

Pervasive Reinforcement and the Role of Sexual Selection in Biological Speciation

EMILY J. HUDSON AND TREVOR D. PRICE

From the School of Biological Sciences, University of Nebraska – Lincoln, 410 Manter Hall, Lincoln, NE 68588-0118 (Hudson); and the Department of Ecology and Evolution, University of Chicago, Chicago, IL (Price).

Address correspondence to Emily J. Hudson at the address above, or e-mail: ehudson@huskers.unl.edu.

Abstract

Sexual selection has been widely implicated as a driver of speciation. However, allopatric forms are often defined as species based on divergence in sexually selected traits and it is unclear how much such trait differences affect reproductive isolation upon secondary contact, the defining feature of biological species. We show that in birds, divergence in song and plumage in allopatry corresponds poorly with whether species mate assortatively in hybrid zones and argue that this is because many other factors besides trait divergence affect propensity to hybridize, including rarity of conspecific mates and choice based on territory rather than male traits. We then present a general model for the establishment of sympatry that assumes a period of differentiation in allopatry followed by secondary contact and often hybridization, with hybridization subsequently reduced by reinforcement of mate preferences. We suggest that reinforcement commonly operates by a narrowing of a “window of recognition” for traits that are different between the species, rather than evolution of the traits themselves. Our arguments imply that it is important to study postmating as well as premating reproductive isolation in limiting sympatry and suggest that studies of reinforcement should focus on evolution of female preferences for diagnostic traits, rather than evolution of traits per se.

Subject areas: Reproductive strategies and kinship analysis; Population structure and phylogeography

Key Words: birds, hybrid zones, postmating isolation, premating isolation, reinforcement, sexual selection, speciation

Sexual selection is an important diversifying force (Andersson 1994; Figure 1). For example, in birds, sexual dimorphism in plumage, assumed to be an index of sexual selection, results in greater interspecific divergence among males than does sexual monomorphism (Seddon et al. 2013). In this paper, we evaluate the role of divergence in male secondary sexual traits as a driver of not only diversification, but also speciation, by which we mean establishment of reproductive isolation between divergent populations (Coyne and Orr 2004). We first consider the problem of defining allopatric taxa as biological species and then present one way to solve this problem, by considering mating patterns in hybrid zones. Based on a review of avian hybrid zones, we find that divergence in sexually selected traits in allopatry does not necessarily correlate with reproductive isolation upon secondary contact. From this observation, we develop 2 arguments. First, postmating barriers are often critical to the establishment of complete sympatry because they drive reinforcement of premating isolation. Second, premating isolation may often depend on mechanisms that result in trait discrimination, rather than evolution of the traits themselves.

In Figure 1, we show 2 classic examples of male variation across sexually dimorphic taxa, chosen for the historical interest. The Bellbirds (genus *Procnias*) from South America were at the forefront of the renaissance of sexual selection in the 1970s (West Eberhard 1983): Snow (1976, p. 88), suggested “some very powerful and arbitrary selective force is continually acting on the males.” The Golden Whistler (*Pachycephala pectoralis*) was termed the “great speciator” by Mayr and Diamond (2001, p. 143), for its tremendous variation in male plumages across South Pacific islands. Besides illustrating diversification, these 2 examples also exemplify the perennial question for students of speciation: when should differentiated allopatric forms be considered different biological species (Coyne and Orr 2004; Tobias et al. 2010)? Under the biological species concept species are “groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups” (Mayr 1942, p. 120). However, it is not known if the named forms in the examples of Figure 1 would interbreed if found together in sympatry. Nor is it often possible to resolve the question by experiments in the lab, because many sympatric

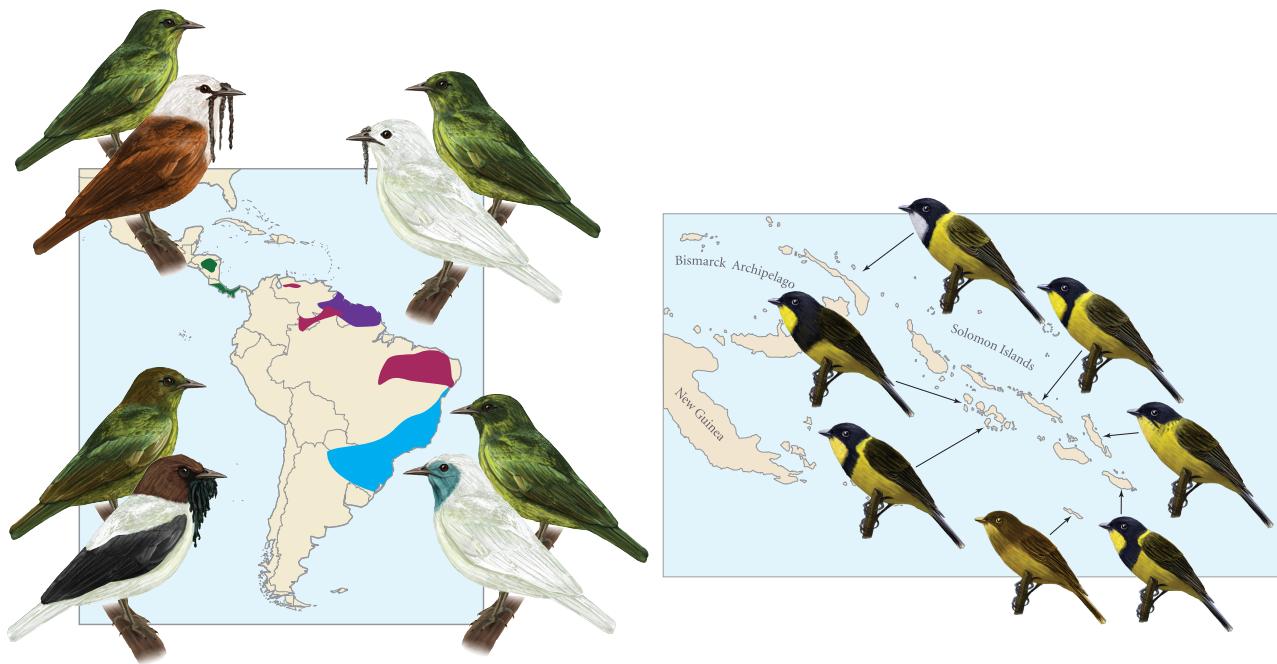


Figure 1. Left: The 4 species of bellbirds (genus *Procnias*). Females of all species are greenish and similar. Clockwise from top left, 3-wattled bellbird (*Procnias tricarunculatus*), White bellbird (*Procnias albifrons*), Bare-throated bellbird (*Procnias nudicollis*), Bearded bellbird (*Procnias averano*). Right: Males of several subspecies of the Golden Whistler (*Pachycephala pectoralis*). Isolated Rennell Island contains a particularly distinctive form (left at bottom), and in this form, the male and female are similar. Emiko Paul drew both these figures for the book “Speciation in Birds” by Price (2008, Roberts and Company).

species can be cross-mated and produce fertile hybrids in captivity (McCarthy 2006). The problem is further exacerbated because allopatric forms are relatively young (Figure 2), reflecting the transitional stage in the process of speciation that is started by divergence between populations experiencing no, or little, gene flow between them. For many of these forms, there is no critical point at which it would be possible to say they are good species.

