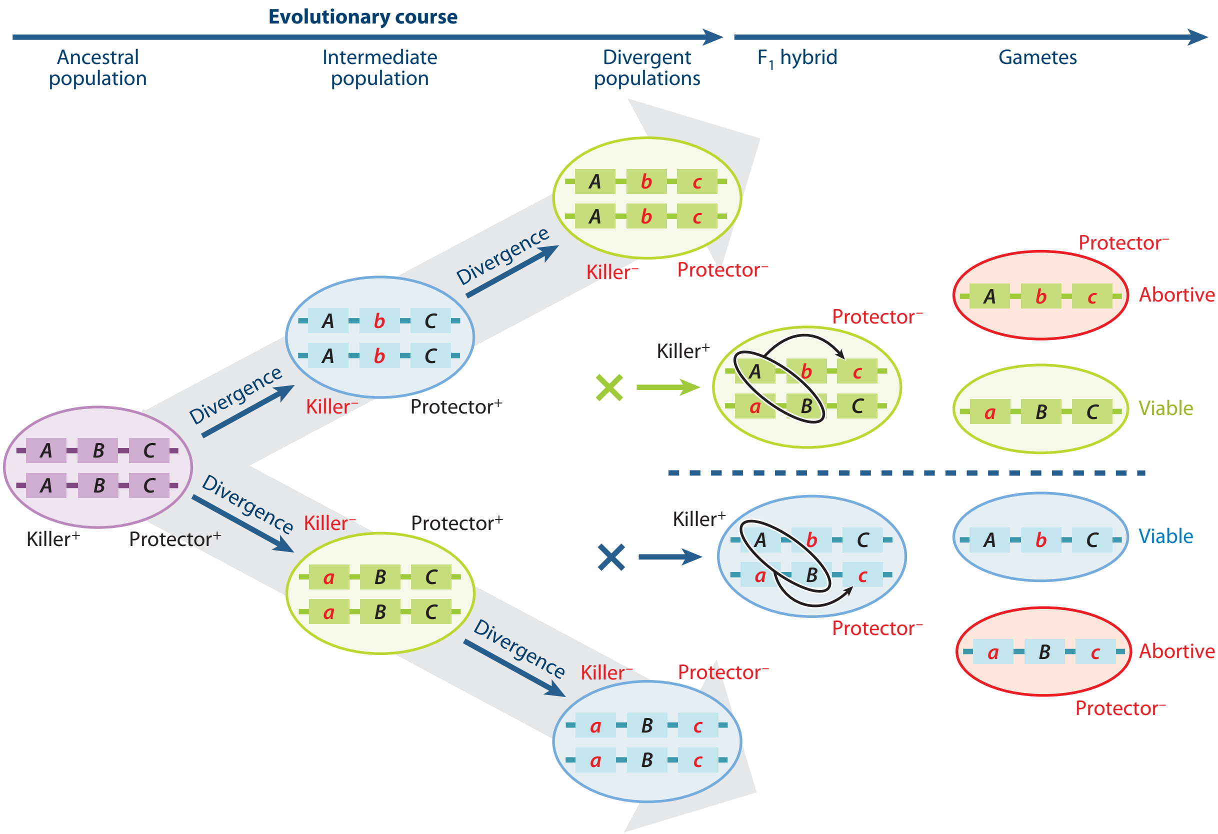

Figure 3

The parallel-sequential divergence model for the genetic architecture of three genes (tightly linked loci) involved in postzygotic reproductive isolation. Crosses are made between the genotypes with circles and backgrounds in the same color (*green AAbbcc* × *green aaBBCC* or *blue AAbbCC* × *blue aaBBcc*). The black arrows indicate the interactions between the killer^{+} and the protector^{-}.

divergence model (**Figure 2**). The parallel-sequential divergence model is more complex: *aa* and *cc* occur and spread in one lineage sequentially, and the similar evolutionary events—the sequential emergence of *bb* and *cc*—are likely to arise parallelly in another population (**Figure 3**).

Second, the fixation time of the two mutations in the parallel divergence model is of no significance. However, the sequence of the two mutations is critical in the sequential divergence model: The *bb* mutation does not occur until the emergence of *aa*. As in the case of the parallel-sequential divergence model, the divergence between *AA* and *BB* occurs stochastically, whereas the *cc* mutation occurs only in the genetic background of either *aa* or *bb* (**Figure 3**).

