

Understanding Reproductive Isolation Based on the Rice Model

Yidan Ouyang and Qifa Zhang

National Key Laboratory of Crop Genetic Improvement, National Center of Plant Gene Research (Wuhan), Huazhong Agricultural University, Wuhan 430070, China;
email: qifazh@mail.hzau.edu.cn

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

Copyright © 2013 by Annual Reviews.
All rights reserved

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.....	112	Perspective of the Proposed Models.....	122
REPRODUCTIVE ISOLATION IN PLANTS.....	113	MOLECULAR EVOLUTIONARY MECHANISMS ILLUSTRATED BY THE HYBRID INCOMPATIBILITY SYSTEMS.....	123
Prezygotic Reproductive Isolation.....	113	The Parallel Divergence Model Features Duplicated Functional Genes.....	123
Postzygotic Reproductive Isolation.....	113	A Precise Mechanism in Each of the Systems Conditioned by Sequentially Diverged Loci....	123
REPRODUCTIVE BARRIERS IN RICE.....	114	Complex Evolutionary Routes of the <i>S5</i> Killer–Protector System.....	124
HYBRID INCOMPATIBILITY GENES IDENTIFIED IN RICE.....	115	Tight Linkage of the Killer and Protector Genes.....	125
The Paralogous <i>DPL1</i> and <i>DPL2</i> Causing Pollen Sterility in an <i>Indica-Japonica</i> Hybrid.....	115	Hybrid Incompatibility Gene Pairs Are Likely Involved in the Same Pathways.....	125
Duplicated Loci <i>S27</i> and <i>S28</i> Causing Pollen Sterility in an Interspecific Hybrid.....	115	Loss-of-Function Mutations in Interactive Gene Pairs Contribute Significantly to Incompatibility.....	126
Interaction of Two Adjacent Genes Leading to Hybrid Male Sterility.....	115	MOLECULAR DIVERSITY OF THE GENES INVOLVED IN REPRODUCTIVE ISOLATION SYSTEMS CONFORMING TO EACH OF THE MODELS.....	126
A Killer–Protector System Involving Three Adjacent Genes Inducing Both Hybrid Female Sterility and Segregation Distortion.....	118	Highly Diverse Molecular Functions of Genes Involved in Incompatibility Systems.....	126
EVOLUTIONARY GENETIC MODELS FOR HYBRID INCOMPATIBILITY SUGGESTED BY THE RICE DATA.....	119	Essential Versus Selfish Genes.....	127
Parallel Divergence Model.....	119	MECHANISMS AND IMPLICATIONS OF WIDE COMPATIBILITY IN CROP BREEDING.....	128
Sequential Divergence Model....	119	FUTURE PERSPECTIVES.....	129
Parallel-Sequential Divergence Model.....	119		
The Commonalities and Differences of the Models.....	120		
Placing Earlier Models for Hybrid Sterility in Rice in the			

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 *bsien* 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 interspecific 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 interspecific hybridization also encountered difficulties in pollen tube growth after pollination. These difficulties resulted in large differences in fertilization rate between intra- and interspecific hybrids, suggesting a different level of affinity between pollen and stigmas in intra- and interspecific pollinations. A subsequent study yielded similar results, with the affinity between pollen and stigmas much lower in interspecific 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						
<i>Oryza sativa</i> ssp. <i>indica</i> × ssp. <i>japonica</i>	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
<i>Oryza sativa</i> × <i>Oryza glumaepatula</i>	S27 S28	S27-T65 ⁺ /S27- <i>glum5</i> ⁸ S28- <i>glum1</i> ⁺ /S28-T65 ⁸	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
<i>Arabidopsis thaliana</i> Columbia-0 × Cape Verde Island accession Cvi-0	HPA1 HPA2	HPA1-Coll/ <i>hpa1</i> -Cvi <i>hpa2</i> -Coll/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
<i>Drosophila melanogaster</i> × <i>Drosophila simulans</i>	<i>Y</i> Alpha	<i>Y</i> Alpha in chromosome 3 of <i>D. simulans</i> <i>Y</i> Alpha in chromosome 4 of <i>D. melanogaster</i>	Transposed genes encoding the catalytic subunit of an Na ⁺ /K ⁺ ATPase	Male sterility	Hybrids homozygous for chromosome 3 of <i>D. melanogaster</i> and chromosome 4 of <i>D. simulans</i> are male sterile One copy of <i>Y</i> Alpha is required for male fertility	57
Sequential divergence model						
<i>Oryza sativa</i> ssp. <i>indica</i> × ssp. <i>japonica</i>	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
<i>Drosophila simulans</i> × <i>Drosophila melanogaster</i>	<i>Lbr</i> <i>Hmr</i>	<i>Lbr-sim</i> / <i>Lbr-mel</i> <i>Hmr-sim</i> / <i>Hmr-mel</i>	Leucine zipper-like structure MADF class of DNA-binding proteins	Hybrid male lethality	<i>Lbr-sim</i> and <i>Hmr-mel</i> can interact negatively to cause lethality in F ₁ hybrid males	9

