

Polymorphism of postmating reproductive isolation within plant species

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Abstract Speciation can be viewed as the evolution of reproductive isolation between formerly interbreeding populations. Recent years have seen great advances in our understanding of the genetic mechanisms underlying postmating reproductive isolation during plant speciation. Nevertheless, little is known about the early stages of species divergence and the evolution of reproductive isolation at the *within* species level. Direct or indirect evidence indicates that intrinsic postzygotic mechanisms are prevalent and often polymorphic among allopatric conspecific populations of plants. We review studies that report direct or indirect evidence for polymorphism of genic (i.e., gene-based) postmating reproductive isolation within species' ranges. Specifically, we focus on three genic mechanisms often held responsible for reproductive isolation between species: Bateson-Dobzhansky-Muller (BDM) incompatibilities and two widespread types of genomic conflict, transmission ratio distortion and cytonuclear interactions. We further highlight the close similarity between reported cases of outbreeding depression among conspecific populations, especially those that correspond to the *intrinsic co-adaptation* model, and the origin of genetic incompatibilities. This association holds great promise to help improve our understanding of the processes involved in the initial stage of speciation, and it highlights the close (and often overlooked) relationship between evolutionary and conservation biology.

Keywords cytoplasmic male sterility; Bateson-Dobzhansky-Muller incompatibilities; outbreeding depression; speciation; transmission ratio distortion

■ INTRODUCTION

Speciation is a key process for the evolution of biological diversity and, under the biological species concept (Mayr, 1942), can be viewed as the evolution of reproductive isolation between formerly interbreeding populations (Coyne & Orr, 2004). The last decade has seen great progress in the study of individual components of reproductive isolation among many plant species pairs (Bradshaw & Schemske, 2003; Marques & al., 2007; Moccia & al., 2007; Pascarella, 2007; Yang & al., 2007; Waelti & al., 2008) and within several plant groups (Moyle & al., 2004; Scopece & al., 2007, 2008) and fascinating insights have recently been obtained into the understanding of the genetic basis of particular isolating barriers (reviewed in Widmer & al., 2009).

For sexually reproducing organisms, such as flowering plants, reproductive isolation is achieved through the joint action of premating and postmating barriers to interspecific gene flow (Grant, 1971). Typically the maintenance of species boundaries is the result of the combination of both processes, but the strength and permeability of premating and postmating barriers are highly variable among species and strongly depend on the degree of floral specialization.

Premating isolation includes geography, habitat, phenology, and other ecological features. In flowering plants, premating isolation is often based on pollinator specialization (Grant, 1971) and pollinator–flower interactions have been emphasized as a driving force for plant species differentiation (Stebbins,

1970). Postmating isolating mechanisms can act both before and after the fusion of parental gametes to form a zygote and are consequently categorized as pre- and postzygotic. Postmating prezygotic barriers include gametic incompatibility or pollen competition, while postzygotic barriers can be further separated into intrinsic (hybrid inviability and sterility) and extrinsic (ecological and behavioural sterility) barriers (Coyne & Orr, 2004). This traditional distinction may be overly simplistic, however, since the breakdown of coadapted gene complexes may affect intrinsic organismal processes in an environment-dependent manner.

While the important role of chromosomal changes in plant speciation is uncontroversial (see recent review by Rieseberg & Willis, 2007), less is known about the potential role of genic (i.e., gene-based) mechanisms in the origin of reproductive isolation. What we know about the genetic basis of postzygotic barriers in plants is that, as in animals, they often evolve according to the Bateson-Dobzhansky-Muller (BDM) model which states that genetic incompatibility in hybrids is produced by deleterious interactions between alleles that have followed independent evolutionary trajectories in each parental lineage (Dobzhansky, 1937; Muller, 1942). Using the adaptive landscapes representation of the relationships between genotypes and reproductive success (Wright, 1932), this model explains how two lineages can cross adaptive valleys without experiencing a substantial reduction of reproductive success and makes epistatic interactions between genes a major theme in speciation research (Orr & Presgraves, 2000). Briefly, in the

BDM model, mutations can accumulate *unopposed by selection* in each of two diverging populations. If these mutant alleles are combined in hybrids upon secondary contact, hybridization will be maladaptive because the new heterospecific allele combinations have never been tested by selection. Thus, reproductive isolation is achieved despite the presence of an adaptive valley separating the ancestral species and the newly arisen daughter species (Coyne & Orr, 2004).