The usual solution to the conundrum of how to classify allopatric forms is to adopt a morphological species concept (Coyne and Orr 2004), whereby a pair of taxa are given species rank if they differ from each other in traits such as plumage coloration and songs to an extent similar to that of sympatric species (Isler et al. 1998; Helbig et al. 2002; Tobias et al. 2010). One difficulty with applying this method is that extremely similar species are often found breeding alongside each other in sympatry (Helbig et al. 2002; Tobias et al. 2010; Tobias et al. 2013). Two examples, again chosen for their historical interest, are illustrated in Figure 3. Both these species pairs breed in the eastern Himalayas. They are altitudinally segregated but with considerable overlap in elevational range (Price et al. 2014). The 2 sexually monomorphic flycatcher warblers (*Seicercus*) were only distinguished in 1999 on the basis of their subtly distinct songs, even though they had been in the same museum drawer for >100 years (Alström and Olsson 1999; Martens et al. 1999). The 2 minivets (*Pericrocotus*), which are sexually dimorphic, with females having a similar pattern to males but with yellow plumage in the places where

males are red, were first separated by Mayr (1940) who noted: “The most interesting aspect of this phenomenon is that the birds themselves are apparently not deceived, even though the taxonomists are. There are no hybrids known between such very similar species, and it seems, therefore, that the small differences in conjunction with certain behavior patterns are specific recognition marks.” Examples such as these led Mayr and Gilliard (1952) and Brown and Wilson (1956) to suggest that degree of divergence in sexually selected traits among allopatric forms is not a strong predictor of reproductive isolation in sympatry.

Species are the basic unit of analysis in fields ranging from macroecology to conservation biology, so the classification of allopatric forms as subspecies or species is critical to many enquiries (Tobias et al. 2010). Long ago, Brown and Wilson (1956, p. 63) stated: “allopatric species or subspecies designated as such on a purely morphological basis frequently enter into theoretical discussions as though they were objectively established realities, when in fact they are usually no more than arbitrary units drawn for curatorial convenience.” One area where this is especially relevant is to the study of a role for sexual selection in speciation. If allopatric forms are defined as species based on degree of divergence in sexually selected traits, then tests that ask if groups with elevated levels of divergence also contain more species (e.g., Barraclough et al. 1995; Seddon et al. 2008) become circular. A way around this is to focus on sympatric assemblages (Price 1998). Few tests have been done as yet, but those that

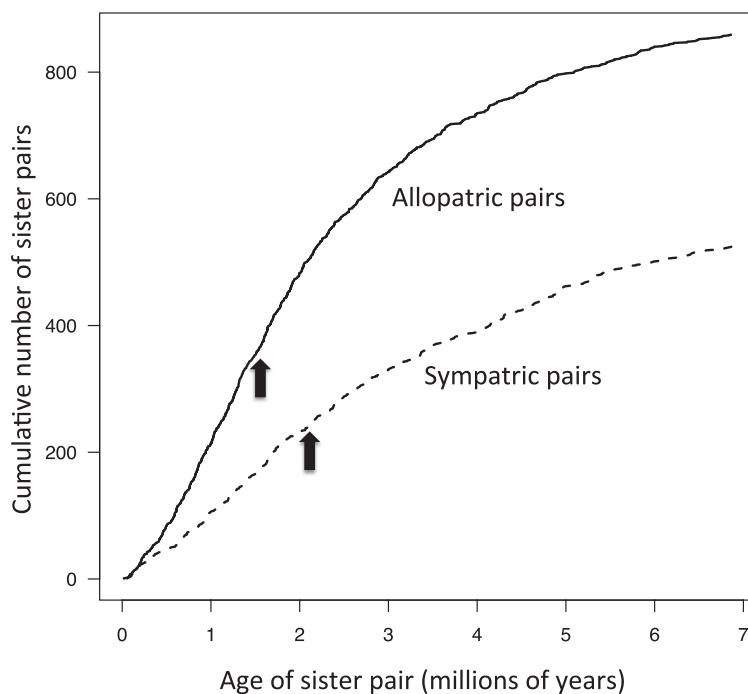


Figure 2. Cumulative frequency of age distributions for oscine (songbird) sister pairs <7 Ma ($N = 1386$ sister pairs) based on the tree of Jetz et al. (2012, [birdtree.org](#)) and range maps available from the [nature reserve](#) website ([birdlife.org](#)). The tree used was the maximum clade credibility tree calculated in TreeAnnotator v1.8.0 (Drummond and Rambaut 2007) from the first 1000 trees on the website (“Hackett backbone”). Note that sequence data are only available for 1765 of the 2772 species (64%); the other species were inserted based on taxonomic relationships. Because of uncertainties in the tree and in the range maps, the differences between allopatric and sympatric pairs (~0.55 millions of years) are almost certainly underestimated.