These three models are further distinguished by the targets on which the evolutionary forces act. In the parallel divergence model, the two derived alleles *aa* and *a′a′* never occur in the same population (**Figure 1**). Therefore, they might induce incompatibility when combined in a hybrid genetic background. Similarly, in the sequential divergence model, interactions between *AA* and *bb* may reduce fitness, and the detrimental combination would be eliminated by natural selection. Thus, the ancestral *AA* is never combined with the mutated *bb* in natural populations, whereas the *AA* and *BB*, *aa* and *BB*, and *aa* and *bb* combinations could occur in the same genetic background (**Figure 2**). The key to the parallel-sequential divergence model is that the combination of *AA* and *BB* never meets the nascent *cc* in natural populations (**Figure 3**). It is highly likely that the individuals carrying *AABBcc* are wiped out during evolution. Therefore, the hybrids are sterile or inviable when the ancestral *A*, *B*, and nascent *c* alleles exist in the same genetic background by hybridization (**Figure 3**).

Furthermore, the two interactive loci in the parallel divergence model evolve independently. In the sequential divergence model, reproductive isolation results from coevolved loci that diverge sequentially, and the emergence of *bb* depends on the divergence from *AA* to *aa*. In the parallel-sequential divergence model, *AA* and *BB* diverge independently, and the emergence of either *aa* or *bb* increases the probability of the emergence of *cc*. Thus one might infer that, in both cases, the ancestral loci are likely to be functionally related.

Placing Earlier Models for Hybrid Sterility in Rice in the Perspective of the Proposed Models

Two models were previously proposed to explain the genetic basis of hybrid sterility in rice. The first is a duplicate gametic-lethal model, which suggests that hybrid fertility in the cross is controlled by two loci: Gametes produced by the hybrid carrying the recessive alleles at both loci are aborted, whereas gametes with at least one dominant allele are fertile (64, 65). The second is the one-locus sporo-gametophytic interaction model, which assumes that hybrid sterility is controlled by a single locus (34). Negative interaction between *indica* and *japonica* alleles at this locus in the hybrid could cause gamete abortion, thus reducing fertility (34). Ikehashi & Araki (27) further developed this model through genetic analysis of a major hybrid sterility locus, *S5*. Based on this analysis, they proposed that there are three alleles at this locus: an *indica* allele (*S5*-i), a *japonica* allele (*S5*-j), and a neutral allele (*S5*-n) [referred to as a wide-compatibility gene (WCG)]. Sterility would occur in hybrids with an *S5*-i/*S5*-j genotype, but this fertility barrier could be overcome by crossing with the varieties carrying *S5*-n (27).

Although these two models seem to be controversial in explaining the genetic architecture of hybrid sterility in rice, they could be well placed into the perspective of the models proposed here. The duplicate gametic-lethal model proposed in rice fits well with the parallel divergence model, as the deleterious interaction occurs between the two mutated alleles in the hybrids (**Figure 1**). In this case, the gametes would need at least one ancestral allele in order to be fertile. The allelic interaction model for rice fits well with both the sequential divergence model (**Figure 2**) and the parallel-sequential divergence model (**Figure 3**) in the sense that interactions of tightly linked genes behave in an apparently single-locus manner. In both cases, the hybrids carrying the heterozygous *indica*/*japonica* genotype would be sterile. The two incompatible alleles from *indica* and *japonica* might correspond to the ancestral *AABB* and derived *aabb* genotypes, respectively, in the sequential divergence model. The deleterious interaction between the *A* and *b* alleles in hybrids might selectively kill the gametes carrying the *b* allele (**Figure 2**). In the parallel-sequential divergence model, the populations with *AAbbCC* and *aaBBcc* might be regarded as the typical *indica* and *japonica* populations, which would produce sterile hybrids when crossed with each other (**Figure 3**).

It should be noted that the parallel divergence model and sequential divergence model are in principle congruent with the Dobzhansky-Muller model of reproductive isolation in the sense that deleterious interactions occur between functionally diverged genes in the hybrids (15, 62). However, the killer–protector system of *S5*, suggesting a parallel-sequential divergence model, presents a scenario different from that expected in the Dobzhansky-Muller model. The gene combination of *A* and *B*, which would cause negative interactions in the hybrids, already existed in the ancestral population, although the effect was apparently not deleterious until the emergence of a loss-of-function mutation in the *C* locus.

MOLECULAR EVOLUTIONARY MECHANISMS ILLUSTRATED BY THE HYBRID INCOMPATIBILITY SYSTEMS

The Parallel Divergence Model Features Duplicated Functional Genes

The present understanding of reproductive isolation confirms the evolutionary corollary of parallel divergence. Reciprocal loss of duplicated genes contributes to reproductive barriers in rice, and such asymmetric resolutions of gene duplicates fit well with the parallel divergence model (**Figure 1**) (60, 98). First, a pair of paralogous genes in different rice populations have undergone divergent resolutions during evolution—i.e., one of the gene copies lost its function in one population, while the other copy retained its function in this population but did not work in another (54). Thus, different mutational events occurred in two allopatric populations. Second, the paralogous genes diverged independently, and the respective mutational events followed a stochastic probability. Therefore, in both cases, the populations carrying either of the functional copies developed properly, whereas in the hybrids, the products of genetic segregation and recombination gave rise to abortive gametes, thus reducing the fitness of the hybrids.

