Genetic mechanisms of postzygotic reproductive isolation: An epistatic network in rice

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Products of interspecific crosses often show abnormal phenotypes such as sterility, weakness and inviability. These phenomena play an important role in speciation as mechanisms of postzygotic reproductive isolation (RI). During the past two decades, genetics studies in rice have characterized a number of gene loci responsible for postzygotic RI. I have identified 10 loci including three sets of epistatic networks in a single intersubspecific cross (Oryza sativa ssp. indica × japonica). These results suggest that RI genes cause developmental dysfunction of vegetative and/or reproductive organs through a variety of molecular pathways. The latest molecular studies demonstrated that hybrid incompatibility is mainly due to deleterious interactions caused by species-specific mutations of two or more genes, mediated by proteins acting within the same molecular pathway. Because genetic interactions provide a perspective on gene function, epistatic networks are a key to the understanding of the molecular basis of postzygotic RI. In this review, I focus on recent progress in postzygotic RI studies in rice and discuss the evolutionary significance as well as implications for improving rice productivity.

Key Words: reproductive isolation, hybrid sterility, epistasis, rice, indica, japonica.

Introduction

The mechanisms of speciation are a central problem in biology. The development of prezygotic and/or postzygotic reproductive isolation (RI) are key steps in speciation. Genetic postzygotic RI mechanisms are categorized into F1 hybrid incompatibility and hybrid breakdown of F2 or later generations. Most cases of postzygotic RI are genetically controlled, and genetic and molecular biological studies have been conducted in a wide variety of animals (Mouse: Bauer et al. 2005, Drosophila: Phadnis and Orr 2009, Wu et al. 1988) and plants (Ichitani et al. 2007, Koide et al. 2008, Rieseberg et al. 1996, Sweigart et al. 2006, Taylor et al. 2009). Since F1 hybrid incompatibility arises in the heterozygous condition while hybrid breakdown requires the recessive homozygous condition for a part of the complementary genes, the molecular mechanisms underlying the two phenomena should be different. For example, the causal recessive gene in hybrid breakdown evokes a loss-of-function mutation of genes essential for normal development, whereas a deleterious heterodimer structure of the causal proteins or a negative interaction between different molecules such as DNA/RNA and proteins are thought to be involved in the F1 incompatibility mechanism. Little is known, however, about the difference on the molecular level, because of our limited understanding of the processes of F1 incompatibility and hybrid breakdown. Elucidating the causal genes and molecular mechanisms involved in the various types of postzygotic RI will have broad implications for evolutionary biology, reproductive and developmental systems, and the improvement of domesticated animals and plants.

Rice belongs to the genus Oryza, which consists of 20 wild and two cultivated species. Asian cultivated rice, Oryza sativa L., was domesticated from the wild species, O. rufipogon, which together are referred to as the “sativa-rufipogon complex”. Oryza rufipogon is widely distributed across Asia to Oceania. The exposure to such a wide spectrum of environmental conditions endowed the sativa-rufipogon complex with an extensive range of ecological, morphological and physiological characters of adaptive significance. The complex therefore represents a reservoir of genes conferring resistance against biotic and antibiotic stresses and genes that control yield components. Due to different morphological and physiological characters, O. sativa is divided into

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two subspecies, *indica* and *japonica* (Kato 1930). Recent phylogenetic and comparative genome studies demonstrated that *indica* and *japonica* were domesticated independently from multiple subpopulations of *O. rufipogon* (Huang et al. 2012, Londo et al. 2006, Wei et al. 2012). With this evolutionary history including different types of ecological adaptation and subsequent domestication, the *indica* and *japonica* subspecies have developed partial postzygotic RI. F1 hybrid sterility is the most commonly observed mechanism of postzygotic RI in *indica/japonica* crosses. So far, more than 40 genes have been reported to be involved in hybrid sterility in rice (Supplemental Table 1). Classical genetic studies proposed two genetic models for F1 hybrid sterility, the interlocus epistasis model also known as sporogametophytic interaction, and the one-locus allelic interaction model also referred to as the Bateson-Dobzhansky-Muller (BDM) model (Bateson 1909, Dobzhansky 1937, Muller 1942), and the one-locus allelic interaction model known as sporogametophytic interaction (Ikehashi and Araki 1986, Rick 1966, Sano 1990). Recent molecular genetic studies of the one-locus model specified the unique properties of its molecular structures. Comparing the classical genetic models, this review focuses on new findings from genetic, molecular and evolutionary studies on postzygotic RI in rice. The mechanisms of F1 hybrid sterility and hybrid breakdown will be described separately, and methodologies for dissecting RI genetic networks and applications in plant breeding will be discussed.