<i>Mus musculus</i> heterozygous <i>t/+</i> males (males heterozygous for the <i>t</i> haplotype)	<i>Tcd1a</i> : <i>Tagap1</i> <i>Tcd2</i> ; <i>Fgd2</i>	<i>Tagap1^{Tcd1a}/Tagap1^{wild}</i> <i>Fgd2^{Tcd2}/Fgd2^{wild}</i>	GTPase-activating protein Rho guanine nucleotide exchange factor Protein kinase Smok1	Males heterozygous for the <i>t</i> haplotype preferentially transmit the <i>t</i> chromosome to their offspring	The preferential inheritance of the <i>t</i> haplotype in heterozygous <i>t/+</i> males is caused by interactions between <i>t</i> -complex distorters (<i>Tcd</i>) and responders (<i>Tcr</i>)	2, 3, 21
<i>Caenorhabditis</i> <i>elegans</i> Hawaii strain × Bristol strain	<i>Ter</i> : <i>Smok1</i> <i>zeel-1</i>	<i>Smok1^{Ter}/Smok1^{wild}</i> <i>zeel-1^{Hawaii}/zeel-1^{Bristol}</i>	<i>zeel-1</i> : homology to the substrate- recognition subunit of a CUL-2-based E3 ubiquitin ligase complex <i>peel-1</i> : not clear	Embryonic lethality	The paternal-effect locus <i>peel-1^{Bristol}</i> can cause lethality in the embryos homozygous for the zygotic <i>zeel-1^{Hawaii}</i>	77
<i>Saccharomyces</i> <i>bayanus</i> × <i>Saccharomyces</i> <i>cerevisiae</i>	<i>peel-1</i> <i>Aep2</i> <i>OLL1</i>	<i>peel-1^{Hawaii}/peel-1^{Bristol}</i> <i>SbAep2/ScAep2</i> <i>ScOLL1/SbOLL1</i>	Nuclear-encoded mitochondrial protein Mitochondrial gene encoding F0-ATP synthase subunit 9	Hybrid sterility	<i>SbAep2</i> and <i>ScOLL1</i> interact negatively, which results in hybrid sterility	43
<i>Saccharomyces</i> <i>cerevisiae</i> × <i>Saccharomyces</i> <i>bayanus</i> or <i>Saccharomyces</i> <i>paradoxus</i>	<i>MRS1</i> <i>COX1</i>	<i>ScMRS1/SpMRS1</i> <i>SpCOX1/ScCOX1</i>	Nuclear-encoded mitochondrial protein Mitochondrial gene encoding subunit I of cytochrome <i>c</i> oxidase	Hybrid lethality	Incompatibility occurs between <i>ScMRS1</i> and <i>SpCOX1</i> , which causes hybrid lethality	13
Parallel-sequential divergence model						
<i>Oryza sativa</i> ssp. <i>indica</i> × ssp. <i>japonica</i>	<i>S5 ORF3</i>	<i>ORF3+/ORF3-</i>	Heat shock protein Hsp70	Embryo-sac sterility and preferential abortion of female gametes with <i>ORF3-</i>	<i>ORF3+</i> in combination with <i>ORF4+</i> can selectively eliminate the female gametes without <i>ORF3+</i>	11, 105
	<i>S5 ORF4</i>	<i>ORF4+/ORF4-</i>	Membrane protein			
	<i>S5 ORF5</i>	<i>ORF5+/ORF5-/ORF5m</i>	Aspartic protease			

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

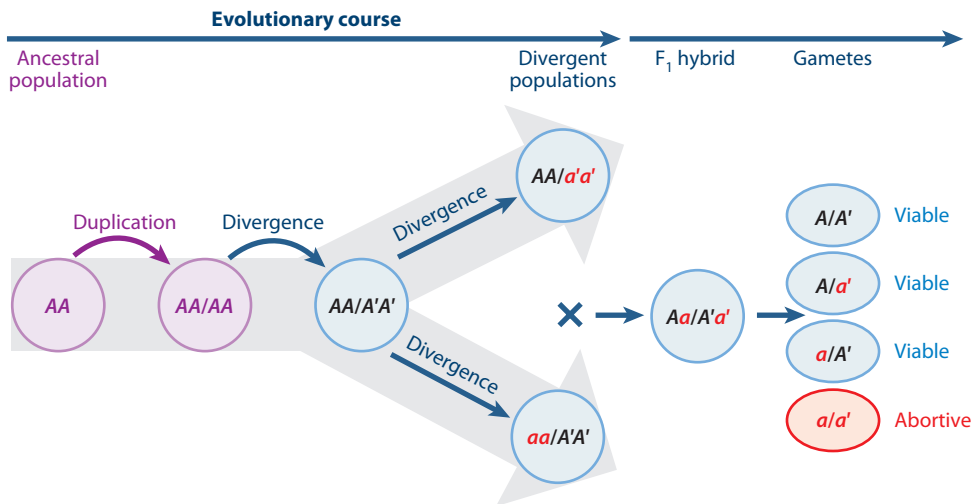


Figure 1

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

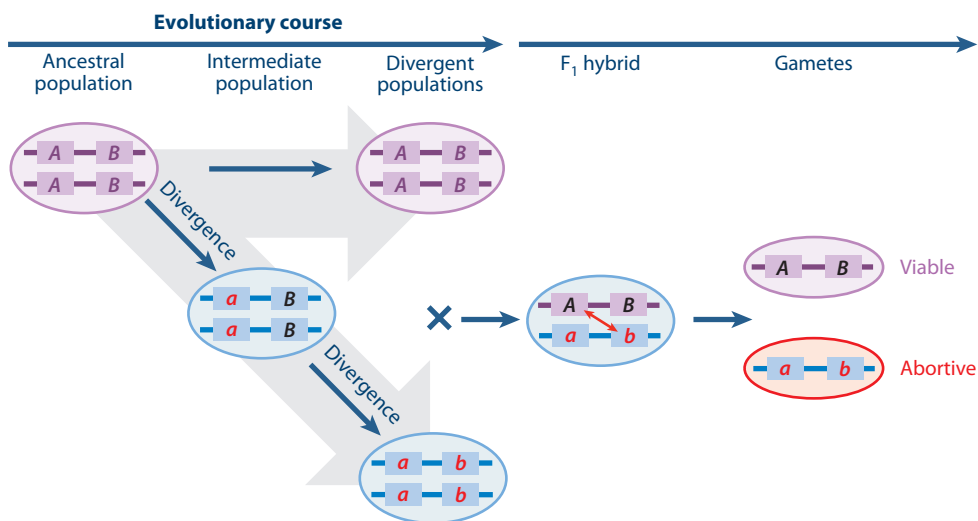


Figure 2

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*.

