The Genetics and Ecology of Reinforcement

Implications for the Evolution of Prezygotic Isolation in Sympatry and Beyond

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Reinforcement, the evolution of prezygotic reproductive barriers by natural selection in response to maladaptive hybridization, is one of the most debated processes in speciation. Critics point to "fatal" conceptual flaws for sympatric evolution of prezygotic isolation, but recent theoretical and empirical work on genetics and ecology of reinforcement suggests that such criticisms can be overcome. New studies provide evidence for reinforcement in frogs, fish, insects, birds, and plants. While such evidence lays to rest the argument over reinforcement's existence, our understanding remains incomplete. We lack data on (1) the genetic basis of female preferences and the links between genetics of pre- and postzygotic isolation, (2) the ecological basis of reproductive isolation, (3) connections between prezygotic isolation between species and within-species sexual selection (potentially leading to a "cascade" of effects on reproductive isolation), (4) the role of habitat versus mate preference in reinforcement, and (5) additional detailed comparative studies. Here, we review data on these issues and highlight why they are important for understanding speciation.

Key words: speciation; reinforcement; reproductive isolation; female preferences; assortative mating; genetic incompatibilities; natural selection

Introduction

Since Darwin, scientists have strived to understand the evolutionary forces that drive speciation (Dobzhansky 1937b; Mayr 1963). In particular, can the formation of new species be favored by natural selection? One popular theory in which natural selection drives speciation is termed "reinforcement." Under this mode of speciation, natural selection strengthens prezygotic reproductive isolation (for example, mate discrimination) as a response to maladaptive hybridization between co-occurring (or sym-

patric) diverging species (Dobzhansky 1940; Howard 1993; Noor 1999). Subsequently, hybridizing populations can achieve full reproductive isolation when alleles that confer mate discrimination spread to the entire species (Servedio & Kirkpatrick 1997). Importantly, many types of costs to hybridization, including both ecological and genetic dysfunctions in hybrid offspring that reduce their fitness, can drive reinforcement. The process of reinforcement is usually detectable before species reach full prezygotic isolation: because selection against hybrids only occurs in sympatry, then pairs of populations in regions where hybridization occurs will exhibit stronger prezygotic isolation than pairs of populations in regions where hybridization is absent (allopatry). This pattern of greater prezygotic isolation in sympatry relative

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to allopatry is one of the main "signatures" of reinforcement and has been termed reproductive character displacement (RCD, Servedio & Noor 2003).

Reinforcement suffers from several major theoretical obstacles, which have been reviewed extensively elsewhere and are therefore treated only briefly here (Felsenstein 1981; Sanderson 1989; Howard 1993; Butlin 1995; Servedio & Noor 2003; Coyne & Orr 2004). Most of these obstacles involve the "swamping" effects of gene flow. In particular, hybridization (with gene flow) between populations or species in regions of sympatry acts as a homogenizing process, which can prevent divergence via reinforcement. In addition, an influx of "less discriminating" alleles from allopatry into sympatry can weaken the strength of discrimination against heterospecific individuals, thereby constraining reinforcement (see Coyne & Orr 2004 for a review). Finally, it can be difficult for alleles contributing to reinforcement to spread out of regions of immediate sympatry because such alleles may be neutral or even disadvantageous in other regions (that is, in allopatry) (Table 3, Fig. 1). Additionally, from an empirical perspective, several processes other than reinforcement can generate the pattern of RCD, and ruling out these alternatives to reinforcement can be difficult because it relies on the presence of stringent—and often unavailable—controls (Rundle & Schluter 1998; Noor 1999).

Despite these obstacles, there are convincing examples that reinforcement operates in nature (Noor 1995; Saetre et al. 1997; Rundle & Schluter 1998; Hoskin et al. 2005; Silvertown et al. 2005; Jaenike et al. 2006; Kronforst et al. 2007; Nosil et al. 2007; Urbanelli & Porretta 2008), and this process appears to have contributed to the origin of diverse lineages of butterflies, fish, fruit flies, birds, walking stick insects, frogs, and flowering plants (e.g., Noor 1997; Lukhtanov et al. 2005; Kay & Schemske 2008). Servedio and Noor (2003) reviewed the empirical and theoretical evidence for reinforcement and highlighted five areas that were particularly poorly understood, and thus war-
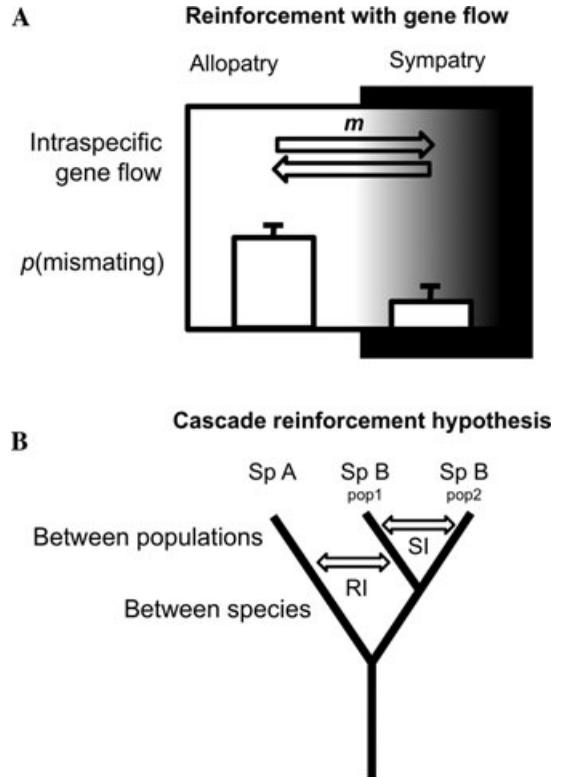
Figure 1. The signatures of speciation by reinforcement. (**A**) Persistence of increased prezygotic isolation in sympatry despite ongoing gene flow between sympatric and allopatric populations of one species. (**B**) The evolution of prezygotic isolation between two sympatric species (RI), could lead to the evolution of sexual isolation among populations within species (SI), despite ongoing gene flow. This would lead to the persistence of the signature of reinforcement, and possibly to the origin of another species (the "cascade effects" hypothesis, see text for details). *m* refers to migration rate among conspecific sympatric and allopatric populations of a species undergoing reinforcement. *p* (mismating) is the probability that a female from a given area mates with a heterospecific male versus a conspecific male.

rant further attention. First, the genetic basis of female preferences and the link between the genetics of prezygotic and postzygotic isolation have only been dissected in a few cases of reinforcement. As discussed below, certain genetic architectures facilitate reinforcement and help overcome many of the theoretical obstacles associated with the homogenizing effects of gene flow on speciation. Second, the

ecological basis of prezygotic and postzygotic reproductive isolation is poorly documented. If ecological sources of selection against hybrids are widespread, then the selection that drives reinforcement itself could be more common than currently thought. Third, connections between the evolution of prezygotic isolation between species and sexual selection within species are likely, but poorly understood. Fourth, habitat preferences might also be reinforced, but the relative roles of habitat versus mate preference in reinforcement are relatively unexplored both theoretically and empirically. In particular, it is unknown how much these different mechanisms of reinforcement might facilitate or constrain one another. Fifth, comparative studies outside of *Drosophila* are scarce, yet are necessary if any broad generalities about patterns for the evolution of reproductive isolation are to be inferred. Here we review and synthesize recent work that addresses these unresolved issues and suggest new avenues for testing the importance of reinforcement on the origins of biodiversity.

The Genetic Basis of Reinforcement: Inversions, Sex Chromosomes, and One-Allele Assortative Mating Mechanisms

Reinforcement in the traditional sense relies on genetic associations between prezygotic isolation (including assortative mating) and postzygotic reproductive isolation (that is, low hybrid fitness). This is because such associations are required for selection against hybrids, to result in the evolution of reproductive prezygotic isolation. Selection against hybrids itself might arise from either extrinsic (ecological) or intrinsic causes (such as genetic incompatibility) (Coyne & Orr 2004). However, reinforcement in the "broad sense" (Servedio & Noor 2003) can arise via any type of reproductive cost driving the evolution of prezygotic isolation, including costs that arise prior to hybrid formation. For example, pure species individ-

uals may waste time or energy courting heterospecifics that always reject them (Schluter 2001), which might have further negative fitness consequences such as increasing predation risk (Albert & Schluter 2004). In these scenarios, failed reproductive attempts before the formation of zygotes can also create enough selective pressure to promote the evolution of prezygotic isolation (Higgie et al. 2000). For simplicity, we discuss in subsequent sections the connections between prezygotic and postzygotic reproductive isolation, but acknowledge that prezygotic isolation might evolve in response to any cost to hybridization, including costs associated with simply attempting to hybridize.