**Allelic interactions responsible for F1 hybrid sterility**

There are numerous studies reporting F1 hybrid sterility in *indica/japonica* crosses (Supplemental Table 1). Most of the occurrences, with a few exceptions, were explained by allelic interaction at a single locus. “Gamete killer” is a typical case of the one-locus model (Rick 1966). Three different alleles, killer (*S*), abortive (*S*′) and neutral (*S*″), comprise the gamete killer system. Gametes carrying the *S*′ allele are abortive in heterozygous hybrids, while gametes carrying *S* are fertile in *S*/S′ heterozygous plants. The neutral allele (*S″*) generates fertile heterozygous hybrids either with *S*′ or *S*″ homozygous parents. Many rice researchers have supported this genetic model in their studies, and have applied the triallelic system to newly identified genes. Recently, cloning and characterization of the *S5* genes unraveled the functioning of the triallelic system at the molecular level (Yang et al. 2012). The *S5* locus contains three genes tightly linked within a 50-kb region and deleterious allelic combinations of these genes causes female gamete abortion via endoplasmic reticulum (ER) stress induction in *indica-japonica* hybrids. Before this study, another hybrid male sterility gene, *Sa*, was also found to interact with two adjacent genes encoding an F-box protein and E3 ligase (Long et al. 2008). These two reports clearly demonstrated that two or more tightly linked genes form functional complexes and that heterozygous polymorphic gene complexes lead to gamete abortion in hybrid plants. Thus, a part of the genetic architecture of the one-locus model is a cluster of functionally related genes that are inherited together like single genes due to their tight linkage. This genetic mechanism substantially corresponds to the BDM model. Another aspect of the one-locus model was revealed in a study of the hybrid sterility gene *S24*. The mode of inheritance of *S24* fits well the one-locus allelic interaction model, as male gametes carrying the *indica* allele, *S24i*, are more frequently transmitted than those carrying the abortive *japonica* allele, *S24j*, in *S24* heterozygotes, while homozygotes for *S24* are fully male fertile (Kubo et al. 2008). Like in the cases of *S5* and *Sa*, *S24* sterility is heterozygous-specific. Kubo et al. (2011) showed that *S24* sterility depends on an unlinked gene named EFS (Epistatic Factor for *S24*) (Fig. 1). *S24* is suppressed in the presence of a dominant *indica* allele of EFS (Efs-i), resulting in good fertility, whereas *S24* is activated and causes male semi-sterility only in plants homozygote for the recessive efs-i. Thus, epistatic regulation is essential for allelic interactions of hybrid sterility genes, a phenomenon I called “epistasis-based allelic interaction”. *S35*, another hybrid male sterility gene, was also demonstrated to be under epistatic control (Kubo et al. 2008). The *S24-i* allele is necessary for *S33* to cause male sterility, and *S33*-bearing male gametes are abortive in an *S33* heterozygous context. Koide et al. (2012) also suggested an involvement of an interaction with unlinked modifiers for the gamete eliminator gene *S6* which was found in an *O. sativa/O. rufipogon* cross. Combined with the other cases (*S5* and *Sa*), these findings suggested that one-locus allelic interaction systems might be specific, complex versions of the BDM interaction, formed by hierarchic components representing killer-protector (Yang et al. 2012), killer-modifier (Koide et al. 2012, Sano 1990) and killer-killer interactions (*S24-S35* interaction in Kubo et al. 2008). In previous studies on hybrid sterility in animals, complex networks of clusters of functionally related genes and unlinked genes have been reported [e.g. segregation distorer in Drosophila by Wu et al. (1988), t-complex in mouse by Bauer et al. (2005)]. These facts suggest a structural commonality between animals and plants with regard to the development of postzygotic RI mechanisms. Consequently, F1 hybrid incompatibility appears generally controlled by epistatic networks involving multiple genes and seems to be controlled rarely by a single gene. The discovery of inconsistent phenotypes of *S24* heterozygotes in *japonica* and *indica* genetic backgrounds led to the identification of EFS (Kubo et al. 2011, see also Fig. 2). In a similar fashion, the killer-type gene *S25* allowed for complete male fertility in the *indica* background (unpublished data), but induced semi-sterility in the *japonica* genetic background (Win et al. 2009). This result suggests that *S25* also is epistatically controlled by an unlinked gene which has not been identified yet and supports the “epistasis-based allelic interaction” model for F1 hybrid sterility. Thus, epistasis generally is involved in what conventionally are assumed to be one-locus allelic interactions.

