

THE BIOLOGY OF SPECIATION

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Since Darwin published the "Origin," great progress has been made in our understanding of speciation mechanisms. The early investigations by Mayr and Dobzhansky linked Darwin's view of speciation by adaptive divergence to the evolution of reproductive isolation, and thus provided a framework for studying the origin of species. However, major controversies and questions remain, including: When is speciation nonecological? Under what conditions does geographic isolation constitute a reproductive isolating barrier? and How do we estimate the "importance" of different isolating barriers? Here, we address these questions, providing historical background and offering some new perspectives. A topic of great recent interest is the role of ecology in speciation. "Ecological speciation" is defined as the case in which divergent selection leads to reproductive isolation, with speciation under uniform selection, polyploid speciation, and speciation by genetic drift defined as "nonecological." We review these proposed cases of nonecological speciation and conclude that speciation by uniform selection and polyploidy normally involve ecological processes. Furthermore, because selection can impart reproductive isolation both directly through traits under selection and indirectly through pleiotropy and linkage, it is much more effective in producing isolation than genetic drift. We thus argue that natural selection is a ubiquitous part of speciation, and given the many ways in which stochastic and deterministic factors may interact during divergence, we question whether the ecological speciation concept is useful. We also suggest that geographic isolation caused by adaptation to different habitats plays a major, and largely neglected, role in speciation. We thus provide a framework for incorporating geographic isolation into the biological species concept (BSC) by separating ecological from historical processes that govern species distributions, allowing for an estimate of geographic isolation based upon genetic differences between taxa. Finally, we suggest that the individual and relative contributions of all potential barriers be estimated for species pairs that have recently achieved species status under the criteria of the BSC. Only in this way will it be possible to distinguish those barriers that have actually contributed to speciation from those that have accumulated after speciation is complete. We conclude that ecological adaptation is the major driver of reproductive isolation, and that the term "biology of speciation," as proposed by Mayr, remains an accurate and useful characterization of the diversity of speciation mechanisms.

KEY WORDS: Ecological niche modeling, ecological speciation, genetic drift, geographic isolation, habitat isolation, isolating barrier, mutation-order, natural selection, polyploid speciation, reciprocal transplants, reproductive isolation, uniform selection.

"No one ought to feel surprise at much remaining as yet unexplained in regard to the origin of species and varieties, if he make due allowance for our profound ignorance in regard to the mutual relations of the many beings which live around us."

Darwin (1859, p. 6)

Since Darwin's publication of the Origin of Species in 1859, the study of speciation has always generated enthusiasm and passionate debate. Interest in the subject continues to grow, as evidenced by an exponential rise in citations of speciation studies over the last three decades (Fig. 1). This is perhaps not surprising,

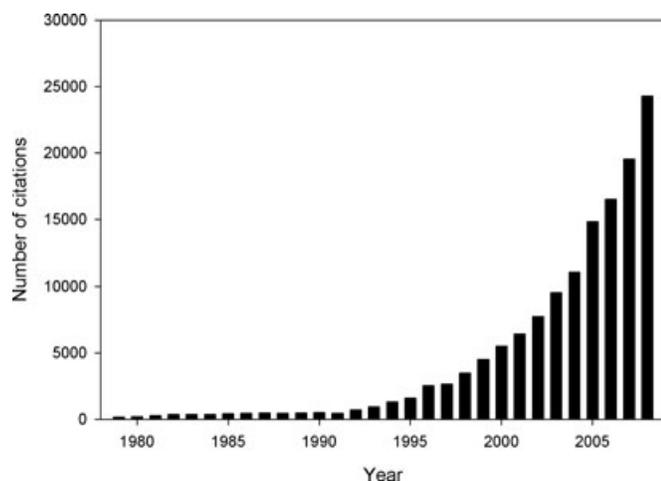


Figure 1. Web of Science literature search for citations of the topic “speciation” for the years 1979–2008, refined to subject areas “evolutionary biology,” “ecology,” or “genetics and heredity.”

given the view espoused by Mayr, and probably held by many researchers, that the origin of species is “. . . the single most important event in evolution” (Mayr 1963, p. 11). Yet, our poor understanding and lack of agreement on seemingly fundamental aspects of the topic is surprising. Despite more than a century of research on the ecological and genetic mechanisms of speciation, major questions remain.

For example, when is speciation nonecological? It was recently suggested that distinguishing ecological from nonecological speciation will improve our understanding of speciation mechanisms (Schluter 2001; Rundle and Nosil 2005; Schluter 2009). Is this a false dichotomy? How is it that we cannot even agree on the terminology of speciation, or when and how ecology is involved?