Known duplicated genes also contribute to reproductive barriers in crosses between strains of *Arabidopsis thaliana* (**Table 1**) (4). The functional copies of *HPA1* and *HPA2* are located at different loci in different accessions. Thus, the combination of two silenced copies in progeny would cause recessive embryo lethality and arrested seed development in the hybrids (4). Reciprocal gene loss might also contribute to multiple rounds of speciation in yeast (76). In this study, a whole-genome duplication event occurred in a shared ancestor of three yeast species. The subsequent losses of duplicated genes differed among the three species at 20% of all loci. The rapid divergence of the three yeast lineages occurred shortly after the whole-genome duplication, during a period of precipitous gene loss. The authors proposed that the reciprocal loss of alternative copies of duplicated genes would lead to reproductive isolation and eventually speciation through the parallel divergence mechanism.

Parallelly diverged loci inducing hybrid incompatibilities can also result from gene movement such as transposition. The original and the transposed loci in different lineages might be regarded as a pair of diverged loci, with each of the gene copies disappearing from their respective populations. Incompatibility would occur with the deficiency of both the original and the transposed loci in the hybrid. A good example of this case is *JYAlpha*, which transposed from chromosome 4 to chromosome 3 during the evolution of the *Drosophila simulans* lineage (**Table 1**) (57). *JYAlpha* gave rise to interspecific hybrid male sterility in *Drosophila*, whereas hybrids lacking both of the gene copies were sterile (57).

Continual occurrence of gene duplication-transposition events provides the supplies for such a reproductive barrier to emerge at a certain frequency. In addition, subfunctionalization and nonfunctionalization are thought to be fairly common fates of one copy of the duplicate genes (54), which also provide abundant sources for the induction of reproductive barriers. Therefore, parallelly diverged loci may represent an important source for driving reproductive isolation in a wide range of organisms.

A Precise Mechanism in Each of the Systems Conditioned by Sequentially Diverged Loci

Although the *Sa* system in rice seems quite complex in its mechanisms at the molecular level, the interaction and divergence of the two components in *Sa* comply with the simple sequential divergence strategy (**Figure 2**). It seems that the hybrid sterility genes in this case are gamete eliminators rather than genes involved in gamete development. *SaF*$^{+}$*SaM*$^{+}$ is proposed to be the haplotype in the ancestor of *Oryza* species (53), which might be regarded as having the *AABB* genotype in **Figure 2**. The intermediate haplotype of *SaF*$^{-}$*SaM*$^{+}$ (*aaBB*) might have acted as a buffer to avoid the elimination of *SaF*$^{-}$*SaM*$^{-}$ (*aabb*) when *SaM*$^{-}$ arose (53). Therefore, the divergence of the mutations is sequential, such that the mutation from *SaF*$^{+}$ to *SaF*$^{-}$ occurred before the emergence of *SaM*$^{-}$. In addition, both of the mutations in *SaM*$^{-}$ and *SaF*$^{-}$ occurred in the same lineage. Thus, the incompatibility is induced by the interaction between the ancestral *SaF*$^{+}$ and the derived *SaM*$^{-}$, which is quite different from the parallel divergence case in which reduced fitness is caused by two derived loci.

Studies in animals have proposed that hybrid incompatibility and segregation distortion are driven by similar systems, which represent a general mechanism to maintain the driving force of genetic differentiation (**Table 1**) (2, 3, 10, 21, 55, 59, 77, 83, 84, 95). In *Caenorhabditis elegans*, embryonic lethality was induced by deleterious interaction between *peel-1*$_{\text{Bristol}}$ (in the Bristol strain) and *zeel-1*$_{\text{Hawaii}}$ (in the Hawaii strain) in the same genetic background (77). The *zeel-1*$_{\text{Bristol}}$/*peel-1*$_{\text{Bristol}}$ haplotype gained a transmission advantage because embryos carrying homozygous *zeel-1*$_{\text{Hawaii}}$ were selectively arrested owing to the presence of *peel-1*$_{\text{Bristol}}$ (77). Transmission ratio distortion in mouse was caused by four *t-complex distorters* (*Tcd1–4*) and a single *t-complex responder* (*Tcr*) in heterozygous *t*/+ males, and sperms carrying *Tcr* were preferentially transmitted into the progeny (2, 3, 21). Incompatible genes in yeast have also provided evidence for the sequential divergence model (13). The mutations in *Saccharomyces cerevisiae MRS1* (*ScMRS1*) occurred after the changes in *ScCOX1*. Incompatibility thus occurs between *ScMRS1* and *Saccharomyces paradoxus COX1* (*SpCOX1*), which contributes to reproductive isolation between yeast species (13). Another pair of sequentially diverged genes in yeast suggest that *Saccharomyces bayanus Aep2* (*SbAep2*) and *ScOLI1* would cause F_2 hybrid sterility (43). Both *SbAep2* and *SbOLI1* are likely to diverge in the *S. bayanus* lineage, although which one mutated earlier remains to be investigated (43). The classical Dobzhansky-Muller *Hmr*/*Lhr* gene pair in *Drosophila* causes incompatibility in F_1 hybrid males (9). The same strategy has been adopted by different organisms during the establishment of reproductive barriers, which suggests that general mechanisms underlying reproductive isolation might exist across different taxa.