A different genetic mechanism of F1 hybrid sterility is the loss of function in duplicate genes that was first demonstrated...
by senior rice geneticist Oka (1974). Cloning and sequencing studies revealed that loss-of-function mutations of duplicate genes cause functional defects in male gametes in rice (Mizuta et al. 2010, Yamagata et al. 2010). Potentially plant F1 hybrid incompatibility could be explained by simple genetic mechanisms involving a small number of loci, such as one-locus allelic interactions or duplicate genes. However, the studies cited above indicate that both genetic models include epistasis-related effects among multiple linked or unlinked loci. It is expected that diversified forms of epistatic interactions will be revealed by future cloning studies of additional F1 sterility genes.

The three F1 sterility genes, S24, S25 and S35, cause developmental defects at mitotic stages of male gametogenesis (Kubo et al. 2008, Win et al. 2009). Interestingly, other rice F1 sterility genes tend to evoke developmental defects at late stages of gametogenesis, while genes that do so at earlier and meiotic stages are rare. The gametophytic sterility due to defects in late gametogenesis has a lesser effect on seed fertility than the sporophytic sterility due to premeiotic and meiotic defects. It is unclear which components of the mechanism lead to such bias, but it is known that the strength of reproductive barriers is proportional to the genetic distance between two species (Coyne and Orr 1989, 1997, Moyle et al. 2004, Presgraves 2002). Actually, there are no cases of F1 hybrid inviability in intraspecific crosses of O. sativa, but interspecific crosses among cultivated and wild rice species have been reported to show F1 inviability (Chu and Oka 1970). Thus, the reproductive isolation is incomplete between indica and japonica.

Complementary genes for hybrid breakdown

Hybrid breakdown is defined as sterility or weakness observed in the F2 or later hybrid generations while the F1 hybrids grow normally with good fertility. In general, fewer case studies of hybrid breakdown than of F1 hybrid incompatibility have been published and therefore the molecular basis of hybrid breakdown remains obscure. The genetics of hybrid breakdown have been studied in rice (Fukuoka et al. 1998, Yamamoto et al. 2010) and a simple genetic mechanism based on duplicate recessive genes (15:1 segregation in F2) has been identified. Examples are the gene pairs hwe1 and hwe2 (hybrid weakness-ε-1 and -ε-2) (Kubo and Yoshimura 2002, see also Fig. 1) and hbd2 and hbd3 (hybrid breakdown 2 and -3) (Yamamoto et al. 2010). The double recessive homozygote for hwe1 and hwe2 causes poor vegetative growth and complete sterility, but the molecular basis has not yet been elucidated. On the other hand, hbd2 and hbd3 were found to encode casein kinase I and NBS-LRR, respectively, and the hybrid breakdown was attributed to an autoimmune response (Yamamoto et al. 2010). Similarly, F1 hybrid necrosis in Arabidopsis, tomato, and lettuce, was found to be due to epistatic interactions between pathogen resistance genes (Alcazar et al. 2010, Kruger et al. 2002, Jeuken et al. 2009). These findings indicated a link between immune response systems and postzygotic RI development in plant evolution. In other cases, complex interactions between three complementary genes occur in indica/japonica crosses (Kubo and Yoshimura 2005). The three genes, hsa1, hsa2 and hsa3 (hybrid sterility-ε-1, -2 and -3), which are located on rice chromosomes 12, 8 and 9, respectively (Fig. 1), showed different inheritance patterns in segregating populations. The recessive gene hsa1 causes sporophytic sterility and sterility segregates at a 3:1 ratio in selfed progeny of the hsa1 heterozygotes. On the other hand, hsa2 and hsa3 cause gametophytic sterility (sterility phenotype determined by gamete genotype) resulting in segregation distortion (nearly equal to a 0.1:1:1 ratio deviating from the expected 1:2:1 ratio). Because the hsa1 gene is recessive, interaction of these genes has no significant effects on F1 hybrid phenotypes, which is why this sterility phenotype is a case of hybrid breakdown. Although the molecular basis remains unknown, the different inheritance modes of the three genes suggest an interaction between different molecules such as different protein family members or DNA/RNA, rather than duplicate genes encoding a single protein.