Since the last part of the 19th century, numerous classical genetic studies (reviewed in Voilokov & Tikhenko, 2009) based on inter- and intraspecific crosses, and on the analysis of phenotypic trait segregation in hybrids, have revealed reproductive barriers not only between but also within species. However, only in recent years we made significant advances in the understanding of the genetic mechanisms underpinning reproductive isolation in plants. Up to now, a handful of potential plant speciation genes, i.e., genes directly involved in the evolution or maintenance of intrinsic postzygotic barriers, have been characterized at the molecular level. These include: hybrid sterility genes contributing to cytoplasmic male sterility (CMS), that is, the disruption of the coordination between nuclear and cytoplasmic genes inherited from different parents (Chase, 2007); genes involved in hybrid necrosis, i.e., autoimmune responses presumably mediated by rapidly evolving pathogen-resistant genes (Bomblies & Weigel, 2007); and transmission ratio distortion (TRD) genes causing hybrid sterility (Koide & al., 2008).

The determination of the genetic mechanisms underlying a reproductive barrier, however, is not sufficient by itself to fully comprehend the events characterizing the speciation process. Indeed, understanding the process of speciation also requires the determination of those isolating mechanisms acting during the first phase of species divergence (Coyne & Orr, 2004). This goal is particularly hard to reach since species are usually separated through a combination of different mechanisms, and the mechanisms maintaining species integrity upon secondary contact of previously diverged lineages do not necessarily reflect those that drove divergence during early stages of speciation. Reproductive isolating mechanisms can indeed evolve subsequently as a consequence of divergent selection or genetic drift or, in secondary contact zones, due to sympatric reinforcement (Coyne & Orr, 2004).

Reproductive isolation starts to arise within species and speciation occurs when one or more reproductive barriers are fixed and impede (or strongly limit) gene exchange between incipient species. The study of isolating mechanisms arising at the *intraspecific* level can thus shed light on the events occurring during early phases of divergence and can potentially help distinguish the genetic mechanisms that are likely to play a major role in speciation.

Despite the dearth of studies directly focused on this early phase of the speciation process in plants, i.e., on the evolution of incipient barriers that arise within species, direct evidence or indirect clues (such as those stemming from ecological studies testing reproductive compatibility of allopatric conspecific populations, i.e., outbreeding depression) indicate that postmating mechanisms can be prevalent and polymorphic among

allopatric conspecific plant populations. While it appears to be relatively easy to identify barriers to reproduction between sister species or at least between reciprocally monophyletic lineages, it is clear that the initial appearance and fixation of genetic factors is a population-level process.

Intrinsic postzygotic barriers caused by chromosomal rearrangements, even at the population level, are well documented within and among plant species (reviewed in Levin, 2002) and their central role in plant speciation has recently been highlighted (Rieseberg & Willis, 2007).

In this review, we will focus on polymorphism in genic (gene-based) isolation factors within species because their roles in plant speciation appear to be less well understood and more controversial than those of chromosomal rearrangements. Also, chromosomal rearrangements can not explain speciation in organismal groups with high levels of synteny suggestive of chromosomal stasis, a situation encountered in numerous enigmatic groups of plants (Lexer & Widmer, 2008) and animals (Ellegren, 2010). Therefore, we here examine those studies that report direct or indirect evidence of polymorphism of genic postmating reproductive isolation within species and especially those cases in which the genetic mechanisms underlying the phenomenon have been identified.

■ POLYMORPHISM OF INCOMPATIBILITY SYSTEMS WITHIN SPECIES

(1) Bateson-Dobzhansky-Muller incompatibilities. — Multilocus interactions (i.e., BDM incompatibilities) are the major source of hybrid inviability and sterility and the effects of nuclear-nuclear epistasis are generally considered to be the primary cause of intrinsic postzygotic reproductive barriers (Coyne & Orr, 2004; Noor & Feder, 2006). Among plants, a number of incompatibility systems have been genetically described in inter- and intraspecific combinations (reviewed in Bomblies & Weigel, 2007; Voilokov & Tikhenko, 2009). However, only a handful of such studies also investigated the allele distribution at the loci involved in this incompatibility offering the opportunity to detect the degree of polymorphism of reproductive barriers between recently diverged lineages. Arguably, among these studies, the model system *Mimulus* offers the most informative results on this topic. The first finding of postzygotic incompatibility in this genus dates back to Vickery (1978) who examined crossing barriers between species showing that interspecific postzygotic barriers were often incomplete and that there was a large variation among populations of the same species in the degree of postzygotic isolation. Indeed, the strength of postzygotic isolation in interspecific crosses could be either higher or lower than that observed in intraspecific crosses. The permeability of these barriers and its variability within species motivated more detailed and direct genetic analysis of hybrid incompatibilities in a population genetic context.

