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Reinforcement's incidental effects on reproductive isolation between conspecifics

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Abstract

Reinforcement—the process whereby maladaptive hybridization leads to the strengthening of prezygotic isolation between species—has a long history in the study of speciation. Because reinforcement affects traits involved in mate choice and fertility, it can have indirect effects on reproductive isolation between populations within species. Here we review examples of these "cascading effects of reinforcement" (CER) and discuss different mechanisms through which they can arise. We discuss three factors that are predicted to influence the potential occurrence of CER: rates of gene flow among populations, the strength of selection acting on the traits involved in reinforcement, and the genetic basis of those traits. We suggest that CER is likely if (1) the rate of gene flow between conspecific populations is low; (2) divergent selection acts on phenotypes involved in reinforcement between sympatric and allopatric populations; and (3) the genetic response to reinforcement differs among conspecific populations subject to parallel reinforcing selection. Future work continuing to address gene flow, selection, and the genetic basis of the traits involved in the reinforcement will help develop a better understanding of reinforcement as a process driving the production of species diversity, both directly and incidentally.

Key words: reinforcement, speciation, reproductive character displacement.

Introduction: Speciation, Reinforcement, and Reproductive Character Displacement

Speciation is the evolutionary process by which one lineage splits into two reproductively isolated groups of organisms (Coyne and Orr 2004). As such, a central goal of speciation research is to understand the processes that drive the evolution of reproductive isolation between different populations or species (Darwin 1859; Dobzhansky 1940; Coyne and Orr 1989, 2004; Nosil 2012). Significant strides have been made towards identifying barriers that generate reproductive isolation between species (hereafter referred to as "isolating barriers" or "barriers"; Coyne and Orr 2004; Harrison 2012), the processes underlying their evolution (Dobzhansky 1937; Liou and Price 1994; Noor 1995; Rundle and Nosil 2005; Seehausen et al. 2008; Maan and Seehausen 2011), and the rate at which they evolve during speciation (Coyne and Orr 1989, 1997; Bolnick and Near 2005; Moyle and Nakazato 2010; Matute et al. 2010).

Barriers to reproduction can be classified depending on where they occur in the reproductive cycle of an organism. Prezygotic barriers occur before the zygote is formed and can be further split into premating and postmating-prezygotic barriers. Premating barriers include ecological and behavioral traits that reduce the likelihood that two individuals will mate, whereas postmating-prezygotic barriers involve interactions between gametes or between sperm and aspects of female reproductive tracts. Finally, postzygotic barriers occur after fertilization, and include any reduction in fitness observed in hybrid organisms.

During speciation, premating barriers are thought to be crucial. Comparative studies have found that premating reproductive isolation evolves faster than postzygotic isolation in areas where species can interbreed (Coyne and Orr 1989, 1997; Yukilevich 2012). Additional phylogenetic studies have shown that the rate of evolution of premating barriers is also comparatively faster than that of postzygotic traits (Coyne and Orr 1989; Yukilevich 2012; Rabosky and Matute 2013), which has led to the hypothesis that premating traits are the first type of reproductive barriers to evolve to substantially high levels and thus are crucial to the process of speciation. The processes that lead to the

evolution of strong premating barriers may therefore be fundamental in generating and maintaining biological diversity (Noor 1999; Kay and Schemske 2008; Hudson and Price 2014).

One process that can drive the evolution of premating barriers is selection either against the formation of maladapted hybrids (Dobzhansky 1940) or against negative fitness effects of hybrid matings on females (Lorch and Servedio 2007). In this process—referred to as reinforcement—indirect selection against maladaptive hybridization results in the evolution of strong prezygotic isolating barriers between individuals of two species in regions where those species co-occur (i.e., sympatry; Dobzhansky 1937, 1940). Reinforcement frequently generates a pattern of reproductive character displacement (RCD; Brown and Wilson 1956), where prezygotic isolation between two hybridizing species is stronger in sympatry when compared with allopatry [see Servedio and Noor (2003) for other patterns generated by reinforcement). Although the importance of reinforcement has been hotly debated (Templeton 1981; Spencer et al. 1986; Butlin 1987; Sanderson 1989), an increasing amount of empirical and theoretical support suggests that it may be a widespread and important process during speciation, capable of promoting the evolution of prezygotic isolation (Koopman 1950; Liou and Price 1994; Noor 1995; Servedio and Noor 2003; Nosil et al. 2003; Kay and Schemske 2008; Matute 2010; Yukilevich 2012; Hudson and Price 2014).