Consider Mayr’s solution to this problem:

The majority of the factors that we have to discuss are environmental, and we might therefore speak of an “ecology of speciation.” However, since we have to include the internal factors (mutability), as well as factors that involve behavior patterns, such as crossability, sexual isolation, pair formation, and the like, it might be preferable to use the broader term, biology. (Mayr 1942, Chapter IX—The Biology of Speciation, p. 216).

Given the long-held view that ecology and divergent selection are major factors in speciation (Dobzhansky 1937, 1951; Mayr 1947, 1963; Stebbins 1950; Grant 1981), one might ask, what does the proposed focus on ecological speciation offer that was lacking in previous treatments?

Even among biologists who believe speciation is sometimes nonecological, there is broad agreement that adaptation plays a significant role in most cases (Templeton 2008). Nevertheless, the mechanisms whereby genetic changes resulting from adaptation contribute to the evolution of reproductive isolation remain

poorly understood. Consider the many ways in which even a single adaptive mutation may affect multiple components of reproductive isolation. For example, adaptation to different habitats may lead to the restriction of gene flow and a gene contributing to this divergence may have pleiotropic effects on other traits, and these may cause more isolation. This leads to the question, how do the genetic and phenotypic changes required for speciation arise, and how do they cause complete reproductive isolation?

The importance of different isolating barriers to speciation remains a topic of considerable debate (Coyne and Orr 2004). How do we estimate total reproductive isolation and which barriers do we include? For example, there is substantial disagreement about how one should measure and interpret isolation that results from differences in geographic distribution. Are genetically based differences in distribution legitimate isolating barriers, as suggested by Schemske (2000), or does the difficulty in distinguishing historical factors from geographic adaptation require that studies of speciation be restricted to sympatric taxa, as suggested by Coyne and Orr (2004)? Can we study speciation in allopatric taxa, and if so, how?

Finally, although most researchers acknowledge that the holy grail of speciation studies is to identify the magnitude and order of appearance of those isolating barriers that have actually contributed to speciation, there is disagreement as to how this can be accomplished. In their landmark papers, Coyne and Orr (1989, 1997) introduced a method in which the isolation contributed by late-acting barriers is “discounted” by that from earlier barriers and the sum of the contributions across stages gives the total isolation. They applied this approach to the study of pre-mating isolation and postzygotic incompatibilities in *Drosophila*, and several studies have since expanded their method to multiple isolating barriers (Ramsey et al. 2003; Husband and Sabara 2004; Kay 2006; Matsubayashi and Katakura 2009). However, others have criticized this method because one cannot assume independence of different isolating barriers (Martin and Willis 2007; Lowry et al. 2008a), and there is some doubt as to the validity of the sequential ordering of individual barriers when estimating their contributions to the total isolation (H. A. Orr, pers. comm.). Hence, there is disagreement on how to test the cornerstone of speciation research, that is, which forms of reproductive isolation are most “important?” Here, we discuss the questions put forth above in light of historical perspective and current practice to develop a research framework for studying the “biology of speciation.” We begin with a brief history of the role of ecological factors in speciation, and then explore the three “nonecological” mechanisms proposed by Schluter (2000, 2001) and Rundle and Nosil (2005), and conclude that ecology is rarely if ever truly absent from speciation. We then discuss the potentially pervasive effect of ecology and natural selection on speciation, emphasizing the potential for both direct and indirect selection to result

in reproductive isolation. We further examine the interaction between adaptation and geography to emphasize the importance of habitat isolation, which has been grossly understudied. Next, focusing on the chronology of isolating barriers, we expand upon the framework developed previously by Coyne and Orr (1989) for assessing the relative importance of different forms of isolation. We conclude by recommending that future speciation studies examine the contribution of all potential isolating barriers, whether they are caused by ecological or nonecological mechanisms, or arise in sympatry or allopatry—an approach first envisioned by Dobzhansky and Mayr, the principal architects of our field.

When is Speciation Nonecological?