Building on these insights, Macnair & Christie (1983) performed the first direct genetic analysis of hybrid incompatibilities in *Mimulus*. They found that interpopulation hybrids

showed a simple genetic control of progressive necrosis, i.e., a gene from a population interacted with a number of genes from the other population. The dominant allele of the major gene was shown to control or to be strongly linked with the gene responsible for the resistance to copper ions. The resistant population occupies the area of a copper mine, and the presumed pleiotropy of the incompatibility factor may indicate fixation of a dominant allele through direct adaptive response. Complementary interaction of two other factors found in *M. guttatus* leads to necrosis of the interpopulation hybrids.

Interestingly, the two populations examined were shown to be polymorphic for incompatibility genes (Christie & Macnair, 1984). Such polymorphism for different incompatibility loci, with variation in more than 90% of the investigated populations, was also found in subsequent larger-scale experiments (Christie & Macnair, 1987). More recently, the existence of a simple genetic mechanism leading to the sterility of hybrids between *M. guttatus* and *M. nasutus* was demonstrated by Sweigart & al. (2006) by using two inbred lines for each species. From these lines, the authors produced near isogenic lines (NILs) carrying heterozygous fragments of the genome of one parent against the homozygous genetic background of the other parent and identified a dominant allele at locus *hms1* (hybrid male sterility 1). This allele, interacting in segregating hybrids with recessive alleles of locus *hms2* (hybrid male sterility 2) from *M. nasutus*, produces complete male and partial female sterility. Surprisingly, when estimating the levels of genetic polymorphism at the *hms1* and *hms2* genes, Sweigart & al. (2007) found that the *hms1* locus was polymorphic in a number of *M. guttatus* populations, while in other populations the incompatibility allele was absent or rare. In contrast, the *hms2* locus was represented in the *M. nasutus* population mainly by the incompatibility allele, which was absent or rare in the *M. guttatus* populations. These results suggest that the potential of *hms1* and *hms2* to form a reproductive barrier between the two species is limited but also that incompatibility loci can be polymorphic within a population or species (Sweigart & al., 2007).

Another thoroughly investigated case of hybrid incompatibility is one occurring in the model species *Arabidopsis thaliana*. Bomblies & al. (2007) found that a small amount (roughly 2%) of intraspecific crosses produced offspring expressing hybrid necrosis. By analysing the genetic architecture of this intraspecific polymorphic reproductive barrier, in one case, they characterized an allele that induces hybrid necrosis, when combined with a specific allele at a second locus (i.e., a simple two-locus BDM incompatibility). The acute *A. thaliana* hybrid necrosis cases can represent the phenotypic extremes of a quantitative distribution of epistatic interactions within populations that can be interpreted as a polymorphic intrinsic postzygotic barrier. In this study, the authors were also able to show that the allele responsible for hybrid necrosis was also involved in the plant immune response, suggesting that selective pressures related to host-pathogen conflict might indirectly cause the evolution of intrinsic postzygotic barriers, a mechanism that may be common among plants (reviewed in Bomblies & Weigel, 2007). Indeed, at such genes involved in biotic interactions, selection is likely to promote intraspecific

polymorphism because the availability of rare alleles confers an advantage to the host whilst pathogens tend to adapt to the more common host genotypes (Stearns & Hoekstra, 2005). Therefore, postzygotic isolation genes involved in plant immunity and other frequency-dependent processes may be particularly prone to be polymorphic within populations and species.

(2) Cytonuclear interactions. — Another potential source of postzygotic isolation is represented by cytonuclear epistatic interactions. Indeed, since cytoplasmic and nuclear genomes have conflicting evolutionary interests and are generally highly coevolved, cytonuclear incompatibilities may be among the first to evolve in recently diverged species (Fishman & Willis, 2006). Furthermore, the uniparental inheritance of cytoplasmic DNA can explain the high frequency of asymmetric reproductive barriers in animals and plants (Tiffin & al., 2001; Scopece & al., 2007; Turelli & Moyle, 2007) with dysfunctions generally arising earlier in males than in females.

