An overview on reproductive isolation in *Oryza sativa* complex

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Abstract. Reproductive isolation is generally regarded as the essence of the speciation process. Studying closely related species is convenient for understanding the genetic basis of reproductive isolation. Therefore, the present review is restricted to the species and subspecies of the *Oryza sativa* complex, which includes the two domestic rice cultivars and six wild species. Although closely related, these rice species are separated from each other by a range reproductive barriers. This review presents a comprehensive understanding of the forces that shaped the formation of reproductive barriers among and between the species of the *O. sativa* complex. We suggest the possibility that domestication and artificial breeding in these rice species can lead to the early stages of speciation. Understanding the evolutionary and molecular mechanisms underlying reproductive isolation in rice will increase our knowledge in speciation and would also offer practical significance for the implementation of crop improvement strategies.

Keywords: Divergent evolution; hybrids; *Oryza*; reproductive barrier.

Introduction

Genetic divergence, reproductive isolation, natural selection and human-assisted artificial speciation are the vital forces that shape population genetics and consequent speciation (Liu et al. 2015; Schulter and Panell 2017). Reproductive isolation is a very important evolutionary phenomena that prevents genome homogenizations and maintains the integrity of species (Bomblies and Weigel 2007; Ouyang and Zhang 2013; Chen et al. 2016). The evolution of reproductive isolation allows differentiation and local adaptations to become fixed in diverging populations.

Rice belongs to the genus *Oryza*, which contains 25 recognized species, of which 23 are wild and 2 are...
domesticated (Vaughan et al. 2003). The genus Oryza has been classified into different species complexes based on their nine distinct genomes, viz., A, B, C, D, E, F, G, H and J (Vaughan et al. 2003). The O. sativa complex belongs to the A genome and contains two domesticated species O. sativa and O. glaberrima and six wild species: O. rufipogon, O. nivara, O. barthii, O. longistaminata, O. meridionalis and O. glumaepatula. These species constitute the primary gene pool of rice (Vaughan et al. 2003; Tripathi et al. 2011). The wild rice O. rufipogon is a perennial, outcrossing species widely distributed in Asia and Oceania. Oryza nivara is an annual, self-fertilized wild rice species mainly found in South and Southeast Asia. Oryza rufipogon and O. nivara are sometimes considered to be separate species and ecotypes of the same species (Sang and Ge 2007; Vaughan et al. 2008). The perennial O. longistaminata and the annual O. barthii (also called O. breviliigulata) are the African wild rice species and can be found growing in the same area. Oryza longistaminata is a rhizomatous and self-incompatible species found to be the most diverged of all the species in the O. sativa complex (Vaughan et al. 2008). The annual O. glumaepatula is widespread in Tropical America, whereas the annual, inbreeding and highly diverged O. meridionalis is endemic to Tropical Africa and is often sympatric with Oryza australiensis (EE genome) in Australia. Oryza sativa is the Asian cultivated rice and is distributed globally, whereas O. glaberrima is the African cultivated rice and is mostly confined to Africa and differs from O. sativa in its morphology and ecology. Oryza sativa has been further subdivided into multiple varietal groups, the major ones being indica and japonica (Garris et al. 2005; Sweeney et al. 2007). In addition, weedy rice (Oryza sativa f. spontanea), which is conspecific and congeneric to cultivated rice, occurs together with cultivated rice in and around the rice fields (Nadir et al. 2017). The weedy rice associated with O. sativa may be called O. sativa, although they are not the crop. Those associated with O. glaberrima were sometimes called O. stapfii (Suh 2008).

The earliest evidence for the domestication of Asian rice, O. sativa found to date was at the region of the Yangtze River valley of China dated back to 11000–12000 BC (Vaughan et al. 2008; Gross and Zhao 2014). The wild Oryza species, O. rufipogon or O. nivara or possibly both of them, are the progenitors of O. sativa (Fig. 1) (Sang and Ge 2007; Vaughan et al. 2008). Apparently, conflicting data are available supporting single and multiple events leading to domestication of O. sativa (Oka 1988; Cheng et al. 2003; Londo et al. 2006; Vaughan et al. 2008; Molina et al. 2011; Huang et al. 2012). Molecular studies based on similarity in the alleles for non-shattering grains and erect growth in indica and japonica subspecies support the hypothesis of single domestication event (Li et al. 2006; Lin et al. 2007; Jin et al. 2008; Tan et al. 2008). These studies suggest that after domestication O. sativa spread and diversified to create divergent subgroups. Other studies based on the biochemical traits, hybrid sterility and subsequently supported by molecular analyses (Cheng et al. 2003; Tripathi et al. 2011) suggest that indica and japonica subspecies originated under separate domestication events from two divergent wild rice species in China and India, respectively (Tripathi et al. 2011). The African rice O. glaberrima was domesticated from O. barthii separately but parallel to the Asian rice in the African continent between 300 BC and 200 BC during a single domestication event (Fig. 1) (Murray 2004; Purugganan 2014). Oryza barthii was introduced from Asia into Africa (Vaughan et al. 2008).

Considerable high genetic variations exist in this primary gene pool of rice. For example, three regional variants of O. glumaepatula, five distinct groups of O. longistaminata and at least two different genetic groups in O. rufipogon, based on their ecology and life histories, have been recognized (Oka 1988; Akimoto et al. 1998; Vaughan et al. 2008). Similarly, the variation between indica and japonica subspecies of O. sativa is well documented (Garris et al. 2005). Differences were also recognized between temperate and tropical japonica varieties as well as within the tropical japonica varieties (Garris et al. 2005; Vaughan et al. 2008). Compared to O. sativa, O. glaberrima has a restricted geographic distribution and consequently lower genetic diversity exists in African rice (Semon et al. 2005). Only a few genetic subgroups have been detected in O. glaberrima, which reflects the ecological differentiation of O. glaberrima in different habitats (Semon et al. 2005).