Reinforcement and the Evolution of Incidental Reproductive Isolation among Conspecific Populations

Reinforcement results in a decrease in the likelihood of maladaptive matings through the evolution of phenotypes that are involved in mate recognition, mate choice, and/or gametic compatibility (i.e., prezygotic traits). When the evolutionary response to reinforcement causes phenotypes to become "mismatched" between populations of the same species, reproductive isolation can evolve between those populations as an incidental effect of reinforcement (Howard 1993; Pfennig and Ryan 2006; Ortiz-Barrientos et al. 2009; Hoskin and Higgie 2010; Abbott et al. 2013). Here, we refer to these effects as the "cascading effects of reinforcement" (CER). The evolutionary consequences of CER differ from those of reinforcement because the former occur between conspecific populations whereas the latter occur between species. The idea that reinforcement can have incidental effects on levels of reproductive isolation between conspecific populations was initially verbalized in the late 1960s (Littlejohn and Loftus-Hills 1968). Nonetheless, this idea was only recently formally put forth in the literature (Howard 1993; Ortiz-Barrientos et al. 2009; Hoskin and Higgie 2010), and it has rarely been tested from a theoretical perspective (however, see Pfennig and Ryan 2006). Below, we summarize two general mechanisms through which reinforcing selection can lead to the incidental evolution of reproductive isolation between conspecific populations and propose a route map for its study.

CER caused by parallel reinforcing selection acting within multiple conspecific populations

The first mechanism that can lead to CER occurs when parallel reinforcing selection, acting within multiple populations of the same species (Abbott et al. 2013; Figure 1A), results in the convergent evolution of reinforced reproductive isolation (RRI). There are two possible outcomes to parallel reinforcing selection acting within conspecific populations. First, these populations can evolve RRI by responding to selection through the use of the same phenotypes and

underlying loci. We do not consider this outcome a CER because the response to reinforcing selection is not expected to generate reproductive isolation between conspecific populations. Second, reinforcement can drive convergent evolution through different traits, phenotypes, and/or loci in the different populations. In this second outcome, reinforcement can lead to phenotypic differentiation between populations, which in turn can lead to reproductive isolation. Hereafter we refer to the effects of this type of CER as convergent-sympatry (c-s) effects (Figure 1A).

C-s effects constitute an example of a mutation order process (Mani and Clarke 1990). Mutation order processes occur when adaptation to similar selective environments (e.g., in populations experiencing parallel reinforcing selection) utilize different genetic or phenotypic pathways due to the stochastic effects of mutation, substitution, and/or the available pool of standing genetic variation (Schluter 2009). Theoretical work has shown that differentiation through mutation order processes (and by extrapolation, c-s effects) is most likely to occur in scenarios where rates of gene flow between conspecific populations are low, as this allows populations to evolve and maintain genetic and phenotypic differences (Nosil and Flaxman 2011).

C-s effects can result in prezygotic isolation, postzygotic isolation, or both evolving between conspecific populations. If mutation order processes resulting from c-s effects lead to prezygotic isolation without any postzygotic isolation, then such differentiation falls into the category of an incidental effect of reinforcement (i.e., a c-s effect). However, mutation order processes could also cause postzygotic isolation through the substitution of different, and incompatible, alleles in different conspecific populations undergoing reinforcement. In this case, mating between conspecific populations would become maladaptive, and reinforcement *sensu stricto* could cascade to populations of the same species. Prezygotic isolation could then evolve as the direct result of reinforcement (i.e., selection against the maladaptive consequences of postzygotic isolation), and the presence of postzygotic isolation would have been an incidental effect of reinforcement that was initially acting independently in parallel populations of the same species. The largest difficulty in identifying c-s effects (as we see it) will be one's ability to causally ascribe parallel reinforcing selection as the mechanism driving the differentiation of conspecific traits.

As one of the few putative examples of a c-s effect, Lemmon (2009) quantified differences in acoustic signals (mating calls) among four populations of the chorus frog *Pseudacris feriarum* undergoing RCD in sympatry with the heterospecific *P. nigrata*. In different regions of sympatry, pulse number and pulse rate of *P. feriarum* calls differ from *P. nigrata* calls. More importantly, the sympatric *P. feriarum* calls also differ from one another. Parallel reinforcing selection has therefore resulted in the diversification of call types among populations of *P. feriarum*. While reproductive isolation between conspecific populations of *P. feriarum* was not explicitly tested in this study, this case may constitute (incipient) mutation order speciation if the differences in conspecific calls lead to reproductive isolation between conspecific populations. This example highlights how the same selective pressure—namely selection against maladaptive hybridization in sympatry—can lead to the incidental differentiation, and diversification, of conspecific traits.

