*Current Zoology*, 2016, 62(2), 131–134
doi: 10.1093/cz/zow011
Special Column: Editorial

Special Column: Editorial

# Reconciling concepts, theory, and empirical patterns surrounding cascade reinforcement

**Rebecca C. Fuller, Editor**

Department of Animal Biology, University of Illinois, Urbana, IL 61801, USA

Email: fuller@life.illinois.edu.

## Introduction

Reinforcement is a critically important process in speciation. It has been credited with often completing speciation, and it is unique in that natural selection directly favors the evolution of reproductive isolating barriers. Reinforcement occurs when selection favors increased prezygotic isolation between two lineages in order to avoid maladaptive hybridization in areas of sympatry (Noor 1999; Servedio and Noor 2003; Coyne and Orr 2004). The classic signature of reinforcement is reproductive character displacement (RCD), where prezygotic isolation is heightened between two lineages in areas of sympatry relative to areas of allopatry. Reinforcement was once considered controversial primarily due to theoretical difficulties (Felsenstein 1981; Spencer et al. 1986; reviewed in Noor 1999, and Coyne and Orr, 2004). However, a wealth of empirical cases demonstrating reinforcement along with renewed theoretical treatments of the process of reinforcement (Liou and Price 1994; Kelly and Noor 1996; Servedio 2000; Kirkpatrick and Ravigne 2002) has led to the conclusion that reinforcement can and does occur in nature. One consequence of reinforcement is that—by altering reproductive isolating traits—reinforcement may also lead to prezygotic isolation among populations within species. Hence, like ecological and sexual selection, reinforcement can have incidental effects upon reproductive isolation (RI). In fact, reinforcement might be particularly likely to have such incidental effects precisely because it alters reproductive isolating traits among species in areas of sympatry.

The process whereby increased prezygotic isolation among populations evolves as an incidental effect of reinforcement has been referred to as "cascade reinforcement" (Ortiz-Barrientos et al. 2009) and as "reproductive character displacement speciation" (Hoskin and Higgie 2010). In this special column, we use the term cascade reinforcement. The idea has been recognized sporadically throughout the history of reinforcement (Zouros and Dentremont 1980; Howard 1993), but has recently received renewed attention (Ortiz-Barrientos et al. 2009; Hoskin and Higgie 2010; Abbott et al. 2013). A substantial number of empirical studies have shown patterns in prezygotic isolation among populations that are consistent with cascade reinforcement (Nosil et al. 2003; Hoskin et al. 2005; Higgie and Blows 2007, Higgie and Blows 2008; Lemmon 2009; Bewick and Dyer 2014; Pfennig and Rice 2014; Kozak et al. 2015).

Two broad classes of cascade reinforcement have been recognized in the literature (Abbott et al. 2013; Comeault and Matute 2016). In this issue, Comeault and Matute (2016) refer to these as "sympatry–allopatry" effects and "convergent-sympatry" effects. To conceptualize these two types of cascade reinforcement, consider Figure 1, which shows a phylogeny with two species (A and B) and two populations within species B (B1 and B2). As noted by Pfennig (2016) in this column, the terminology surrounding these concepts can be confusing. With sympatry–allopatry effects, B1 is sympatric and B2 is allopatric with respect to A, but the two are parapatric to one another. Reinforcement between A and B1 creates preferences and traits that confer RI between them. While the isolating traits in B1 are favored in areas of sympatry with A, they are disfavored in the allopatric population, B2. A number of empirical papers are consistent with this scenario (Bewick and Dyer 2014; Pfennig and Rice 2014; Rundle and Dyer 2015). With convergent-sympatry effects, both B1 and B2 are sympatric with A but are potentially allopatric with one another. Convergent-sympatry effects occur when separate populations within a species experience reinforcement independently and convergent evolution occurs. Reinforcement is convergent in the sense that RI evolves independently between A and both B1 and B2. Reinforcement occurs in both populations but different isolating traits evolve in each, both of which create isolation from A. Because conspecific preference in populations B1 and B2 rely on different isolating traits, prezygotic isolation arises between them and can prevent gene flow upon secondary contact. Empirical work also supports this scenario (Lemmon 2009).