Together with epistasis-based allelic interactions, a variety of other epistatic interactions seem to contribute to postzygotic RI. Many RI genes, which were found in genome-wide surveys including QTL analysis and CSSL analysis, have been detected in a variety of cross combinations between different rice cultivars and species. In contrast, through all my previous studies (Kubo and Yoshimura 2001, 2002, 2005, Kubo et al. 2008, 2011, Win et al. 2009), a total of 10 major gene loci responsible for postzygotic RI have been found in a single cross between Asominori and IR24 (Fig. 1). More recently, my data is suggesting that several genes relating to this genetic network still remain to be identified. Because all these genes were identified in an intraspecific cross, an equal number or more genes should be expected to be involved in postzygotic RI of crosses between more remotely related parents. However, there are no reports of similar gene numbers in other cross combinations, suggesting that we have identified only a very small fraction of the genes involved in plant postzygotic RI. It is intriguing that such a large number of RI genes have developed at subspecies level and how they may have contributed to rice speciation.

Toward a better understanding of a complex genetic network

Epistasis has become a central genetic concept in understanding postzygotic RI as well as other quantitative traits. Despite this importance, epistasis has been elusive. Among several types of epistasis, digenic interaction has often been observed as digenic segregation patterns like 15:1 or 11:5 (Fukuoka et al. 1998, 2005, Kubo et al. 2002, Yamamoto et al. 2010). However, there are few studies that have characterized multiple-gene interactions with more than two unlinked loci. Furthermore, we have never established exactly
how many genes were involved in individual incompatible phenotypes. Almost all cases of postzygotic RI studies have focused on only one or two genes/chromosome region(s) that evoke a major phenotypic effect, and have ignored other unlinked interacting or cryptic factors with minor effects in a given genetic background. The reason is that dissecting complex genetic networks requires hard work and a lot of time. How can such networks be untangled?

Chromosome segment substitution lines (CSSLs) have been developed in many plant species to facilitate the discovery of genes/QTLs responsible for natural variation (Eshed and Zamir 1995, Kubo et al. 2002, Ramsay et al. 1996). To date, CSSL have proven to be a powerful tool for the positional cloning of genes, and the evaluation of gene-gene as well as gene-environment interactions for many types of traits (Doi et al. 2004, Wei et al. 2010, Yu et al. 2007). Multiple concurrent introgressions produced by intercrossing different CSSLs provide an important source of segregating populations to evaluate multiple-gene interactions. Additionally, reciprocal sets of CSSL series are useful for solving the complex network problem and enable us to specify the number of participant genes in a network. If a sterility phenotype is masked in a certain genetic background, we may postulate the existence of additional interacting genes (Fig. 2). Conversely, if the same phenotype is consistently observed in two alternative genetic backgrounds, we may conclude that the genes under study represent the complete set, and no more genes may be required to control the sterility phenotype. Actually, this step-by-step approach using reciprocal CSSLs already has led to the identification of two sets of epistatic networks, namely hsa1–has2–hsa3 and EFS–S24–S35 (Kubo and Yoshimura 2005, Kubo et al. 2011).

Gene clusters putatively related to indica-japonica differentiation

The effects of adaptive evolution on gene sequences, gene expression levels and the resulting molecular pathways, and how RI between divergent populations develops in this evolutionary process are questions of general biological interest. A tendency toward linkages between major morphological genes and RI genes has been observed in the analysis of series of CSSLs. For example, the replacement of a chromosome segment in the Asominori genome by the corresponding segment from the cultivar IR24 that contained the RI genes S24 and S35, introduced IR24-like morphological traits including narrow grains and increased number of grains per panicle (Fig. 3). These morphological changes probably were caused by the genes qSW5/GW5 for grain width (Shomura et al. 2008, Weng et al. 2008) and Gn1a for grain number per panicle (Ashikari et al. 2005), because of the similar positions on the chromosomes. Additionally, S24 was very tightly linked to another hybrid female sterility locus, S31 (Zhao et al. 2007). Both qSW5/GW5 and S31 were identified using CSSLs derived from Asominori and IR24 (Weng et al. 2008, Zhao et al. 2007). Other RI genes also tend to tightly or loosely link with major trait genes such as heading date (Hd3a, Hd1 and S26 on chromosome 6, DTH8 and hsa2 on chromosome 8) (Kojima et al. 2002, Kubo and Yoshimura 2001, Yano et al. 2000, Wei et al. 2010) and blast resistance and brown planthopper resistance genes (Pi-ta and hwe1, Bph26 and hsa1 on chromosome 12) (Bryan et al. 2000, Yara et al. 2010). Among 10 RI genes mapped on rice chromosomes (Fig. 1), eight were linked with some major trait gene(s). Traits such as heading date and biotic stress resistance probably had a significant role in rice evolution. The linkages between incompatibility genes and favorable trait genes likely reflect a hitchhiking effect associated with natural selection and/or domestication. These gene clusters appear related to the indica-japonica differentiation and
therefore should become a focus of molecular studies into rice evolution and domestication. Similarly, linkages between morphological genes and viability genes (referred to as “M-V linkage”) has been observed in several plant species, as reviewed by Grant (1967). Because M-V linkages in plants are responsible for linkage drag, their knowledge is critical to crop breeding.