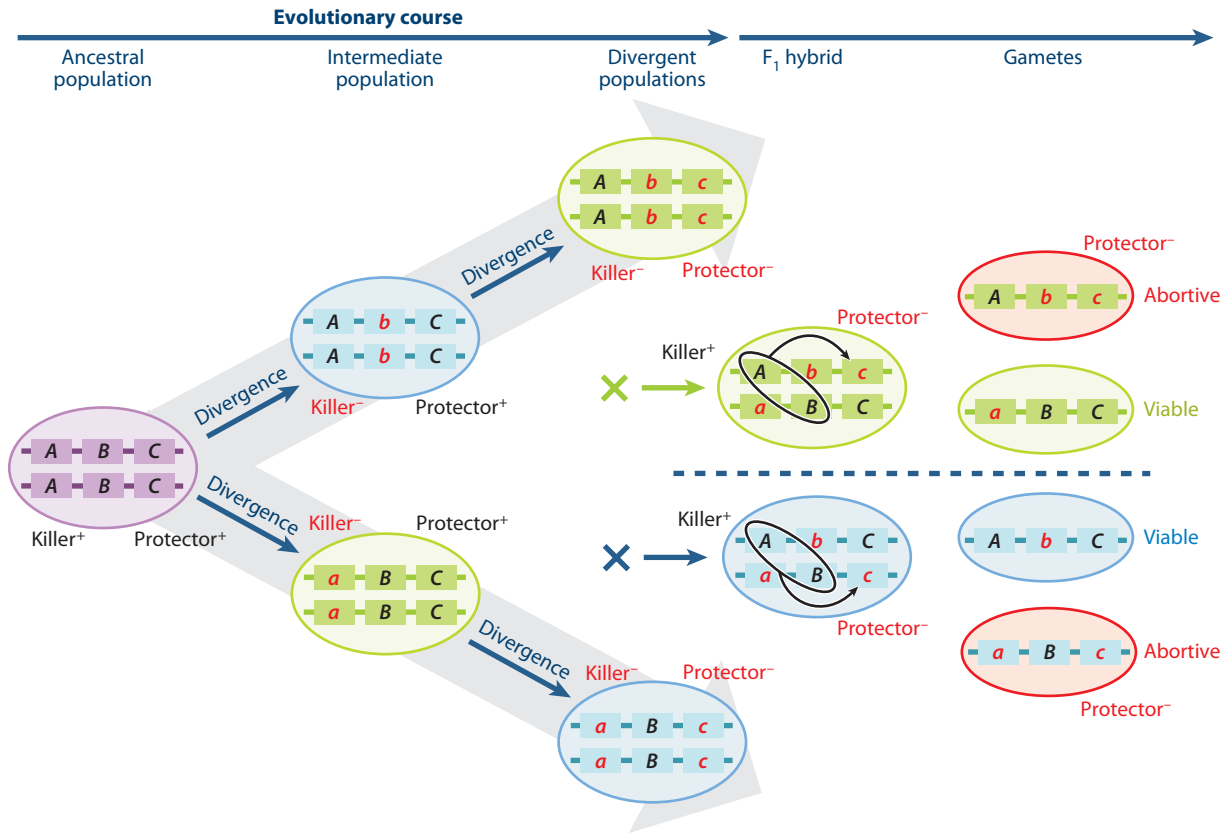


Figure 3

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.

Parallely 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, parallely 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*_{Bristol} (in the Bristol strain) and *zeel-1*_{Hawaii} (in the Hawaii strain) in the same genetic background (77). The *zeel-1*_{Bristol}/*peel-1*_{Bristol} haplotype gained a transmission advantage because embryos carrying homozygous *zeel-1*_{Hawaii} were selectively arrested owing to the presence of *peel-1*_{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/Lbr* 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, *SbOL11* and its translation regulator *SbAep2* have evolved during adaptation to nonfermentable carbon sources. *SbAep2* was thus unable to regulate the translation of *SbOL11* 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 *ScOL11* 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 non-functional 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 *Ter* 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 *OLII* 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 *COXI*, 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 interspecific 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-1*_{Bristol}/*peel-1*_{Bristol} (77) or the sperms carrying *Smok1*^{Ter} 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.

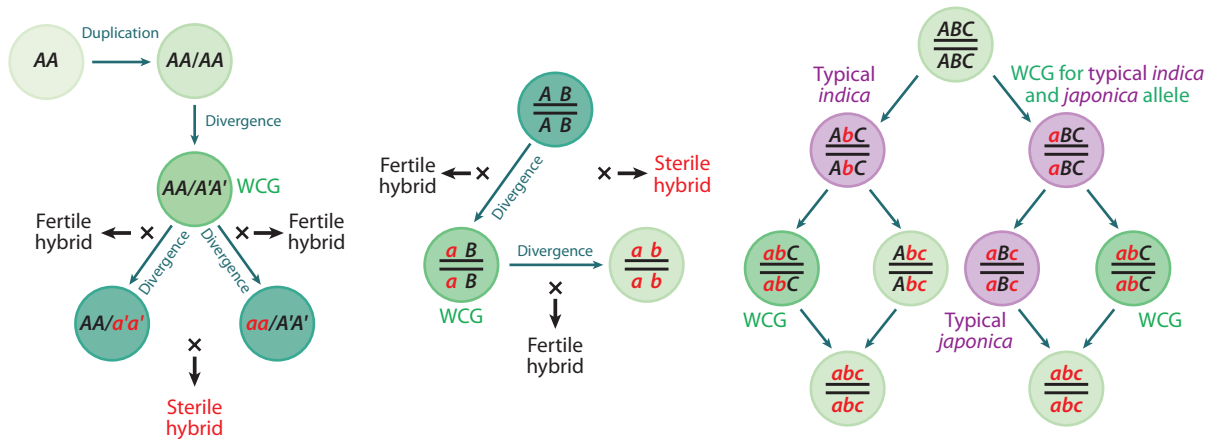
MECHANISMS AND IMPLICATIONS OF WIDE COMPATIBILITY IN CROP BREEDING

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 4a**). 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 4b**). Such WCVs seem to act as an intermediate buffer between the ancestral populations and the nascent ones (**Figure 4b**). 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

a Parallel divergence model**b Sequential divergence model****c Parallel-sequential divergence model****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 4c). 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 4c). 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.