Complex Evolutionary Routes of the *S5* Killer–Protector System

The killer–protector system at *S5* expanded the scope of the mechanism for reproductive isolation by involving three genes in the system, which suggested a parallel-sequential divergence model. Whether such a system has generality in reproductive isolation remains to be investigated. It can be speculated that establishment of the system involved two major steps: parallel divergence between the two components of the killer, and sequential divergence of the protector following nonfunctional mutation(s) of the killer. The haplotype *ORF3+ORF4+ORF5+* (which might be regarded as *AABBCC*), representing a balance between killing and protecting of the gametes according to the genetic model in the *S5* system, is the most likely ancestral type (**Figure 3**) (105). The parallel divergence between *ORF4* and *ORF5* leads to the breakdown of the killer by mutations in *ORF4* and/or *ORF5*. Populations carrying *ORF3+ORF4−ORF5+* (which might be regarded as *AAbbCC*) and *ORF3+ORF4+ORF5−* (which might be regarded as *aaBBCC*) are able to produce fertile offspring when hybridized (**Figure 3**). However, the parallel divergence is critical for the establishment of reproductive isolation, because once the killer is nonfunctional, the protector is no longer required for the gametes to survive and thus is free to evolve. Therefore, the nonfunctional mutations in the protector would occur in the genetic background of a loss-of-function killer, resulting in sequential divergence of *ORF3* following the parallel divergence of *ORF4* and *ORF5*. Consequently, a large number of genotypes formed of various three-gene combinations could occur during evolution (**Figure 3**). Hybridization between populations carrying *ORF3+ORF4−ORF5+* (a typical *indica*-like haplotype, which might be regarded as *AAbbCC*) and *ORF3−ORF4+ORF5−* (a typical *japonica*-like haplotype, which might be regarded as *aaBBcc*) would lead to *indica-japonica* hybrid sterility owing to the deleterious interaction between ORF5+ and ORF4+ without protection by ORF3 (ORF3−).

Tight Linkage of the Killer and Protector Genes

Natural selection favors the formation of closely linked killer and protector genes, as recombination between them is likely to induce the suicidal killer/nonprotector haplotype in a hybrid and thus results in a breakdown of the system (10). For instance, the two genes coding for the killer and one gene for the protector at the *S5* locus exist as physically adjacent genes (105). Similar situations are found in systems described under the sequential divergence model, such as the two adjacent genes of *SaM* and *SaF* at the *Sa* locus (53) and the tightly linked *zeel-1* and *peel-1* in *C. elegans* (77). In other cases, the killer might be near a centromere that was selected by reduced recombination during evolution (10). Similarly, inversions that block recombination between the components of the gamete-eliminator system are also likely to be favored by selection (10). Burt & Trivers (10) proposed that such tight linkage of the killer and protector facilitates the spread of the killer, while the recombination acts as a filter for the evolution of the gamete-eliminator system.

Hybrid Incompatibility Gene Pairs Are Likely Involved in the Same Pathways

In the parallel divergence model, gene pairs involved in reproductive isolation favor duplicated or transposed loci generated by genome evolution. Thus, the genes in such hybrid incompatibility systems are likely to have analogous (or redundant) functions, although divergent populations have retained different functional copies after divergence.

Sequentially diverged loci might be involved in the same biological pathway, as illustrated by the *Sa* system in rice. In mouse, the *Tcd* genes function as signaling molecules acting upstream of the responder gene *Tcr* (2, 3, 21). In yeast, *MRS1* is required for the splicing of specific introns in *COX1* (13); functional changes in *MRS1* are therefore a result of coevolution with changes in the *COX1* introns within species, and incompatibility occurs between pairs that are not coevolved in different species (13). Similarly, *SbOLI1* and its translation regulator SbAep2 have evolved during adaptation to nonfermentable carbon sources. SbAep2 was thus unable to regulate the translation of *ScOLI1* mRNA, which resulted in hybrid incompatibility (43).