One of the fundamental problems of the theory of reinforcement is explaining how such genetic associations between prezygotic and postzygotic isolation are maintained when confronted with the homogenizing effects of gene flow (Felsenstein 1981). Certain genetic scenarios mitigate this problem. For example, regions of low recombination, like sex chromosomes (Lemmon & Kirkpatrick 2006; Saether et al. 2007), and chromosomal inversions (Noor et al. 2001b), can help maintain linkage disequilibrium among genes within them (Ortiz-Barrientos et al. 2002; Butlin 2005). Thus, reinforcement is predicted to be possible when genes affecting hybrid fitness and assortative mating reside within regions of reduced recombination.

There are other genetic scenarios in which linkage disequilibrium between forms of reproductive isolation is maintained. One stems from the conceptual distinction between one-allele and two-allele models described by Felsenstein (1981) (see Servedio 2009 for a detailed review of these mechanisms). In a one-allele mechanism, reproductive isolation is caused by the same allele fixing in both populations (for example, an allele causing individuals to prefer mates phenotypically similar to themselves). Such alleles cause nonrandom mating regardless of genetic background (assuming the populations differ already in the trait that is being used as a mating cue; for instance courtship
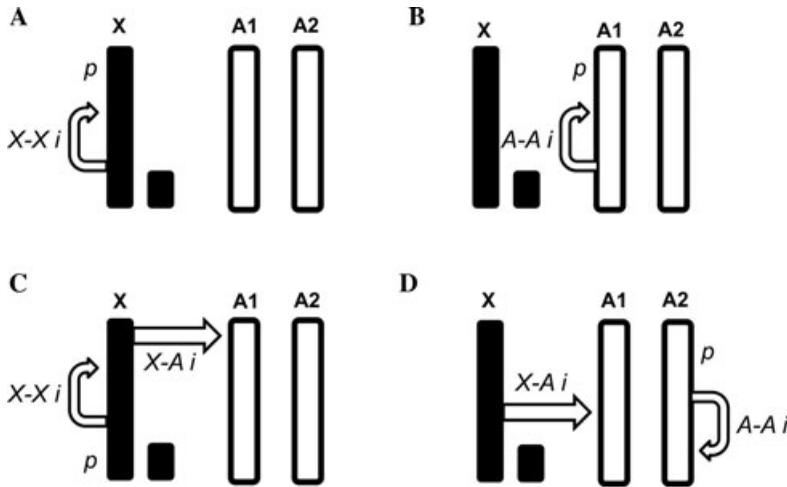
Figure 2. The most conducive genetic architectures for the evolution of prezygotic isolation in response to maladaptive hybridization (following Lemmon & Kirkpatrick 2006). X and A denote sex and autosomal chromosomes, respectively, *p* the female preference, and X-A, A-A, or X-X genetic incompatibilities between species, whose interaction is denoted with an arrow. (**A, B**) Both preference and genetic incompatibilities have the same mode of inheritance; (**C, D**) X-A genetic incompatibilities are required regardless of the location of the preference. Note, however, that the second type of genetic incompatibility (X-X or A-A) is favorable depending on the location of the preference.

song, body size, or pheromones might already differ between the two hybridizing species). In a two-allele mechanism, different alleles fix in each population (for example, a preference allele for large individuals in one population and a different preference allele for small individuals in the other).

This distinction is important when considering the effects of recombination: recombination in a two-allele mechanism breaks down linkage disequilibrium, weakening the genetic association between genes affecting pre- and postzygotic isolation, while in a one-allele mechanism, linkage disequilibrium between pre- and postzygotic isolation exists automatically and cannot be reduced by recombination. Similarly, alleles that affect a trait which in turn affects both post- and prezygotic isolation (or "magic traits" [Gavrilets 2004]) also facilitate the evolution of prezygotic isolation in the face of gene flow. In both the one-allele and magic-trait scenarios, linkage disequilibrium between forms of reproductive isolation is unopposed by recombination (Servedio 2009). Thus, it is possible, for instance, that a single allele can spread be-

tween diverging populations and complete the speciation process.

Figure 2 depicts some of the most conducive genetic architectures for reinforcing prezygotic isolation (based on Lemmon & Kirkpatrick 2006). When genetic incompatibilities and mate preference genes share the same inheritance (i.e., are on the same chromosome; Fig. 2A, B), or when genetic incompatibilities and mate preference genes are between the sex chromosome and an autosome (Fig. 2C, D), prezygotic isolation usually spreads (Kelly & Noor 1996; Lemmon & Kirkpatrick 2006). This suggests a special role for the X (or Z) chromosome in reinforcement, most likely due to its reduced recombination rate and because of its pronounced effects on the heterogametic sex (because recessive genetic incompatibilities are covered in heterozygotes, but expressed phenotypically in homozygotes).

Although few studies have looked in detail at the genetics of reinforcement, several studies have looked at the genetics of hybrid sterility (Table 1 and references therein) or inviability (Adam et al. 1993; Barbash et al. 2000;

TABLE 1. Studies Examining the Genetic Basis of Hybrid Sterility

System	Methodology	Findings	Implications	Reference
Ceratopteris richardii races	Mendelian segregation tests throughout the genome of hybrid populations of haploid lines and F2s	A large number of loci departed from Mendelian segregation, but a single QTL for spore viability was identified	Genetic incompatibilities, possibly involving cytoplasmic effects, cause hybrid sterility	(Nakazato et al. 2007)
Drosophila simulans	Molecular characterization of a sex-ratio distortion/ suppressor system	X-linked distorter evolved from retroposition from another gene on the X. Sex-ratio suppressor is an autosomal retroposon gene of the distorter. RNAi is a likely mechanism of suppression.	The genetics of hybrid sterility may result from genetic incompatibilities evolving by intragenomic conflict within species	(Tao et al. 2001; Tao, Araripe, et al. 2007; Tao, Masly, et al. 2007)
D. mauritiana x D. sechellia	Introgression of *D. mauritiana* chromosome segments into *D. sechellia*	Most introgression segments cause hybrid male sterility (compared to female sterility and general hybrid inviability) and act recessively. There is a disproportional contribution of the X to hybrid sterility.	Haldane's rule: Both dominance and faster-male theories are supported; Large X effect: sex-ratio distortion, dosage compensation or X inactivation	(Masly & Presgraves 2007)
D. pseudoobscura	Introgression experiments of X-linked regions between subspecies of *D. pseudoobscura*	Sterility and segregation distortion map to the same genetic locus	Genetic incompatibilities between species and suppressed systems of meiotic drive within species share genetic basis	(Orr & Irving 2005; Orr et al. 2007)
D. simulans x D. mauritiana	Introgression of *D. mauritiana* 3rd chromosome segments into *D. simulans*	Identification of a dominant autosomal suppressor of an X-linked sex-ratio distortion in *D. simulans* that also causes hybrid male sterility	Genetic incompatibilities between species and suppressed systems of meiotic drive within species share genetic basis	(Tao, Araripe, et al. 2007; Tao, Masly, et al. 2007)
D. simulans x D. mauritiana	Introgression of *D. mauritiana* chromosome segments into *D. simulans*	Identification of a rapidly evolving homeobox gene	Divergence in both expression and DNA sequence contributes to hybrid sterility	(Ting et al. 1998; Sun et al. 2004)
D. simulans x D. mauritiana, D. sechellia	Comparison of spermatogenesis genes expression profiles between parentals and their hybrids	Significant overlap on missexpression profiles between hybrids between different pairs of species. Spermatogenesis disruption appear to be postmeiotic.	Regulatory divergence between species in spermatogenesis genes may lead to transcriptional failure in their hybrids	(Moehring et al. 2007)