CER caused by divergent selection acting between conspecific populations undergoing reinforcement and those found in allopatry

The most common (or at least detectable) signature of reinforcement is RCD (Howard 1993; Lemmon et al. 2004). However, the factors
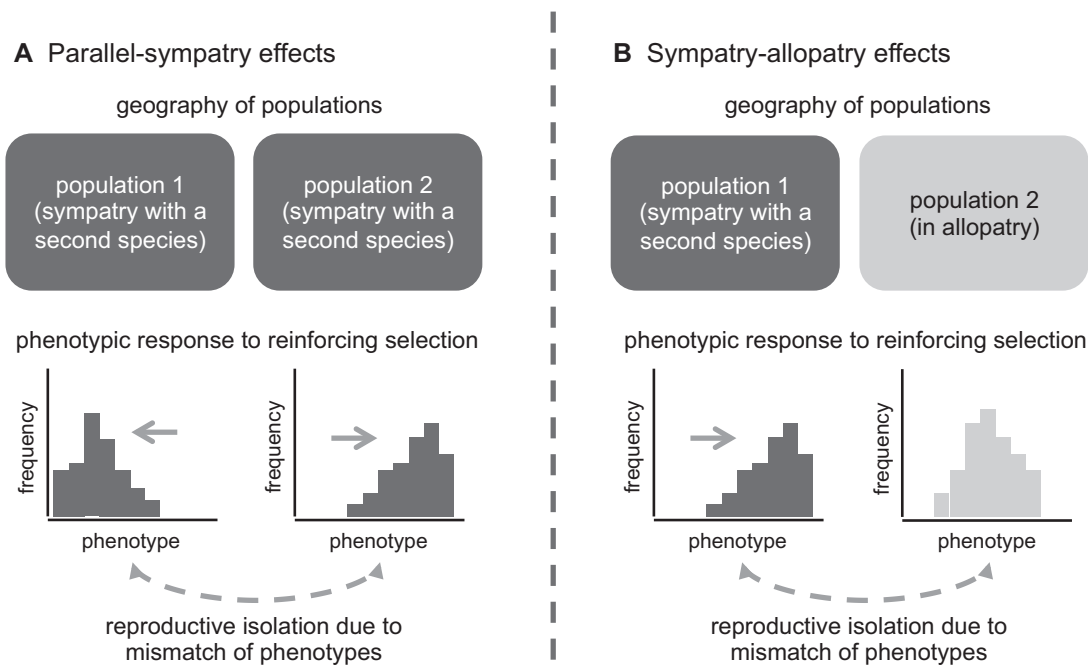
A Parallel-sympatry effects

geography of populations

| population 1 (sympatry with a second species) | population 2 (sympatry with a second species) |

phenotypic response to reinforcing selection

reproductive isolation due to mismatch of phenotypes

B Sympatry-allopatry effects

geography of populations

| population 1 (sympatry with a second species) | population 2 (in allopatry) |

phenotypic response to reinforcing selection

reproductive isolation due to mismatch of phenotypes

Figure 1. Cascading effects of reinforcement that generate reproductive isolation (RI) among conspecific populations. (A) C–s effects are the result of reinforcing selection acting in multiple, geographically isolated populations of the same species. The response to reinforcing selection in these populations can differ (as indicated by the gray arrows showing shifts in phenotypes) and this can in turn lead to reproductive isolation. The word "phenotype" in the bottom histograms could have just the same been replaced with "genotype". (B) S–a effects arise between populations undergoing reinforcement (dark gray box) and those found in allopatry (light gray box). In one scenario, if reinforcing selection causes phenotypes favored in sympatry to be disadvantageous in allopatry, reinforcement can lead to reproductive isolation between sympatric and allopatric populations. See text for further scenarios and descriptions.

that generate or maintain RCD among conspecific populations are frequently unknown. For example, RCD can arise as a result of geographic isolation that exists between populations of a species undergoing reinforcement or divergent selection acting on the phenotype(s) involved in reinforcement between sympatric and allopatric regions of a species' range (Higgie et al. 2000; Hopkins et al. 2014; Pfennig and Rice 2014). Here we focus on the latter of these two mechanisms because divergent selection can result from the action of reinforcement, whereas geographic isolation can be due to other ecological or environmental factors.