Fig. 2. Dissecting the epistatic network by using reciprocal CSSLs. The example of the three complementary genes, hsa1, hsa2 and hsa3 is used; the gene set of hsa1-i hsa2-j hsa3-j is incompatible and causes high seed sterility. Once a digenic interaction is identified, it is necessary to confirm the resulting sterile phenotype in the alternative genetic background. If the same phenotype is observed, the identified genes probably represent the complete gene set causing sterility. If not, additional interacting gene(s) can be expected (in this case, a restorer is hidden in the indica background). hsa1-i and hsa2-j denote the indica allele of hsa1 and the japonica allele of hsa2, respectively. For details, refer to Kubo and Yoshimura (2005).

Conclusions and future perspective

Molecular genetics studies in rice have revealed that clusters of adjacent interacting genes and epistatic interactions are involved in the mechanism of F1 hybrid sterility. These studies suggest that the gradual accumulation of mutations of functionally related genes promoted the development of RI in the evolution of diverging populations. Consequently, the genetic network underlying RI evolution appears more complex than generally thought. The causal molecular pathways are likely to be different in each case as molecules of different functions are involved, such as heat shock protein and aspartic protease (Yang et al. 2012), E3-like ligase and F-box protein (Long et al. 2008), mitochondrial L27 protein.
(Yamagata et al. 2010) and casein kinase and NBS-LRR (Yamamoto et al. 2010). My previous studies have revealed various types of incompatibility phenotypes and genetic mechanisms even in a single cross of indica and japonica rice. It is worth noting that there is no report, verified by molecular analysis, of a RI gene that is sufficient to cause hybrid incompatibility by itself in any plant species. Since most postzygotic RI traits are due to defects in reproductive development, the study of functional molecules and their networks that control reproductive development will foster a better understanding of the mechanisms of RI. The molecular pathways of plant reproductive development have remained largely unknown, but the genes and a part of the molecular networks have become clearer in recent years through mutant and transcriptome analyses (Aya et al. 2011, Fujita et al. 2010, Kubo et al. 2013, Li et al. 2011, Moon et al. 2013, Tan et al. 2012). Coupled with new findings in reproductive development, future analyses will elucidate the molecular basis of RI and reproductive systems in plants.

Plant breeders have long been facing the challenge of developing high yielding and stress-tolerant varieties. Because the rice germplasm including its wild relatives presents a wide genetic diversity and sources of heterosis, hybrid rice breeding has a large potential for the improvement of yield, stress tolerance and other agronomical traits. However, the actual utilization of the rice germplasm has been limited to combinations of elite varieties that lack sterility barriers. Understanding RI genes and their molecular function will help to remove this limitation. Physical mapping of RI genes will facilitate the breakage of linkage drag by marker-assisted selection. In addition, the understanding of epistatic interactions can be utilized to mask the harmful effects of RI genes.

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Literature Cited

Alcazar, R., A.V. Garcia, I. Kronholm, J.de Meaux, M.Koornneef, J.E.Parker and M.Reymond (2010) Natural variation at Strubbelig
Receptor Kinase 3 drives immune-triggered incompatibilities between *Arabidopsis thaliana* accessions. Nat. Genet. 42: 1135–1139.

Ashikari, M., H. Sakakibara, S. Lin, T. Yamamoto, T. Takashi, A. Nishimura, E.R. Angeles, Q. Qian, H. Kitano and M. Matsuoka (2005) Cytokinin oxidase regulates rice grain production. Science 309: 741–745.

Aya, K., G. Suzuki, K. Suwabe, T. Hobo, H. Takahashi, K. Shiono, K. Yano, N. Tsutsumi, M. Nakazono, Y. Nagamura *et al.* (2011) Comprehensive network analysis of anther-expressed genes in rice by the combination of 33 laser microdissection and 143 spatiotemporal microarrays. *PLoS One* 6: e26162.

Bateson, W. (1909) Heredity and variation in modern lights. In: Seward, A.C. (ed.) Darwin and Modern Science, Cambridge University Press, Cambridge.