TABLE 1. *Continued*

System	Methodology	Findings	Implications	Reference
D. simulans x D. melanogaster	Homozygous introgression of *D. simulans* chromosome segments into a *D. melanogaster* background and deficiency mapping of sterility gene	Gene, JYAlpha, locates to different chromosomes in the two species. Sterility results from the absence of the gene in the homozygous introgression line.	Mechanisms, other than typical genetic incompatibilities, may cause hybrid sterility	(Masly et al. 2006)
D. yakuba x D. santomea	Backcross genetic mapping of hybrid sterility loci	Disproportional contribution of the X, and colocalization of hybrid sterility loci with those causing pigmentation differences between species	Large X-effect and possible adaptive evolution of pigmentation genes contribute to hybrid sterility	(Moehring et al. 2006a, 2006b)
Mimulus guttatus x M. nasutus	Backcross and nearly isogenic lines genetic mapping of sterility loci	A two-locus dominant–recessive incompatibility causes nearly complete male sterility	In contrast to *Drosophila* studies, the genetic basis of sterility is simple	(Sweigart et al. 2006)
Mus musculus and *M. domesticus*	Reciprocal introgression of X-chromosomes between 2 species	Asymmetric X-linked hybrid sterility. QTL on X contains few genes including a fast-evolving spermatogenesis gene	Possible large-X effect; recessive X-linked genetic incompatibilities, some polymorphic	(Good et al. 2008a, 2008b)
Oryza sativa x O. japonica	Map-based cloning of hybrid sterility genes	Three alleles from an aspartic protease contributes to patterns of female gamete abortion in hybrids	Incipient speciation shows genetic variability for alleles causing reproductive isolation	(Chen et al. 2008)
Saccharomyces cerevisiae	Experimental evolution of the early stages of speciation	Evolved sterility involves genetic incompatibilities and not underdominant effects or antirecombinational effects	Ecological adaptation can lead to the evolution of postzygotic isolation	(Dettman et al. 2007)
S. cerevisiae x S. paradoxus	Introgression of *S. paradoxus* chromosomes into *S. cerevisiae* background	No chromosomal introgression caused death of hybrid gametes	Recessive genetic incompatibilities do not cause yeast hybrid sterility	(Greig 2007, 2008)
Solanum pennellii, S. habrochaites x S. lycopersicum	Nearly isogenic lines mapping	Pollen and seed sterility	Similar number of genomic regions contributing to pollen sterility and other hybrid dysfunctions; QTL for pollen and seed sterility are colocalized	(Moyle & Graham 2005; Moyle & Nakazato 2008)
X. laevis and *X. muelleri*	Micro-RNA expression profiling in parentals and their hybrids	Hybrid testis are depleted of micro-RNAs relative to parentals	Imprinting and transgenerational effects may lead to hybrid sterility	(Michalak & Malone 2008)

TABLE 2. Concurrent Exploration of the Genetic Architecture of Prezygotic and Postzygotic Isolation

System	D	Trait	Autosomal	Sex-linked	Locus effect	Number of QTL	Epistasis	References
					Prezygotic isolation			
Solanum lycopersicum x S. esculentum, S. habrochaites	~1 MY	Floral morphology	Yes	N.A.	Weak	2–6	N.A.	(Moyle 2005; Moyle 2007; Moyle & Nakazato 2009)
Drosophila melanogaster Z and M races	~0.1 MY	Cuticular hydrocarbons	Yes	No	One major single locus	3	Significant but weak effects	(Takahashi et al. 2001; Greenberg et al. 2003)
D. pallidosa x D. ananassae	Recent	Female choice	Yes	Yes	One major autosomal locus	3	Not detected	(Shug et al. 2007; Doi et al. 2001; Sawamura et al. 2006b)
D. santomea x D. yakuba	~0.4 MY	Components of male and female discrimination	Yes	No	Few autosomal with moderate to large effects; X-effects negligible	At least 3 for discrimination and 3 for male mating success	Not detected	(Moehring et al. 2006)

System	D	Trait	Single incompatibilities	Autosomal	Sex-linked	Locus effect	Negative epistasis	References
					Postzygotic isolation			
Solanum lycopersicum x S. esculentum, S. habrochaites	> 1 MY	Pollen and seed fertility	Possibly few	4–11 QTL	N.A.	Strong	Possibly rare	(Moyle & Graham 2005; Moyle 2007; Moyle & Nakazato 2008)

Continued

TABLE 2. *Continued*

System	D	Trait	Single incompatibilities	Postzygotic isolation		Locus effect	Negative epistasis	References
				Autosomal	Sex-linked			
D. mauritiana x *D. sechellia*	~0.3 MY	Hybrid viability and fertility	Unknown	3 viability and 8 sterility QTL	1 viability and at least 9 sterility QTL	Strong and fairly recessive	Common	(Masly & Presgraves 2007)
D. pallidosa x *D. ananassae*		Hybrid breakdown	Unknown	2 QTL	Not detected	Possibly moderate recessive–recessive interactions	Present	(Sawamura et al. 2006)
D. santomea x *D. yakuba*	~0.4 MY	Hybrid behavioral sterility	Unknown	3 QTL	Possibly 1 QTL when X is from *D. santomea*	Autosomal effects are strong	Not detected	(Moehring et al. 2006a)

D, divergence time; MY, million years before the present; N.A., not applicable; QTL, Quantitative Trait Loci.

Presgraves et al. 2003; Presgraves & Stephan 2007), and the genetics of female preferences in general (Table 2 and references therein, see also Etges & Noor 2002; Arbuthnott in press). Many studies on the genetics of hybrid sterility have found strong genetic effects on the X chromosome, as well as multiple genetic incompatibilities that involve the sex chromosome and an autosome. Similarly, many studies tend to find a disproportionate genetic effect of the sex chromosome on female mate preferences. Although none of these studies deals directly with a case of reinforcement, they do suggest that the overall genetic variability conducive for reinforcement exists in nature (Fig. 2).

Two major studies on the genetics of reinforcement illustrate how suitable genetic architectures promote speciation with gene flow. *Drosophila pseudoobscura* and *D. persimilis* are hybridizing species of fruit flies that produce sterile F1 hybrids (Dobzhansky 1937a). They co-occur (sympatry) in the northwest coast of the United States and western Canada, with *D. pseudoobscura* extending its range into central United States and Central America (allopatry). Females derived from sympatry consistently copulate more readily with males from the same species than with males from the other species (that is, prezygotic isolation has evolved between the two species). Conversely, females derived from areas where *D. pseudoobscura* occurs alone tend to mate as readily with heterospecific as with conspecific males in a laboratory setting (Noor 1995). This type of reproductive character displacement (in the presence of maladaptive hybridization) is the signature of speciation by reinforcement. Genetic incompatibilities causing hybrid sterility, as well as basal female preferences for conspecifics (those present between allopatric populations of the two species), consistently map to chromosomal inversions located on the X and 2nd chromosomes (Noor et al. 2001a, 2001b). These observations are consistent with Lemmon and Kirkpatrick's (2006) predictions that the most conducive genetic architecture for reinforcement involves

interactions between a sex (X, or W) and an autosomal chromosome (Fig. 2), as well as with chromosomal models of speciation where the antirecombinational effects of chromosomal rearrangements maintain genetic associations between forms of reproductive isolation (Noor et al. 2001b; Rieseberg 2001; Ortiz-Barrientos et al. 2002; Navarro & Barton 2003; Kirkpatrick & Barton 2006). All together, these data suggest that the observed genetic architecture in *D. pseudoobscura* and *D. persimilis* is favorable for reinforcement (Fig. 3A, B).

The genetic basis of female preferences between sympatric and allopatric populations of *D. pseudoobscura* also reveals fascinating insights about reinforcement. Ortiz-Barrientos and colleagues (Ortiz-Barrientos et al. 2002, 2004) found two quantitative trait loci (QTL), *Coy-1* and *Coy-2*, that contribute to the differences in mating discrimination between *D. pseudoobscura* females derived from sympatry (which exhibit high mate discrimination) and from allopatry (low mate discrimination). These QTL contained as few as five genes and were located in two chromosomal regions. *Coy-1* was located next to a chromosomal inversion on the right arm of the X-chromosome, with the antirecombinational effects of the adjacent inversion facilitating its persistence (Machado et al. 2007; Noor et al. 2007, but see; Yatabe et al. 2007). In contrast, the location of *Coy-2* was not near or within an inversion, which suggested that some reinforcement genes might have experienced high levels of gene flow between the two hybridizing species. This implied that the effects of these genes on mating discrimination could operate regardless of genetic background, as occurs in one-allele models. Consistent with this suggestion, Ortiz-Barrientos & Noor (2005) showed that alleles conferring strong or weak assortative mating in *D. pseudoobscura* produce the same relative strength of assortative mating when inserted into *D. persimilis.* This suggests that the same assortative mating locus occurs in both species (a one-allele mechanism).