Almost all kinds of reproductive barriers so far reported in plants have been found in O. sativa complex and these include reduced cross-fertility, low germinability of F₁ seeds, F₁ inviability, F₁ pollen and embryo sac sterility, and a sporo-phytic sterility and weakness in F₂ generations also known as hybrid breakdown (Fig. 2) (Amemiya and Akemine 1963; Chu and Oka 1969, 1970; Ichtiani et al. 2007, 2011; Chen et al. 2008, 2014). Despite the occurrence of many barriers to hybridization, introgression among these Oryza species is common. Gene flow between O. sativa and O. rufipogon was identified in a number of studies (Pusadee et al. 2016; Wang et al. 2017). Gene flow has also been reported between O. rufipogon and O. nivara as well as between the indica and japonica subspecies of O. sativa (Zheng and Ge 2010; Yang et al. 2012). Hybridization between O. sativa and African wild and cultivated rice at varying level has also been reported (Jones et al. 1997).
Previously, comprehensive literature has been presented on the reproductive isolation in model as well as non-model organisms which has extending our understanding of reproductive isolation (Coyne and Orr 2004; Bombiles and Weigel 2007; Lowry et al. 2008; Ouyang and Zhang 2013; Baack et al. 2015). The topic of reproductive isolation in rice (O. sativa) has been reviewed in previous studies (Ouyang et al. 2010; Ouyang and Zhang 2013) but these studies have focused only on the hybrid sterility observed in indica–japonica hybridization. As rice is one of the better developed systems for understanding the evolution of reproductive isolation, we suggest that a broader view of the various factors that cause the reproductive isolation offers the opportunity to thoroughly understand the phenomena of reproductive isolation in rice. Here, we present a comprehensive study of closely related species of O. sativa complex and attempt to identify all reproductive barriers limiting hybridization. Understanding the molecular basis and the evolutionary forces that caused these barriers to evolve will increase our knowledge of reproductive isolation.

**Divergence Patterns and the Evolution of Reproductive Barriers in Rice**

**Domestication: selection by early humans**

Domestication is the result of a selection process by early farmers that led to the increased adaptation of a plant to cultivation and utilization by humans (Gross and Oslen 2010). The domestication process involves the repeated selection for desirable traits, resulting in the responsible gene mutations becoming fixed in the populations. In some cases, these mutations may lead to divergence and acquiring of variable degrees of isolation from their wild ancestors (Milla et al. 2015). After divergence, the domesticated species became dependent on humans for their reproduction and geographical spread (Milla et al. 2015).

Rice domestication under early human selection led to the intense morphological and physiological variations from its wild ancestors (Table 1) (Sweeney and McCouch 2007; Asano et al. 2011). These alterations resulted in the creation of high-yielding, uniform-germinating and densely planting present-day cultivated rice varieties. The most striking impact of domestication, which differentiated wild and cultivar populations into different reproductive and ecological realms, was changing rice from an outcrossing to an inbreeding crop under human selection for uniform traits. The wild species O. rufipogon and O. barthii are outcrossing, while O. sativa and O. glaberrima are almost entirely self-pollinated.

**Oryza sativa** has a limited degree of outcrossing owing to the short style and stigma, short anthers, limited pollen viability and the brief period between flower opening and pollen release (Tripatti et al. 2011). However, the wild ancestor O. rufipogon have large stigma, and long anthers (Fig. 3). The shift to selfing is associated with changes in flower morphology, matting patterns and reproductive investment that can in turn affect the extent of hybridization and gene flow between populations (Wendt et al. 2002; Coyne and Orr 2004; Martin and Willis 2007; Sicard and Lenhard 2011; Wright et al. 2013). This suggests that different suites of genes and
the corresponding positive mutations that accumulated in the domesticated genotype would be absent from the wild genotype. Therefore, when crosses are made between the domestic and wild populations, domestication-related loci may not interact well with each other.

**Crop improvement: selection by breeders**

Crop improvement involves the incorporation of as many desirable characteristics as possible into a single variety to make it a superior variety. Rice crop improvement tends to produce new varieties with increased yields, enhanced grain characteristics and nutritional values, and an increased resistance against various biotic and abiotic factors. Selective breeding is also associated with the spreading of genes among/or between populations (Chen et al. 2016). As an example, the domestication allele of the reduced shattering gene sh4 in rice originated in the japonica group and spread to the indica group through selective breeding (Gross and Olsen 2010). Selective breeding favours the white grains of domesticated varieties compared with the pigmented grains of wild rice. The gene OsC1 is responsible for the grain pigmentation (Saitoh et al. 2004). OsC1 is tightly linked with the sterility locus S5 (Saitoh et al. 2004). S5 is a major locus controlling embryo-sac fertility and is responsible for the low fertility observed in the indica–japonica hybridizations. The S5 locus contains three alleles: an indica allele (S5i), japonica allele (S5j) and a neutral allele (S5n) (Chen et al. 2008). S5i and S5j are each compatible with S5n, but the combination of S5i and S5j leads to hybrid sterility. Independent mutations to OsC1 leading to lighter grain colour occurred on the background of S5i and S5j, respectively. Selection for the light-colour OsC1 allele indirectly led to increased frequencies of the S5i and S5j alleles at the S5 locus. Thus, selective breeding for OsC1 results in the increase in frequency of S5 genes in the population and an increase in sterility barrier which indicates that S5 locus and post-zygotic isolation can arose as a by-product of genetic hitchhiking between tightly linked genes in rice (Saitoh et al. 2004). Another well-documented example results from the tight linkage between the yield establishment gene Gna1 and the sterility locus S35. Again, selection for the yield increasing gene might have indirectly favoured the retention of a
Table 1. Morphological changes associated with domestication in rice.

| Trait               | Wild rice                           | Domestic rice                       |
|---------------------|-------------------------------------|-------------------------------------|
| Plant height        | Tall                                | Medium to short                     |
| Growth habit        | Creeping                            | Erect                               |
| Tiller number       | Multiple spreading tillers          | Reduced tillers                     |
| Breeding system     | Outbreeding                         | Self-fertilized                     |
| Yield               | Low                                 | High                                |
| Seed quality        | Non-glutinous                       | Glutinous                           |
| Seed dormancy       | High seed dormancy                  | Low seed dormancy                   |
| Seed shattering     | High shattering                     | Non-shattering                      |
| Floral structure    | Long anthers long stigma            | Short anthers short stigma          |
| Panicle shape       | Open panicle                        | Closed panicle                      |
| Grain size          | Small                               | Variable                            |
| Awns                | Long awns                           | Short awns                          |
| Hulls               | Dark/black coloured                 | Straw coloured                      |
| Pericarp/seed coat  | Pigmented                           | Most Asian cultivars lack pigmentation, but many African cultivars retain |