Interspecific *indica-japonica* hybrids show strong heterosis, which has displayed great promise for further increasing rice yield potential. Utilization of interspecific 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.

LITERATURE CITED

1. Alcazar R, Garcia AV, Parker JE, Reymond M. 2009. Incremental steps toward incompatibility revealed by *Arabidopsis* epistatic interactions modulating salicylic acid pathway activation. *Proc. Natl. Acad. Sci. USA* 106:334–39
2. Bauer H, Veron N, Willert J, Herrmann BG. 2007. The *t*-complex-encoded guanine nucleotide exchange factor *Fgd2* reveals that two opposing signaling pathways promote transmission ratio distortion in the mouse. *Genes Dev.* 21:143–47
3. Bauer H, Willert J, Koschorz B, Herrmann BG. 2005. The *t* complex-encoded GTPase-activating protein Tagap1 acts as a transmission ratio distorter in mice. *Nat. Genet.* 37:969–73
4. Bikard D, Patel D, Le Mette C, Giorgi V, Camilleri C, et al. 2009. Divergent evolution of duplicate genes leads to genetic incompatibilities within *A. thaliana*. *Science* 323:623–26
5. Bomblies K. 2009. Too much of a good thing? Hybrid necrosis as a by-product of plant immune system diversification. *Botany* 87:1013–22
6. Bomblies K. 2010. Doomed lovers: mechanisms of isolation and incompatibility in plants. *Annu. Rev. Plant Biol.* 61:109–24
7. Bomblies K, Lempe J, Epple P, Warthmann N, Lanz C, et al. 2007. Autoimmune response as a mechanism for a Dobzhansky-Muller-type incompatibility syndrome in plants. *PLoS Biol.* 5:e236
8. Bomblies K, Weigel D. 2007. Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. *Nat. Rev. Genet.* 8:382–93
9. Brideau NJ, Flores HA, Wang J, Maheshwari S, Wang X, Barbash DA. 2006. Two Dobzhansky-Muller genes interact to cause hybrid lethality in *Drosophila*. *Science* 314:1292–95
10. Burt A, Trivers R. 2006. *Genes in Conflict: The Biology of Selfish Genetic Elements*. Cambridge, MA: Belknap
11. Chen J, Ding J, Ouyang Y, Du H, Yang J, et al. 2008. A triallelic system of *S5* is a major regulator of the reproductive barrier and compatibility of *indica-japonica* hybrids in rice. *Proc. Natl. Acad. Sci. USA* 105:11436–41
12. Chen J, Jiang L, Wang C, Ikehashi H, Zhai H, Wan J. 2006. Mapping of loci for pollen sterility of *indica-japonica* hybrids in rice (*Oryza sativa* L.). *Acta Agron. Sin.* 32:515–21
13. Chou JY, Hung YS, Lin KH, Lee HY, Leu JY. 2010. Multiple molecular mechanisms cause reproductive isolation between three yeast species. *PLoS Biol.* 8:e1000432
14. Coyne JA, Orr HA. 2004. *Speciation*. Sunderland, MA: Sinauer
15. Dobzhansky T. 1937. *Genetics and the Origin of Species*. New York: Columbia Univ. Press
16. Du H, Ouyang Y, Zhang C, Zhang Q. 2011. Complex evolution of *S5*, a major reproductive barrier regulator, in the cultivated rice *Oryza sativa* and its wild relatives. *New Phytol.* 191:275–87
17. Feng Q, Zhang Y, Hao P, Wang S, Fu G, et al. 2002. Sequence and analysis of rice chromosome 4. *Nature* 420:316–20
18. Garris AJ, Tai TH, Coburn J, Kresovich S, McCouch S. 2005. Genetic structure and diversity in *Oryza sativa* L. *Genetics* 169:1631–38
19. Glaszmann JC. 1987. Isozymes and classification of Asian rice varieties. *Theor. Appl. Genet.* 74:21–30
20. Han B, Xue Y. 2003. Genome-wide intraspecific DNA-sequence variations in rice. *Curr. Opin. Plant Biol.* 6:134–38
21. Herrmann BG, Koschorz B, Wertz K, McLaughlin KJ, Kispert A. 1999. A protein kinase encoded by the *t complex responder* gene causes non-mendelian inheritance. *Nature* 402:141–46
22. Huang X, Wei X, Sang T, Zhao Q, Feng Q, et al. 2010. Genome-wide association studies of 14 agronomic traits in rice landraces. *Nat. Genet.* 42:961–67
23. Huang X, Zhao Y, Wei X, Li C, Wang A, et al. 2012. Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. *Nat. Genet.* 44:32–39