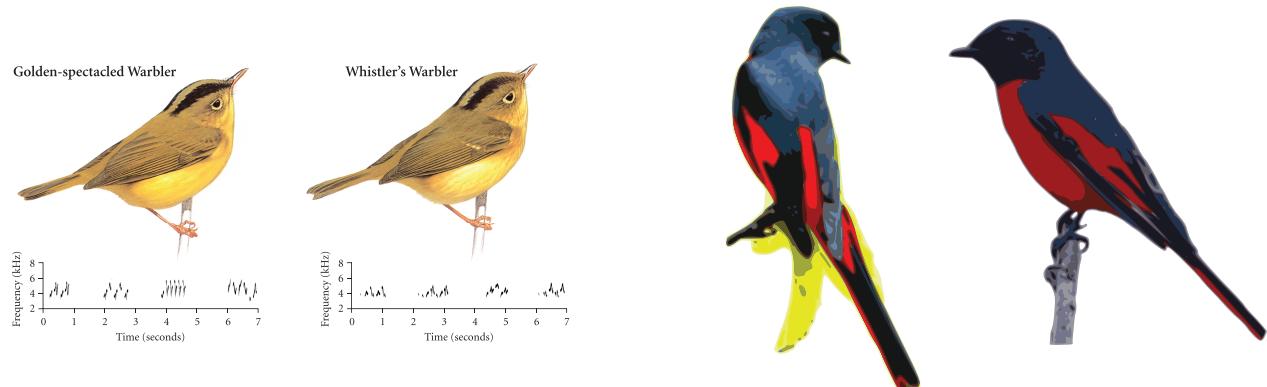


Figure 3. Left: 2 sympatric species of flycatcher-warbler (genus *Seicercus*, from Alström and Olsson, 1999, painted by Ian Lewington). Songs (illustrated) are less variable in the Whistler's warbler (*Seicercus whistleri*). Right: 2 sympatric species of minivet (*Pericrocotus*). The short-billed minivet (*Pericrocotus brevirostris*), right, has an extended patch of red on the secondary flight feathers; at left, long-tailed minivet (*Pericrocotus ethologus*). Minivet images adapted from photographs courtesy of Ramki Sreenivasan/Conservation India and © [www.clementfrancis.com](#).

have find little evidence that divergence in sexually selected traits correlates with number of sympatric species (Price 1998; Pfennig and Hurlbert 2012). In this paper, we more directly assess correlates of reproductive isolation and trait divergence. We consider bird songs and bird plumage colors,

2 traits that have been widely implicated as targets of mate choice in sexual selection (Andersson 1994; Wilkins et al. 2013), in species recognition (Clayton 1990; Price 2008) and in the designation of allopatric forms as good species (Isler et al. 1998; Tobias et al. 2010).

The examples of Figures 1 and 3 are chosen to contrast large differences in sexually selected traits between allopatric forms with often-small differences between sympatric species. They illustrate variable rates of divergence with time (neither of the 2 sympatric pairs in Figure 3 consist of sisters and each are divergent by >4 millions of years; Price et al. 2014). However, on average, allopatric sister pairs are less divergent than sympatric sister pairs in both song (Weir and Wheatcroft 2011; Tobias et al. 2013) and plumages (Martin et al. 2010) and this divergence has been suggested to be instrumental to their coexistence (Martin et al. 2010). Instead the greater divergence among sympatric sisters may reflect the fact that allopatric species are in the first stage of speciation and thus are generally younger than sympatric ones (Figure 2; Appendix). Sympatry often appears to be established only after a long period of divergence in allopatry (Weir and Price 2011). During the waiting time in allopatry, traits that affect both pre- and postmating isolation accumulate, held in association with each other purely as a result of the geographical barrier between the populations (Kirkpatrick and Ravigné 2002). Thus, while divergence in songs and plumages may make assortative mating more likely when ranges of different taxa expand into contact, divergence in factors affecting postmating isolation should also make hybrids less fit than parentals. We argue that while both these factors increase reproductive isolation, postmating isolation is a critical and often overlooked component, to be discussed further below in the section “Postmating isolation.”

We envisage secondary contact as being initiated along the range edges of each incipient species, creating a zone of parapatry. One possible outcome of such a meeting is that populations are already fully reproductively isolated, with no reproductive impediments to further range expansions. However, often some hybridization is expected (Mallet 2005) with theoretical outcomes differing depending both on the amount of hybridization and degree of postmating isolation, as summarized in Table 1 (in this table, following Servedio, 2001, we consider any postmating barriers, whether prezygotic or postzygotic, to act in similar ways with respect to the evolution of premating isolating mechanism). First, when both postmating and premating isolation are not strong, the differentiated forms collapse into a hybrid swarm. Second, conditions of strong premating isolation and weak postmating isolation are often implicit in considerations of how divergence in sexually selected traits may drive speciation (Edwards et al. 2005; Seddon et al. 2013), but we suggest that this should also eventually lead to a breakdown into a hybrid swarm, following occasional

hybridization events. A third outcome is when hybrids have low fitness but mating is not strongly assortative; here the rarer species goes extinct because females from the rarer form tend to mate frequently with males of the more common form (Liou and Price 1994).

These alternative outcomes are consistent with patterns in present-day avian hybrid zones, in which by definition, premating isolation is incomplete. Hybrid zones seem to fall into 2 classes (Figure 4): either narrow between old species (here individuals crossing the zone mate with members of the other taxon, and we assume because the taxa are old, hybrids have low fitness) or wide between young taxa, which we assume have relatively low postmating isolation. Note that these hybrid zone patterns persist only because of spatial structure: individuals and genes from pure populations on either side continually disperse into the zone. Complete sympathy would not be possible, either because the populations would collapse into a hybrid swarm or one or the other would go extinct.

A final possibility is that both postmating and premating isolation are already well established (Table 1). In this case, premating isolation may be further strengthened to the point of completion of reproductive isolation (Bank et al. 2012) in the process known as reinforcement (Coyne and Orr 2004, Chapter 10), which occurs concomitantly with the establishment of complete sympathy. We suggest this is a common process. Reinforcement arises in hybridizing taxa when females that mate with members of their own taxon have relatively higher reproductive success than those that mate with males from the other taxon; hence females with strong conspecific mating preferences are favored. Theoretical studies generally show that both strong pre- and postmating isolation are required on secondary contact for reinforcement to happen (Liou and Price 1994; Servedio 2000 [here we consider reinforcement to include the case where postmating isolation is complete, although this is sometimes separated from reinforcement and termed reproductive character displacement, e.g., Butlin 1987; Armbruster and Muchhal 2009]).