Figure 3C shows the contrasting predictions of one- and two-allele models for a gene

contributing to the evolution of prezygotic isolation when species exchange genes (Ortiz-Barrientos & Noor 2005). In the one-allele model scenario, the high mating discrimination allele derived from *D. pseudoobscura* in sympatry is expected to cause assortative mating when introgressed into a *D. persimilis* genetic background. The low discrimination allele from allopatry is expected to have a neutral effect on mating discrimination or perhaps increase the frequency of matings with heterospecifics. In a 2-allele model, in contrast, the allele derived from sympatric *pseudoobscura* is expected to confer stronger female preference for a *D. pseudoobscura* phenotype. As such, a *D. persimilis* female carrying such allele should also prefer such phenotype and thus mate more often with *D. pseudoobscura* males than if carrying an allele derived from allopatric populations of *D. pseudoobscura*. The *D. pseudoobscura* data strongly suggest that a one-allele model might have operated during reinforcement between this species and *D. persimilis*

The genetic basis of reinforcement has also been studied in great detail in the European flycatcher birds, *Ficedula hypoleuca* and *F. albicolis* (Saetre et al. 1997). Males of both species (that, for the most part, have allopatric ranges) have black and white plumage. However, in zones of contact, the males of the two species vastly differ in plumage coloration. Plumage differences appear to be derived characters within each of the two species, suggesting that they evolved in sympatry, possibly in response to maladaptive hybridization (especially given that hybridization between these species is common and that F1 hybrids have low fitness compared to the parental species). Females in sympatry display stronger preferences for the distinct, and conspecific, flycatcher males, leading to assortative mating. Using a series of experiments in the field and in the lab, Saether et al. (2007) compared expectations of one-allele models versus recombination–suppression models (specifically, mechanisms invoking chromosomal inversions or sex chromosomes) in the evolution of assortative mating. The authors contrasted
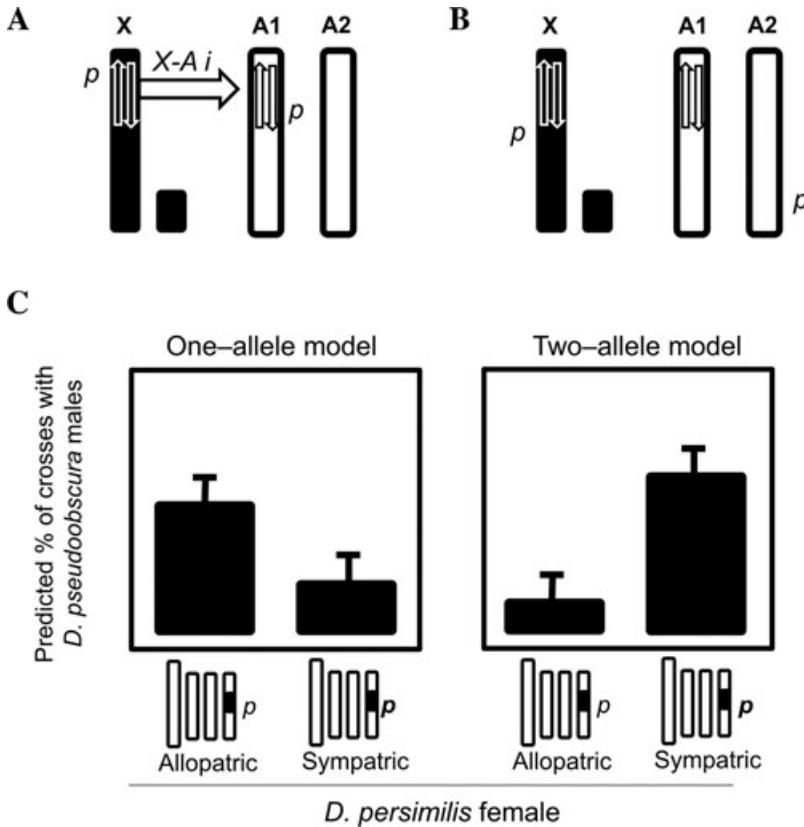
Figure 3. The genetics of reinforcement in *D. pseudoobscura*. (**A**) Genetic architecture of genetic incompatibilities and female preferences between *D. pseudoobscura* and *D. persimilis*, (**B**) genetic architecture of reinforcement in *D. pseudoobscura*, (**C**) predictions derived from one-allele versus two-allele models in *D. pseudoobscura* and *D. persimilis*. *D. persimilis* females carried either a chromosomal segment with discriminating alleles derived from sympatric *D. pseudoobscura* females, or carried one with less discriminating alleles derived from allopatric *D. pseudoobscura* females. In the one-allele model, the discriminating alleles favor assortative mating, and therefore *D. persimilis* females carrying the *D. pseudoobscura* sympatric alleles would tend to discriminate strongly against *D. pseudoobscura* males. In contrast the two-allele model predicts that *D. persimilis* females carrying the *D. pseudoobscura* sympatric alleles would have a strong preference for a trait specific to *D. pseudoobscura*. Therefore, such females would tend to prefer *D. pseudoobscura* to *D. persimilis* males. X and A denote sex and autosomal chromosomes respectively, *p* the female preference, and X-A, A-A, or X-X genetic incompatibilities between species, whose interaction is denoted with an arrow.

the effects of imprinting (a one-allele model) with the effects of sex linkage on the Z chromosome (a recombination–suppression model) on female preferences. Since both genes for genetic incompatibilities and genes for female mate preferences map to the Z chromosome, they are subjected to rounds of recombination only in females. However, because females inherit the Z chromosome from their fathers both recombination–suppression on the Z chromo-

some or paternal imprinting of preferences on female offspring could explain sympatric female preferences for distinct plumages. Using a combination of both behavioral and genetic experiments, Saether et al. (2007) found conclusive evidence that Z-linkage of genes contributing to various forms of reproductive isolation, and not imprinting, facilitated the evolution of prezygotic isolation in hybridizing flycatchers (see also Albert 2005).

Together, the cases of reinforcement in *Drosophila* and in *Ficedula* illustrate the genetic conditions under which natural selection can more easily complete speciation in the face of gene flow: genetic incompatibilities can involve both the sex and autosomal chromosomes (Noor et al. 2001a; Ortiz-Barrientos et al. 2004; Saether et al. 2007), but regions of the genome that prevent the formation of recombinant hybrids (like chromosomal inversions and sex-chromosomes) play a key role in the strengthening of prezygotic isolation as they maintain genetic associations between genetic incompatibilities and sexual preferences. Sexual preferences appear to have the same mode of inheritance as genetic incompatibilities. Finally, the evolution of assortative mating in some cases involves one-allele mechanisms, but the prevalence of these in nature is unknown (Ortiz-Barrientos & Noor 2005). Overall, these processes generate genomic divergence that is heterogeneous, with some regions causing reproductive isolation, and with others freely exchanged between species (Saetre et al. 1999; Noor et al. 2001a), possibly a genetic signature of speciation with gene flow (Via & West 2007).

The Ecological Basis of Prezygotic and Postzygotic Reproductive Isolation

We cover three topics here pertaining to how ecological differences between populations might contribute to reinforcement. First, ecological differences between populations can affect postzygotic isolation; namely, ecological divergence can result in hybrids exhibiting low fitness (for example, if an intermediate hybrid phenotype renders them ecologically maladapted to either parental environment, and an intermediate niche is unavailable [Rundle & Whitlock 2001]). Indeed there are some examples where reinforcement is likely driven by ecological causes, that is, ecologically dependent selection against hybrids. In ecotypes of *Gasterosteus aculeautus* stickleback fishes and

Timema cristinae walking stick insects, intrinsic genetic incompatibilities in hybrids are lacking and sources of selection against hybrids appear primarily (if not solely) ecological. In both systems, there is evidence for RCD and reinforcement (Rundle & Schluter 1998; Hatfield & Schluter 1999; Nosil et al. 2003, 2007).

Second, ecological differences between populations might also affect prezygotic isolation. For example, during the process of "sensory drive" mating signals evolve to be most detectable in the environment they are transmitted in, and sensory/perceptual systems evolve to be tuned to these signals (Endler & Basolo 1998 for review; Boughman 2002; Nosil, et al. 2003). As a consequence, fine-tuning of signals to divergent environments (adaptive divergence) has the potential to produce differences in mating signals, perceptual abilities, and mating preferences between populations. Reproductive barriers can evolve as a byproduct of this adaptive divergence in mating signals. The contributions of sensory drive to reinforcement are poorly studied, although in some cases both processes are known to operate within the same system (e.g., stickleback fishes, see Boughman 2001).