24. Ichitani K, Namigoshi K, Sato M, Taura S, Aoki M, et al. 2007. Fine mapping and allelic dosage effect of *Hwc1*, a complementary hybrid weakness gene in rice. *Theor. Appl. Genet.* 114:1407–15
25. Ichitani K, Takemoto Y, Iiyama K, Taura S, Sato M. 2012. Chromosomal location of *HCA1* and *HCA2*, hybrid chlorosis genes in rice. *Int. J. Plant Genomics* 2012:649081
26. Ikehashi H. 1991. Genetics of hybrid sterility in wide hybridization in rice. In *Rice*, Biotechnol. Agric. For. 14, ed. YPS Bajaj, pp. 113–27. Berlin: Springer-Verlag
27. Ikehashi H, Araki H. 1986. Genetics of F₁ sterility in remote crosses of rice. In *Rice Genetics: Proceedings of the International Rice Genetics Symposium*, pp. 119–30. Manila: Int. Rice Res. Inst.
28. Ikehashi H, Zou JS, Huhn PM, Maruyama K. 1994. Wide compatibility gene and *indica-japonica* heterosis in rice for temperate countries. In *Hybrid Rice Technology: New Developments and Future Prospects*, ed. SS Virmani, pp. 21–31. Manila: Int. Rice Res. Inst.
29. Jeuken MJ, Zhang NW, McHale LK, Pelgrom K, den Boer E, et al. 2009. *Rin4* causes hybrid necrosis and race-specific resistance in an interspecific lettuce hybrid. *Plant Cell* 21:3368–78
30. Ji Q, Lu J, Chao Q, Gu M, Xu M. 2005. Delimiting a rice wide-compatibility gene *S5ⁿ* to a 50 kb region. *Theor. Appl. Genet.* 111:1495–503
31. Jiang W, Chu SH, Piao R, Chin JH, Jin YM, et al. 2008. Fine mapping and candidate gene analysis of *bwh1* and *bwh2*, a set of complementary genes controlling hybrid breakdown in rice. *Theor. Appl. Genet.* 116:1117–27
32. Jing W, Zhang W, Jiang L, Chen L, Zhai H, Wan J. 2007. Two novel loci for pollen sterility in hybrids between the weedy strain Ludao and the *japonica* variety Akihikari of rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 114:915–25
33. Kato S, Kosaka H, Hara S. 1928. On the affinity of rice varieties as shown by fertility of hybrid plants. *Bull. Sci. Fac. Agric. Kyushu Univ.* 3:132–47
34. Kitamura E. 1962. Genetics studies on sterility observed in hybrids between distantly related varieties of rice, *Oryza sativa* L. *Bull. Chugoku Agric. Exp. Stn. A* 8:141–205
35. Koide Y, Ikenaga M, Sawamura N, Nishimoto D, Matsubara K, et al. 2008. The evolution of sex-independent transmission ratio distortion involving multiple allelic interactions at a single locus in rice. *Genetics* 180:409–20
36. Kruger J, Thomas CM, Golstein C, Dixon MS, Smoker M, et al. 2002. A tomato cysteine protease required for Cf-2-dependent disease resistance and suppression of autonecrosis. *Science* 296:744–47
37. Kubo T, Yamagata Y, Eguchi M, Yoshimura A. 2008. A novel epistatic interaction at two loci causing hybrid male sterility in an inter-subspecific cross of rice (*Oryza sativa* L.). *Genes Genet. Syst.* 83:443–53
38. Kubo T, Yoshimura A. 2001. Linkage analysis of an F₁ sterility gene in *Japonica/Indica* cross of rice. *Rice Genet. Newsl.* 18:52–53
39. Kubo T, Yoshimura A. 2002. Genetic basis of hybrid breakdown in a Japonica/Indica cross of rice, *Oryza sativa* L. *Theor. Appl. Genet.* 105:906–11
40. Kubo T, Yoshimura A. 2005. Epistasis underlying female sterility detected in hybrid breakdown in a Japonica–Indica cross of rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 110:346–55
41. Kubo T, Yoshimura A, Kurata N. 2011. Hybrid male sterility in rice is due to epistatic interactions with a pollen killer locus. *Genetics* 189:1083–92
42. Lai Z, Nakazato T, Salmaso M, Burke JM, Tang S, et al. 2005. Extensive chromosomal repatterning and the evolution of sterility barriers in hybrid sunflower species. *Genetics* 171:291–303
43. Lee HY, Chou JY, Cheong L, Chang NH, Yang SY, Leu JY. 2008. Incompatibility of nuclear and mitochondrial genomes causes hybrid sterility between two yeast species. *Cell* 135:1065–73
44. Li D, Chen L, Jiang L, Zhu S, Zhao Z, et al. 2007. Fine mapping of *S32(t)*, a new gene causing hybrid embryo sac sterility in a Chinese landrace rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 114:515–24
45. Li WT, Zeng RZ, Zhang ZM, Ding XH, Zhang GQ. 2006. Fine mapping of locus *S-b* for F₁ pollen sterility in rice (*Oryza sativa* L.). *Chin. Sci. Bull.* 51:675–80
46. Li WT, Zeng RZ, Zhang ZM, Ding XH, Zhang GQ. 2008. Identification and fine mapping of *S-d*, a new locus conferring the partial pollen sterility of intersubspecific F₁ hybrids in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 116:915–22
47. Li Z, Pinson SR, Paterson AH, Park WD, Stansel JW. 1997. Genetics of hybrid sterility and hybrid breakdown in an intersubspecific rice (*Oryza sativa* L.) population. *Genetics* 145:1139–48