Bauer, H., J. Willert, B. Koschorz and B.G. Herrmann (2005) The t complex-encoded GTPase-activating protein Tagap1 acts as a transmission ratio distorting in rice. Nat. Genet. 37: 969–973.

Bryan, G.T., K.S. Wu, L. Farrall, Y. Jia, H.P. Hershey, S.A. McAdams, K.N. Faulk, G.K. Donaldson, R. Tarchini and B. Valent (2000) tA single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene Pi-ta. Plant Cell 12: 2033–2046.

Chu, Y.E. and H.I. Oka (1970) The genetic basis of crossing barriers between *Oryza perennis* subsp. *barthii* and its related taxa. Evolution 24: 135–144.

Coyne, J.A. and H.A. Orr (1989) Patterns of speciation in Drosophila. Evolution 43: 362–381.

Coyne, J.A. and H.A. Orr (1997) “Patterns of speciation in Drosophila” Revisited. Evolution 51: 295–303.

Dobzhansky, T. (1937) Genetics and the Origin of Species. Columbia Univ. Press, New York.

Doi, K., T. Izawa, T. Fuse, U. Yamanouchi, T. Kubo, Z. Shimatani, M. Yano and A. Yoshimura (2004) *Ehd1*, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of *Hd1*. Genes Dev. 18: 926–936.

Eshed, Y. and D. Zamir (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141: 1147–1162.

Fujita, M., Y. Horiiuchi, Y. Ueda, Y. Mizuta, T. Kubo, K. Yano, S. Yamaki, K. Tsuda, T. Nagata, M. Niihama *et al.* (2010) Rice expression atlas in reproductive development. Plant Cell Physiol. 51: 2060–2081.

Fukuoka, S., H. Namai and K. Okuno (1998) RFLP mapping of the genes controlling hybrid breakdown in rice (*Oryza sativa* L.). Theor. Appl. Genet. 97: 446–449.

Fukuoka, S., M.C.V. Newingham, M. Ishitaq, T. Nagamine, M. Kawase and K. Okuno (2005) Identification and mapping of two new loci for hybrid breakdown in cultivated rice. Rice Genet. NewsL. 22: 29–31.

Grant, V. (1967) Linkage between morphology and viability in plant species. Amer. Nat. 101: 125–139.

Huang, X., N. Kurata, X. Wei, Z.X. Wang, A. Wang, Q. Zhao, Y. Zhao, K. Liu, H. Lu, W. Li *et al.* (2012) A map of rice genome variation reveals the origin of cultivated rice. *Nature* 490: 497–501.

Ichitani, K., K. Namigoshi, M. Sato, S. Taura, M. Aoki, Y. Matsumoto, T. Saitou, W. Marubashi and T. Kuboyama (2007) Fine mapping and allelic dosage effect of *Hwc1*, a complementary hybrid weakness gene in rice. Theor. Appl. Genet. 114: 1407–1415.

Ikehashi, H. and H. Araki (1986) Genetics of *F1* sterility in remote crosses of rice. In: Khush, G.S. (ed.) Rice Genetics, International Rice Research Institute. Manilla, pp. 119–130.

Jeukens, M.J., N.W. Zhang, L.K. McHale, K. Pelgrom, E. den Boer, P. Lindhout, R.W. Michelmore, R.G. Visser and R.E. Niks (2009) *Rin4* causes hybrid necrosis and race-specific resistance in an interspecific lettuce hybrid. Plant Cell 21: 3368–3378.

Kato, S. (1930) On the affinity of the cultivated rice varieties of rice plants, *Oryza sativa* L. J. Dept. Agr. Kyushu Imp. Univ. 2: 241–275.

Koide, Y., K. Onishi, D. Nishimoto, A.R. Banah, A. Kanazawa and Y. Sano (2008) Sex-independent transmission ratio distortion system responsible for reproductive barriers between Asian and African rice species. New Phytol. 179: 888–900.

Koide, Y., Y. Shinya, M. Ikenaga, N. Sawamura, K. Matsubara, K. Onishi, A. Kanazawa and Y. Sano (2012) Complex genetic nature of sex-independent transmission ratio distortion in Asian rice species: the involvement of unlinked modifiers and sex-specific mechanisms. Heredity 108: 242–247.

Kojima, S., Y. Takahashi, Y. Kobayashi, L. Monna, T. Sasaki, T. Araki and M. Yano (2002) *Hd3a*, a rice ortholog of the Arabidopsis FT gene, promotes transition to flowering downstream of *Hdl* under short-day conditions. Plant Cell Physiol. 43: 1096–1105.