In the next section, we ask whether divergence in song and plumage is an important predictor of assortment (i.e., premating isolation) in avian hybrid zones. We find that it is not and suggest this is because many other factors besides trait divergence affect probability of hybridization. We then review the mechanism of species recognition in birds. Both females and males have a “window of recognition” on conspecific characteristics that include both sexually and naturally selected traits (Figure 5). The window is defined by the range in values of a trait that leads to the individual being recognized as conspecific. The window of recognition is easily modified by learning, and probably genetically as well. These observations lead to a model of reinforcement whereby female preferences for conspecifics become rapidly strengthened in sympatry, based on one or more diagnostic differences between forms, without any necessary further divergence in traits. We argue that reinforcement by discrimination is likely to be common and hence that postmating reproductive isolation is an essential contributor to

Table 1 Possible outcomes in sympatry in the presence of hybridization, based on Liou and Price (1994)

	Strong premating isolation	Weak premating isolation
Strong postmating isolation	Speciation by reinforcement	Rarer species goes extinct
Weak postmating isolation	Gradual collapse to hybrid swarm	Hybrid swarm

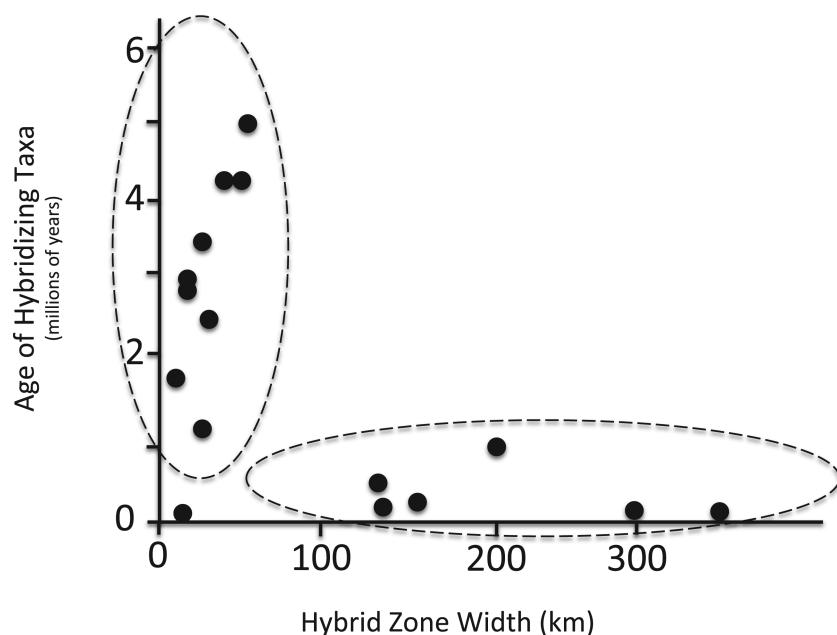


Figure 4. Age of divergence between hybridizing species pairs and corresponding hybrid zone widths. As indicated by the ellipses, hybrid zones between young species pairs tend to be wide, while those between older pairs are narrow. In this figure, taxa with historically recent secondary contact (<100 years ago) are not shown. Figure adapted from Price (2008, Chapter 15).

the establishment of sympatry, but degree of divergence in traits that affect premating isolation need not be.

Assortment in Hybrid Zones

Methods

To assess the importance of signal divergence as a reproductive barrier, we measured the degree to which divergence in plumage color and song in allopatric populations predicts premating isolation (i.e., assortative mating) in 17 well-studied avian hybrid zones. We chose hybridizing species or subspecies pairs documented in Price (2008, Chapter 15) based on the availability of reliable assessments of assortative mating from observations of breeding pairs. Assortative mating was assessed either with a chi-square test of heterospecific and conspecific pairings of pure parental types or by Pearson correlation of hybrid phenotypes within observed pairs (see Price 2008, p. 350). Because of these different approaches, and also because of uncertainty in field assessments, here we used a dichotomous classification into random mating, or significant deviation from random mating (assortment). All species and associated data are listed in Supplementary Table 1 online. For each of the species in each pair, we used several easily quantified song characteristics, and color of 10 or 11 standard plumage patches (Supplementary Table 1 online), as indicators of divergence in traits that are considered relevant to species recognition. Although we did not ask if these traits were under sexual selection in the species pairs studied, the importance of song and plumage for mate choice in birds is generally well supported (reviewed in Price 2008, Chapter 9).

Song

For each species, we obtained 3–5 vocalizations, each from a different individual, from [xeno-canto.org](https://xenocanto.org) or the Macaulay Library (macaulaylibrary.org). Wherever possible, songs were taken from allopatric regions of the species' range and from different dates and locations to insure the same individual was not included twice. Details of song recordings can be found in [Supplementary Materials](#) online. Following the methods of Weir and Wheatcroft (2011), we defined a song as “a discrete sequence of one or more syllables followed by a period of silence before another song is given.” We defined a syllable (unit) as the smallest repeating sequence of notes: either a single note or a sequence whose constituent parts were never repeated alone.

We analyzed songs in Raven Pro version 1.5 ([Bioacoustics Research Program 2014](#)). We averaged center, high and low, first quartile, and third quartile frequencies over the songs of each species and calculated the absolute difference between the pair of species. We also compared the total number of notes, syllables, and types of syllables for each song between each pair of species.

Plumage

We measured plumage color with an Ocean Optics USB2000+ spectrometer using SpectraSuite software (Ocean Optics). Bird skins were obtained courtesy of the Chicago Field Museum of Natural History and the American Museum of Natural History. Wherever possible, we measured adult breeding males from allopatric portions of the species' ranges. Using the R package `tetracolorspace` ([Stoddard and Prum](#)

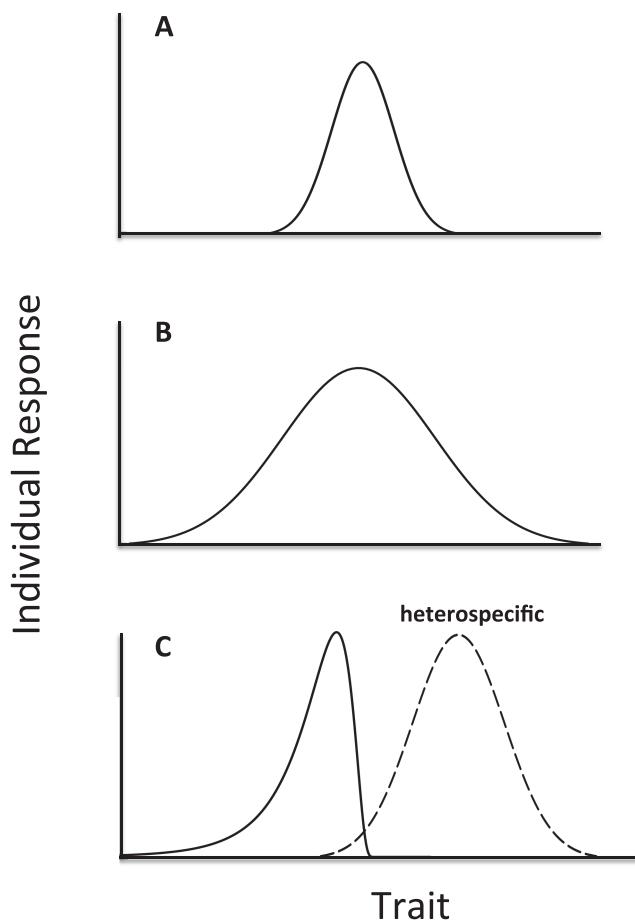


Figure 5. A female's hypothetical response to conspecific trait values throughout her lifetime. We refer to the width of this curve as the window of recognition. **(A)** The young female associates a narrow range of traits with her individual parents. **(B)** The female generalizes her response to include a wider variety of conspecific trait values over time. **(C)** The presence of a heterospecific with overlapping trait values can cause the female preference function to contract, reducing responses on the right side of the distribution. Figure adapted from Irwin and Price (1999).