In the parallel-sequential divergence model, the three components of the *S5* killer–protector system are involved in different stages of the ER stress–induced PCD pathway (105). The interaction between ORF4 and ORF5 triggers the signal to induce the ER stress pathway, and the protector ORF3 responds to the ER stress, which turns the switch for the premature PCD pathway downstream on or off, ultimately resulting in embryo-sac abortion (105). The three genes work together in the same pathway without direct physical interactions but with a significant amount of signal transduction and communication. The function of the protector depends on its corresponding killer, as the coevolution between the killer and protector within the population determines the fate of the gametes. After the killer diverges, selection pushes the protector to evolve, maintaining a balance within the organism. Consequently, the divergence that occurs in the protector is also in accordance with the mutation in the killer gene. When a nonkiller emerges, the nonprotector could arise in a relaxed background, whereas incompatibility occurs between the diverged pairs in different populations.

Thus, the genes in the hybrid incompatibility system are likely to be involved in the same pathways and to coevolve. In addition, the divergences of these interaction genes do not occur independently of one another, and the evolution of one locus is expected to be conditional on the evolution of another.

Loss-of-Function Mutations in Interactive Gene Pairs Contribute Significantly to Incompatibility

Reproductive isolation is induced by the continuous accumulation of incompatibilities, which act as a by-product of the speciation genes (14). The establishment of reproductive barriers or speciation involves the divergence of multiple loci between closely related populations. A large number of mutations that contribute to reproductive barriers appear to be loss-of-function mutations that emerged and became fixed at random during evolution.

In the parallel divergence model, genes that have significant effects on fertility or viability might undergo duplication events in ancestral species. Subsequent loss-of-function mutations might occur randomly in different copies of these essential genes. The mutations also occur at different levels, which might be caused by the deficiency of the duplicated copy, the failure of expression, or the absence of the protein (4, 60, 98). Thus, paralogs that have been reciprocally silenced or lost would result in a lack of both of the gene copies in the gametes produced by the hybrids, which would display hybrid incompatibility and reproductive isolation. The large numbers of duplication or transposition events therefore provide gene sources for reproductive barriers.

Loss-of-function mutations also occur frequently in the sequential divergence model. The *zeel-1* locus was identified as deleted in the *C. elegans* Hawaii strain, which confirmed a loss-of-function event in the divergence process of *peel/zeel* gene pairs (77). One might infer that wild-type alleles of $Tagap1^{wild}$ and $Fgd2^{wild}$ in mouse represent the loss-of-function alleles compared with the $Tagap1^{Tcd1a}$ and $Fgd2^{Tcd2}$ type (2, 3, 21). The *SbAep2* loss-of-function allele fails to translate the *ScOLI1* mRNA (43), whereas *ScMRS1* loses the ability to splice the intron of *SpCOX1* (13). The emergence of these loss-of-function mutations offers possible opportunities to cause incompatibility in hybrids.

The establishment of reproductive isolation induced by the parallel-sequential divergence model comprises two key steps. The loss-of-function mutations in the killer component provide a chance to break down the balanced system within the population, and the nonfunctional mutation in the protector would arise only in the genetic background with a loss-of-function killer.

The divergence process does not stop after loss-of-function mutations occur. Divergence of related genes might be accelerated by natural selection and ultimately lead to reproductive isolation after the accumulation of a series of genetic incompatibilities. The occurrence of reproductive isolation would therefore be irreversible, causing speciation between divergent populations.

MOLECULAR DIVERSITY OF THE GENES INVOLVED IN REPRODUCTIVE ISOLATION SYSTEMS CONFORMING TO EACH OF THE MODELS

Highly Diverse Molecular Functions of Genes Involved in Incompatibility Systems

The duplication and subsequent parallel divergence of the gene pairs have contributed to reproductive isolation in a wide spectrum of organisms. The independent transmission provided a chance for the mutated forms to meet in the hybrid and then segregate into the gametes, causing negative interaction. These loci are physically unlinked, which allows free recombination and independent segregation in the gametes. Despite the conformity in genetics, the duplicated gene pairs involved in reproductive isolation systems are highly diverse (**Table 1**). DPL1 and DPL2 in rice are plant-specific small proteins (60), whereas *S27* and *S28* encode mitochondrial proteins, which are conserved among both prokaryotes and eukaryotes (98). The duplicated paralogs that result in intraspecific genetic incompatibilities within *A. thaliana* encode the histidinol-phosphate aminotransferase, which catalyzes an important step in the biosynthetic pathway leading to histidine (4). The transposed *JYAlpha* associated with the sterility of hybrids between two *Drosophila* species encodes a transmembrane protein, which appears to be the alpha subunit of Na^+ and K^+ ATPase (57). The whole-genome duplication and divergent resolution of duplicated genes in yeasts resulted in reciprocal gene-loss loci with diverse functions, which are likely involved in conserved biological processes (76).