Selection acting against phenotypes favored by reinforcing selection in allopatric regions of a species' range can generate divergent selection on those phenotypes and can drive RCD (Walker 1974; Price and Liou 1994). Here, we collectively refer to the effects caused by divergent selection between sympatry and allopatry as "sympatry–allopatry" or "s–a" effects (Figure 1B). S–a effects can manifest as a result of selection acting directly against reinforced phenotypes in allopatry (Hopkins et al. 2014) or against pleiotropic or correlated phenotypes that have evolved with the reinforced phenotypes (Comeault et al. 2015). The Texas wildflower *Phlox drummondii* provides a good example of a system where a reinforced phenotype is directly selected against in allopatric regions of the species' range. Hopkins et al. (2014) showed that *P. drummondii* with red colored flowers are favored by reinforcing selection in regions where *P. drummondii* co-occurs with the blue-flowered species *P. cuspidata*. Blue flowers, on the other hand, are favored in allopatric regions of *P. drummondii*'s range. Clines in flower color have been used to show that divergent selection limits the spread of different colored flowers (and their underlying alleles) between sympatric and allopatric regions of *P. drummondii*'s range (Hopkins and Rausher 2014). This example represents an s–a effect of reinforcement because

reinforcement has led to the diversification of flower color in *P. drummondii* and this phenotypic diversification is predicted to generate selection against migrants (a form of reproductive isolation) between allopatric and sympatric regions of *P. drummondii*'s range.

S–a effects can also occur when reinforcement indirectly drives the evolution of traits or phenotypes that are correlated with those undergoing reinforcement. This can occur through genetic linkage or additive genetic co-variation between traits. An example of this type of indirect cost has been observed in the fruit fly *Drosophila yakuba*. Female *D. yakuba* that are sympatric with their sister species *D. santomea* show evidence for reinforcement and lay fewer eggs when mated to male *D. santomea* than allopatric females. In addition to this female trait, male *D. yakuba* collected from sympatric populations have lower fertility when mated to conspecific females from allopatric populations (Comeault et al. 2015). Comeault et al. (2015) used experimental evolution to show that this s–a effect is likely the result of a correlated evolutionary response in male gametes due to reinforcing selection that acts on an unidentified trait in the female reproductive tract. These results show how reinforcing selection acting on females can drive a correlated evolutionary response in male traits that are selected against in allopatry.

In general, s–a effects of reinforcement have received more attention in the literature than c-s effects. Table 1 highlights this and, to our knowledge, only three of the examples in this table suggest that c-s effects could be occurring: those of the stick insect *Timema cristinae* (Nosil et al. 2003), the chorus frog *P. feriarum* (Lemmon 2009), and the killifish *Lucania parva* (Kozak et al. 2015). However, c-s and s–a effects are not mutually exclusive. In the chorus frog *P. feriarum*, for example, c-s effects are the result of acoustic signal traits that are favored by reinforcement but are predicted to increase the energetic cost of signaling relative to allopatric signal types

Table 1. Systems where cascading effects of reinforcement have been implicated in within species differentiation