2008; Schultz and Rudy 2011), we projected the patches into a measure of avian color space and generated a Euclidean distance value for all 10 patches for each species pair (11 patches for some pairs; see *Supplementary Data* online), as a method to assess chromatic differences. Different methods have been used to estimate brightness (e.g., Maia et al. 2013). Here, we averaged the area under the reflectance curve for each patch for each species, then found the absolute value of the difference between each patch's brightness value for each pair.

Genetic distances in Price (2008) were used when available; for the remaining species pairs, mitochondrial DNA sequences were obtained from Genbank, aligned with MAFFT, and distances were calculated using the R package ape (Paradis et al. 2004) with the substitution model TN93 (Tamura and Nei 1993). The approximate age of divergence in millions of years was then calculated assuming 2% per million years.

Results

We found that assortatively mating and randomly mating species pairs showed a large degree of overlap in trait

differences, with phenotypically distinct pairs no more likely to mate assortatively than pairs that are more similar in signal space. Neither differences in song complexity (as measured by song length and number of syllables, Figure 6A), song frequency (Figure 6B), plumage color space distance (Figure 6C), nor plumage brightness difference (Figure 6D) appear to correlate with assortative mating. There is also no obvious trade-off between signal similarity in one modality and the other. Randomly and assortatively mating species pairs showed little difference in age (mean age for randomly mating pairs = 1.501 millions of years \pm 0.585 SE, $N = 7$; for assortatively mating pairs, mean age = 1.488 ± 0.396 , $N = 9$).

One might expect trait divergence to correlate with assortment, but our findings suggest that this is overwhelmed by other factors. We consider that most hybridization results out of mate choice rules that have evolved in the context of conspecific choice. In this case, as females search and fail to find a suitable mate, they are expected to become less choosy (Real 1990), leading to the expectation that they will enter into hybrid pairings. Identified correlates of hybridization

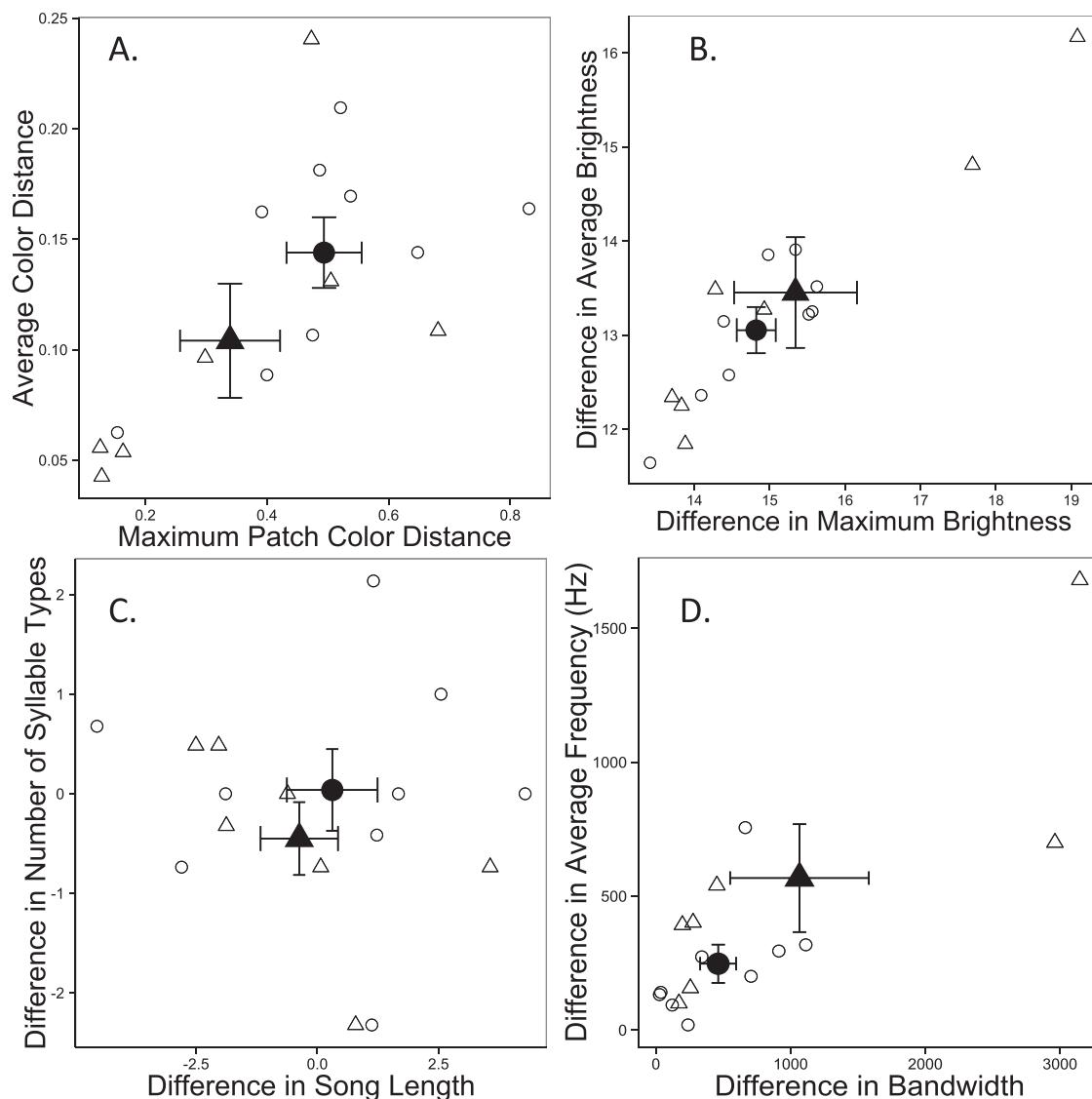


Figure 6. Difference in signaling trait values for hybridizing species pairs. Randomly mating pairs are designated with open triangles, assortatively mating pairs with open circles. Means and standard errors are designated with closed shapes and cross bars, respectively. **(A)** Euclidean distance in color space between each species pair; *y* axis shows the distance averaged between all 10 plumage patches, while the *x* axis shows the greatest distance between the pair in any one patch. **(B)** Average and maximum distance in plumage patch brightness (total reflectance) for each species pair. **(C)** Difference in song length (seconds) and number of unique syllable types for each species pair. **(D)** Difference in average center frequency (Hz) and average bandwidth (highest minus lowest frequency) for each species pair. A, B, and C are displayed in log scale.

include rarity (Grant and Grant 1997), choice focused on territory of the male (Vallin et al. 2012) and differences in arrival time of migratory forms (Rolshausen et al. 2010).