The sequentially diverged *SaM* and *SaF* in rice encode a small ubiquitin-like modifier E3 ligase-like protein that is unique in rice and an F-box protein carrying a plant-specific F-box protein domain, respectively (53). The *zeel-1* locus also belongs to a lineage-specific gene family in *Caenorhabditis* with homology to *zyg-11*, the substrate-recognition subunit of a CUL-2-based E3 ubiquitin ligase complex (77). Molecular dissection suggests that the responder gene of *Tcr* encodes a dominant-negative form of the protein kinase Smok1, whereas *Tcd1a* and *Tcd2* encode a Tagap1 GTPase-activating protein and a Rho guanine nucleotide exchange factor, respectively (2, 3, 21). It should be noticed that the functions of incompatibility genes in the sequential divergence model also involve mitochondrial proteins (13, 43). The nuclear-encoded Aep2 works with the mitochondrial *OLI1* gene, which encodes F0-ATP synthase subunit 9 (43). Another case identified in yeast suggests that a nuclear gene product of *Mrs1* is required for intron splicing of the mitochondrial *COX1*, which encodes subunit I of cytochrome *c* oxidase (13). Such nuclear-mitochondrial conflict reflects a functional bias toward genes for generating hybrid incompatibility.

There has been only one case identified for the parallel-sequential divergence model, which comprises a killer–protector system conditioning intersubspecific hybrid female sterility in rice (105). The protector encoding the heat shock protein Hsp70 counterbalances the harmful effect of the killer, which comprises an aspartic protease in combination with a membrane protein (105).

In conclusion, genes involved in reproductive isolation systems are highly diverse in their biological functions, ranging from enzymes such as proteases, transferases, ATPases, kinases, synthases, and oxidases to structural proteins and transcription factors. It is also notable that these genes are either lineage specific or conserved among both prokaryotes and eukaryotes, encode either mitochondrial or nuclear proteins, and have distinct cellular locations in the cell.

Essential Versus Selfish Genes

Incompatible interactions induced by parallelly diverged loci could occur at any developmental stage, depending on the primary function of the original copy of the gene. The ancestral gene must play an essential role in the life of the organism before duplication, i.e., development of the gametes, viability of the organisms, or other critical pathways. A functional bias in genome-wide reciprocal gene-loss loci in yeasts was found that favored those involved in fundamental processes, thus increasing the potential contribution to reproductive isolation (76). Therefore, deficiency in both of the functional copies impairs the organism's survival, and the phenotype of the incompatibilities in hybrids depends largely on the function of the genes.

However, in some cases the genes for hybrid incompatibility do not seem essential for the organisms. When this happens, the hybrid incompatibility genes act as selfish genetic elements only for their own good. For example, in the case of *S5*, mutations in any of the three genes, individually or in combination, do not seem to affect the growth and development of the plants. However, there is a selective advantage to the killer, which leads to segregation distortion in its favor in a heterozygous genetic background. Conflict can thus be induced by these selfish genetic elements, which results in a cost to the protector. The gametes carrying *ORF3–* are selectively eliminated, leading to preferential transmission of those carrying *ORF3+* (105). Moreover, *ORF4+* in *japonica* varieties may constitute a functional killer in hybrids with the *indica*-derived *ORF5+*, which could kill the gametes carrying the *japonica* haplotype, therefore running the risk of causing hybrid sterility and losing itself in the offspring (105). In other words, *ORF4+* in *japonica* varieties increases the probability of killing itself. One might infer that such a nonegoistical existence of *ORF4+* and the maintenance of the killer–protector system must be highly dependent on geographical isolation. In other cases, the *indica* type of $SaM^{+}SaF^{+}$ could be transmitted to the progeny more frequently than the expected 50%, at the expense of SaM^{-} (53). In addition, either the Bristol haplotype of $zeel\text{-}1_{\text{Bristol}}$/$peel\text{-}1_{\text{Bristol}}$ (77) or the sperms carrying $Smok1^{Tcr}$ gain a transmission advantage (2, 3, 21). Therefore, the selfish elements can spread in the populations regardless of the reduced fertility or viability of the offspring. All this molecular evidence supports the idea that genetic conflict might be the evolutionary force driving gene pairs involved in the sequential divergence and parallel-sequential divergence models.

MECHANISMS AND IMPLICATIONS OF WIDE COMPATIBILITY IN CROP BREEDING

Reduced fitness in a heterozygote can be rescued by a specific combination of alleles, or WCGs, at the hybrid incompatibility loci. Individuals carrying one or more WCGs can produce fertile offspring when crossed with individuals carrying either of the incompatible alleles. The molecular mechanisms of WCGs take diverse forms owing to the divergent strategies in reproductive barriers.