Figure 3. Wild and cultivated rice phenotypes. (A) and (B) represent comparative phenotypes of wild and cultivar open florets. Black arrows indicate anthers and white arrows indicate stigmas.

sterility locus in the *indica* and *japonica* subspecies (Chen et al. 2016; Kubo et al. 2016). Generally, the wild relatives of rice crops are an important reservoir of genetic variability for various economic characteristics, such as disease and insect resistance, tolerance to abiotic stresses, male sterility, increased biomass, grain yield and improved quality characteristics (Xiao et al. 1998).

However, the hybridization between cultivated and wild species is often limited by linkages between desirable and undesirable genes that may hinder the production of lines with desirable agronomic characteristics (Xiao et al. 1998). For example, many desirable agronomic traits in wild rice are often linked with the easy shattering phenotype (Xiao et al. 1998). This suggests that linkage
between favourable and unfavourable alleles may limit introgression.

Reproductive Barriers in Rice

Pre-zygotic reproductive barriers

In some well-studied cases of plants, pre-zygotic reproductive barriers are found to be stronger and lead to nearly complete reproductive isolation (Lowry et al. 2008). The adaptation of species or subspecies to different environments has been recognized as an important reproductive isolating barrier (Baack et al. 2015). Local adaptation involves the evolution of traits in response to different environmental conditions (Baack et al. 2015). These traits include abiotic stress tolerance, breeding times, changes in floral characteristics, flowering time and gametes compatibility that can create reproductive incompatibility between populations (Baack et al. 2015). The sympatrically growing species O. barthii–O. glaberrima and O. nivara–O. rufipogon are precluded from hybridization by the differences in flowering time (Zheng and Ge 2010; Liu et al. 2015). Incompatibility in pollen–pistil interactions provides a strong pre-zygotic reproductive barrier (Oka 1988; Harushima et al. 2002; Lowry et al. 2008). Crosses between O. nivara–O. sativa and O. breviligulata–O. glaberrima showed embryo sac sterility (Chu and Oka 1970).

Post-zygotic reproductive barriers

Post-zygotic reproductive barriers preclude the development of viable fertile hybrid progeny. These barriers in rice begin at hybrid seed development and continue until the hybrid plant reaches the seed-producing stage (Tiffin et al. 2001). At each developmental stage in a plant’s life cycle, a barrier prevents the hybrid progeny from reaching the next developmental stage. These post-zygotic barriers are further classified based on the stage of occurrence.

Hybrid inviability: an endosperm-based hybridization barrier

The endosperm is the basic nutrient source for the developing embryo (Lafon-Placette and Köhler 2016). Any abnormality during endosperm development ultimately leads to embryo arrest and seed failure (Lafon-Placette and Köhler 2016). In dicot species like Arabidopsis, the endosperm supports embryonic growth and disappears soon after cellularization (Bushell et al. 2003). In monocot species, like rice, the endosperm continues to proliferate and support seedling growth even after germination (Sekine et al. 2013). Seeds that lack properly developed endosperm fail to germinate. The endosperm is a highly sensitive tissue and requires a relative maternal to paternal genome dosage ratio of 2:1 for successful development and requires a highly specific balanced gene expression (Lafon-Placette and Köhler 2016). Defects in parental genome dosages, caused by either interspecific crosses or interploidy crosses, are the main reasons for hybrid endosperm defects (Ishikawa et al. 2011; Sekine et al. 2013). Genomic imprinting, whereby some genes are expressed in parental origin-specific manners, serves as the molecular basis for parental genome dosage effects (Matsubara et al. 2003; Ishikawa et al. 2011; Chen et al. 2016).

Generally, outbreeding is thought to increase the intensity levels of the parental genome conflict and the associated genomic imprinting, while inbreeding is thought to reduce those (Lafon-Placette and Köhler 2016). Interspecific hybridizations between O. rufipogon × O. sativa and O. langistamanita × O. sativa resulted in dosage imbalance in the hybrid endosperm leading to its developmental defects (Matsubara et al. 2003; Ishikawa et al. 2011). Similar endosperm development defects were observed in F₁ embryos obtained from O. barthii × O. sativa, O. barthii × O. glaberrima hybridizations (Chu and Oka 1970). Parental genome conflicts arising from ploidy variations have also been reported in a cross between diploid and tetraploid japonica rice (Sekine et al. 2013). Endosperm-based hybridization barriers are the evolutionary forces associated with parental genome conflict, which might arose as a result of a shift in mating processes (i.e. from outbreeding to inbreeding) and thus established a barrier to interploidy and interspecific hybridization in rice.

Hybrid weakness: a post-embryonic-stage hybridization barrier

Hybrid weakness also known as hybrid necrosis is a post-embryonic-stage barrier frequently observed in plant taxa (Bomblies and Weigel 2007). The phenotype of hybrid weakness is similar to the symptoms associated with disease responses (Bomblies and Weigel 2007). Hybrid weakness has been reported in many crosses involving O. rufipogon × O. sativa, O. barthii × O. glaberrima as well as between the subspecies of O. sativa (indica × indica and japonica × japonica hybridizations) (Ichitani et al. 2011; Zhang et al. 2012; Chen et al. 2013, 2014). Early embryogenesis is normal, and normal seedlings are established (Chen et al. 2013). However, at later stages, the seedlings fail to grow properly. The hybrid seedlings have retarded growth rates, with a pale yellow phenotype, and they undergo wilting and necrosis (Chen et al. 2013, 2014). Although,
many cases of hybrid weakness have been reported in rice, the exact molecular mechanism underlying hybrid weakness is still not well known. Previous studies have suggested that hybrid weakness is under the control of complementary gene either dominant or recessive (Amemiya and Akemine 1963; Chu and Oka 1970; Ichitani et al. 2007, 2011; Saito et al. 2007; Kuboyama et al. 2009; Chen et al. 2014). Some studies have suggested that hybrid weakness is due to the activity of defence-related genes which was further confirmed by the high activity of F1 hybrids against pathogens (Bombiles and Weigel 2007; Chen et al. 2014). Recently, genes conferring hybrid weakness in O. rufipogon × O. sativa F1 hybrids have been cloned (Chen et al. 2014). Molecular studies identifying the casual gene of hybrid weakness will help in detailed understanding of the phenomena.