Third, ecology affects numerous processes other than reinforcement. These other processes can either confound or promote the effects of reinforcement. One example is competition for resources, which can generate accentuated divergence between sympatric versus allopatric taxa, a pattern referred to as "ecological character displacement" (ECD, Schluter 2000 for review). Separating the effects of reinforcement and competition on levels of prezygotic isolation may be difficult; both occur in sympatry from the interaction of populations and can produce the same evolutionary outcome—stronger prezygotic isolation between sympatric populations (Servedio & Noor 2003). The extent of this problem will not be known until we determine how frequently prezygotic isolation is strengthened as a byproduct of ecological character displacement. The problem could be serious, as
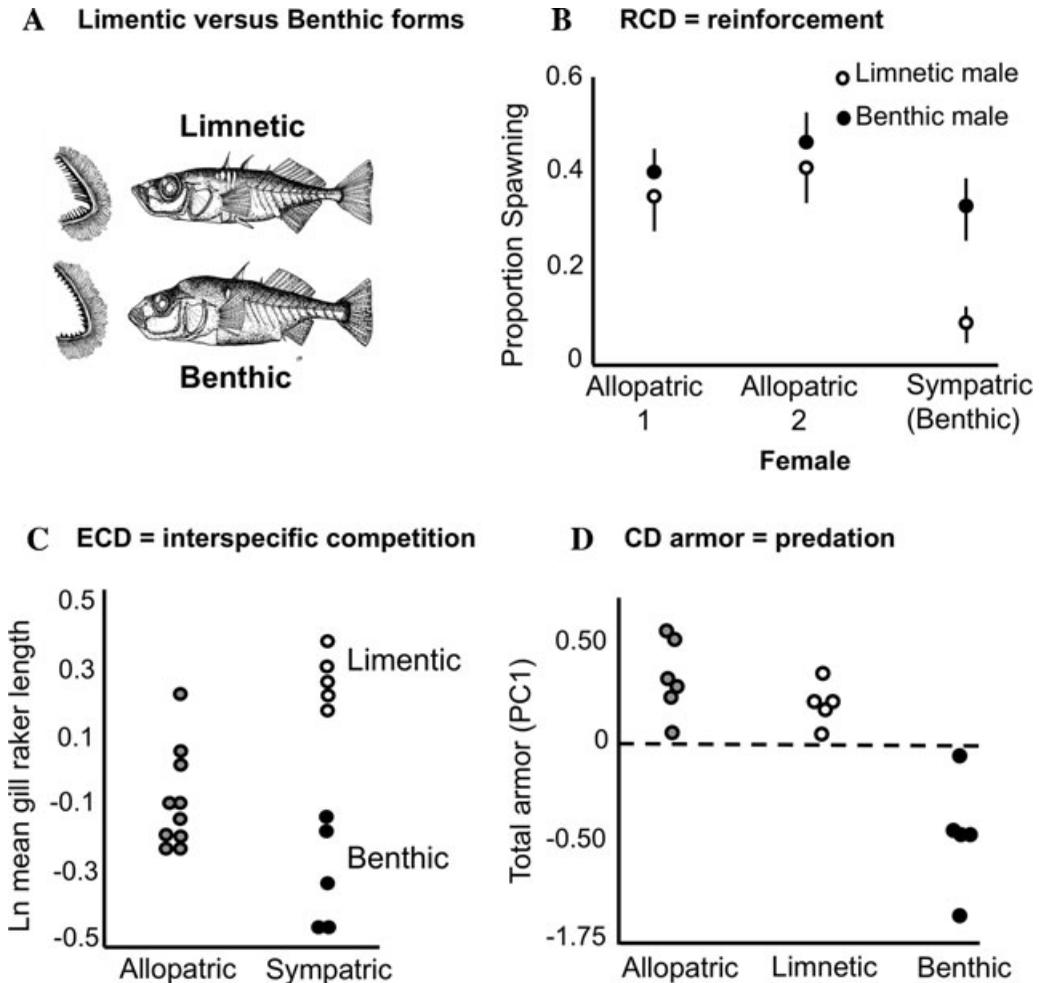
Figure 4. Multiple types of interactions between species might contribute to phenotypic divergence and speciation within a single system, in this case speciation between sympatric limnetic and benthic forms of *Gasterosteus aculeatus* stickleback fishes. (**A**) Illustrations of the two forms (courtesy of L. Nagel). (**B**) Reproduction character displacement (RCD) due to reinforcement. Modified from Rundle and Schluter (1998) and reprinted with permission of Wiley-Blackwell. (**C**) Ecological character displacement (ECD) in trophic traits due to interspecific competition. Modified from Schluter and McPhail (1993) and reprinted with permission of Elsevier. (**D**) Character displacement (CD) in defensive armor due to predation. Modified from Vamosi and Schluter (2004) and reprinted with permission of Wiley-Blackwell.

exemplified by studies of *Gasterosteus aculeatus* stickleback fishes, which show that multiple processes can simultaneously contribute to character displacement: reinforcement was implicated in reproductive character displacement of mating preferences (Rundle & Schluter 1998; Albert & Schluter 2004); competition was implicated in ecological character displacement of trophic traits (Schluter & McPhail 1992; Schluter 1994; Schluter 2003); and pre-

dation was implicated in character displacement of armor (Vamosi & Schluter 2004) (Fig. 4). How much the latter two forms of displacement affect the first is unknown, although the tests of reinforcement in this system did control for ecological character displacement (Rundle & Schluter 1998).

In summary, because different processes can yield similar patterns, they can confound each other's effects. Alternatively and on the upside,

the different processes might also complement one another. For example, ecological character displacement due to competition could result in reduced hybrid fitness (e.g., Pfennig & Rice 2007), which then drives the process of reinforcement. Future studies of reinforcement should consider the different effects of ecological divergence on prezygotic and postzygotic isolation, as well as the effects of different types of ecological interactions.

Connections between the Evolution of Prezygotic Isolation between Species and Sexual Selection within Species

Although species recognition and sexual selection within species are inherently related (Ryan & Rand 1993 for a review), how they interact during speciation has received little attention (Howard 1993; Higgie & Blows 2007; Lemmon 2009). This is a gross oversight, as the evolution of prezygotic isolation in response to maladaptive hybridization between incipient species might clearly affect mating patterns, and even the evolution of prezygotic isolation, within species. For instance, if female mate recognition systems are under strong stabilizing selection in sympatry, then choosy females that preferentially mate with conspecifics in sympatry may delay their mating with highly suitable conspecifics in allopatry (Ortiz-Barrientos pers. observ. and Nosil 2007). Thus, sympatric females might incur a cost in allopatry for being "choosier." Overall, the net gain in fitness of being choosy is high in sympatry because maladaptive hybrids are not produced, but low (or even negative) in allopatry.

A major trend across studies examining links between reinforcement and sexual isolation within species is that mating discrimination evolving in response to maladaptive hybridization induces widespread effects on mate choice within species (Table 3, Fig. 1). For instance, in frogs from the genus *Spea*, sympatric females of *S. multiplicata* reject allopatric males of the same species despite such males having higher

fitness than their sympatric counterparts. However, these females are better at distinguishing conspecific males from heterospecific males and thus do not incur the hybridization costs that allopatric migrants (to regions of sympatry with the other species) do (Pfennig & Simovich 2002; Pfennig 2003; Pfennig & Pfennig 2005; Pfennig 2007). In contrast, females of allopatric *Drosophila serrata* reject conspecifics from the area of sympatry with *D. birchii*, possibly because both male mating cues and female preferences are tuned optimally (Higgie & Blows 2007).

Whether this kind of conspecific rejection can lead to the formation of new species has rarely been explored. An exception concerns different species of frogs from the genus *Litoria*. These species appear to have speciated by reinforcement, but, because of increases in prezygotic isolation due to reinforcement, they have also evolved divergent mate preferences between populations within species (Hoskin et al. 2005). A similar pattern has been reported in *Timema cristinae* walking stick insects. In this system, although females are selected to be more discriminating against males from a single adjacent population that is adapted to feeding on a different host plant species, this selection has indirectly resulted in increased mating discrimination against foreign males from multiple other populations, including even males from other populations that use the same host (Nosil et al. 2003; Nosil 2007).

We propose that this mechanism—by which the effects of reinforcement within a particular taxon pair (e.g., a species pair) cascade to incidentally result in reproductive isolation among other taxon pairs (e.g., between sympatric and allopatric populations within the species in the aforementioned pair)—be termed the "cascade reinforcement hypothesis" (Fig. 1). We further postulate that this hypothesis will often involve sexual selection within species. Such cascade effects of reinforcement on the evolution of reproductive isolation within species may be due to females recognizing and preferring males from their own population based on a

population-specific trait, instead of an ecology-specific or species-specific trait (Zouros & D'Entremont 1980; Kelly & Noor 1996; Higgie et al. 2000; Hoskin et al. 2005; Lemmon 2009). If such cascade effects are common, then reinforcement could contribute to speciation between ecologically similar pairs of populations, between populations that are geographically separated from one another, and between conspecific populations (Pfennig & Ryan 2006).

The cascade reinforcement hypothesis is likely to operate when recognition systems in females act in similar genetic ways between and within species (for example, stickleback fish tend to use body size as cue for choosing mates both between and within species [Schluter 2001]). However, changes in other fitness components between species (e.g., due to adaptation to the environment) could also incidentally drive the evolution of reproductive isolation among conspecific populations. Therefore, a tendency for different traits and genes to be involved in between- versus within-species mate choice (Arbuthnott 2009), would not preclude the possibility of cascading reinforcement. However, these differences in mate choice genetics make a simple prediction: when females use the same cues between and within species, conspecific reproductive isolation will tend to also be behavioral. In contrast, when interspecific cues are different from those used within species, reproductive isolation among conspecific populations might have been based on traits involved in divergent ecological adaptation (such as morphological or physiological traits).