Kruger, J., C.M. Thomas, C. Golstein, M.S. Dixon, M. Smoker, S. Tang, L. Mulder and J.D. Jones (2002) A tomato cysteine protease required for *Cf2*-dependent disease resistance and suppression of autonecrosis. Science 296: 744–747.

Kubo, T., Y. Aida, K. Nakamura, H. Tsunematsu, K. Doi and A. Yoshimura (2002) Reciprocal chromosome segment substitution series derived from japonica and indica cross of rice (*Oryza sativa* L.). Breed. Sci. 52: 319–325.

Kubo, T., M. Fujita, H. Takahashi, M. Nakazono, N. Tsutsumi and N. Kurata (2013) Transcriptome analysis of developing ovules in rice isolated by laser microdissection. Plant Cell Physiol. 54: 750–765.

Kubo, T., Y. Yamagata, M. Eguchi and A. Yoshimura (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: 443–453.

Kubo, T. and A. Yoshimura (2001) Linkage analysis of an *F1* sterility gene in Japonica/Indica cross of rice. Rice Genet. NewsL. 18: 52–54.

Kubo, T. and A. Yoshimura (2002) Genetic basis of hybrid breakdown in a Japonica/Indica cross of rice, *Oryza sativa* L. Theor. Appl. Genet. 105: 906–911.

Kubo, T. and A. Yoshimura (2005) Epistasis underlying female sterility detected in hybrid breakdown in a Japonica-Indica cross of rice (*Oryza sativa* L.). Theor. Appl. Genet. 110: 346–355.

Kubo, T., A. Yoshimura and N. Kurata (2011) Hybrid male sterility in rice is due to epistatic interactions with a pollen killer locus. Genetics 189: 1083–1092.

Li, H., Z. Yuan, G. Vizcay-Barrena, C. Yang, W. Liang, J. Zong, Z.A. Wilson and D. Zhang (2011) PERSISTENT TAPETAL CELLS1 encodes a PHD-finger protein that is required for tapetal cell death and pollen development in rice. Plant physiol. 156: 615–630.

Li, Y., C. Fan, Y. Xing, Y. Jiang, L. Luo, L. Sun, D. Shao, C. Xu, X. Li, J. Xiao *et al.* (2011) Natural variation in *G55* plays an important role in regulating grain size and yield in rice. Nat. Genet. 43: 1266–1269.

Londo, J.P., Y.C. Chiang, K.H. Hung, T.Y. Chiang and B.A. Schaal (2006) Phylogeography of Asian wild rice, *Oryza rufipogon*, reveals multiple independent domestications of cultivated rice,
Oryza sativa. Proc. Natl. Acad. Sci. USA 103: 9578–9583.

Long,Y., L.Zhao, B.Niu, J.Su, H.Wu, Y.Chen, Q.Zhang, J.Guo, C.Zhuang, M.Mei et al. (2008) Hybrid male sterility in rice controlled by interaction between divergent alleles of two adjacent genes. Proc. Natl. Acad. Sci. USA 105: 18871–18876.

Mizuta,Y., Y.Harushima and N.Kurata (2010) Rice pollen hybrid incompatibility caused by reciprocal gene loss of duplicated genes. Proc. Natl. Acad. Sci. USA 107: 20417–20422.

Moyle, H. (1942) Isolating mechanisms, evolution, k and temperature. Biol. Symp. 6: 71–125.

Moyle, L.C., M.S.Olson and P.Tiffin (2004) Patterns of reproductive isolation in three angiosperm genera. Evolution 58: 1195–1208.

Oka, H. (1974) Analysis of genes controlling F1 sterility in rice by the use of isogenic lines. Genetics 77: 521–534.

Phadnis, N. and H.A.Orr (2009) A single gene causes both male sterility and segregation distortion in Drosophila hybrids. Science 323: 376–379.

Presgraves, D.C. (2002) Patterns of postzygotic isolation in Lepidoptera. Evolution 56: 1168–1183.

Ramsay, L.D., D.E.Jennings, M.J.Kearsey, D.F.Marshall, E.J.Bohuon, A.E.Arthur and D.J.Lydiate (1996) The construction of a substitution library of recombinant backcross lines in Brassica oleracea for the precision mapping of quantitative trait loci. Genome 39: 558–567.

Rick, C.M. (1966) Abortion of male and female gametes in the tomato determined by allelic interaction. Genetics 53: 85–96.