**Hybrid sterility: a reproductive-stage hybridization barrier**

Hybrid sterility is the most common form of postzygotic hybridization barrier in plants (Ouyang et al. 2010). A well-documented example is the hybrid sterility observed in crosses between O. sativa and O. meridionalis, O. sativa and O. glaberrima and between indica and japonica subspecies of O. sativa (Ouyang et al. 2010; Yu et al. 2018). The hybrids obtained are robust in their vegetative growth; however, the progeny are often sterile and cannot produce the next generation. The physiological determinants of reproductive failure in rice include female gamete abortion, pollen sterility, reduced affinity between the uniting gametes, panicle growth rate and ovary growth (Chen et al. 2008; Long et al. 2008; Mizuta et al. 2010). Approximately 50 loci have been identified as being involved in the control of indica-japonica hybrid sterility (Chen et al. 2008; Long et al. 2008; Yamagata et al. 2010; Yang et al. 2012a; Kubo et al. 2016; Li et al. 2017). Several sterility loci have been identified and mapped as a single genetic loci in O. glaberrima (Hu et al. 2006; Li et al. 2011). Further studies relating to gene characterization will help in understanding the molecular mechanism underlying hybrid sterility. Recently, Yu et al. (2018) mapped a sterility locus that contains two tightly linked open reading frames (ORFs) that confers hybrid sterility in F1 hybrids derived from crossing O. sativa × O. meridionalis. One of the ORFs encodes a toxin, which affects the development of pollen, and the other encodes an antidote, which is required for pollen viability. Hybrid breakdown is the weakness and sporophytic sterility found in the F1 and advanced generations, and can be genetically different from F1 weakness or sterility (Okuno and Fukuoka 1999). Hybrid breakdown has been detected in many crosses of rice (Okuno and Fukuoka 1999).

**Genetic Models for the Evolution of Reproductive Barriers in Rice**

Three genetic models have been developed to explain the kinds of genetic changes that occur to cause reproductive isolation in rice.

**Complementary epistasis interaction between two loci**

The simplest genetic explanation of the complementary epistatic interactions between two loci that lead to hybrid inferiority was proposed by Bateson, Dobzhansky and Muller and termed the BDM model (Coyne and Orr 2004). These two loci may be duplicate gene copies or the same locus evolving repeatedly in one species or differently in two species. Figure 4A show a model representation of two-locus interaction causing hybrid incompatibility. Reviewing the literature of hybrid incompatibility cases in rice, a two-locus interaction seems to be the most common cause. A two-locus interaction leading to hybrid weakness was recently reported in O. rufipogon and O. sativa (indica) hybridization (Chen et al. 2014). The hybrids were found to have elevated immune responses similar to previously reported in Arabidopsis (Bombiles and Weigel 2007). The genes in hybrid weakness were found to encode defence-related proteins against pathogens and that incompatible allelic combinations appear to induce autoimmune responses (Chen et al. 2014). Similar two-locus interaction between pathogen resistance genes and their interacting partners has been implicated in hybrid weakness between the indica and japonica subspecies of cultivated rice (Yamamoto et al. 2010).

**Differential silencing**

Molecular divergence owing to the divergent evolution of duplicated genes has been identified as a potential source of reproductive isolation (Werth and Windham 1991). After the duplication event, it is highly probable that both or one of the genes will mutate (Prince and Pickett 2002). Therefore, it can be assumed that an independent mutational event causes a pair of paralogous genes to undergo divergent evolutionary paths and becomes fixed in the two diverging populations. When the two populations hybridize, the populations carrying either of the functional copies develop properly, whereas the hybrids receiving the two silenced copies will have reduced fitness levels. The loss of the
duplicated gene that encodes an essential protein for pollen development causes pollen sterility in the F₁ interspecific hybrids of *O. sativa* and *O. glumaepatula* (Yamagata et al. 2010). Additionally, the independent disruption of duplicated genes *DOPPELGANGER1* and *DOPPELGANGER2* in the *indica* and *japonica* cultivars, respectively, causes pollen sterility in inter-subspecific F₁ hybrids (Fig. 4B) (Mizuta et al. 2010). Likewise, duplication and the reciprocal silencing due to loss-of-function mutation of duplicate genes *S27* and *S28*, encoding a mitochondrial protein, were observed in the F₁ interspecific hybrids of *O. sativa* and *O. glumaepatula*.
(Yamagata et al. 2010). Thus, gene duplication and subsequent mutations may play important roles in establishing reproductive barriers in rice.

**Genic interactions**

A well-documented example of genic interactions leading to hybrid sterility comes from the tight linkage of three genes at the S5 locus causing female gamete abortion and hybrid sterility in *indica japonica* hybridization (Yang et al. 2012b). Two of these three genes constitute the killer system which will preferentially kill the gametes which lack the protector. The divergence in any of these three genes does not occur independently and the evolution of one gene is expected to be conditional on the evolution of another gene. Therefore, a non-functional mutation in the killer would not cause the loss-of-function mutation in the protector. Rather, once the loss-of-function mutation in the killer exists, loss-of-function mutation in the protector would no longer be deleterious and might drift to fixation. Subsequent evolution in the *indica* and *japonica* subspecies has silenced different parts of the killer/protector system and neither subspecies has a functional killer phenotype. In heterozygotes, different genotypes will be formed of these three-gene combinations, hybrid sterility will appear owing to the deleterious interaction between the killer loci without protection by the protector (Yang et al. 2012) (Fig. 4C). Similar findings have been reported by Yu et al. (2018) where a selfish genetic element that encodes for a toxin and antidote that affects pollen viability causes hybrid sterility in *O. meridionalis* and *O. sativa* hybrids.