Faster male sterility in animals is often due to interactions of genes on the X chromosomes with other genes located on autosomes and/or in cytoplasmic genomes. In hermaphroditic flowering plants, despite the lack of sex chromosomes, male sterility often evolves before female sterility in numerous wild and crop species (reviewed in Schnable & Wise, 1998).

Up to now, most quantitative trait locus (QTL) studies have not been designed to detect cytonuclear interactions and thus their importance could have been underestimated, particularly in incipient species. A polymorphic cytoplasm-dependent fertility barrier, yielding anther sterility, has recently been described in hybrids between the closely related *Mimulus guttatus* and *M. nasutus* (Fishman & Willis, 2006). In this study, the polymorphism of this cytonuclear barrier observed in hybrids could reflect the presence of cryptic cytoplasmic male sterility (CMS) factors that are fixed and masked by a restorer system within the parental populations.

The degree of polymorphism of reproductive isolation mechanisms produced by the disruption of cytonuclear interactions within species has been assessed only in a small number of cases. In this context, Levy (1991) found a highly variable asymmetric barrier, resulting in reduced pollen fertility, among different recognized or putative varieties of the *Phacelia dubia* complex. Intraspecific polymorphic cytonuclear barriers have also been found in *Campanulastrum americanum* (Galloway & Fenster, 1999) and *Plantago* (Van Damme & al., 2004). Similarly, at least one incompatibility involving interactions between cytoplasmic and nuclear genes was found by Skrede & al. (2008) in *Draba nivalis*. Wade & Goodnight (2006) suggested that the evolution of cytonuclear barriers might be accelerated in selfing species, such as *Draba*, because selection on cytonuclear combinations is more efficient if they are inherited together. *Draba nivalis* apparently evolved during the Pleistocene, thus the incompatibility barriers evolved quickly and exhibit extensive intraspecific polymorphism (Grundt & al., 2006). The cryptic arctic *Draba* species are not ecologically or morphologically differentiated, thus suggesting that genetic drift due to the strongly selfing breeding system rather than selection is responsible for the evolution of sterility barriers (Grundt & al., 2006). Furthermore, being autogamous, *Draba* species are not

exposed to the filter of interspecific mating that can promote (or impede) the fixation of reproductive incompatibilities (Wu & Ting, 2004). As a consequence, a high level of polymorphism of reproductive barriers is expected between closely related taxa. Such polymorphism is confirmed by the finding of several plants producing no pollen among the intraspecific hybrids in their crossing experiments (Grundt & al., 2006).

Gynodioecious species, i.e., species in which male sterile (functionally female) and hermaphroditic morphs coexist within populations, represent a good model for studying the evolution of CMS. Indeed, the evolution and maintenance of this reproductive system depends on the persistent polymorphisms of CMS genes and of nuclear restorers of male fertility. In this context, Landergott & al. (2009) found that sex-determining alleles in *Thymus praecox* were geographically widespread, but within-population crosses produced significantly higher proportions of hermaphrodites compared with among-population crosses, suggesting the local variability of restorer frequencies in allopatric populations.

In a second gynodioecious species, *Silene vulgaris*, Charlesworth & Laporte (1998) showed that the genetic basis of sex determination is cytonuclear and is complicated by the likelihood that multiple restorers exist within populations as is expected, since once populations with CMS factors have been restored, they are susceptible to invasion of new CMS factors (Taylor & al., 2001). Molecular genetic studies of male sterility suggest that CMS factors are usually mitochondrial mutants (Saumitou-Laprade & al., 1994) and Taylor & al. (2001) found a strong variance in mitochondrial haplotype frequencies among populations of *S. vulgaris*. The assessment of polymorphism in polygenic traits such as CMS in *S. vulgaris* is difficult, but intraspecific crosses over large geographical distances detected differences in nuclear restorer frequencies, thus suggesting significant genetic structure for genes that determine sex, likely reflecting invasion events of migrants from different populations (Bailey & McCauley, 2005). Even if this mechanism occurs *within* gynodioecious species, similar types of genomic conflict can potentially be responsible of the insurgence of reproductive barriers between incipient non-gynodioecious species. Indeed, while in gynodioecious species CMS genes and nuclear restorers of male fertility are persistently polymorphic within species, in non-gynodioecious species, CMS-induced genomic conflict can result in the evolution of reproductive incompatibility between diverging populations (Futuyama, 2009).