Species	Trait	Selection	Gene flow?	Genetic basis	References
Drosophila serrata	Male cuticular hydrocarbons (CHCs) have diverged between sympatry and allopatric populations. Female preference is based on CHCs and has also diverged.	Divergent natural between sympatry and allopatry. Sexual selection opposes reinforcing selection	Unknown	Differences in cuticular hydrocarbon profiles are explained by a few loci of large effect; one known locus: mFAS (12–30% of PV)	Higgie et al. (2000); Higgie and Blows (2008); Hine et al. (2011); Chung et al. (2014)
Drosophila mojavensis	Female preferences differ between sympatric and allopatric populations. Male CHCs are hypothesized to have diverged as well.	Unknown	Moderate to high among populations of D. mojavensis. Early in the divergence between D. arizonae and D. mojavensis	Hypothesized to be mono- or oligogenic. Male and female courtship behaviors might be under the control of separate alleles or sex-specific behaviors are controlled by the same gene or genes acting in a sex-specific manner. The specific details remain unknown	Wasserman and Koepfer (1977); Zouros and D'Entremont (1980); Markow (1991); Massie and Markow (2005); Ross and Markow (2006); Lohse et al. (2015) Jennings and Etges 2010
Drosophila subquinaria	Both male and female CHCs differ between sympatric and allopatric populations.	Clines in mate discrimination and genetic markers suggest reinforcing selection is advantageous only in sympatry	Reduced gene flow inferred from clines, but not directly measured.	Unknown	Jaenike et al. (2006); Curtis et al. (2013); Giglio and Dyer (2013); Bewick and Dyer (2014); Dyer et al. (2014)
Drosophila yakuba	Gametic incompatibility between sympatric and allopatric populations. Female traits involved in retention or use of sperm.	Clines in gametic isolation suggest reinforcing selection is advantageous only in sympatry.	Mitochondrial introgression in the São Tomé hybrid zone. Moderate evidence of nuclear introgression between species and extensive gene flow between allopatric and sympatric lines	Unknown	Llopart et al. (2005); Bachtrog et al. (2008); Beck et al. (2015)
Litoria verreauxii	Male call differs between sympatric and allopatric populations.	Unknown	Unknown	Unknown	Littlejohn and Loftus-Hills (1968)
Lucania parva	Unknown, but potentially linked to body length and size of anal fin	Unknown	Unknown	Unknown	Kozak et al. (2015)
Pseudacris feriarum	Male call and female preference differ among multiple hybrid zones	Not measured, but aspects of mating signal might be costly in sympatry favored in allopatry. Possible case of convergent reinforcing selection	Unknown	Unknown	Lemmon (2009)
Litoria genimaculata	Male call differs among populations.	Individuals from contact zones are discriminated upon by allopatric individuals	Unknown	Unknown	Hoskin et al. (2005)
Spea multiplicata	Male and female choice have evolved via reinforcement in areas where S. bombifrons is present	Unknown	Gene flow is slightly reduced among sympatric and allopatric populations. No information of gene flow between S. multiplicata and S. bombifrons	Unknown	Pfennig and Simovich (2002); Pfennig and Pfennig (2005); Pfennig and Rice (2014)
Gryllus fultoni	Calling song/female preference		Unknown	Unknown	Jang and Gerhardt (2006a, b)

(continued)

Table 1. Continued

Species	Trait	Selection	Gene flow?	Genetic basis	References
Magicicada neotredecim	Calling song/female preference	Not measured yet, but females prefer local song types. Possible case of a one-allele mechanism. Not measured, but females prefer local song types. Possible case of a one-allele discrimination model	Unknown	Unknown	Cooley et al. (2006)
Phlox drummondii	Flower color and hue have evolved under reinforcing selection in areas where *P. cuspidata* is also present. Reproductive isolation between red and blue morphs of *P. drummondii* driven by selection against migrants.	Population genetic model of phenotypic/ genotypic clines demonstrates that there is selection against red colors in allopatry. Source of selection remains unknown.	High	Flower color variation is completely explained by regulatory changes in two genes (flavanone-3′ 5′-hydroxylase, R2R3-Myb) controlling the type and amount of anthocyanin in flowers	Hopkins and Rausher (2011, 2014), Hopkins et al. (2014)
Timema cristinae	Female mate choice	Sexual selection favoring local male types. Females discriminate against "foreign" males	High	Unknown	Nosil et al. (2003, 2012)

For each system we give the species, the putative trait subject to reinforcing selection, whether selection has been measured for that trait between conspecific populations occurring in sympatry and allopatry (with respect to a second species driving reinforcement), if gene flow has been estimated between populations found in sympatry and allopatry (Gene flow?), and whether the genetic basis of those traits is known. This table was inspired by, and shows some overlap with table 3 of Ortiz-Barrientos et al. (2009); however, we have included additional cases (e.g., *D. mojavensis*, *P. drummondii*, and *D. yakuba*). References for each of the systems are given in the final column.

(Lemmon 2009). Energetic costs such as these could result in selection against reinforced signal phenotypes in allopatry. If this were the case, then parallel divergence in these traits (i.e., c-s effects) would also result in s–a effects. In order to fully appreciate the diversity and frequency of CER, research will need to continue to identify the phenotypes involved in reinforcement and determine how they are favored (or disfavored) by selection across a species' range.

A (Verbal) Population Genetics Model of CER

Factors predicted to affect the likelihood of CER

The examples presented above and in Table 1 suggest that CER are feasible. However, there is currently little formal quantitative/population genetic theory available to predict when CER should occur (but see Yukilevich and Aoki, this column). Below, we discuss how pre-existing theory developed to understand the parameters affecting local adaptation and reinforcement may be applicable to CER.