**Introgression across Barriers**

**Crop-to-weed gene flow**

Weedy rice is one of the troublesome weeds that grows sympatrically with the cultivated rice in rice fields (Nadir et al. 2017). Variable level of gene flow has been recorded to occur between cultivated rice and weedy rice (Langvin et al. 1990; Gealy et al. 2003; Nadir et al. 2017). One study has reported that these two sympatric populations can hybridize with each other freely without any sterility issue which indicates that fewer postzygotic barriers exist between them (Craig et al. 2014). Furthermore, gene flow in reverse order from weed to crop has also been reported (Burgos et al. 2008; Serrat et al. 2013). Gene flow in either direction may have detrimental environmental consequence such as the evolution of undesired agronomic characters in rice crop or the evolution of more aggressive weeds that are difficult to control.

**Crop-to-wild gene flow**

Domesticated rice mate with wild relatives and variable level of gene flow has been reported to occur (Langvin et al. 1990; Majumder et al. 1997; Song et al. 2002). *Oryza sativa* frequently hybridize with *O. rufipogon*. The morphological differences in floral traits suggest that gene flow will predominantly be from *O. sativa* to *O. rufipogon* and the gene flow shows distinct geographic patterns and varies with the *O. sativa* subspecies (Wang et al. 2017). The gene flow from domestic to wild rice has the potential to affect the genetic structure of wild rice (Wang et al. 2017). Wild-to-crop gene flow can be expected even though the frequency is lower than that of crop-to-wild gene flow. The asymmetric gene flow recorded in the *O. rufipogon* and *O. sativa* should be further explored to gain an insight into the forces and mechanism that determine reproductive isolation. These data can also help us in understanding the patterns of population-specific reproductive isolation in rice.

**Crop-to-crop gene flow**

The two domestic rice species *O. sativa* and *O. glaberrima* represent parallel domestications from different progenitors on two different continents assisted by nearly same set of genes (Purugganan 2014). This suggests a high level of homology between the genomes of these *Oryza* species and the possibility to carry out gene transfer between them but this is difficult because of strong reproductive barriers between them (Chu and Oka et al. 1969). Similarly, gene exchange between *indica* and *japonica* subspecies of *O. sativa* has also been reported (Yang et al. 2012b). Gene exchange between these rice subspecies would be highly beneficial to rice breeding practices, but the sterility barriers hinder the exchange (Chen et al. 2008).

In this condition, we may assume that a certain rate of gene exchange takes place across the complex of isolating barriers (Zheng and Ge 2010). Perhaps, a barrier protects populations from reproductive waste, whereas introgression across the barrier offers genetic variability to the populations, giving rise to a balance between isolation and hybridization (Chu et al. 1969). With the rapid advances in transgenic biotechnology, several transgenic crop varieties have been developed and released into markets (Kwit et al. 2011). Synthetic genotypes with desired properties have been developed in rice (Croughan 1998; Paine et al. 2005). Crop-to-weed or crop-to-wild gene flow is of special concern when transgenes are involved (Lu and Snow 2005). These transgenes can escape and introgress into the wild and weedy population with potentially serious consequences (Lu and Snow 2005). The development of genetically modified
crops has been accompanied by efforts to development of intrinsic genetic barriers to prevent transgene flow between synthetic and wild/weedy populations. These include interfering with pollination and fertilization using maternal inheritance and male sterility, terminating transgenic fruit/seed development (seed sterility), selectively terminable transgenic lines or compromising the fitness of hybrids that have acquired positive survival traits from crop genes through introgression (transgenic mitigation) (Suh 2008; Kwit et al. 2011; Nadir et al. 2017). This may also have the side effect of constructing intrinsic barriers to gene flow between independently synthesized transgenic lines that originate from the same ancestral forms (Schluter and Pennel 2017).

**Future Research Perspective**

Rice serves as an excellent model for understanding speciation, and some species (O. rufipogon and O. nivara) are in the early stages of divergence. Therefore, a comprehensive understanding of all the forces and mechanisms that drive reproductive isolation is required. Some additional studies needed in this field are listed below:

1. Analysis of the quantification of the strength of the individual barrier and its contribution to the reproductive isolation in rice would allow us to better evaluate the importance of gene flow and reproductive isolation.
2. Although species of different ploidy are generally reproductively isolated from each other, gene flow across ploidy barriers has been reported in many plant taxa (Chapman and Abbott 2010; Pinheiro et al. 2010). However, in rice introgression across ploidy barrier is not well studied. Understanding the gene flow in ploidy variation and its impact on the morphology and ecology of rice species will be helpful in understanding the diversification and evolution of rice.
3. In addition to the crop-wild (interspecific) and indica–japonica (inter-subspecific) reproductive barriers, some barriers also exist between intra-subspecific hybridizations (indica–indica and japonica–japonica) (Zhang 2012; Fu et al. 2013). The hybrid weakness observed in the intra-subspecific hybridization (indica–indica and japonica–japonica) needs evolutionary as well as molecular analysis.
4. Few genes involved in reproductive isolation in rice have been identified and cloned (Chen et al. 2008; Long et al. 2008; Chen et al. 2013, 2014; Yu et al. 2018). However, the biochemical and molecular mechanisms, and the relationships between the causal genes are still largely unknown. For a complete understanding of these processes, further efforts are needed to characterize the mechanisms that control how gene products function to induce hybrid dys-function at the molecular, cellular and organ levels. These studies will not only improve our understanding of reproductive isolation but also help to improve crop breeding strategies.

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**Conflict of Interest**

None declared.

**Literature Cited**

Akimoto M, Shimamoto Y, Marishima H. 1998. Genetic differentiation in Oryza glumaepatula and its phylogenetic relationships with other AA genome species. *Rice Genetic Newsletter* 14:37–39.

Amemiya A, Akemine H. 1963. Biochemical genetic studies on the root growth inhibiting complementary lethals in rice plant. *Bulletin of the National Institute of Agricultural Sciences (Japan) D10*:139–226 (in Japanese with English summary).