The cascade reinforcement hypothesis predicts that the evolution of prezygotic isolation between sympatric species can lead to the evolution of reproductive isolation within species. Interestingly, because reinforcement affects only prezygotic isolation, the process of cascade speciation among conspecific populations could proceed in the absence of postzygotic isolation among these conspecific populations, or it could allow for the evolution of postzygotic isolation as a byproduct of the evolution of prezygotic isolation among conspecific populations. Presumably, this newly evolved postzygotic isolation would feed back on strengthening prezygotic isolation. These types of feedback loops between prezygotic and postzygotic isolation have been noted before (see Servedio 2009 for a review), but they have not been extended to the geography of speciation.

The effects of reinforcement on mating behavior within species also provide insights into our ability to detect reinforcement. For example, in species experiencing high levels of gene flow between sympatric and allopatric populations of the same species, we should expect alleles causing increased prezygotic isolation between species (such as female mate discrimination against males from other species) to spread over the entire species range relatively quickly, erasing the pattern of reproductive character displacement that is traditionally used to detect reinforcement. This is particularly likely to occur if mating discrimination, which evolved in sympatry, has little or no cost in areas of allopatry (for example, if prezygotic isolation between species is neutral with respect to sexual selection within species). It is in such scenarios that cases of reinforcement may go unrecognized (Walker 1974). Why then, have researchers typically found signatures of reinforcement in many instances of divergence (Fig. 1)?

One answer is that alleles conferring prezygotic isolation between species fail to spread to the rest of the species range (i.e., to the allopatric range). Such a failure of the spread of alleles conferring discrimination could occur for numerous reasons including strong asymmetrical gene flow going from allopatry into sympatry, physical linkage of genes affecting mate preference and local adaptation to sympatric ecological conditions, and assortative mating alleles that are advantageous in sympatry but disadvantageous in allopatry (such as rejection of suitable mates in allopatry (Moore 1957; Barton & Hewitt 1985; Butlin 1993; Howard 1993). In particular, mating discrimination alleles can be disadvantageous in allopatry

TABLE 3. Mating Discrimination Effects on Mate Choice Within Species

System	Selective agent studied	Hybrids in the wild	Intrinsic postzygotic isolation effects	Preferred male trait between and within species	Female preference effects	Implications	Gene flow between populations	References
Spea multiplicata and *S. bombifrons*	Hybrid inviability	Commonly found	Reduced fitness if *S. multiplicata* is the mother; facultative overdominance if *S. bombifrons* is the mother	Call, indirect selection on body size	Sympatric females reject allopatric males despite high fitness	Reinforcement leads to sexual selection within species	Shared haplotypes	(Pfennig & Pfennig 2005; Pfennig 2007; Pfennig 2008; Reyer 2008)
Litoria genimaculata forms N and S	Hybrid inviability	Commonly found	Hybrid lethality when *L. genimaculata* form S is the mother, and normal hybrid development if the N form is the mother	Call, indirect selection on body size	Sympatric females reject allopatric males	Reinforcement creates new allopatric species	Low	(Hoskin et al. 2005; Smadja & Butlin 2006)
Drosophila serrata and *D. birchii*	Wasted time attempting to mate heterospecifically	Unknown	Absent	Cuticular hydrocarbons	Allopatric females reject sympatric males	There exists a sexual selection optimum in allopatry	High	(Higgie et al. 2000; Higgie & Blows 2007; Higgie & Blows 2008)

Continued

TABLE 3. *Continued*

System	Selective agent studied	Hybrids in the wild	Intrinsic postzygotic isolation effects	Preferred male trait between and within species	Female preference effects	Implications	Gene flow between populations	References
D. subquinaria and *D. recens*	Wolbachia induced hybrid inviability	Evidence of hybridization in the past	Hybrid lethality when *D. recens* is the father, and hybrid male sterility when *D. subquinaria* is the father	Unknown	Sympatric females reject allopatric males	Reinforcement leads to sexual selection within species	High	(Telschow et al. 2005; Jaenike et al. 2006)
D. pseudoobscura and *D. persimilis*	F1 hybrid male sterility	Rarely found	Hybrid male sterility is slightly stronger when *D. pseudoobscura* is the mother	Courtship song, but unknown within species	Sympatric females court slowly allopatric males*	Reinforcement leads to sexual selection within species	High	(Noor 1995; Noor et al. 2001a, 2001b; Ortiz-Barrientos et al. 2004; Ortiz-Barrientos & Noor 2005 unpublished results*)
Timema cristinae host plant ecotypes	Extrinsic hybrid inferiority	Commonly found	Absent	Body size	Sympatric females reject foreign males regardless of host association	Reinforcement leads to sexual selection within species	High	(Nosil et al. 2002; Nosil et al. 2003; Nosil 2007)

because: (1) they cause increased mating discrimination against suitable conspecific males in allopatry, (2) allopatric females could reject sympatric males that have changed their morphology or behavior as a correlated response to the evolution of female prezygotic isolation, (3) females may have traded their ability to choose the fittest conspecific mates for the ability to discriminate against heterospecifics so that in allopatry they choose less fit males (which is disadvantageous), or (4) they traded their own fecundity/survival (or the fecundity/survival of their offspring) for the ability to choose conspecific mates, which would make them less fit in allopatry (so the alleles that confer discrimination are selected against in allopatry). Finally, even if alleles for increased mating discrimination are neutral outside of sympatric regions, it may take a long time for them to spread out of sympatry and across the species range, particularly when effective population sizes are large and hybrid zones narrow. In all such cases, we expect the pattern of reproductive character displacement to linger for longer periods of time, although these conditions might actually interfere with the completion of speciation (that is, the spread of between-species mating discrimination throughout the species range, including allopatry).

In conclusion, the consequences of geographically localized reproductive isolation are vast, and they include the rapid formation of new subspecies, the evolution of strong sexual isolation within species, and the evolution of cascading discrimination. Similarly, changes in sexual selection as a byproduct of the evolution of prezygotic isolation can explain why sometimes the signature of reproductive character displacement persists despite extensive gene flow between populations.

The Role of Habitat versus Mate Preference in Generating Prezygotic Isolation

Research on reinforcement has primarily focused on divergent mating preferences between sympatric species. However, selection might favor the evolution of other forms of prezygotic isolation, such as various migration modification behaviors (Mayr 1942; Fisher 1958; Balkau & Feldman 1973; Yukilevich & True 2006). An example of such a behavior is habitat preference, which reduces movement and thus gene flow between habitats (Coyne & Orr 2004). Like mate preference, migration modification can evolve to avoid maladaptive hybridization. In addition to selection against hybrids, selection might often act on immigrant parental genotypes that migrate to an alternative habitat (Hendry 2004; Nosil et al. 2005). Whether scenarios in which selection acts against immigrants should be considered examples of reinforcement in the traditional sense is debatable (Butlin 1995). However, selection against hybrids and immigrants can clearly drive the evolution of both types of prezygotic isolation: mate preference and migration modification (such as habitat preference).

The relative importance of these two different mechanisms of reinforcement, and how they interact, remains unclear. A recent theoretical model examined relative fixation probabilities for mate preference (their assortative mating) and migration modification when the two evolved simultaneously (Yukilevich & True 2006). An extension of this model examined allele frequencies in polymorphic populations that had yet to fix either form of prezygotic isolation (that is, the early stages of the speciation process) (Nosil & Yukilevich 2008). Both models found that the relative likelihood of reinforcement via mate preference versus migration modification was highly dependent on selection strength.

Under weak selection, reinforcement by either mechanism is unlikely. Under intermediate selection, the conditions favoring the rise and fixation of one mechanism favored the rise and fixation of the other. However, mate preference evolved somewhat more readily than migration modification. Populations of *T. cristinae* walking sticks, that exhibit polymorphism in habitat and

migration preferences and experience such intermediate selection, supported these predictions (Nosil & Yukilevich 2008). Specifically, both mate and habitat preference are subject to reinforcement in the walking stick system, but mate preference shows more strongly accentuated divergence in the face of gene flow (see also Nosil et al. 2006). Under strong selection, the results were very different. The evolution of migration modification generally interfered with the evolution of mate preference, by decreasing migration between populations and thereby reducing reinforcing selection for assortative mating. Thus, fixation probabilities and allele frequencies for mate preference versus migration modification were negatively correlated when selection was strong (Fig. 5). In essence, divergence in habitat preference can result in a lack of selection to avoid maladaptive hybridization in geographically sympatric populations, because such hybridization no longer occurs.