(3) Transmission ratio distortion. — The life cycle of sexually reproducing organisms begins with the fusion of male and female gametes to form a zygote. Transmission of alleles in the offspring progeny generally follows Mendelian rules. However, under different circumstances, one of a pair of alleles can be preferentially recovered in the progeny of a heterozygote. Distortion factors typically modify Mendelian ratios to their own advantage (Lyttle, 1991) and, due to the loss of certain gamete haplotypes, impose a fertility cost on their bearers. The genes that underlie this reduction of fitness are considered to be involved in reproductive barriers, which might be important in driving incipient species to become an independent genetic entity (Coyne & Orr, 2004; Wu & Ting, 2004).

Transmission ratio distortion associated with gametic dysfunction has frequently been detected in inter- and intraspecific hybrids of plants (Crow, 1991; Lyttle, 1991) and increasing evidence testifies of genetic markers showing significant TRD across a wide range of taxonomic groups (see Moyle & Graham, 2006). Nonetheless, there is currently little empirical evidence for the predominance of active segregation distortion mechanisms as a direct cause of hybrid sterility or inviability in plants and for their polymorphism within species. So far, the best contribution to understanding the role of TRD in plant evolution is that from Koide & al. (2008) who reported an analysis of the fine-scale structure of a transmission ratio distortion locus that causes both pollen and ovule sterility. These authors showed that this locus contributes to F_1 semi-sterility between Asian and African rice species complexes (respectively, *Oryza glaberrima* and *O. sativa*). However, whether hybrid sterility is induced by a given locus depends on its allelic state, and patterns of allelic differentiation (i.e., allele frequency distributions) have rarely been investigated to date. TRD was frequently reported in the S1 region in the cross combination between *O. glaberrima* and *O. sativa* (Yabuno, 1990; Doi & al., 1998; Lorieux & al., 2000). In a survey of allelic diversity at the S1 locus, at least for the major component of the locus that causes male TRD (mTRD), all varieties of the Asian rice species examined bore the allele, while the S1 locus was polymorphic in the African rice species. Because it was fixed in the Asian species, the S1 locus potentially causes F_1 hybrid sterility in any pairwise combination, which leads to a reproductive barrier between these rice species.

Even if this case represents an outstanding contribution to the current understanding of the genetic underpinnings of TRD loci contributing to postzygotic reproductive barriers among plant species, the importance of this mechanism in the early phases of species divergence in plants still remains unproved (Moyle, 2008). Some clues suggest that TRD mechanisms can be polymorphic within species even if a direct link with offspring fertility has not been established. This is the case, for instance, of different varieties of *Oryza sativa* where it has been shown that the fertility of inter-variety hybrids can vary from fully fertile to completely sterile (Liu & al., 1996; Zhang & al., 1997) and segregation distortion was observed at several loci (Lin & al., 1992; Kinoshita, 1995; Lu & al., 2000). Another case of TRD polymorphism within species has been found between two ecotypes of *Silene vulgaris* with different levels of tolerance to heavy metals (Bratteler & al., 2006), suggesting that the number of loci deviating from a proposed Mendelian ratio can be correlated with increased levels of divergence among parents (see also Jenczewski & al., 1997; Whitkus, 1998).

To date, several loci with significantly distorted transmission ratios have been detected in a wide range of plant species but, in most cases, their role in the evolution of postzygotic mechanisms is still unclear. In *Draba nivalis*, for instance, no association between TRD loci and fertility QTLs was found (Skrede & al., 2008) suggesting that several different genetic mechanisms underlie intrinsic postzygotic isolation in this arctic species, including underdominant QTLs, cytonuclear and nuclear-nuclear epistatic interactions. Further investigations

testing the correlation between TRD and QTLs for fertility are thus needed to understand the relative importance of this mechanism in the evolution of hybrid sterility or inviability.