Baack E, Melo MC, Rieseberg LH, Ortiz-Barrientos D. 2015. The origins of reproductive isolation in plants. *The New Phytologist* 207:968–984.

Bombilies K, Weigel D. 2007. Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. *Nature Reviews Genetics* 8:382–393.

Bushell C, Spielman M, Scott RJ. 2003. The basis of natural and artificial postzygotic hybridization barriers in *Arabidopsis* species. *The Plant Cell* 15:1430–1442.

Burgos NR, Norsworthy JK, Scott RC, Smith KL. 2008. Red rice status after five years of ClearfieldTM rice technology in Arkansas. *Weed Technology* 22:200–208.

Chapman MA, Abbott RJ. 2010. Introgression of fitness genes across a ploidy barrier. *The New Phytologist* 186:63–71.

Chen C, Chen H, Lin YS, Shen JB, Shan JX, Qi P, Shi M, Zhu MZ, Huang XH, Feng Q, Han B, Jiang L, Gao P, Lin HX. 2014. A twolocus interaction causes interspecific hybrid weakness in rice. *Nature Communications* 5:3357.

Chen C, Chen H, Shan JX, Zhu MZ, Shi M, Gao JP, Lin HX. 2013. Genetic and physiological analysis of a novel type of interspecific hybrid weakness in rice. *Molecular Plant* 6:716–728.