The empirical evidence for cascade reinforcement has preceded the theoretical treatment of the phenomenon, a situation reminiscent of the earlier situation with general reinforcement. Cascade reinforcement has, until now, relied primarily on verbal models (Ortiz-Barrientos et al. 2009; Hoskin and Higgie 2010). However, two studies are noteworthy. McPeek and Gavrilets (2006) created a model that showed that interactions with other species could rapidly create impressive variation among populations within a species in female preference functions, which they assumed to be vital for the early stages of speciation. One could consider this as an early model of convergent-sympatry effects. However, this study assumed that speciation was completed with the interacting species (i.e., hybrid fitness was zero) and did not allow

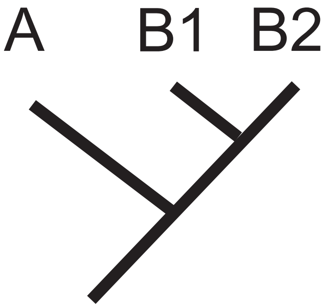

**Figure 1**. Reinforcement between A and B can affect RI between B1 and B2.

for gene flow among the diverging allopatric populations. As we have seen in the past, gene flow and recombination create problems for speciation. The other notable work stems from neural network studies by Pfennig and Ryan (2006). Pfennig and Ryan explicitly simulated evolution in sympatric and allopatric populations of neural networks. Sympatric neural networks had to identify conspecific over heterospecific calls. The study found that RCD also created RI among allopatric and sympatric populations of networks. However, this study did not allow for gene flow among populations. Hence, the complications presented by gene flow were precluded. As an aside, it is worth noting that a number of papers have used neural networks to examine the consequences of selection for species recognition on mating preferences among conspecifics (Phelps and Ryan 1998; Phelps and Ryan 2000; Phelps et al. 2001). These papers address a fundamental assumption of cascade reinforcement, which is that selection for species recognition has correlated effects that alter preferences at the within species level.

## Contributions to this Issue

The papers in this column dramatically extend our understanding of cascade reinforcement and set the stage for compelling lines of future research. First, Yukilevich and Aoki (2016) present an individual-based, population genetic model of sympatry–allopatry effects that seeks to determine the conditions under which stable RI can evolve between sympatric and allopatric populations (B1 and B2) as a function of cascade reinforcement. The take home message from this study is that RI between sympatric and allopatric populations is only stable when the effective number of migrants from sympatric to allopatric populations is low. Specifically, the authors found that RI was only maintained when the effective number of migrants from sympatry to allopatry was less than one ($Nm < 1$) where $N$ is the population size and $m$ is the proportion of individuals migrating per generation. Interestingly, the migration rate from allopatry to sympatry had little effect on the maintenance of RI between allopatric and sympatric populations, provided that it was not so high as to prevent reinforcement between A and B1. Above the threshold for stable RI ($Nm > 1$), the isolating traits conferring RI with A spread beyond the zone of sympatry resulting in species wide reinforcement with A and no RI between B1 and B2. In effect, gene flow from sympatry to allopatry erased the signature of RCD and precluded cascade reinforcement. In contrast, cascade reinforcement coupled with ecological selection could readily generate stable RI when $Nm > 1$, but the unique contribution of cascade reinforcement to this process varied.

Of course, this is a single model that considers a specific scenario. Yukilevich and Aoki (2016) considered a two-allele system for preference where stabilizing sexual selection in allopatry is posited to prevent the spread of isolating traits from sympatry to allopatry. Ortiz-Barrientos et al. (2009) present a long list of mechanisms that could potentially cause selection against isolating traits in allopatric populations and thus prevent species wide reinforcement. Clearly, not all of these have been the subject of theoretical models. Additionally, one-allele mechanisms where the same allele is fixed in both populations and confers RI among species (and potentially populations) may make cascade reinforcement more likely (Kirkpatrick and Ravigne 2002). One-allele mechanisms include phenomena such as phenotypic matching, parental imprinting, and increased self-fertilization (see Castillo et al. 2016, this issue).

This theoretical framework provides a strong vantage point for considering the two empirical papers (Humphreys et al. 2016; Rice 2016) found in this issue. Humphreys et al. (2016) consider cascade reinforcement within the *Drosophila subquinaria*–*D. recens*–*D. transversa* system. This system arguably represents one of our most complete examples of cascade reinforcement. Past work has shown very strong, asymmetrical reinforcement between *D. subquinaria* and *D. recens* due to the presence of Wolbachia in *D. recens* and its absence in *D. subquinaria* that has created a pattern of RCD (Jaenicke et al. 2006). Females of *D. subquinaria* from sympatric populations discriminate strongly against *D. recens* males; females from allopatric populations mate more readily with *D. recens* males. Dyer and colleagues have shown a pattern consistent with cascade reinforcement and sympatry-allopatry effects where female *D. subquinaria* from sympatric populations also discriminate against conspecifics from allopatric populations. Likewise, male *D. subquinaria* from sympatric populations are not favored as mates by allopatric females. These effects are very robust, as they have been demonstrated multiple times (Bewick and Dyer 2014; Dyer et al. 2014; Rundle and Dyer 2015; Humphreys et al. 2016). Furthermore, both the female preferences and the male target traits have been quantified (Rundle and Dyer 2015).