The widespread finding of TRD among plants however is difficult to explain only with meiotic drive factors. Indeed, in plants, another remarkable source of transmission ratio distortion is represented by both male–male and male–female gametophytic interactions. Such interactions are particularly important among plants since, in contrast to animals, haploid gene expression in gametophytes is common (e.g., approximately 20% of genes are expressed in the pollen of *Arabidopsis thaliana*: Becker & al., 2003; Bernasconi & al., 2004). This pattern highlights the potential relevance of male–male and male–female interactions during the gametophytic phase in plants and their potential contribution to the origin of transmission ratio distortion and, as a consequence, of reproductive isolation mechanisms.

Competitive gametophytic interactions among plants, referred to as conspecific pollen precedence (CPP: Howard, 1999), have been reported among species of *Helianthus* (Rieseberg & al., 1995), *Iris* (Carney & Arnold, 1997), *Mimulus* (Ramsey & al., 2003), *Senecio* (Chapman & al., 2005), *Ipomopsis* (Aldridge & Campbell, 2006), *Zea* (Kermicle, 2006), *Phlox* (Ruane & Donohue, 2008), and *Silene* (Rahme & al., 2009). In most of these cases the contribution of CPP to total reproductive isolation between closely related taxa has been found to be among the most important (reviewed in Lowry & al., 2008). Such gametophytic interactions have long been recognized as a potentially major source of species barriers (Dobzhansky, 1937) and pollen-specific mediated TRD often explains a large proportion of non-Mendelian segregation between and within plant species (see Fishman & al., 2008).

In plants, the molecular background of the species-specificity of male–female gametophyte interactions is well established in *Arabidopsis* (Swanson & al., 2004), but its evolutionary significance has been little explored. Genetic factors involved in this kind of barriers have been mapped in several plants. Fishman & al. (2008) found that CPP in *Mimulus* hybrids is polygenic and is the main cause of interspecific TRD genome-wide, and that several genomic regions contribute to the transmission advantage of *M. guttatus* pollen on *M. guttatus* styles. Furthermore, the authors found that these male-specific transmission ratio distorting loci (TRDLs) could account for all the observed precedence of *M. guttatus* pollen over *M. nasutus* pollen in mixed pollinations of *M. guttatus*. These findings suggest that species-specific differences in pollen tube performance accumulate gradually and may have been driven by coevolution between pollen and style in the outcrossing *M. guttatus*. Furthermore, this polygenic background suggests that CPP is potentially polymorphic in *Mimulus* populations. This evidence of a polymorphism in genic isolating barriers has also been interpreted as prove of the minor relevance of genic mechanisms in plant speciation, as opposed to the hypothesized primary role of chromosomal rearrangements (Rieseberg & Willis, 2007).

The role of male–female gametophyte interactions within species has been investigated in a few taxa. In this context,

Kermicle (2006) found that some populations of the teosintes of Mexico (all subspecies of *Zea mays*) were unreceptive to the conspecific maize pollen because, when present in the pistil, several maize genes discriminate against or exclude pollen lacking the same allele. An analogous gene was found in several teosinte populations, but never in sympatric or parapatric maize populations. Interestingly, this gene (*Tcb1-s*) was polymorphic among wild populations of teosinte, but regularly present in populations growing in close association with maize, suggesting that, in the absence of maize, *Tcb1-s* is not strongly associated with fitness, while in the presence of maize, *Tcb1-s* appears to have been co-opted to provide reproductive isolation as a response to maladaptive hybridization. These results show that natural selection on an intraspecific polymorphic reproductive barrier, under particular circumstances, could lead to its fixation.

(4) Outbreeding depression as a potential source of data

for future research. — To date, only a limited number of studies have investigated the genetic basis of reproductive barriers within species. However during the last two decades, with the increasing interest in ecological restoration programs, a noteworthy amount of data on the crossing compatibility of different conspecific populations has been accumulated in the scientific literature (reviewed in Hufford & Mazer, 2003). Although such studies often report a great reduction of fertility among conspecific geographically isolated populations (the so-called outbreeding depression), they have generally been ignored by evolutionary biologists. Also, the genetic basis of outbreeding depression often remained undetermined, because it was not the primary scope of these studies. Nonetheless, some cases of outbreeding depression can be interpreted as incipient isolating barriers, and can thus be studied in order to understand early phases of reproductive isolation. Furthermore, outbreeding depression is typically variable (i.e., a proportion of fertility still exists) suggesting that the reduction of fertility in different populations of a species can represent a polymorphic stage of reproductive isolation that, depending on different factors, can either be fixed (leading to speciation) or reabsorbed (leading to the maintenance of species integrity). Independently from its subsequent fate, the evolution of outbreeding depression among populations can be a consequence of different kinds of interactions between genotypes and, in a few cases, outbreeding depression has been shown to be correlated with some of the genetic mechanisms reviewed here. For instance, Etterson & al. (2007) found that different populations of the autotetraploid species *Campanulastrum americanum* showed strongly asymmetric outbreeding depression and attributed these asymmetries to cytoplasmic and/or cytonuclear interactions.