Chen J, Ding J, Ouyang Y, Du H, Yang J, Cheng K, Zhao J, Qiu S, Zhang X, Yao J, Liu K, Wang L, Xu C, Li X, Xue Y, Xia M, Ji Q, Lu J, Xu M, Zhang Q. 2008. A triallelic system of S5 is a major regulator of the reproductive barrier and compatibility of indica-japonica hybrids in rice. *Proceedings of the National Academy of Sciences of the United States of America* 105:11436–11441.
Chen C, E Z, LinHX. 2016. Evolution and molecular control of hybrid incompatibility in plants. Frontiers in Plant Science 7:1208.
Cheng CY, Motohashi R, Tsuchimoto S, Fukuta Y, Ohtsubo H, Ohtsubo E. 2003. Polyphylectic origin of cultivated rice based on the interspersion pattern of SINEs. Molecular Biology and Evolution 20:67–75.
Chu Y, Morishima H, Oka HI. 1969. Reproductive barriers distributed in cultivated rice species and their wild relatives. Japanese Journal of Genetics 44:207–223.
Chu YE, Oka HI. 1970. Introggression across isolating barriers in wild and cultivated oryza species. Evolution 24:344.
Coyne JA, Orr HA. 2004. Speciation. Sunderland, MA: Sinauer Associates.
Craig SM, Reagan M, Resnick LE, Caicedo AL. 2014. Allele distributions at hybrid incompatibility loci facilitate the potential for gene flow between cultivated and weedy rice in the US. PLoS ONE 9:e86647. doi:10.1371/journal.pone.0086647
Croughan TP. 1998. Herbicide resistant rice. US Patent 5773704.
Fu CY, Wang F, Sun BR, Liu WG, Li JH, Deng RF, Liu DL, Liu ZR, Zhu MS, Liao YL, Chen JW. 2013. Genetic and cytological analysis of a novel type of low temperature-dependent intrasubspecific hybrid weakness in rice. PLoS ONE 8:e73886.
Garris AJ, Tai TH, Coburn J, Kresovich S, McCouch S. 2005. Genetic structure and diversity in Oryza sativa L. Genetics 169:1631–1638.
Gealy DR, Mitten DH, Rutger JN. 2003. Gene flow between red rice (Oryza sativa) and herbicide resistant rice (O. sativa): implications for weed management. Weed Technology 17:627–645. doi:10.1614/WT02-100
Gross BL, Olsen KM. 2010. Genetic perspectives on crop domestication. Trends in Plant Science 15:529–537.
Gross BL, Zhao Z. 2014. Archeological and genetic insights into the origins of mesticated rice. Proceedings of the National Academy of Sciences of the United States of America 111:6190–6197.
Harushima Y, Nakagahara M, Yano M, Sasaki T, Kurata N. 2002. Diverse variation of reproductive barriers in three intraspecific rice crosses. Genetics 160:313–322.
Huang P, Molina J, Flowers JM, Rubinstein S, Jackson SA, Purugganan MD, Schaal BA. 2012. Phylogeography of Asian wild rice, Oryza rufipogon: a genome-wide view. Molecular Ecology 21:4593–4604.
Hu F, Xu YP, Deng XN, Zhou JW, Li J, Tao DY. 2006. Molecular mapping of a pollen killer gene S29(t) in Oryza glaberrima and co-linear analysis with S22 in O. glumaepatula. Euphytica 151:273–278.
Ishikawa R, Ohnishi T, Kinoshita Y, Eiguchi M, Kurata N, Kinoshita T. 2011. Rice interspecies hybrids show precocious or delayed developmental transitions in the endosperm without change to the rate of syncytial nuclear division. The Plant Journal 65:798–806.
Ichitani K, Namigoshi K, Sato M, Taura S, Aoki M, Matsumoto Y, Saitou T, Marubashi W, Kuboyama T. 2007. Fine mapping and allelic dosage effect of Hwc1, a complementary hybrid weakness gene in rice. Theoretical and Applied Genetics 114:1407–1415.
Ichitani K, Taura S, Tezuka T, Okiyama Y, Kuboyama T. 2011. Chromosomal location of HWA1 and HWA2, complementary hybrid weakness genes in rice. Rice 4:29–38.
Jin J, Huang W, Gao JP, Yang J, Shi M, Zhu MZ, Luo D, LinHX. 2008. Genetic control of rice plant architecture under domestication. Nature Genetics 40:1365–1369.
Jones MP, Dingkuhn M, Aluko GK, Semon M. 1997. Interspecific Oryza sativa L. × O. glaberrima Steud. progenies in upland rice improvement. Euphytica 94:237–246.
Kubo T, Yoshimura A, Kurata N. 2016. Pollen killer gene S35 function requires interaction with an activator that maps close to S24, another pollen killer gene in rice. Genes, Genomes, Genetics 6:1459–1468.
Kuboyama T, Saito T, Matsumoto T, Wu J, Kanamori H, Taura S, Sato M, Marubashi W, Ichitani K. 2009. Fine mapping of HWC2, a complementary hybrid weakness gene, and haplotype analysis around the locus in rice. Rice 2:93–103.
Kwitt C, Moon HS, Warwick SJ, Stewart CN Jr. 2011. Transgene introgression in crop relatives: molecular evidence and mitigation strategies. Trends in Biotechnology 29:284–293.
Lafon-Placette C, Köhler C. 2016. Endosperm-based postzygotic hybridization barriers: developmental mechanisms and evolutionary drivers. Molecular Ecology 25:2620–2629.
Langevin SA, Clay K, Grace JB. 1990. The incidence and effects of hybridization between cultivated rice and its related weed red rice (Oryza sativa L.). Evolution 44:1000–1008.
Li G, Li X, Wang Y, Mi J, Xing F, Zhang D, Dong Q, Li X, Xiao J, Zhang Q, Ouyang Y. 2017. Three representative inter and intra-subspecific crosses reveal the genetic architecture of reproductive isolation in rice. The Plant Journal 92:349–362.
Li F, Liu FH, Morinaga D, Zhao Z. 2011. A new gene for hybrid sterility from a cross between Oryza sativa and O. glaberrima. Plant Breeding 130:165–171.
Li C, Zhou A, Song T. 2006. Rice domestication by reducing shattering. Science 311:1936–1939.
Lin Z, Griffith ME, Li X, Zou Z, Tan L, Fu Y, Zhang W, Wang X, Xie D, Sun C. 2007. Origin of seed shattering in rice (Oryza sativa L.). Planta 226:11–20.
Liu R, Zheng XM, Zhou L, Zhou HF, Ge S. 2015. Population genetic structure of Oryza rufipogon and Oryza nivara: implications for the origin of O. nivara. Molecular Ecology 24:5211–5228.
Londo JP, Chiang YC, Hung KH, Chiang TY, School BA. 2006. Phylogeography of Asian wild rice, Oryza rufipogon, reveals multiple independent domestications of cultivated rice, Oryza sativa. Proceedings of the National Academy of Sciences of the United States of America 103:9578–9583.
Long Y, Zhao L, Niu B, Su J, Wu H, Chen Y, Zhang Q, Guo J, Zhuang C, Mei M, Xia J, Wang L, Wu H, Liu YG. 2008. Hybrid male sterility in rice controlled by interaction between divergent alleles of two adjacent genes. Proceedings of the National Academy of Sciences of the United States of America 105:18871–18876.
Lowry DB, Modliszewski JL, Wright KM, Wu CA, Willis JH. 2008. The strength and genetic basis of reproductive isolating barriers in flowering plants. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 363:3009–3021.
Lu BR, Snow AA. 2005. Gene flow from genetically modified rice and its environmental consequences. Bioscience 55:669–678.
Majumder, ND, Ram T, Sharma AC. 1997. Cytological and morphological variation in hybrid swarms and introgressed population of interspecific hybrids (Oryza rufipogon Griff. × O. sativa L.)
and its impact on evolution of intermediate types. *Euphytica* 94:295–302.
Martin NH, Willis JH. 2007. Ecological divergence associated with mating system causes nearly complete reproductive isolation between sympatric *Mimusopsis* species. *Evolution* 61:68–82.
Matsubara K, Khin-Thidar, Sano Y. 2003. A gene block causing cross-incompatibility hidden in wild and cultivated rice. *Genetics* 165:343–352.
Milla R, Osborne CP, Turcotte MM, Violette C. 2015. Plant domestication through an ecological lens. *Trends in Ecology & Evolution* 30:463–469.
Mizuta Y, Harushima Y, Kurata N. 2010. Rice pollen hybrid incompatibility caused by reciprocal gene loss of duplicated genes. *Proceedings of the National Academy of Sciences of the United States of America* 107:20417–20422.
Molina J, Sikora M, Garud N, Flowers JM, Rubinstein S, Reynolds A, Huang P, Jackson S, Schoal BA, Bustamante CD, Boyko AR, Purugganan MD. 2011. Molecular evidence for a single evolutionary origin of domesticated rice. *Proceedings of the National Academy of Sciences of the United States of America* 108:8351–8356.
Murray SS. 2004. Searching for the origins of African rice domestica- tion. *Antiquity*. 78:1–3. http://antiquity.ac.uk/pjg/jall/murray/index.html.
Nadir S, Xiong HB, Zhu Q, Zhang XL, Wu HY, Li J, Dongchen W, Henry D, Guo XQ, Khan S, Suh HS, Lee DS, Chen LJ. 2017. Weedy rice in sustainable rice production—a review. *Agronomy for Sustainable Development* 37:46.
Oka HI (ed.). 1988. *Origin of cultivated rice*. Amsterdam: Elsevier.
Okuno K, Fukuzo S. 1999. Distribution and RFLP mapping of complementary genes causing hybrid breakdown in Asian cultivat- ed rice, *Oryza sativa L.* *Japan Agricultural Research Quarterly* 33:1–6.
Ouyang Y, Liu YG, Zhang Q. 2010. Hybrid sterility in plant: stories from rice. *Current Opinion in Plant Biology* 13:186–192.
Ouyang Y, Zhang Q. 2013. Understanding reproductive isolation based on the rice model. *Annual Review of Plant Biology* 64:111–135.
Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL, Drake R. 2005. Improving the nutritional value of golden rice through increased pro-vitamin A content. *Nature Biotechnology* 23:482–487.
Prince VE, Pickett FB. 2002. Splitting pairs: diverging fates of duplicated genes. *Invited review, Nature Reviews Genetics* 3:827–837.
Pinheiro F, De Barros F, Palma-Silva C, Meyer D, Fay MF, Suzuki RM, Lexer C, Cozzolino S. 2010. Hybridization and introgression across different ploidy levels in the Neotropical orchids *Epidendrum fulgens* and *E. puniceoluteum* (Orchidaceae). *Molecular Ecology* 19:3981–3994.
Purugganan MD. 2014. An evolutionary genomic tale of two rice species. *Nature Genetics* 46:931–932.
Pusadee T, Jamjod S, Rerkasem B, Schoal B. 2016. Life-history traits and geographical divergence in wild rice (*Oryza rufipogon*) gene pool in Indochina Peninsula region. *Annals of Applied Biology* 168:52–65.
Saito T, Ichitan K, Suzuki T, Marubashi W, Kuboyama T. 2007. Developmental observation and high temperature rescue from hybrid weakness in a cross between Japanese rice cultivars and Peruvian rice cultivar “Jamaica.” *Breeding Science* 57:281–288. doi:10.1270/jsbbs.57.28
Saitoh K, Onishi K, Mikami I, Thidar K, Sano Y. 2004. Allelic diversification at the C (OsC1) locus of wild and cultivated rice: nucleotide changes associated with phenotypes. *Genetics* 168:997–1007.
Sang T, Ge S. 2007. The puzzle of rice domestication. *Journal of Integrative Plant Biology* 49:760–768.
Schluter D, Perrnell MW. 2017. Speciation gradients and the distribu- tion of biodiversity. *Nature* 546:48–55.
Sekeine D, Ohnishi T, Furumui H, Ono A, Yamada T, Kurata N, Kinoshita T. 2013. Dissection of two major components of the postzygotic hybridization barrier in rice endosperm. *The Plant Journal* 76:792–799.
Semon M, Nielsen R, Jones MP, McCouch SR. 2005. The population structure of African cultivated rice *Oryza glaberrima* (Steud.): evidence for elevated levels of linkage disequilibrium caused by admixture with *O. sativa* and ecological adaptation. *Genetics* 169:1639–1647.
Serra X, Esteve R, Peñas G, Catala MM, Melé E, Messegue J. 2013. Direct and reverse pollen-mediated gene flow between GM rice and red rice weed. AoB Plants 5:plt050; doi:10.1093/aobpla/plt050.
Sicard A, Lenhard M. 2011. The selfing syndrome: a model for studying the genetic and evolutionary basis of morphological adap- tation in plants. *Annals of Botany* 107:1433–1443.
Song Z, Lu B, Zhu Y, Chen J. 2002. Pollen competition between culti- vated and wild rice species (*Oryza sativa* and *O. rufipogon*). *New Phytologist* 153:289–296.
Suh HS. 2008. Weedy rice. Korea: Wild Crop Germplasm Bank, Yeungnam University.
Sweeney M, McCouch S. 2007. The complex history of the domestica- tion of rice. *Annals of Botany* 100:951–957.
Tan L, Li X, Liu F, Sun X, Li C, Zhu Z, Fu Y, Cai H, Wang X, Xie D, Sun C. 2008. Control of a key transition from prostrate to erect growth in rice domestication. *Nature Genetics* 40:1360–1364.
Tiffin P, Olson MS, Mylo LC. 2001. Asymmetrical crossing barriers in angiosperms. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 268:861–867.
Tripathi KK, Warrier R, Govila OP, Ahuja V. 2011. Biology of *Oryza Sativa L.* (rice). India: Series of Crop Specific Biology Documents, Ministry of Science and Technology.
Vaughan DA, Lu BR, Tomooka N. 2008. The evolving story of rice evolution. *Plant Science* 174:394–408.
Vaughan DA, Morishima H, Kadowaki K. 2003. Diversity in the Orzya genus. *Current Opinion in Plant Biology* 6:139–146.
Wong H, Vieira FG, Crawford JE, Chu C, Nielsen R. 2017. Asian wild rice is a hybrid swarm with extensive gene flow and feralization from domesticated rice. *Genome Research* 27:1029–1038.
Wendt T, Canela BF, Klein DE, Rios RI. 2002. Selfing facilitates repro- ductive isolation among three sympatric species of *Pitcairnia* (Bromeliaceae). *Plant Systematic Evolution* 232:201–212.
Wright CR, Windham MD. 1991. A model for divergent, allopatric speciation of polyploid pteridophytes resulting from silencing of duplicate-gene expression. *American Naturalist* 137:515–526.
Wright SJ, Kalisz S, Slotte T. 2013. Evolutionary consequences of self-fertilization in plants. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 280:20130–20133.
Yamagata Y, Yamamoto E, Aya K, Win KT, Doi K, Sobrizal, Ito T, Kanamori H, Wu J, Matsumoto T, Matsuoka M, Ashikari M, Yoshimura A. 2010. Mitochondrial gene in the nuclear genome induces reproductive barrier in rice. Proceedings of the National Academy of Sciences of the United States of America 107:1494–1499.