In the current study, Humphreys et al. (2016) extend this work to consider a third species, *D. transversa*, which is allopatric to *D. subquinaria* but shares a zone of sympatry with *D. recens*. This study shows that the high levels of female discrimination in sympatric populations of *D. subquinaria* also alter female discrimination against male *D. transversa*. Females of *D. subquinaria* that are sympatric with *D. recens* discriminate against male *D. transversa*, but allopatric females do not. Hence, reinforcement between *D. subquinaria* and *D. recens* has also led to increased RI between sympatric populations of *D. subquinaria* and another allopatric species (*D. transversa*). There are two implications of this study. First, cascade reinforcement may cause RI between allopatric taxa that would prevent hybridization upon secondary contact. Second, reinforcement may alter mating preferences more broadly than previously expected such that RI is generated—not only among populations within species—but also at multiple taxonomic levels.

Rice (2016) takes a slightly different approach and considers the effects of cascade reinforcement on levels of gene flow among populations in spadefoot toads *Spea multiplicata*. Previous studies have shown that reinforcement between *S. multiplicata* and *S. bombifrans* has created a pattern of RCD that has resulted in cascade reinforcement. Females of *S. multiplicata* that occur in sympatry with *S. bombifrans* have heighted preferences for conspecifics over heterospecifics and also prefer males from their own populations over males from allopatric populations, which results in reduced gene flow between sympatric and allopatric populations (Pfennig and Rice 2014; Pfennig and Simovich 2002). The current study extends this analysis by considering a larger suite of populations. The main result is that gene flow is reduced between sympatric versus allopatric populations, but the result is small compared to the overall effects of distance.

Castillo et al. (2016) provide a unique contribution to this column and consider the question of whether reinforcing selection can favor increased rates of self-fertilization. Self-fertilization can potentially act as a one-allele mechanism. While self-fertilization and uniparental reproduction is studied most often in plants, it also occurs in animal taxa (Charnov 1982). The complicating issue with self-fertilization is that it potentially results in as much as a two-fold increase in fitness depending on the levels of inbreeding depression. Castillo et al. (2016) modeled the evolution of self-fertilization as an effect of reinforcing selection and inbreeding depression. They found self-fertilization readily evolved provided that inbreeding depressions did not reduce fitness by more than 50% (as expected). However, they also considered the role of reinforcing selection in these dynamics. When inbreeding depression was low (and the inherent advantage of self-fertilization was high), then reinforcing selection had little effect on the rates at which selfing alleles fixed within populations. In contrast, when inbreeding depression was high (and the inherent advantage of self-fertilization was low, but still above 0), then reinforcing selection caused selfing alleles to fix more rapidly within populations. When they allowed for both self-fertilization and assortative mating to both evolve, they found that self-fertilization often precluded assortative mating. They then analyzed two clades with multiple transitions to between uniparental reproduction (such as selfing) to determine whether increased rates of lineage diversification were associated with selfing. The results were mixed with one clade (*Silene*) showing no evidence of such an effect while another (Nematoda) showed a trend towards increased diversification with transitions to uniparental reproduction. There are three take home messages from this study. First, reinforcing selection can play a role in the evolution of self-fertilization when the inherent fitness advantage to self-fertilization is low. Second, self-fertilization can often preclude assortative mating. Third, self-fertilization can provide a unique mechanism that increases RI between species and populations.

Finally, this special column concludes with two reviews/perspectives of cascade reinforcement. While hitting upon many of the same topics, the two articles focus on slightly different aspects of cascade reinforcement. Comeault and Matute (2016) focus specifically on the two classes of cascade reinforcement and provide the terms of "sympatry–allopatry effects" and "convergent-sympatry effects", which I have adopted in this editorial. They then provide a verbal model for each type of effect and provide guidance for future research endeavors. Pfennig (2016) focuses on the factors that promote and prevent cascade reinforcement via sympatry–allopatry effects. This is a lucid discussion of the roles of gene flow and biogeography, sexual selection, and the potential asymmetrical nature of mate choice on cascade reinforcement. Pfennig points out that many of the difficulties raised with testing reinforcement are also present for cascade reinforcement. Pfennig also notes that divergence will be problematic with asymmetric isolation where individuals in sympatric populations reject allopatric mates, but individuals in allopatric populations accept sympatric mates. In *Lucania* killifish, there is evidence that such dynamics are important in the geographic pattern of preference (Berdan and Fuller 2012; Gregorio et al. 2012; Kozak et al. 2015). Distantly allopatric populations mate at random, sympatric populations have high levels of preference for conspecifics and natives (i.e., individuals from their own populations), and allopatric populations that are geographically close to sympatric populations show high levels of preference for conspecifics and natives. This highlights the recurring difficulty of gene flow from sympatry to allopatry. Pfennig (2016) also provides recommendations for experimental design and future directions.