Rieseberg, L.H., B.Sinervo, C.R.Linder, M.C.Ungerer and D.M.Arias (1996) Role of gene interactions in hybrid speciation: Evidence from ancient and experimental hybrids. Science 272: 741–745.

Sano, Y. (1990) The genetic nature of gamete eliminator in rice. Genetics 125: 183–191.

Shomura, A., T.Izawa, K.Ebana, T.Ebitani, H.Kanegae, S.Konishi and M.Yano (2008) Deletion in a gene associated with grain size increased yields during rice domestication. Nat. Genet. 40: 1023–1028.

Sweigart, A.L., L.Fishman and J.H.Willis (2006) A simple genetic incompatibility causes hybrid male sterility in mimulus. Genetics 172: 2465–2479.

Tan, H., W.Liang, J.Hu and D.Zhang (2012) MTR1 encodes a secretory fasciclin glycoprotein required for male reproductive development in rice. Dev. Cell 22: 1127–1137.

Taylor, S.J., M.Arnold and N.H.Martin (2009) The genetic architecture of reproductive isolation in Louisiana irises: hybrid fitness in nature. Evolution 63: 2581–2594.

Wei, X., J.Xu, H.Guo, L.Jiang, S.Chen, C.Yu, Z.Zhou, P.Hu, H.Zhai and J.Wan (2010) DTH8 suppresses flowering in rice, influencing plant height and yield potential simultaneously. Plant Physiol. 153: 1747–1758.

Wei, X., W.H.Qiao, Y.T.Chen, R.S.Wang, L.R.Cao, W.X.Zhang, N.N.Yuan, Z.C.Li, H.L.Zeng and Q.W.Yang (2012) Domestication and geographic origin of Oryza sativa in China: insights from multilocus analysis of nucleotide variation of O. sativa and O. rufipogon. Mol. Ecol. 21: 5073–5087.

Win, K.T., T.Kubo, Y.Miyazaki, K.Doi, Y.Yamagata and A.Yoshimura (2009) Identification of two loci causing F1 pollen sterility in inter- and intraspecific crosses of rice. Breed. Sci. 59: 411–418.

Wy, C.L., T.W.Lyttle, M.L.Wu and G.F.Lin (1988) Association between a satellite DNA sequence and the Responder of Segregation Distorer in D. melanogaster. Cell 54: 179–189.

Yamagata,Y., E.Yamamoto, K.Aya, K.T.Win, K.Doi, Sobrizal, T.Ito, H.Kanamori, J.Wu, T.Matsuo et al. (2010) Mitochondrial gene in the nuclear genome induces reproductive barrier in rice. Proc. Natl. Acad. Sci. USA 107: 1494–1499.

Yamamoto, E., T.Takashi, Y.Morinaka, S.Lin, J.Wu, T.Matsumo, H.Kitano, M.Matsuoka and M.Ashikari (2010) Gain of deleterious function causes an autoimmune response and Bateson-Dobzhansky-Muller incomparability in rice. Mol. Genet. Genomics 283: 305–315.

Yang, J., X.Zhao, K.Cheng, H.Du, Y.Ouyang, J.Chen, S.Qiu, J.Huang, Y.Liang, L.Jiang et al. (2012) A killer-protector system regulates both hybrid sterility and segregation distortion in rice. Science 337: 1336–1340.

Yano, M., Y.Katayose, M.Ashikari, U.Yamanouchi, L.Monna, T.Fuse, T.Baba, K.Yamamoto, Y.Umehara, Y.Nagamura et al. (2000) Hdl1, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene CONSTANS. Plant Cell 12: 2473–2484.

Yara, A., C.N.Phi, M.Matsumura, A.Yoshimura and H.Yasui (2010) Development of near-isogenic lines for BPH25(t) and BPH26(t), which confer resistance to the brown planthopper, Nilaparvata lugens (Stål.) in indica rice ‘ADR52’. Breed. Sci. 60: 639–647.

Yu, B., Z.Lin, H.Li, X.Li, J.Li, Y.Wang, X.Zhang, Z.Zhu, W.Zhai, X. Wang et al. (2007) TAC1, a major quantitative trait locus controlling tiller angle in rice. Plant J. 52: 891–898.

Zhao, Z.G., L.Jiang, W.W.Zhang, C.Y.Yu, S.S.Zhu, K.Xie, H.Tian, L.L.Liu, H.Ikehashi and J.M.Wan (2007) Fine mapping of S31, a gene responsible for hybrid embryo-sac abortion in rice (Oryza sativa L.). Planta 226: 1087–1096.