Yamamoto E, Takashi T, Morinaka Y, Lin S, Wu J, Matsumoto T, Kitano H, Matsuoka M, Ashikari M. 2010. Gain of deleterious function causes an autoimmune response and Bateson-Dobzhansky-Muller incompatibility in rice. Molecular Genetics and Genomics 283:305–315.

Yang J, Zhao X, Cheng K, Du H, Ouyang Y, Chen J, Qiu S, Huang J, Jiang Y, Jiang L, Ding J, Wang J, Xu C, Li X, Zhang Q. 2012b. A killer-protector system regulates both hybrid sterility and segregation distortion in rice. Science 337:1336–1340.

Yu X, Zhao Z, Zheng X, Zhou J, Kong W, Wang P, Bai W, Zheng H, Zhang H, Li J, Liu J, Wang Q, Zhang L, Liu K, Yu Y, Guo X, Wang J, Lin Q, Wu F, Ren Y, Zhu S, Zhang X, Cheng Z, Lei C, Liu S, Liu X, Tian Y, Jiang L, Ge S, Wu C, Tao D, Wang H, Wan J. 2018. A selfish genetic element confers non-Mendelian inheritance in rice. Science 360:1130–1132.

Zhang H. 2012. Phenotypic characterization and molecular bases of hybrid weakness in japonica rice (Oryza sativa L.). PhD Thesis, Yunnan Agriculture University, Kunming, China.

Zheng XM, Ge S. 2010. Ecological divergence in the presence of gene flow in two closely related Oryza species (Oryza rufipogon and O. nivara). Molecular Ecology 19:2439–2454.