In conclusion, this special column illustrates that there are many critical questions remaining for cascade speciation. Two issues are particularly noteworthy. First, the model by Yukilevich and Aoki (2016) suggests that cascade reinforcement may not produce stable RI between sympatric and allopatric populations in the face of substantial gene flow from sympatry to allopatry. Yet, both spadefoot toads and *D. subquinaria* provide evidence for substantial RI between sympatric and allopatric populations. Furthermore, population structure calculations in spadefoot toads suggest a potential for gene flow between sympatric and allopatric populations, (Rice, 2016), although it is difficult to confidently estimate Nm without better marker coverage. Dyer and colleagues also provide evidence that Nm is greater than 1 between populations (Dyer, personal communication; Bewick and Dyer 2014, see Table S3), although the confidence intervals may overlap 1. Second, and related, is the notion repeatedly espoused in verbal models and empirical work that differences in the direction of sexual selection and female mating preferences may maintain RI between sympatric and allopatric populations provided that the traits favored in sympatry are disfavored in allopatry (Comeault and Matute 2016; Pfennig 2016). The subtle disconnect between theory, verbal models, and empirical pattern suggests that there is interesting work to be done. As was the case with reinforcement, cascade reinforcement presents both a theoretical and an empirical challenge to evolutionary biologists. I hope that this special column highlights areas where progress will be made and represents a substantial step forward in this field.

## Funding

This work was supported by NSF (0953716 to R.C.F.).

## Acknowledgments

I thank Zhi-Yun Jia and Maria Servedio for inviting me to be a guest edit for this special column in *Current Zoology*. It's been a very enjoyable experience. I also thank the contributors for their fine manuscripts and other valuable contributions to the special column. Jeff Birdsley provided helpful comments on this editorial.

## References

Abbott R, Albach D, Ansell S, Arntzen JW, Baird SJE et al., 2013. Hybridization and speciation. *J Evol Biol* **26**:229–246.

Berdan EL, Fuller RC, 2012. A test for environmental effects on behavioral isolation in two species of killifish. *Evolution* **66**:3224–3237.

Bewick ER, Dyer KA, 2014. Reinforcement shapes clines in female mate discrimination in *Drosophila subquinaria*. *Evolution* **68**:3082–3094.

Castillo DM, Gibson AK, Moyle LC, 2016. Assortative mating and self-ertilization differ in their contributions to reinforcement, cascade speciation, and diversification. *Curr Zool* **62**:145–154.

Charnov EL, 1982. *The Theory of Sex Allocation*. Princeton: Princeton University Press.

Comeault A, Matute D, 2016. Reinforcement's incidental effects on reproductive isolation between conspecifics. *Curr Zool* **62**:135–144.

Coyne JA, Orr HA, 2004. Speciation. Sunderland: Sinauer.

Dyer KA, White BE, Sztepanacz JL, Bewick ER, Rundle HD, 2014. Reproductive character displacement of epicuticular compounds and their contribution to mate choice in *Drosophila subquinaria* and Drosophila recens. *Evolution* **68**:1163–1175.

Felsenstein J, 1981. Skepticism towards Santa Rosalia, or why are there so few kinds of animals. *Evolution* **35**:124–138.

Gregorio O, Berdan EL, Kozak GM, Fuller RC, 2012. Reinforcement of male mate preferences in sympatric killifish species *Lucania goodei* and Lucania parva. *Behav Ecol Sociobiol* **66**:1429–1436.

Higgie M, Blows MW, 2007. Are traits that experience reinforcement also under sexual selection? *Am Nat* **170**:409–420.

Higgie M, Blows MW, 2008. The evolution of reproductive character displacement conflicts with how sexual selection operates within a species. *Evolution* **62**:1192–1203.