48. Liu A, Zhang Q, Li H. 1992. Location of a gene for wide-compatibility in the RFLP linkage map. *Rice Genet. Newsl.* 9:134–36
49. Liu HY, Xu CG, Zhang Q. 2004. Male and female gamete abortions, and reduced affinity between the uniting gametes as the causes for sterility in an *indica/japonica* hybrid in rice. *Sex. Plant Reprod.* 17:55–62
50. Liu KD, Wang J, Li HB, Xu CG, Liu AM, et al. 1997. A genome-wide analysis of wide compatibility in rice and the precise location of the *S5* locus in the molecular map. *Theor. Appl. Genet.* 95:809–14
51. Liu KD, Yang GP, Zhu SH, Zhang Q, Wang XM, Saghai Maroof MA. 1996. Extraordinary polymorphic ribosomal DNA in wild and cultivated rice. *Genome* 39:1109–16
52. Liu KD, Zhou ZQ, Xu CG, Zhang Q, Saghai Maroof MA. 1996. An analysis of hybrid sterility in rice using a diallel cross of 21 parents involving *indica*, *japonica* and wide compatibility varieties. *Euphytica* 90:275–80
53. Long Y, Zhao L, Niu B, Su J, Wu H, et al. 2008. Hybrid male sterility in rice controlled by interaction between divergent alleles of two adjacent genes. *Proc. Natl. Acad. Sci. USA* 105:18871–76
54. Lynch M, Force AG. 2000. The origin of interspecific genomic incompatibility via gene duplication. *Am. Nat.* 156:590–605
55. Lyttle TW. 1991. Segregation distorters. *Annu. Rev. Genet.* 25:511–57
56. Mackill DJ. 1995. Classifying *japonica* rice cultivars with RAPD markers. *Crop Sci.* 35:889–94
57. Masly JP, Jones CD, Noor MA, Locke J, Orr HA. 2006. Gene transposition as a cause of hybrid sterility in *Drosophila*. *Science* 313:1448–50
58. Matsubara K, Ando T, Mizubayashi T, Ito S, Yano M. 2007. Identification and linkage mapping of complementary recessive genes causing hybrid breakdown in an intraspecific rice cross. *Theor. Appl. Genet.* 115:179–86
59. Merrill C, Bayraktaroglu L, Kusano A, Ganetzky B. 1999. Truncated RanGAP encoded by the *Segregation Distorter* locus of *Drosophila*. *Science* 283:1742–45
60. Mizuta Y, Harushima Y, Kurata N. 2010. Rice pollen hybrid incompatibility caused by reciprocal gene loss of duplicated genes. *Proc. Natl. Acad. Sci. USA* 107:20417–22
61. Morinaga T, Kuriyama H. 1958. Intermediate type of rice in the subcontinent of India and Java. *Jpn. J. Breed.* 7:253–59
62. Muller HJ. 1942. Isolating mechanisms, evolution, and temperature. *Biol. Symp.* 6:71–125
63. Nakano M, Yoshimura A, Iwata N. 1992. Phylogenetic study of cultivated rice and its wild relatives by RFLP. *Rice Genet. Newsl.* 9:132–34
64. Oka HI. 1957. Genetic analysis for the sterility of hybrids between distantly related varieties of cultivated rice. *J. Genet.* 55:397–409
65. Oka HI. 1974. Analysis of genes controlling F₁ sterility in rice by the use of isogenic lines. *Genetics* 77:521–34
66. Oka HI. 1988. *Origin of Cultivated Rice*. Tokyo: Jpn. Sci. Soc. Press
67. Orr HA. 2005. The genetic basis of reproductive isolation: insights from *Drosophila*. *Proc. Natl. Acad. Sci. USA* 102(Suppl. 1):6522–26
68. Orr HA, Masly JP, Phadnis N. 2007. Speciation in *Drosophila*: from phenotypes to molecules. *J. Hered.* 98:103–10
69. Orr HA, Masly JP, Presgraves DC. 2004. Speciation genes. *Curr. Opin. Genet. Dev.* 14:675–79
70. Ouyang Y, Chen J, Ding J, Zhang Q. 2009. Advances in the understanding of inter-subspecific hybrid sterility and wide-compatibility in rice. *Chin. Sci. Bull.* 54:2332–41
71. Ouyang Y, Liu YG, Zhang Q. 2010. Hybrid sterility in plant: stories from rice. *Curr. Opin. Plant Biol.* 13:186–92
72. Presgraves DC. 2010. Darwin and the origin of interspecific genetic incompatibilities. *Am. Nat.* 176(Suppl. 1):S45–60
73. Qiu SQ, Liu K, Jiang JX, Song X, Xu CG, et al. 2005. Delimitation of the rice wide compatibility gene *S5^w* to a 40-kb DNA fragment. *Theor. Appl. Genet.* 111:1080–86
74. Rieseberg LH, Blackman BK. 2010. Speciation genes in plants. *Ann. Bot.* 106:439–55
75. Sang T, Ge S. 2007. Genetics and phylogenetics of rice domestication. *Curr. Opin. Genet. Dev.* 17:533–38
76. Cannell DR, Byrne KP, Gordon JL, Wong S, Wolfe KH. 2006. Multiple rounds of speciation associated with reciprocal gene loss in polyploid yeasts. *Nature* 440:341–45