In hybrid zones, reinforcement of preferences may not happen because only a small fraction of the total species population enters into hybrid matings. However, when species spread into extensive sympatry, a large fraction of each species is exposed to the other, and this should then strongly favor reinforcement when postmating isolation is strong. In the following sections, we develop the argument that discrimination of diagnostic traits is an important part of the

establishment of complete sympatry and that this is driven by postmating isolation.

Species Recognition

In birds and many other animals, species recognition begins with the process of imprinting, whereby young birds learn features of their parents (Clayton 1990; ten Cate and Vos 1999). They then generalize out from these features to recognize other conspecifics, using multiple cues to define a “window of recognition,” beyond which sufficiently different

individuals are not considered suitable mates (Figure 5). The width of the window appears to vary substantially across species. For example, each male Darwin's Medium Ground finch on I. Daphne Major in the Galápagos Islands sings a single song. Different individuals can sing quite different songs, with variation apparently larger than that seen across species in some cases (Millington and Price 1985; Grant and Grant 1996). However, individuals respond to playbacks of conspecifics equally, whether or not they sing a similar or different song (Ratcliffe and Grant 1985). The presumed mechanism whereby different songs are recognized as conspecific is through generalization out from morphology (beak and body size and shape), which is diagnostically different among species on the island. This is an example where large differences in vocalizations among males become recognized as conspecific. Similarly, plumage color polymorphisms within species may exceed differences between species (Gray and McKinnon 2007).

Studies of geographical variation in the window of recognition have been conducted largely by recording responses of males to playback of song. They have shown discrimination often varies in a way that corresponds to the presence or absence of related species (Gil 1997; Irwin and Price 1999; Sedlacek et al. 2006). In some cases, this variation is clearly a consequence of learning, for individuals only a few hundred meters apart respond differently. In Figure 7, we show one example. Male Chaffinches, *Fringilla coelebs*, in the Canary Islands that have not encountered Blue Chaffinches, *Fringilla teydea*, respond to Blue Chaffinch song but those that are familiar with Blue Chaffinches do not respond to Blue Chaffinch song (Lynch and Baker 1991). The assumption is that Chaffinch males in sympatry generalize from Blue Chaffinch color, which they recognize as different, to Blue Chaffinch song. In this case, learning plays an important role

in narrowing the song recognition window; however, the (presumably innate) mechanisms that allow the chaffinch to correctly categorize heterospecific color are unknown.

Chaffinches and other species use diagnostic traits to distinguish conspecifics from heterospecifics and to generalize to other traits. We suggest that the limit to generalization is often genetic and set by selection against hybridization, that is, reinforcement. However, other selection pressures in the environment also narrow windows of recognition (Gröning and Hochkirch 2008). For example, species may narrow windows of song recognition in order to detect signal over background noise (Wiley 2006) or because they develop search images for certain colors of prey, which then spill over to preferences during mate choice (Rodd et al. 2002). Galbraith (1956) and Noor (1997) suggested that narrowing windows might be the result of encounters with multiple species in the environment, from which the focal species is fully reproductively isolated, but toward which responses are maladaptive; this could lead to a relatively narrow window reducing hybridization with other species that spread into the focal species range. The relative role of these processes and reinforcement per se remains to be assessed.

Trait Evolution

Although our analysis of hybrid zones suggests the effect of trait differences on assortment may be small and overwhelmed by other factors, we expect trait divergence to contribute to premating isolation to some extent, with greater divergence lessening the likelihood of females mating with heterospecifics. Upon establishment of sympatry, socially selected traits may diverge or converge, as a result of competition for territories or other resources (Brown and Wilson 1956; Grether et al. 2009) and this may then increase or



Figure 7. Playbacks of song of Blue chaffinch (*Fringilla teydea*), a species endemic to the higher elevations of 2 Canary Islands to the Chaffinch (*Fringilla coelebs*), which is found elsewhere on the Canary Islands. In locations where the 2 species have overlapping territories, the Chaffinch does not respond to playback, but in areas where the Chaffinch occurs alone, the males respond by aggressively approaching the speaker (based on a total of 19 experiments). Similar results were found when Chaffinch song is played to Blue Chaffinches. From Lynch and Baker (1991), with illustrations of the birds from the public domain.

decrease the propensity for individuals to mate assortatively. It is unclear how strong a contribution this makes to assortment, given our general assessment in Figure 6. For example, in zones of overlap between the Pied Flycatcher, *Ficedula hypoleuca*, and the Collared Flycatcher, *Ficedula albicollis*, male Pied Flycatchers diverge from Collared Flycatchers in plumage, but converge in song, and these changes are thought to be favored in Pied Flycatcher territory establishment and maintenance (Vallin et al. 2012). However, in this system, female choice of territories rather than males may drive much of the hybridization. In the antbirds *Hypocnemis peruviana* and *Hypocnemis subflava*, male songs converge in sympatry, and males of both species are indiscriminate in their aggressive responses to either song. However, female antbirds show strong discrimination and only respond to conspecific songs (Seddon and Tobias 2010). In both cases, divergence in traits does not seem to correlate with (female) discrimination in a straightforward way.

Finally, it is difficult to ascertain how frequently sympatry is associated with divergence in sexually selected traits. Studies have found that in comparisons of sister pairs, sexually selected traits are more divergent in sympatry than they are among allopatric closely related species (Seddon 2005; Martin et al. 2010). Trait divergence has been attributed either to trait evolution in sympatry (i.e., character displacement, Brown and Wilson 1956), or to sorting, in which only taxa that are sufficiently different are able to establish in sympatry (Martin et al. 2010). However, a great difficulty in ascribing sympatry as the factor affecting trait divergence comes from controlling for time (Appendix). Allopatric sister pairs are generally younger than sympatric sister pairs (Martin et al. 2010; Weir and Price 2011; Pigot and Tobias 2013; Tobias et al. 2013; Figure 2). It may therefore be that it is the older age of sympatric species that drives their greater differences, rather than any effect of sympatry on divergence (see Appendix for a more detailed exposition). This means the extent to which trait divergence is essential to achieving sympatry is hard to assess from sister pair comparisons, because time is correlated with divergence in other traits, such as those affecting postmating isolation (e.g., Price and Bouvier 2002). For example, although song divergence increases with time in antbirds, Tobias et al. (2013) found that very old sympatric sister pairs (>6 millions of years) are actually less divergent in song than similarly aged allopatric pairs. They suggested song divergence is not critical to the establishment of sympatry.