Hoskin CJ, Higgie M, McDonald KR, Moritz C, 2005. Reinforcement drives rapid allopatric speciation. *Nature* **437**:1353–1356.

Hoskin CJ, Higgie M, 2010. Speciation via species interactions: the divergence of mating traits within species. *Ecol Lett* **13**:409–420.

Howard DJ, 1993. Reinforcement: origin, dynamics, and fate of an evolutionary hypothesis. In: Harrison RG, editor. *Hybrid Zones and the Evolutionary Process*. New York: Oxford University Press, 46–69.

Humphreys DP, Rundle HD, Dyer KA, 2016. Patterns of reproductive isolation in the *Drosophila subquinaria* complex: can reinforced premating isolation cascade to other species? *Curr Zool* **62**:183–192.

Kelly JK, Noor MAF, 1996. Speciation by reinforcement: a model derived from studies of *Drosophila*. *Genetics* **143**:1485–1497.

Kirkpatrick M, Ravigne V, 2002. Speciation by natural and sexual selection: models and experiments. *Am Nat* **159**:S22–S35.

Kozak GM, Roland G, Rankhorn C, Falater A, Berdan EL et al., 2015. Behavioral isolation due to cascade reinforcement in *Lucania* killifish. *Am Nat* **185**:491–506.

Lemmon EM, 2009. Diversification of conspecific signals in sympatry: geographic overlap drives multidimensional reproductive character displacement in frogs. *Evolution* **63**:1155–1170.

Liou LW, Price TD, 1994. Speciation by reinforcement of premating isolation. *Evolution* **48**:1451–1459.

McPeek MA, Gavrilets S, 2006. The evolution of female mating preferences: differentiation from species with promiscuous males can promote speciation. *Evolution* **60**:1967–1980.

Noor MAF, 1999. Reinforcement and other consequences of sympatry. *Heredity* **83**:503–508.

Nosil P, Crespi BJ, Sandoval CP, 2003. Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. *Proc R Soc B Biol Sci* **270**:1911–1918.

Ortiz-Barrientos D, Grealy A, Nosil P, 2009. The genetics and ecology of reinforcement: implications for the evolution of prezygotic isolation in sympatry and beyond. In: Schlichting CD, Mousseau TA, editors. *Year in Evolutionary Biology*, 156–182.

Pfennig K, 2016. Reinforcement as an initiator of population divergence and speciation. *Curr Zool* **62**:145–154.

Pfennig KS, Simovich MA, 2002. Differential selection to avoid hybridization in two toad species. *Evolution* **56**:1840–1848.

Pfennig KS, Ryan MJ, 2006. Reproductive character displacement generates reproductive isolation among conspecific populations: an artificial neural network study. *Proc R Soc B Biol Sci* **273**:1361–1368.

Pfennig KS, Rice AM, 2014. Reinforcement generates reproductive isolation between neighbouring conspecific populations of spadefoot toads. *Proc R Soc B Biol Sci* **281**:20140949.

Phelps SM, Ryan MJ, 1998. Neural networks predict response biases of female tungara frogs. *Proc R Soc B Biol Sci* **265**:279–285.

Phelps SM, Ryan MJ, 2000. History influences signal recognition: neural network models of tungara frogs. *Proc R Soc B Biol Sci* **267**:1633–1639.

Phelps SM, Ryan MJ, Rand AS, 2001. Vestigial preference functions in neural networks and tungara frogs. *Proc Natl Acad Scie USA* **98**:13161–13166.

Rice AM, 2016. Population differentiation at a regional scale in spadefoot toads: contributions of distance and divergent selective environments. *Curr Zool* **62**:193–206.

Rundle HD, Dyer KA, 2015. Reproductive character displacement of female mate preferences for male cuticular hydrocarbons in *Drosophila subquinaria*. *Evolution* **69**:2625–2637.

Servedio MR, 2000. Reinforcement and the genetics of nonrandom mating. *Evolution* **54**:21–29.

Servedio MR, Noor MAF, 2003. The role of reinforcement in speciation: theory and data. *Ann Rev Ecol Evol Syst* **34**:339–364.

Spencer HG, McArdle BH, Lambert DM, 1986. A theoretical investigation of speication by reinforcement. *Am Nat* **128**:241–262.

Yukilevich R, Aoki F, 2016. Is cascade reinforcement likely when sympatric and allopatric populations exchange migrants? *Curr Zool* **62**:155–168.

Zouros E, Dentremont CJ, 1980. Sexual isolation among populations of *Drosophila mojavensis*: response to pressure from a related species. *Evolution* **34**:421–430.