As discussed above, reinforcement can lead to local adaptation in prezygotic traits, resulting in differentiation among conspecific populations. In general, differentiation between populations subject to divergent selection (as when s–a effects are observed) represents a balance of multiple factors including selection favoring the local adaptation (s_1), selection against the adaptation outside of regions where it is advantageous (s_2), gene flow among populations (m; realized migration), and the effective population size (N_e) (Slatkin 1975, 1987; Barton and Bengtsson 1986; Yeaman and Otto 2011). However, RRI differs from other adaptations in the sense that there are two levels of gene flow (m) that are important for the evolution of the trait: gene flow between the hybridizing species (m_1), and gene flow between allopatric and sympatric populations of the same species (m_2). The role of selection and m_1 in reinforcement has been dealt with extensively elsewhere (Liou and Price 1994; Kelly and Noor 1996; Servedio and Kirkpatrick 1997; Servedio and Noor 2003). In general, these studies show that reinforcement is more likely to lead to speciation when hybrid offspring have very low fitness and when there is a mechanism causing linkage disequilibrium (LD) between the alleles underlying hybrid dysfunction and those controlling prezygotic isolation (Servedio 2009).

Explicit treatments of the factors affecting CER remain much less common than those affecting reinforcement (however, see Pfennig and Ryan 2006; McPeek and Gavrilets 2006). On the other hand, the general roles of selection and gene flow in facilitating adaptive differentiation between populations have received much more attention. Theoretical studies of local adaptation and hybrid zones are particularly germane to CER because they provide a framework for understanding the geographical distribution of an allele that is locally adaptive in a particular location, but neutral or deleterious elsewhere (e.g., Haldane 1948; Barton and Bengtsson 1986; Slatkin 1987). Additional work has addressed the role of genetics in facilitating (or constraining) differentiation in the face of gene flow (Yeaman and Otto 2011; Yeaman and Whitlock 2011; Bank et al. 2012; Yeaman 2015). These bodies of theory could be used to help predict the conditions most conducive to CER because they deal with the roles of selection and gene flow in promoting and maintaining differentiation between populations (see reviews by Kawecki and Ebert 2004; Nosil et al. 2009; Savolainen et al. 2013).

Two factors—selection and migration—have arguably received the most theoretical attention with respect to their role in the evolution of phenotypic differentiation and reproductive isolation between populations, especially in the context of clines and hybrid zones (Slatkin 1975, 1987; Barton and Bengtsson 1986; Hendry

et al. 2002; Moore et al. 2007; Bank et al. 2011, 2012). These studies describe how differentiation can be maintained among conspecific populations when the strength of selection acting among populations is greater than rates of gene flow (Haldane 1948; Barton 1979; Szymura and Barton 1986; Jiggins and Mallet 2000). In neutral cases, we expect population differentiation if m is much smaller than 1/4Ne (for diploid populations; Slatkin and Maruyama 1975). Generally speaking, these results mean that s_1, s_2, and m_2 will all influence the likelihood of CER and highlight the importance of understanding their magnitude in natural populations.

A third factor—the genetic basis of traits—has received significant attention in the reinforcement literature (Liou and Price 1994; Servedio and Noor 2003). With respect to reinforcement, a genetic mechanism linking the traits generating prezygotic isolation and those causing hybrid dysfunction can help to facilitate the evolution of strong prezygotic isolation and speciation (see Servedio and Noor 2003 and references therein for details). Both the physical clustering of loci in the genome and assortative mating driven by the action of a single allele have been discussed as mechanisms that can facilitate this LD. The former mechanism can promote speciation by reinforcement because it reduces recombination between loci involved in prezygotic and postzygotic isolation (Felsenstein 1981; Liou and Price 1994). The latter mechanism (frequently referred to as the "one-allele" model) can promote reinforcement because reproductive isolation is the result of a single allele that causes assortative mating irrespective of the genetic background it is found in (e.g., an allele causing individuals to mate with individuals more phenotypically similar to themselves or to reject heterospecifics in general). Since recombination cannot affect reproductive isolation caused by a one-allele mechanism, this mechanism has been considered particularly powerful for speciation by reinforcement (Ortiz-Barrientos et al. 2004; Ortíz-Barrientos and Noor 2005; Bank et al. 2012).