A final point is that congruence of the results for allele frequencies versus fixation probabilities suggests that similar patterns of reinforcement are expected during different stages of the speciation process. These studies assumed a one-allele scenario, and further work with a two-allele scenario is required, as are analytical solutions to verify the simulation results.

Comparative Studies: The Effects of Geography and Gene Flow

Comparative studies of reinforcement, which examine numerous population or species pairs, are extremely rare, but are necessary for inferring broad generalities about reinforcement. The most well known example concerns *Drosophila*, and revealed that prezygotic isolation evolves faster than postzygotic isolation, but that this pattern is driven almost entirely by accentuated prezygotic isolation in sympatric taxa (Coyne & Orr 1988). Although this important finding rekindled enthusiasm for rein-
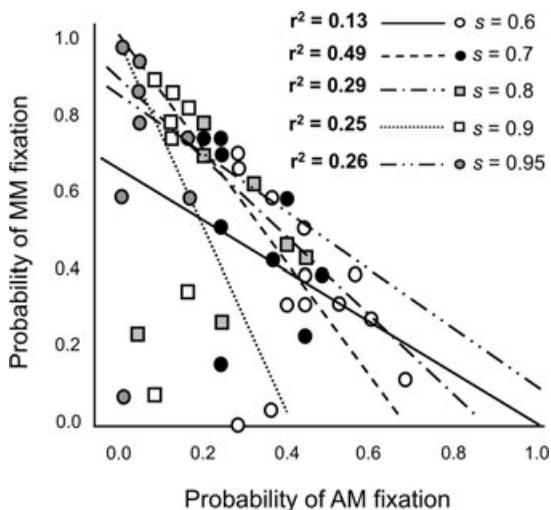
Figure 5. Correlation between the probabilities of fixation for assortative mating (divergent mate preferences) versus migration modification (divergent habitat preferences) when selection is strong (*s* in the graphs represents selection strength against immigrant and hybrid matings in both niches). The scenario depicted represents the simultaneous evolution of each form of reproductive isolation under a niche-based isolation model where selection against immigrants and hybrids is ecological in nature. Data are from (Nosil et al. 2005). Under strong selection, as depicted here, the evolution of migration modification generally interfered with the evolution of assortative mating by decreasing migration between populations thereby reducing selection for assortative mating. Such patterns were observed for both allele frequencies in polymorphic populations and for fixation probabilities, suggesting that similar patterns are expected for different stages of the speciation process. Modified from Nosil & Yukilevich 2008 and reprinted with permission of the Linnean Society.

forcement (see Coyne & Orr 2004 for a review), few other comparative studies have been conducted. There are some notable exceptions, of which we consider three.

The first example stems from a comparative analysis of song structure in 163 species of antbird (Thamnophilidae). In this study, habitat differences and reinforcement were both found to contribute to divergent song evolution (Seddon 2005). Specifically, songs differed between habitats and were more divergent between sympatric than allopatric species pairs. Although mate preference itself was not

measured, if song affects mate choice then differences between habitats and reinforcement could combine to promote speciation. Similarly, Lukhtanov and colleagues (Lukhtanov et al. 2005) showed that the sympatric distribution of 15 relatively young sister taxa of *Agrodiaetus* butterflies strongly correlates with difference in male wing color, and that this pattern most likely results from reinforcement. Again though, mate preference itself was not examined.

The final example considers multiple conspecific populations of *Timema cristinae* walking stick insects and addresses theoretical issues relating to how relative population sizes and levels of gene flow can affect reinforcement. Numerous theoretical models have demonstrated that high levels of gene flow between diverging populations can erode the effects of reinforcing selection, and thus prevent reinforcement (Sanderson 1989; Servedio & Kirkpatrick 1997; Cain et al. 1999; Servedio 2004). However, gene flow also generates the opportunity for selection against hybridization to occur. Thus migration can exert a dual effect during reinforcement. As noted by Coyne and Orr (2004, pp. 371) "reinforcement requires some gene flow, but not too much."

A potential prediction is that the effects of reinforcement are maximized when gene flow is intermediate. Indeed, several theoretical models have observed this to be the case, and in particular Kirkpatrick (2000) gave the dual effects of migration on reinforcement explicit theoretical consideration. Nosil and colleagues (2003) examined the effects of migration on the outcome of reinforcement in natural populations of walking stick insects. The results demonstrate the dual effects of gene flow: the magnitude of female mating discrimination against males from other populations was greatest when gene flow between populations adapted to alternate host plants was intermediate (Fig. 6). In essence, reinforcement was maximized when gene flow was high enough to allow the evolution of reinforcement, but low enough to prevent gene flow from eroding adaptive divergence in mate
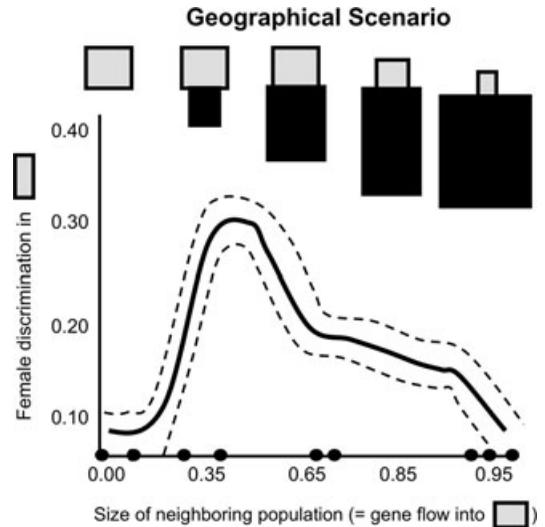
Figure 6. Among twelve populations of *Timema cristinae* walking stick insects, female mating discrimination against males from other populations is strongest when gene flow into the population is intermediate. Shown here is the size the host patch occupied by a population that is adjacent to a study population, relative to the study population itself (gray boxes, study population; black boxes, population adjacent to study population). X-axis values represent the proportion of total area, study population plus adjacent population, that is occupied by the adjacent population (actual values denoted by black circles on the axis). Host plant patch size and insect population size are known to be strongly, positively correlated ($r > 0.50$, Sandoval 1994). In turn, molecular, morphological, and behavioral data all indicate that the relative size of the population adjacent to a study population is significantly positively correlated with the level of gene flow into the study population (Nosil et al. 2003; Nosil 2007, 2008 for details). The y-axis is female mating discrimination against foreign males from other populations that use the alternative host (absolute value of mean copulation frequency with foreign males minus mean copulation frequency with males from the female's own population). Boxes illustrate the different geographical scenarios. The curve was estimated using the nonparametric cubic spline (dashed lines show standard errors from 1,000 bootstrap replicates) (Schluter 1988). Modified from Nosil et al. (2003) and reprinted with permission of the Royal Society of London.

choice (i.e. when the sizes of coexisting populations are similar).

The generality of the walking stick results is unknown, although at least three other studies

have documented stronger effects of reinforcement on the less abundant species within a hybridizing pair, which undergoes more frequent encounters with heterospecifics (Waage 1975; Noor 1995; Peterson et al. 2005). Further studies are required to generate insight into whether migration will tend to facilitate divergence via increased opportunity for reinforcing selection or to act as a homogenizing force due to gene flow. In particular, some recent models have examined the dynamics of reinforcement or character displacement along clines or gradients (Lemmon et al. 2004; Goldberg & Lande 2006) rather than between discrete patches, and empirical reinforcement studies considering gene flow along clines are lacking.

Reproductive Character Displacement, Maternal Investments, and Reinforcement in Plants

Most data on reinforcement comes from animal studies (Coyne & Orr 2004). The few carried out in plants have provided mixed results. Moyle and co-workers (2004) found that in three genera of angiosperms, prezygotic and postzygotic isolation evolved at similar rates in both allopatry and sympatry (that is, sympatry did not accentuate the rate of evolution). In contrast, Kay and Schemske (2008) found that in a genus of tropical gingers, prezygotic isolation evolved faster in sympatric pairs than in allopatric pairs, possibly in response to poor growth of F1 individuals. As in the case of gingers, where style length seems to explain most of the variation in prezygotic isolation, several other studies have shown that the position of anthers (a male trait) may evolve to specify precise pollen placement on pollinators (Levin 1978). Modification of female and male organs in plants might suggest that behavioral preferences could evolve in plants and perhaps be selected during reinforcement.

Understanding how plant traits affect "mate choice" is crucial for studying reinforcement in plants. For instance, flowering plants have the potential to evolve prezygotic isolation in similar ways to marine organisms with open fertilization, in which gametic interactions are key to mate choice (Swanson & Vacquier 2002; Palumbi 2008). Plants also exhibit great variation in flowering time, pollinator behavior, and mating systems (including the evolution of selfing), and as such, these traits also could potentially be recruited when evolving reproductive isolation in sympatry in response to reductions in hybrid fitness. Because many of these traits occur at different stages of their life cycle, plants that shift from one reproductive stage to another could possibly escape the costs of maladaptive hybridization.