Two main models have been proposed for the evolution of outbreeding depression (i.e., *under-dominance* and *intrinsic co-adaptation*: Hufford & Mazer, 2003). Outbreeding depression can arise between populations that experienced divergent natural selection, and which have become fixed for different alleles (Roff, 1998; Fenster & Galloway, 2000a,b; Montalvo & Ellstrand, 2000). In such a situation, F_1 hybrids heterozygous for locally adapted alleles at different loci can suffer a dilution of each differently adapted parental genome. The resulting

Table 1. Studies to detect outbreeding depression in hybrid generations beyond the F₁.

Species	Hybrid generation	Traits involved in hybrid breakdown	Reference
<i>Agrostemma githago</i>	F ₂	Shoot biomass	Keller & al., 2000
<i>Anchusa crispera</i>	F ₂	Flowers per cyme and number of cymes	Quilichini & al., 2001
<i>Avena barbata</i>	F ₆	Hybrid fitness	Johansen-Morris & Latta, 2006
<i>Campanulastrum americanum</i>	F ₂	Seed weight, days to germination, leaf size	Etterson & al., 2007
<i>Chamaecrista fasciculata</i>	F ₂ , F ₃	Fruit production	Fenster & Galloway, 2000a
<i>Papaver rhoeas</i>	F ₂	Hybrid survival and shoot biomass	Keller & al., 2000
<i>Silene alba</i>	F ₂	Seed and shoot biomass	Keller & al., 2000

under-dominance (i.e., heterozygote disadvantage) can thus be expressed in F₁ hybrids in the native environments of both parents. In this context, a prominent role is thus supposed to be played by divergent natural selection acting upon isolated populations. This kind of outbreeding depression has been widely reported and, depending on the strength and the direction of natural selection can also occur within the same (subdivided) population (Grindeland, 2008). This *under-dominance* model provides interesting insights for the understanding of the action of natural selection in isolated populations but is likely to be of limited interest in a speciation context. The allele segregation in F₂ and subsequent hybrid generations can quickly restore homozygosity and thus raise offspring fitness to normal levels.

Most studies on outbreeding depression only report F₁ performance and thus its relative occurrence in later hybrid generations may be seriously underestimated. However, in the literature there is clear evidence for a decrease of fertility in hybrid generations subsequent to the F₁ generation, affecting different life history traits (see Table 1). This type of outbreeding depression follows a different mechanism, namely the hybrid breakdown caused by the loss of co-adapted gene complexes (i.e., *intrinsic co-adaptation*: Templeton, 1986; Fenster & Galloway, 1997, 2000a; Roff, 1998). Hybrid breakdown results from the recombination or shuffling of adaptive multi-locus gene combinations during sexual reproduction and is generally not expressed until the F₂ generation or later because the F₁ hybrids still possess two intact, adaptive multi-locus gene combinations (one from each parent). Hybrid breakdown occurs in the second or later hybrid generations, when recombination between the different co-adapted gene complexes can occur. Ultimately, under this model, hybrid breakdown is the reduction in fitness associated with the disruption of epistatic interactions among loci (Hufford & Mazer, 2003). This mechanism corresponds well to the BDM model of interspecific genetic incompatibility which states that postzygotic isolation arises in allopatry as a secondary effect of evolutionary divergence. Thus, cases of outbreeding depression fitting with this model can be of major interest for studies on the evolution of postzygotic isolation due to epistatic gene interactions (such as those reviewed in Bomblies & Weigel, 2007).

Assessing the genetic background of outbreeding depression can be crucial in order to understand its potential role in the evolution of reproductive isolation. Moreover, the distinction between the *under-dominance* and *intrinsic co-adaptation* models can help to identify new model systems for further and

more detailed studies. Finally, the close relationship between outbreeding depression among conspecific populations and the origin of incompatibilities during speciation highlights the need to bridge such seemingly disparate fields as restoration ecology and evolutionary genomics, for the benefit of both.

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