In the case of parallel divergence, varieties carrying functional alleles at both loci could compensate for the abnormal phenotypes caused by the deficiency of the essential copy. The WCG might therefore be regarded as an ancestral type comprising both functional copies, which could rescue the hybrid incompatibility caused by deficiency of the functional loci (**Figure 4*a***). Sequencing and expression analysis in *DPL1* and *DPL2* suggested that 16 of the accessions investigated have functional alleles at both loci (60). These 16 varieties might be regarded as the ancestral populations and be considered WCVs at *DPL* loci. However, the WCVs in this model involve loci located in distinct positions or in different chromosomes. This would cause difficulty in utilizing such WCGs in rice breeding.

In the sequential divergence model, the ancestral *A* allele was proposed to interact negatively with the derived *b* allele, thus causing hybrid sterility. Therefore, the WCG should be the intermediate type of *aaBB*, which facilitates hybridization with varieties carrying the other two types (*AABB* and *aabb*) (**Figure 4*b***). Such WCVs seem to act as an intermediate buffer between the ancestral populations and the nascent ones (**Figure 4*b***). The $SaM^{+}SaF^{-}$ WCG has been identified at the *Sa* locus and is compatible with both $SaM^{-}SaF^{-}$ (a typical *japonica* haplotype) and $SaM^{+}SaF^{+}$ (a typical *indica* haplotype) owing to the absence of SaF^{+} or SaM^{-} (53). Because of the tight linkage between the two genes in this system, such WCGs would be practically useful for breaking the fertility barrier of intersubspecific hybrids. Interestingly, similar wide-compatibility haplotypes also exist in animals. In *C. elegans*, a doubly compatible wild strain has been identified that shows no lethality in crosses with either the Bristol or Hawaii strain (77). This compatible strain carries a Bristol-like allele of *zeel-1* that is functional in antidote activity; it might also carry a Hawaii-like allele of *peel-1* that fails to induce lethality in crosses with the Hawaii strain (77). This evidence suggests that WCGs may occur in all organisms.

For the killer–protector system as illustrated by *S5*, WCGs are predicted to be the haplotypes

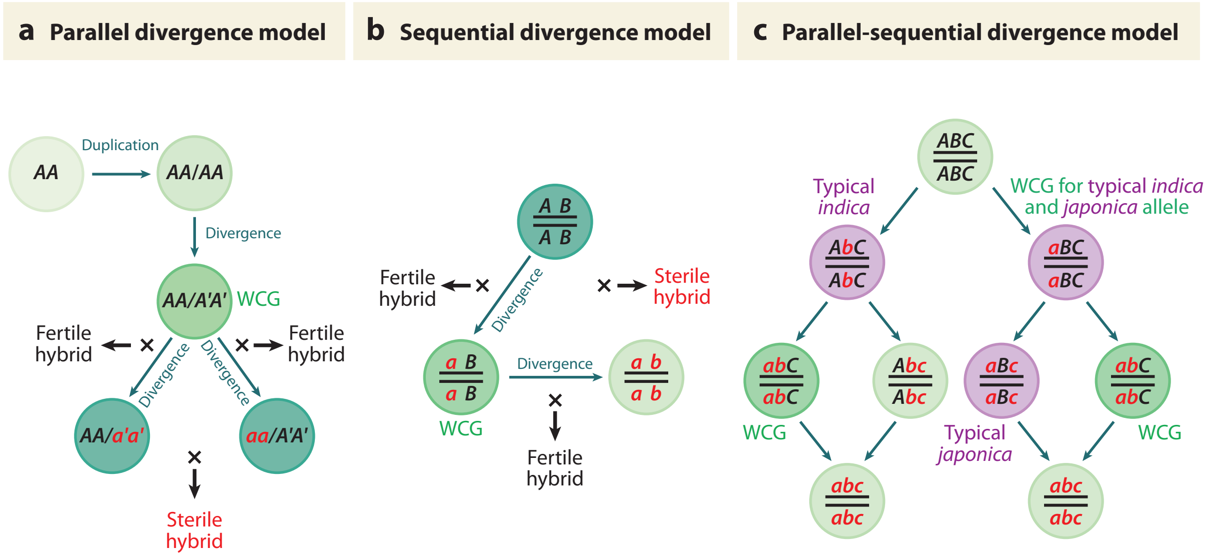

Figure 4

Generation of wide-compatibility genes (WCGs) during evolution and the fertility of their hybrids with other genotypes in (*a*) the parallel divergence model, (*b*) the sequential divergence model, and (*c*) the parallel-sequential divergence model.

with a functional *ORF3* protector and nonfunctional *ORF4* and *ORF5*: *ORF3+ORF4−ORF5−* and *ORF3+ORF4−ORF5n*, which would not induce negative interactions in crosses with any genotypes (**Figure 4*c***). The remaining genotypes would produce hybrids with reduced compatibility if crossed to one or more of the other genotypes. These two WCG haplotypes could occur in both lineages (corresponding to *indica* and *japonica*) as the intermediate products of the parallel-sequential evolution. For breeding applications, however, haplotypes *ORF3+ORF4+ORF5−* and *ORF3+ORF4+ORF5n* would also be compatible with typical *indica* and *japonica* rice varieties and thus may also be regarded as WCGs. An interesting consequence of such divergent evolution is that the projected intermediate and even end products may be highly similar or essentially the same with respect to their functionality in the two lineages (**Figure 4*c***). This may eventually cause a breakdown of the reproductive barrier unless there is a very strong selective force to maintain it.