Unlike reinforcement, the genetics of CER does not require LD between the alleles generating hybrid dysfunction and those underlying prezygotic isolation. Therefore, it is unknown whether genetic mechanisms favoring reinforcement, such as the one-allele mechanism, are likely to lead to CER. For example, if reinforcement causes sympatric populations to differentiate from allopatric populations with respect to a trait crucial for the recognition of conspecific mates, then strong-LD or one-allele mechanisms could promote CER. By contrast, a "discrimination" allele that strengthens one's ability to actively locate and secure conspecific mates could be favored in all populations and rapidly spread throughout a species' range. This second scenario would not constitute a CER because there would be no reproductive isolation among conspecific populations. Dissecting the genetic basis of the traits involved in reinforcement and CER provides an exciting opportunity to clarify the genetic mechanisms most likely to lead to CER and connect microevolutionary processes such as selection on particular alleles with macroevolutionary processes such as speciation.

Factors affecting c-s effects

C-s effects rely on the convergent evolution of populations experiencing parallel reinforcing selection. This is because different phenotypic and genetic solutions are required to facilitate the differentiation of conspecific traits, and different genetic variants fixed in different populations will potentially be incompatible and generate RI when brought together. Theoretical work explicitly testing the demographic and genetic factors affecting the likelihood of c-s effects are needed, however others have discussed how the genetic basis of parallel phenotypic adaptation will depend on genetic, mutational, and demographic factors (Stern and Orgogozo 2008, 2009; Ralph and Coop 2015). For example, recent theoretical work has shown how parallel adaptation can frequently occur through different genetic mechanisms when population sizes are large, there are many mutational targets underlying adaptive phenotypic variation (Ralph and Coop 2015), and rates of gene flow between the populations adapting in parallel are low (Nosil and Flaxman 2011; Ralph and Coop 2015). In some ways these results suggest that the "simple" genetic control predicted for reinforced traits may result in c-s effects being less likely. For example, a trait under simple genetic control will have fewer mutation targets than a highly polygenic trait. However, this depends on the dimensionality of phenotypic adaptation, and large mutational targets can be manifest through either polygenic control of as single trait, or simple, and independent, control of many traits (Nosil and Hohenlohe 2012). These predictions and scenarios highlight how identifying the traits and genes that are targets of reinforcement will help us to better understand and predict reinforcement's evolutionary consequences, especially with respect to genetic differentiation among conspecific populations.

Factors affecting s–a effects

S–a effects require the alleles underlying reinforced phenotypes to be under divergent selection between sympatric and allopatric regions of a species's range (i.e., traits are favored in sympatry but disadvantageous in allopatry, a type of Genotype × Environment interaction). There are three parameters that are therefore expected to influence the magnitude of s–a effects: s_1, s_2, and m_2 (Haldane 1948; Barton 1983). When reinforced phenotypes are advantageous in sympatry but deleterious in allopatry ($s_1 > 0$ and $s_2 < 0$), their underlying alleles are expected to be structured geographically depending on the relative magnitudes of s_1, s_2, and the rate of migration between populations [m_2; a full theoretical treatment on the subject can be found in Bank et al. (2012)]. Direct measurements of selection acting on the phenotypes involved in reinforcement in sympatric and allopatric regions of the same species' range (i.e., s_1 and s_2) remain rare (however, see: Bewick and Dyer 2014; Hopkins and Rausher 2014; Hopkins et al. 2014). Studies of the Texas wildflower *P. drummondii* arguably provide the best example of how characterizing selection, gene flow, and the genetic basis of reinforced phenotypes can help explain their observed geographic distributions and evolutionary relevance (Hopkins and Rausher 2014; Hopkins et al. 2014). Estimating these parameters in other systems undergoing reinforcement are required to verify whether observed clines in reinforced phenotypes (i.e., a pattern of RCD) are simply due to geographical isolation present between conspecific populations or reflect true CER.

Future Directions and Conclusion

Our understanding of CER could be strengthened through the development of three avenues of research. First, we need to establish how frequently reinforcement has cascading effects among populations of the same species and quantify the magnitude of those effects. A considerable proportion of the systems where reinforcement is acting have also shown evidence of CER (see Table 1 for examples). However, for many of these cases we do not know the magnitude of RI that exists between allopatric and sympatric populations of conspecifics, or the specific phenotypes involved in generating RI, making it difficult to establish the importance of reinforcement in incidentally promoting differentiation and ultimately speciation among conspecifics.