Consider two hybridizing plant species that produce hybrid offspring with reduced fitness, with one of them incurring cost to heterospecific pollination in mixed pollen loads (for example, heterospecific pollen grains consistently outcompete or swamp conspecific pollen grains (e.g., Fishman & Wyatt 1999). If there is variation in the population for flowering time or mating system, then alleles responsible for poor competitive ability might become associated with alleles contributing to early flowering or selfing (Vallejo-Marin & Uyenoyama 2004). As a result, poor competitors increase fitness. These associations might be enough to trigger or complete speciation by reinforcement. This mechanism predicts that variation for mating system or RCD for flowering time exists in sympatric areas of hybridizing species. Further, it predicts that in heterospecific matings between selfers and outcrossers, the selfers should be poorer competitors in heterospecific pollen competition experiments. If selfers happen to be strong pollen competitors, then most likely selfing occurs at an appreciable frequency in sympatry for reasons other than unpredictable pollination environments (Kalisz et al. 2004).

As mentioned in a previous section, the genetic architecture underlying postzygotic and prezygotic isolation, and their correlations, will determine the likelihood of reinforcement. A recent study in morning glory species found

selection for reproductive character displacement in areas of overlap between *Ipomea hederacea* and *I. purpurea*. Specifically, in sympatric conditions *I. hederacea* plants with anthers clustered around the stigma are less susceptible to heterospecifics pollination from *I. purpurea* than plants with anthers of different heights (Smith & Rausher 2008). However, floral traits in *I. hederacea* constrain character displacement: positive genetic correlations between anther height and floral traits constrain the response to selection for anther clustering (selection for the tallest anther to decrease in size, and selection for the shortest anther to increase in size). This study demonstrates how correlations among traits under selection, and maternal investment in producing hybrids, can adversely affect the likelihood of reinforcement despite favorable genetic architectures, particularly, when such correlations constrain selection trajectories (note that this applies to both animals and plants).

Quantitative measures of costs to hybridization are usually lacking, and therefore it is difficult to evaluate empirically the effects of such costs on the success of reinforcement (for a notable exception see Pfennig & Simovich 2002). Maternal investment in hybrid production and its added cost to hybridization remains largely unexplored in reinforcement. All things being equal, if two pairs of hybridizing species incur similar costs to hybridizing (males are sterile, for example), then species whose females invest relatively more in producing such hybrids would experience greater selection against choosing heterospecifics. This difference could increase the chances of evolving prezygotic isolation in sympatry. Although this is applicable to both animals and plants, an obvious trait for increased plant maternal investment is seed mass. Many plants produce few, but large seeds, whereas others produce many, but small seeds. Most likely, plants producing few, large seeds incur greater costs associated with maladaptive hybridization and therefore might be, on average, more prone to undergo reinforcement (Coyne & Orr 2004).
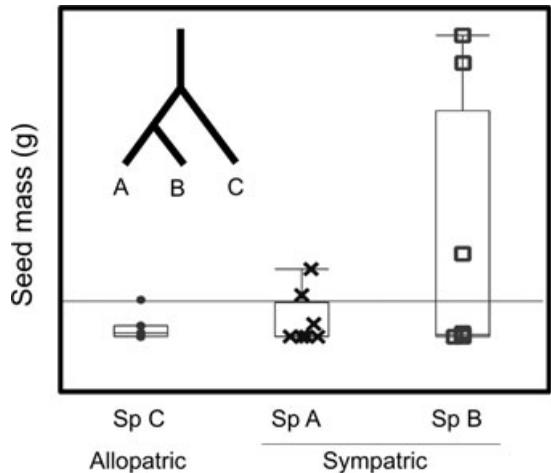
Figure 7. Patterns of reproductive character displacement in Australian angiosperm genera. In four out of seven genera, a sympatric sister species produces larger seeds than the other sympatric pair and the allopatric sister taxa. Species comparisons involve three sister taxa of which one is allopatric to the other two; in this case, taxon C is allopatric to taxa A and B. Each symbol in the graph represents the mean value for seed mass for one of the species used in a comparison. Boxes show the 95% quantile distribution when considering all species simultaneously.

Two major predictions follow from the previous discussion. First, sister plant species that produce large seeds are more likely to be found in sympatry than in allopatry, whereas sister plants species with smaller seeds are equally likely to be found in sympatry or in allopatry. In a more general sense, as maternal investment in hybrids increases, the tendency for sister species to be found in sympatry should increase. Second, we expect to detect reproductive character displacement for seed mass. Preliminary data derived from seven genera of Australian flowering plants show that mean seed mass is greater for sympatric sister species than for the sister allopatric species (Fig. 7), consistent with reproductive character displacement for maternal investment. However, maternal investment differences could have evolved before sympatry. In this case, differences in seed mass would set an asymmetry in costs associated with maladaptive hybridization and lead to the

evolution of prezygotic isolation only in one of the two hybridizing species.

Asymmetries in prezygotic and postzygotic isolation are common in both plants and animals (Turelli & Moyle 2007), and perhaps the evolution of maternal investment differences is at the heart of such phenomena. In its simplest form, asymmetric postzygotic isolation during reinforcement predicts also asymmetric evolution of prezygotic isolation in sympatry. This is seen in some systems, as in plants from the genus *Anigozanthos*, where crossing pairs with greater pollen sterility in one direction of the cross produce fewer seeds than the reciprocal cross that enjoys higher pollen fertility (Steve Hopper pers. comm.). Alternatively, seed mass differences could have evolved in response to maladaptive hybridization. Counterintuitively, females that already incur hybridization costs would possibly reduce their chances of hybridizing by increasing the actual costs of producing a hybrid; although this appears to be selection for postzygotic isolation, we should note that this would be a case for increased maternal investment, which, as mentioned above, increases the overall selective cost associated with hybridization. Subtle similarities can be found in insects, for instance, where some females spend more energy delaying copulation possibly at the expense of their own foraging for food or mating with conspecifics. In general, maternal investment could modulate hybridization costs and possibly lead to the faster evolution of prezygotic isolation in sympatry.

Conclusions

Divergence in the face of gene flow can be unlikely (Felsenstein 1981), yet many cases of sympatric speciation and reinforcement have been uncovered. Although we still remain largely ignorant about the frequency of these processes, it is evident that both the genetic architecture of postzygotic and prezygotic isolation and the demographic and ecological conditions for speciation with gene flow exist

in nature. Examples include one-allele models, genes for reproductive isolation residing in genomic regions exhibiting reduced recombination, genetic correlations between preferences and alleles causing genetic incompatibilities, and widespread ecologically based selection against hybrids.

It has only recently become apparent that speciation by reinforcement brings unexpected consequences. For example, an important observation is that evolving prezygotic isolation between species can lead to strong effects on sexual selection within species, perhaps to the point of cascading into speciation events between sympatric and allopatric populations of a single diverging species (we have called this the cascade reinforcement hypothesis). Therefore, we suggest that studies examining prezygotic isolation between species should make additional efforts to consistently measure its incidental effects on mating behavior between conspecific populations. This phenomenon also might explain why we detect reinforcement or reproductive character displacement despite some gene flow between sympatric and allopatric portions of the species range. These speciation dynamics suggest that female preferences often act in similar ways genetically between and within species (or between and within sympatric versus allopatric populations). However, this need not be the case for the cascade hypothesis to operate, and the supposition actually runs counter to reports that traits involved in between- versus within-species mate choice are often not one and the same (Arbuthnott 2009 for review). This discrepancy requires further attention. In general, to better understand the causes and consequences of reinforcement, more data are required on the trade off between how enhanced mating discrimination (the evolution of prezygotic isolation) increases fitness in sympatry versus how such discrimination might reduce fitness in allopatric areas.

Finally, speciation by reinforcement in plants remains quite controversial, despite the obvious favorable conditions for its evolution:

multiple paternity, formation of hybrid zones, variable maternal investment in hybrid offspring, unpredictable pollination environments, and fertilization assurance mechanisms. Genetic correlations between selected traits showing reproductive character displacement and other traits under different selection regimes could heavily constrain the evolution of prezygotic isolation in sympatry. In conclusion, although reinforcement remains an active area of research, much work remains to be done before even single cases are understood well, and even then it is only through comparative studies that we will be able to disentangle the anecdotal from the recurrent. The good news is that the conceptual framework and tools to address gaps in our understanding exist.

Acknowledgments

Conflicts of Interest

The authors declare no conflicts of interest.

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