The coexistence of genetic differentiation by reproductive isolation mechanisms and coherence by WCGs suggests a dynamic process of reproductive isolation and speciation. Hybrid incompatibility genes may promote genetic differentiation and maintain population distinctions during evolution, whereas WCGs that enable gene flow and hybridization provide a suppression force for genetic isolation and speciation. Thus, the different strategies for inducing WCGs also reflect the complex regulation and feedback mechanisms involved in reproductive isolation and speciation.

FUTURE PERSPECTIVES

The reproductive barriers and hence their genes may function cumulatively in hybrids, which further complicates the process of reproductive isolation. Recent molecular characterizations of hybrid sterility genes in rice have helped provide insight into this process. Although the genetic basis of reproductive isolation seems simple by classical genetic analysis, the molecular mechanisms underlying each of the loci are complex. Reproductive isolation begins with somewhat incidental mutations, and speciation will inevitably occur once the process proceeds by the accumulated effects of multiple diverged genes.

A mechanistic understanding of incompatibilities will therefore help to predict the candidates for speciation genes and to reveal the molecular pathways that regulate the phenotype. Our present understanding may enable the prediction of hybrid incompatibility genes. First, genes under rapid evolution undergo drastic sequence divergence between closely related populations. These genes might be more prone to generating incompatibilities when placed in the genetic background of hybrids. Second, loss-of-function mutations and copy-number variations are common strategies adopted by many organisms during evolution and frequently contribute to reproductive barriers. Third, genes causing hybrid sterility are likely responsible for some pathways in reproduction development. In rice, approximately 50 loci for hybrid sterility have been genetically identified, and the four cases of cloned genes characterized so far have already revealed three fundamentally different strategies for reproductive isolation. Diverse mechanisms will likely be revealed as genes at more loci and their underlying mechanisms are unveiled. Further cloning of genes involved in hybrid sterility or other forms of hybrid abnormalities may lead to the identification of different molecular mechanisms and evolutionary strategies of reproductive isolation.

The hybrid sterility genes contribute significantly to maintaining the distinctions between the *indica* and *japonica* subspecies. The classification of these two rice groups using traditional criteria can be confusing, because the criteria used are not clear cut and intermediate types exist with respect to genomic composition (22, 23). The best criterion should probably be hybrid fertility based on test crosses, which may conform to the definition of subspecies. Therefore, an understanding of rice hybrid sterility may provide reference systems for classifying *indica* and *japonica*. This information may also provide guidance in hybridization for rice breeding.

Intersubspecific *indica-japonica* hybrids show strong heterosis, which has displayed great promise for further increasing rice yield potential. Utilization of intersubspecific heterosis has been proposed as an important strategy for rice breeding (26, 28, 107). The hybrid sterility or reproductive isolation discussed here has impeded the exploration of heterosis in breeding programs. Thus, accurate prediction of potential WCVs may greatly facilitate such breeding programs. At least three strategies can be used to break the reproductive barrier between *indica* and *japonica*. In the first strategy, WCGs can be utilized for fertility restoration through their incorporation into the elite breeding lines whose hybrids show high-yield heterosis. The second strategy may involve the development of *indica*-compatible *japonica* lines by using a backcrossing approach to introduce *indica* alleles into *japonica* backgrounds such that crossing the resulting lines with *indica* varieties produces high-fertility *indica-japonica* hybrids (109), or vice versa. In the third strategy, suppressing the expression of hybrid sterility genes using RNA interference, microRNA, or gene-editing technology might provide genetic materials to overcome hybrid sterility. A combination of these strategies and the development of cultivars harboring more than one WCG may provide better assurance of hybrid fertility. Zhang (111) has proposed a new goal, referred to as Green Super Rice, for global rice genetic improvement for sustainable rice production. Increasing yield potential through *indica-japonica* hybridization would contribute to this goal.

DISCLOSURE STATEMENT

A patent was filed based on work on *S5* by the authors.

ACKNOWLEDGMENTS

This work was supported by grants from the National Natural Science Foundation (31130032, 30921091) and the 863 Project (2012AA100103). We apologize to those colleagues whose work could not be cited because of space limitations.

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Volume 64, 2013

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