A second avenue of research is the development of alternative approaches for detecting CER. All cases in which CER have been detected rely on the observation of RCD in sympatry and differentiation between conspecific populations. Nonetheless, we know that reinforcement does not always generate RCD (i.e., stronger reproductive isolation spreads from the hybrid zone throughout the whole range of a species; Noor 1997) and that not all RCD is caused by reinforcement (Brown and Wilson 1956; Walker 1974). A simple, but not always feasible, approach to detecting CER is the use of time-series data collected to measure how the magnitude of reproductive isolation and levels of hybridization change through time (Pfennig 2003). This, of course, will only be possible for organisms with relatively short generation times, rapid evolutionary responses to reinforcing selection (Pfennig 2003), and/or those that are amenable to experimental evolution in the lab (e.g., Drosophilid flies, Matute 2010; Comeault et al. 2015b; *Neurospora,* Turner et al. 2010; *Saccharomyces,* Murphy and Zeyl 2015).

The development of phylogenetic comparative or population genetic methods for detecting CER would be a more generalizable approach. Reinforcement can leave a signature on phylogenies. Noor (1997) proposed a simple yet powerful test to compare the magnitude of reproductive isolation in a phylogenetic framework. The premise of this approach is to use three closely related species (2 sister species "A" and "B", and an outgroup "C"). The triad must fulfill the following criteria: one of the sister species (A) must be sympatric with the outgroup (C), and the other (B) must be allopatric to both species (A and C). If reinforcement has played a role in the evolution of reproductive isolation in the sympatric species (A), then it should show greater species discrimination toward (C) than the allopatric species (B). Noor (1997) used this approach and found that reinforcement has played a role in 21% of the *Drosophila* species examined. This type of approach does not exist for detecting CER and its development could be critical for organisms in which measuring reproductive isolation in the laboratory is not feasible. While controlling for the divergence time between sister species, the expectation of a phylogenetic approach for detecting CER would be that if CER were common, levels of differentiation among conspecific populations (measured as genetic or phenotypic differentiation) should be greater in those systems where reinforcement occurs when compared with those systems where it does not. The challenge to this type of approach will be in determining the systems (and populations) where reinforcement is or is not occurring. However, patterns such as asymmetries in the cost of hybrid matings can be used to define species that are more likely to be affected by reinforcement (Yukilevich 2012). This information could then be used to predict the species that are most likely to show CER.

A third avenue of research that would benefit the study of CER would be to determine the genetics of the traits involved in reinforcement. The genetics of RRI remains largely unknown, and even less is known regarding whether the traits that respond to reinforcing selection are the same as those that lead to CER. Disentangling whether RRI toward heterospecifics shares the same genetic basis as its cascading effects is crucial to understanding the mechanism leading to both reproductive isolation between heterospecifics and that observed between conspecifics. For example, this would allow us to determine whether CER are a consequence of direct selection or selection through linkage (i.e., hitchhiking).

CER represent a mechanism through which reinforcement can not only complete speciation after secondary contact, but can also promote additional speciation events between conspecific populations. Current evidence indicates that CER can lead to the origin of moderate levels of reproductive isolation (see examples in Table 1). Speciation, however, involves the origin of new barriers and the persistence of reproductively isolated lineages. It remains to be shown whether the CER can indeed drive the diversification of lineages that persist over time or whether they are transient patterns observed during the completion of speciation by reinforcement.

Glossary of Terms

Reinforcement: the process where selection against maladapted hybrid offspring indirectly selects for, and causes the evolution of, increased prezygotic isolation between two co-occurring species.

RCD: a pattern generated when competition between two species causes phenotypes to shift in one or both interacting species such that competition is minimized. RCD is commonly (but not always) generated by reinforcement and is frequently identified by comparing the distribution of phenotypes observed in populations of a species that co-occur with a competing species with those of conspecific populations that are found in allopatry. In reproductive character displacement, the phenotypes that are displaced in sympatry affect phenotypes used in mate recognition, mate choice, or fertility.

CER: incidental effects that reinforcement can have on levels of reproductive isolation between populations of the same species.

RRI: isolation between two species that evolves as a result of reinforcing selection.

C-s effects: CER that are the result of convergent responses to reinforcing selection acting in parallel in multiple populations of the same (focal) species.

S–a effects: CER that are the result of alleles or phenotypes that are favored by reinforcing selection in the sympatric (with respect to a second species) part of a species' range, but selected against in allopatric regions of that same species' range.

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