77. Seidel HS, Rockman MV, Kruglyak L. 2008. Widespread genetic incompatibility in *C. elegans* maintained by balancing selection. *Science* 319:589–94
78. Skrede I, Brochmann C, Borgen L, Rieseberg LH. 2008. Genetics of intrinsic postzygotic isolation in a circumpolar plant species, *Draba nivalis* (Brassicaceae). *Evolution* 62:1840–51
79. Song X, Qiu SQ, Xu CG, Li XH, Zhang Q. 2005. Genetic dissection of embryo sac fertility, pollen fertility, and their contributions to spikelet fertility of intersubspecific hybrids in rice. *Theor. Appl. Genet.* 110:205–11
80. Sweeney M, McCouch S. 2007. The complex history of the domestication of rice. *Ann. Bot.* 100:951–57
81. Sweigart AL, Fishman L, Willis JH. 2006. A simple genetic incompatibility causes hybrid male sterility in *Mimulus*. *Genetics* 172:2465–79
82. Sweigart AL, Mason AR, Willis JH. 2007. Natural variation for a hybrid incompatibility between two species of *Mimulus*. *Evolution* 61:141–51
83. Tao Y, Araripe L, Kingan SB, Ke Y, Xiao H, Hartl DL. 2007. A *sex-ratio* meiotic drive system in *Drosophila simulans*. II: an X-linked distorter. *PLoS Biol.* 5:2576–88
84. Tao Y, Masly JP, Araripe L, Ke Y, Hartl DL. 2007. A *sex-ratio* meiotic drive system in *Drosophila simulans*. I: an autosomal suppressor. *PLoS Biol.* 5:2560–75
85. Ting Y. 1949. Chronological studies of the cultivation and the distribution of rice varieties, Keng and Sen. *Agric. Bull. Coll. Agric. Sun Yatsen Univ.* 6:1–32 (In Chinese)
86. Ting Y. 1957. The origin and evolution of cultivated rice in China. *Acta Agron. Sin.* 8:243–60 (In Chinese)
87. Wan J, Ikehashi H. 1995. Identification of a new locus *S-16* causing hybrid sterility in native rice varieties (*Oryza sativa* L.) from Tai-hu lake region and Yunnan province, China. *Breed. Sci.* 45:461–70
88. Wan J, Ikehashi H, Sakai M, Horisue H, Imbe T. 1998. Mapping of hybrid sterility gene *S17* of rice (*Oryza sativa* L.) by isozyme and RFLP markers. *Rice Genet. Newsl.* 15:151–54
89. Wan J, Yamaguchi Y, Kato H, Ikehashi H. 1996. Two new loci for hybrid sterility in cultivated rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 92:183–90
90. Wan J, Yanagihara S, Kato H, Ikehashi H. 1993. Multiple alleles at a new locus causing hybrid sterility between a Korean *indica* variety and a *javanica* variety in rice (*Oryza sativa* L.). *Jpn. J. Breed.* 43:507–16
91. Wang C, Zhu C, Zhai H, Wan J. 2005. Mapping segregation distortion loci and quantitative trait loci for spikelet sterility in rice (*Oryza sativa* L.). *Genet. Res.* 86:97–106
92. Wang GW, He YQ, Xu CG, Zhang Q. 2006. Fine mapping of *f5-Du*, a gene conferring wide-compatibility for pollen fertility in inter-subspecific hybrids of rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 112:382–87
93. Wang J, Liu KD, Xu CG, Li XH, Zhang Q. 1998. The high level of wide-compatibility of variety “Dular” has a complex genetic basis. *Theor. Appl. Genet.* 97:407–12
94. Widmer A, Lexer C, Cozzolino S. 2009. Evolution of reproductive isolation in plants. *Heredity* 102:31–38
95. Wu C-I, Lyttle TW, Wu M-L, Lin G-F. 1988. Association between a satellite DNA sequence and the responder of *segregation distorter* in *D. melanogaster*. *Cell* 54:179–89
96. Xu CG. 1995. Adsorption and germination of pollen in crosses within and between *indica* and *japonica* rice. *J. Huazhong Agric. Univ.* 14:421–24 (In Chinese with English abstract)
97. Xu X, Liu X, Ge S, Jensen JD, Hu F, et al. 2012. Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. *Nat. Biotechnol.* 30:105–11
98. Yamagata Y, Yamamoto E, Aya K, Win KT, Doi K, et al. 2010. Mitochondrial gene in the nuclear genome induces reproductive barrier in rice. *Proc. Natl. Acad. Sci. USA* 107:1494–99
99. Yamamoto E, Takashi T, Morinaka Y, Lin S, Kitano H, et al. 2007. Interaction of two recessive genes, *bbd2* and *bbd3*, induces hybrid breakdown in rice. *Theor. Appl. Genet.* 115:187–94
100. Yamamoto E, Takashi T, Morinaka Y, Lin S, Wu J, et al. 2010. Gain of deleterious function causes an autoimmune response and Bateson-Dobzhansky-Muller incompatibility in rice. *Mol. Genet. Genomics* 283:305–15
101. Yanagihara S, Kato H, Ikehashi H. 1992. A new locus for multiple alleles causing hybrid sterility between an *Aus* variety and *javanica* varieties in rice (*Oryza sativa* L.). *Jpn. J. Breed.* 42:793–801
102. Yanagihara S, McCouch SR, Ishikawa K, Ogi Y, Maruyama K, Ikehashi H. 1995. Molecular analysis of the inheritance of the *S-5* locus, conferring wide compatibility in *indica/japonica* hybrids of rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 90:182–88

103. Yang C, Chen Z, Zhuang C, Mei M, Liu Y. 2004. Genetic and physical fine-mapping of the *Sr* locus conferring *indica-japonica* hybrid sterility in rice (*Oryza sativa* L.). *Chin. Sci. Bull.* 49:1718–21
104. Yang GP, Saghai Maroof MA, Xu CG, Zhang Q, Biyashev RM. 1994. Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. *Mol. Gen. Genet.* 245:187–94
105. Yang J, Zhao X, Cheng K, Du H, Ouyang Y, et al. 2012. A killer-protector system regulates both hybrid sterility and segregation distortion in rice. *Science* 337:1336–40
106. You X. 1995. *History of Rice Crop in China*. Beijing: China Agric. Press (In Chinese)
107. Yuan LP. 1994. Increasing yield potential in rice by exploitation of heterosis. In *Hybrid Rice Technology: New Developments and Future Prospects*, ed. SS Virmani, pp. 1–6. Manila: Int. Rice Res. Inst.
108. Zhang GQ, Lu YG. 1996. Genetics of F₁ pollen sterility in *Oryza sativa*. In *Rice Genetics III: Proceedings of the Third International Rice Genetics Symposium*, ed. G Khush, pp. 418–22. Manila: Int. Rice Res. Inst.
109. Zhang GQ, Lu YG. 1999. Breeding of the *indica-compatible japonica* lines and their use in the breeding of super-high-yield hybrid rice. *Hybrid Rice* 14:3–5 (In Chinese with English abstract)
110. Zhang GQ, Lu YG, Zhang H, Yang JC, Liu GF. 1994. Genetic studies on the hybrid sterility in cultivated rice (*Oryza sativa*) IV. Genotypes for F₁ pollen sterility. *Acta Genet. Sin.* 21:34–41 (In Chinese with English abstract)
111. Zhang Q. 2007. Strategies for developing Green Super Rice. *Proc. Natl. Acad. Sci. USA* 104:16402–9
112. Zhang Q, Liu KD, Yang GP, Saghai Maroof MA, Xu CG, Zhou ZQ. 1997. Molecular marker diversity and hybrid sterility in *indica-japonica* rice crosses. *Theor. Appl. Genet.* 95:112–18
113. Zhang Q, Saghai Maroof MA, Lu TY, Shen BZ. 1992. Genetic diversity and differentiation of *indica* and *japonica* rice detected by RFLP analysis. *Theor. Appl. Genet.* 83:495–99
114. Zhang ZS, Lu YG, Liu XD, Feng JH, Zhang GQ. 2006. Cytological mechanism of pollen abortion resulting from allelic interaction of F₁ pollen sterility locus in rice (*Oryza sativa* L.). *Genetica* 127:295–302
115. Zhao ZG, Jiang L, Zhang WW, Yu CY, Zhu SS, et al. 2007. Fine mapping of *S31*, a gene responsible for hybrid embryo-sac abortion in rice (*Oryza sativa* L.). *Planta* 226:1087–96
116. Zhao ZG, Wang CM, Jiang L, Zhu S, Ikehashi H, Wan JM. 2006. Identification of a new hybrid sterility gene in rice (*Oryza sativa* L.). *Euphytica* 151:331–37
117. Zhu SS, Jiang L, Wang CM, Zhai HQ, Li DT, Wan JM. 2005. The origin of weedy rice Ludao in China deduced by a genome wide analysis of its hybrid sterility genes. *Breed. Sci.* 55:409–14
118. Zhu SS, Wang CM, Zheng TQ, Ikehashi H, Wan J. 2005. A new gene located on chromosome 2 causing hybrid sterility in a remote cross of rice. *Plant Breed.* 124:440–45
119. Zhu WY, Li WT, Ding XH, Zhang ZM, Zeng RZ, et al. 2008. Preliminary identification of F₁ pollen sterility gene *S-e* in *Oryza sativa*. *J. South China Agric. Univ.* 29:1–5 (In Chinese with English abstract)
120. Zhuang C, Zhang G, Mei M, Lu Y. 1999. Molecular mapping of the *Sa* locus for F₁ pollen sterility in cultivated rice (*Oryza sativa* L.). *Acta Genet. Sin.* 26:213–18 (In Chinese with English abstract)