Postmating Isolation

Postmating isolation is essential to the process of reinforcement. Thus, if reinforcement is widespread, as we argue, the establishment of sympatric forms requires not only premating isolation, but also postmating isolation. We believe that both extrinsic and intrinsic postmating factors act in similar ways to promote reinforcement, although extrinsically arising postmating isolation is less stable, in that it can collapse if the environment changes, as described in Darwin's finches, below.

Assessment of the strength of postmating isolation between sympatric forms is difficult, because in nature, most sympatric species do not hybridize. Some authors have noted that old, often sympatric, species can produce fertile hybrids in the lab and used this to argue for the primacy of premating isolation in bird speciation (Edwards et al. 2005). We believe this to be misleading. First, the majority of sympatric species are distantly related to their closest relative (Figure 2), by which time hybrids have low fitness in the lab, even if a few fertile hybrids can be produced (Price and Bouvier 2002). In the wild, the fitness of these hybrids is likely to be negligible, because of various other intrinsic incompatibilities that reduce general health (Price and Bouvier 2002; Price 2008). Second, in nature, hybrids may fall between the ecological niches of the parental species, suffering reduced fitness as a consequence, part of the paradigm of ecological speciation (Schluter 2009). This is illustrated by long-term studies of Darwin's finches, which are one of the few bird groups with multiple young sympatric species. On I. Daphne Major, it was found that when ecological conditions are such that hybrids have low fitness, a low level of hybridization is maintained, but when conditions changed so that hybrid fitness increased, a pair of species began a process of collapse into one, even as hybridization remained at a few percent (reviewed in Grant and Grant 2014).

Discussion

In this paper, we have argued that reinforcement is widespread, but that this is often a result of the strengthening of conspecific preferences for diagnostic traits, rather than evolution of the traits themselves. Although only birds are examined here, we believe that our conclusions are likely general to many animals in which the sexes can interact directly. For example, in *Drosophila*, strong evidence for reinforcement has been obtained from laboratory studies, which have investigated mate choice rather than signal evolution (Coyne and Orr 1989; Yukilevich 2012). Further, selection experiments have rapidly altered discrimination windows, not only in *Drosophila* (e.g., Matute 2010) but also young birds (Kovach 1990), without detectable effects on traits. Reinforcement on traits used in mate choice results from postmating isolation and this includes both postmating prezygotic isolation and postzygotic isolation. Postmating prezygotic isolation should contribute to reinforcement of mate preferences in a similar way to that of postzygotic isolation (Servedio 2001); however postmating prezygotic isolation can itself be reinforced by postzygotic isolation, and this is a relatively unexplored area (Matute 2010).

These arguments may apply to plants as well as animals, where postmating prezygotic isolation as well as premating isolation may often be reinforced as a result of postzygotic isolation (Armbruster and Muchhal 2009; Hopkins 2013). However, in animal-pollinated plants, premating isolation cannot be reinforced by a direct narrowing of discrimination windows, but instead is effected by trait evolution that shapes pollinator preferences (Levin 1970; Hopkins 2013). Armbruster and Muchhal (2009) emphasized the importance

of near complete postmating isolation and subsequent reinforcement in plants (which they term reproductive character displacement, following [Butlin 1987](#)). They noted that heterospecific pollen regularly lands on flowers, making for strong selection favoring reinforcing mechanisms.

Returning to animals, if trait divergence is less important than trait discrimination in reinforcement and establishment of sympatry, as we argue, it should be possible to test the model by quantifying trait differences between sympatric forms, then comparing behavioral discrimination of these traits with discrimination between allopatric forms that show a similar degree of trait divergence. We predict that allopatric forms will be less discriminating in their responses to heterospecifics than equally diverged sympatric forms, due to the absence of reinforcement. In birds, some qualitative support for this prediction is summarized in [Price \(2008, Chapters 10 and 14\)](#).

The main alternative to reinforcement in the production of fully sympatric species is that the forms are already completely reproductively isolated before coming into contact. Although this likely occurs sometimes, we consider that hybridization may often follow secondary contact, before being reduced through reinforcement as species spread from parapatry into full sympatry. First, the observation that some species in hybrid zones are old (millions of years, [Weir and Price 2011](#)) implies that hybridization potential may often be lost only over long timescales; this is supported by the age of species which can hybridize in captivity ([Price and Bouvier 2002](#); [Coyne and Orr 2004](#)). Second, windows of recognition are demonstrably broad for some species, suggesting that these species would respond to quite different forms on first contact. Indeed, our examination of hybrid zones suggests that individuals may frequently accept as mates individuals with trait values outside of conspecific distributions. Third, various factors that increase possibilities of hybridization, such as rarity of a species or presence of heterospecifics in the same habitat, may be particularly prevalent on first contact.

Speciation and Sexual Selection

Although traits like bird song and plumage are clearly shaped by sexual selection, we should be cautious in attributing divergence in signaling traits to sexual selection exclusively, when ecological and other factors may often work in concert with sexual selection to create diverging signals ([Wilkins et al. 2013](#)). Whatever the primary driving force behind divergence in traits, it is not clear that this divergence corresponds closely to reproductive isolation. Several studies have used song playbacks and/or responses to artificial mounts to test significance of trait divergence between allopatric populations (e.g., [Nelson 1998](#); [Irwin et al. 2001](#)) and/or infer reproductive isolation (e.g., [Uy et al. 2009](#)). A general finding has been that the more divergent the traits between allopatric forms, the weaker the responses to each other. This has been used as support for the idea that premating isolation evolves as a correlated response to trait divergence, and that trait divergence is a good measure of speciation ([Irwin et al. 2001](#); [Uy et al. 2009](#)). If responses regularly change upon establishment of sympatry, this conclusion is less certain. We

suggest that while divergence in sexually selected traits likely contributes to some assortment on first contact between species, it has a relatively minor influence on whether species will expand their ranges to coexist in sympatry.

Here, we have followed general discussions of a role for sexual selection in speciation (at least in birds) in assuming that divergence in secondary sexual traits primarily affects premating reproductive isolation. However, divergence in secondary sexual traits may also have some effect on postmating isolation, if hybrids appear dramatically different from their parental forms and hence not chosen as mates. If this is an important effect, it could conceivably contribute to reinforcement ([Kirkpatrick and Servedio 1999](#)), implying that strong sexual selection can increase rates of sympatry, even as postmating isolation remains an essential ingredient. We doubt that sexual selection against hybrids drives postmating isolation and reinforcement to a great extent. In particular, hybrid females should be able to find mates. Further, most sympatric species are old ([Figure 2](#)), and this implies postmating isolation is likely present for multiple other reasons.

Defining Allopatric Forms as Species

[Brown and Wilson \(1956, p. 63\)](#) noted that “the degree of observed difference between sympatric species cannot be considered a reliable yardstick for measuring the real status of related allopatric populations, nor can the differences among the latter be taken too seriously as indications of their relationships.” Our analysis supports this statement. If trait divergence is a poor predictor of biological speciation, we are again left with the difficulty of how to define allopatric forms as species. One route is to use an arbitrary cutoff in terms of species age, generally correlated with postmating isolation ([Price and Bouvier 2002](#)). We suggest a value of 2 millions of years (~4% divergence in mtDNA; [Price 2008](#)), given the age of hybridizing forms in hybrid zones ([Weir and Price 2011](#)). This is likely to be at least as controversial as other definitions and leads to a reduction in the number of allopatric forms being confirmed as species ([Figure 2](#)). However, it does place an emphasis on a role for postmating isolation as a critical factor in the establishment of sympatry.

Supplementary Material

Supplementary material can be found at <http://www.jhered.oxfordjournals.org/>.

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Appendix

Controlling for time in assessments of divergence in sympatry

Sympatric sister pairs are often more divergent than allopatric ones, not only in plumage and song, but also in morphological

traits (e.g., Martin et al. 2010; Weir and Wheatcroft 2011; Pigot and Tobias 2013; Tobias et al. 2013). Counter to our arguments here, this divergence has been taken as an essential step in the achievement of sympatry (Martin et al. 2010; Pigot and Tobias 2013). However, a general problem is that sympatric sister pairs are older than allopatric ones (Martin et al. 2010; Weir and Price 2011; Pigot and Tobias 2013; Tobias et al. 2013; Figure 2), and it is not clear if it is trait divergence or some other correlate of time that is the essential factor. The common approach to control for time is to use multiple regression of the trait difference between sister pairs on 1) allopatry/sympatry and 2) genetic distance, which then sometimes shows an effect of sympatry on divergence (e.g., Martin et al. 2010), although not always (Tobias et al. 2013). A difficulty arises because genetic distance is not time, but instead an *estimate* of time. If time itself were in the model, the importance of sympatry in multiple regression models must be reduced (Figure A1).

To illustrate this problem, we reanalyzed Martin et al.'s (2010) study, which found that sympatry was associated with increased color divergence among North American birds. In the original data, no effect of sympatry is detectable unless family membership is included in the model. Therefore, we followed Martin et al. (2010) and used the residuals from an analysis of variance of color on family membership and latitude. These residuals are henceforth termed "color." The correlations for 78 sister comparisons are: $r_{gs} = 0.143$, $r_{sc} = 0.35$, $r_{gc} = 0.296$, where the subscripts, g, s, and c indicate genetic distance, sympatry, and color, respectively. The relatively high correlation of color with sympatry results in a significant partial regression coefficient for color on sympatry ($P = 0.003$) and a nonsignificant partial regression coefficient for color on genetic distance ($P = 0.1$).

Standardized regression coefficients for color on time and sympatry can be obtained from the solution to 3 simultaneous equations, by assuming various values for the correlation of time with genetic distance (α) (Figure A1): $r_{gs} = \alpha r_{st}$, $r_{ge} = \alpha \beta_{cs} + r_{gs} \beta_{cs}$, $r_{se} = \beta_{cs} + r_{st} \beta_{ct}$. Some results are in Table A1. When the correlation of genetic distance with time is above about 0.8, results on the importance of sympatry hold, but if the correlation is less than 0.5, the importance of sympatry becomes very small. Correlations of time and genetic distance are generally unknown. Weir and Schlüter (2008) found a correlation of 0.8, but this was estimated over 10 million

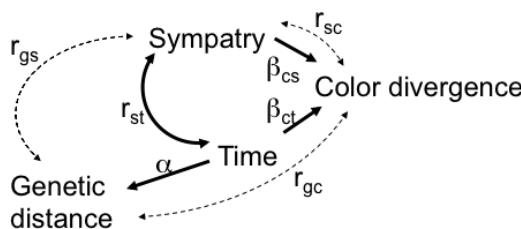


Figure A1. Path diagram of hypothesized relationships between genetic distance, time, sympatry, and color. Dashed lines are measured correlations. $\hat{\alpha}$ are the 20 standardized partial regression coefficients.

Table A1 Standardized partial regression coefficients of color on sympatry and time, as estimated for 78 sister taxa comparisons in Martin et al. (2010), but assuming different correlations of time with genetic distance

Time and genetic distance, α	Color and sympatry (β_{cs})	Color and time (β_{ct})
1	0.31	0.25
0.8	0.29	0.32
0.5	0.20	0.54
0.4	0.10	0.71
0.35	0.01	0.84

years, not the shorter timescales relevant to sister species analysis (e.g., Figure 2). It is also worth noting that if genetic distance is considered a surrogate for postmating isolation, and that is a critical factor in the establishment of sympatry, as we have argued here, the correlation between genetic distance and postmating isolation is likely to be subject to even more error than the correlation between genetic distance and time. Arguments associated with measurement error only apply if sympatry is associated with increased divergence. If sympatry is associated with *reduced* divergence, it is difficult to argue this is an effect of time (provided a linear model, as in multiple regression is appropriate). Tobias et al. (2013) found that after including genetic divergence in the model, antbird song was less divergent in sympatry than allopatry; however, this effect is attributable to some very old (>7 millions of years) allopatric sister pairs, so it is uncertain if these findings are relevant to the attainment of sympatry, which often happens earlier than this (Figure 2).

We argue that assessment of any role for sympatry in the evolution of color or other traits is important, but controlling for genetic distance using multiple regression is not likely to produce a strong result. Two other approaches hold promise. The first is to use triplets, where an outgroup species is in sympatry with one member of a sister pair by not the other member (Noor 1997; Coyne and Orr 2004, p. 364). The second is to study patterns of within-species geographical variation as we have described in the text and in Figure 7.

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