Contents

Benefits of an Inclusive US Education System <i>Elisabeth Gantt</i>	1
Plants, Diet, and Health <i>Cathie Martin, Yang Zhang, Chiara Tonelli, and Katia Petroni</i>	19
A Bountiful Harvest: Genomic Insights into Crop Domestication Phenotypes <i>Kenneth M. Olsen and Jonathan F. Wendel</i>	47
Progress Toward Understanding Heterosis in Crop Plants <i>Patrick S. Schnable and Nathan M. Springer</i>	71
Tapping the Promise of Genomics in Species with Complex, Nonmodel Genomes <i>Candice N. Hirsch and C. Robin Buell</i>	89
Understanding Reproductive Isolation Based on the Rice Model <i>Yidan Ouyang and Qifa Zhang</i>	111
Classification and Comparison of Small RNAs from Plants <i>Michael J. Axtell</i>	137
Plant Protein Interactomes <i>Pascal Braun, Sébastien Aubourg, Jelle Van Leene, Geert De Jaeger, and Claire Lurin</i>	161
Seed-Development Programs: A Systems Biology–Based Comparison Between Dicots and Monocots <i>Nese Sreenivasulu and Ulrich Wobus</i>	189
Fruit Development and Ripening <i>Graham B. Seymour, Lars Østergaard, Natalie H. Chapman, Sandra Knapp, and Cathie Martin</i>	219
Growth Mechanisms in Tip-Growing Plant Cells <i>Caleb M. Rounds and Magdalena Bezanilla</i>	243
Future Scenarios for Plant Phenotyping <i>Fabio Fiorani and Ulrich Schurr</i>	267

Microgenomics: Genome-Scale, Cell-Specific Monitoring of Multiple Gene Regulation Tiers <i>J. Bailey-Serres</i>	293
Plant Genome Engineering with Sequence-Specific Nucleases <i>Daniel F. Voytas</i>	327
Smaller, Faster, Brighter: Advances in Optical Imaging of Living Plant Cells <i>Sidney L. Shaw and David W. Ehrhardt</i>	351
Phytochrome Cytoplasmic Signaling <i>Jon Hughes</i>	377
Photoreceptor Signaling Networks in Plant Responses to Shade <i>Jorge J. Casal</i>	403
ROS-Mediated Lipid Peroxidation and RES-Activated Signaling <i>Edward E. Farmer and Martin J. Mueller</i>	429
Potassium Transport and Signaling in Higher Plants <i>Yi Wang and Wei-Hua Wu</i>	451
Endoplasmic Reticulum Stress Responses in Plants <i>Stephen H. Howell</i>	477
Membrane Microdomains, Rafts, and Detergent-Resistant Membranes in Plants and Fungi <i>Jan Malinsky, Miroslava Opekarová, Guido Grossmann, and Widmar Tanner</i>	501
The Endodermis <i>Niko Geldner</i>	531
Intracellular Signaling from Plastid to Nucleus <i>Wei Chi, Xuwu Sun, and Lixin Zhang</i>	559
The Number, Speed, and Impact of Plastid Endosymbioses in Eukaryotic Evolution <i>Patrick J. Keeling</i>	583
Photosystem II Assembly: From Cyanobacteria to Plants <i>Jörg Nickelsen and Birgit Rengstl</i>	609
Unraveling the Heater: New Insights into the Structure of the Alternative Oxidase <i>Anthony L. Moore, Tomoo Shiba, Luke Young, Shigeharu Harada, Kiyoshi Kita, and Kikukatsu Ito</i>	637
Network Analysis of the MVA and MEP Pathways for Isoprenoid Synthesis <i>Eva Vranová, Diana Coman, and Wilhelm Grussem</i>	665

Toward Cool C ₄ Crops <i>Stephen P. Long and Ashley K. Spence</i>	701
The Spatial Organization of Metabolism Within the Plant Cell <i>Lee J. Sweetlove and Alisdair R. Fernie</i>	723
Evolving Views of Pectin Biosynthesis <i>Melani A. Atmodjo, Zhangying Hao, and Debra Mohnen</i>	747
Transport and Metabolism in Legume-Rhizobia Symbioses <i>Michael Udvardi and Philip S. Poole</i>	781
Structure and Functions of the Bacterial Microbiota of Plants <i>Davide Bulgarelli, Klaus Schlaeppi, Stijn Spaepen, Emiel Ver Loren van Themaat, and Paul Schulze-Lefert</i>	807
Systemic Acquired Resistance: Turning Local Infection into Global Defense <i>Zheng Qing Fu and Xinnian Dong</i>	839

Indexes

Cumulative Index of Contributing Authors, Volumes 55–64	865
Cumulative Index of Article Titles, Volumes 55–64	871

Errata

An online log of corrections to *Annual Review of Plant Biology* articles may be found at <http://www.annualreviews.org/errata/arplant>