... virtually all barriers can be considered ecological in the sense that they arise from environmentally imposed selection.
(Coyne and Orr 2004, p. 179)

Darwin (1859) developed the theory of natural selection to explain how populations diverge morphologically and ecologically as they adapt to local environmental conditions. To Darwin, speciation was simply the end point of a chronological series of steps, beginning with variation among individuals within species to the production of adaptively differentiated varieties and eventually to new species, with extinctions occurring along the way. Darwin's view that species originate by adaptive divergence is perhaps best illustrated in the section titled "The Probable Effects of the Action of Natural Selection through Divergence of Character and Extinction, on the Descendants of a Common Ancestor," in chapter IV of the "Origin." Here he illustrates how natural selection acts to differentiate varieties that ultimately evolve to become different species. He concludes, "The complex and little known laws governing the production of varieties are the same, as far as we can judge, with the laws which have governed the production of distinct species" (p. 648). Darwin's views on the role of ecological divergence in the origin of species were articulated most clearly in his unpublished "Big Book," where, as an example, he describes how selection on drought tolerance in a group of plants might contribute to adaptive divergence and speciation (Diagram I in Stauffer 1975). Although he did little to connect natural selection with reproductive isolation, it is clear that Darwin viewed ecological adaptation as the key to understanding the origin of species.

Since Darwin, our understanding of speciation has been improved dramatically by the adoption of Mayr's (1942) biological species concept (BSC), which defines species as interbreeding natural populations. The first author to propose a species definition based on interbreeding was Poulton (1908), who not only defined species by reproductive isolation, but also addressed the relative importance of pre- versus postmating barriers. He clearly

viewed premating barriers as vital to the speciation process, saying, "It will be argued that the true interspecific barrier is not sterility but Asyngamy—the cessation of interbreeding—but that the first will inevitably follow, sooner or later, as the incidental consequence of the second" (Poulton 1908, p. 65). He continues by offering a list of these premating reproductive barriers including "asyngamy" as a consequence of allopatry, mechanical isolation, and preferential mating.

Dobzhansky provided the first complete list of possible isolating barriers between species (1937, p. 231–232), and included "ecological isolation" as an example of reproductive (genetically based) isolation, in which "Representatives of the populations occur in different habitats in the same general region." Dobzhansky placed seasonal and temporal isolation in a separate category, although these clearly fall under the rubric of ecological isolation. In addition to ecological barriers that act prior to hybrid formation, Dobzhansky also acknowledged the role of ecology and natural selection in postzygotic isolation, "... the genotype of a species is an integrated system adapted to the ecological niche in which the species lives. Gene recombination in the offspring of species hybrids may lead to formation of discordant gene patterns. This decreases the reproductive potentials of both interbreeding species" (1951, p. 208).

Mayr (1942, 1947) judged ecological factors as the major drivers of speciation. In his classic paper "Ecological factors in speciation," Mayr (1947) concluded that geographic isolation leads to the formation of segregated populations that experience different ecological conditions, leading to evolutionary divergence. In *Animal Species and Evolution*, Mayr (1963, p. 556) devoted an entire chapter to the role of ecology in speciation, and began the second paragraph as follows: "An exhaustive treatment of the indicated subject matter would require an entire book, for there is hardly an ecological factor that does not affect speciation directly or indirectly, actually or potentially." Many other evolutionary biologists have also supported the notion that ecological divergence of populations is typically required for speciation. Simpson (1953, p. 234n) concluded "... speciation, the basic process of radiation, is normally adaptive," and Grant (1981) provided numerous examples in which ecological factors are the primary isolating barriers between species.

Our brief summary illustrates that natural selection and ecological factors have been at the center of discussions of speciation mechanisms since the inception of the field. In Table 1, we compile more recent examples in which ecology is either demonstrated or strongly implicated in the evolution of reproductive isolation. These include studies of phenotypes and behavior, such as Hatfield and Schluter's (1999) demonstration of extrinsic postzygotic isolation in sticklebacks, Ramsey et al.'s (2003) study of ecogeographic and pollinator isolation in *Mimulus*, and Rundle et al.'s (2005) investigation of sexual isolation via

Table 1. Reproductive isolation caused by ecological factors.

Reproductive barrier	System	Role of ecology	References
Habitat isolation	<i>Lucania</i> (killifish)	<i>L. goodie</i> and <i>L. parva</i> display reduced survival to adulthood when reared at nonnative salinity levels. Natural distributions along salinity gradients generally correspond to fitness differentiation.	Fuller et al. 2007
	<i>Drosophila</i> (fruit fly)	<i>D. santomea</i> and <i>D. yakuba</i> inhabit distinct habitats based on ecological conditions associated with elevation. Both species exhibit reduced survival to adulthood and fertility when reared at nonnative temperatures, and each species chooses its native temperature range when placed on a temperature cline.	Matute et al. 2009
	<i>Mimulus</i> (mon-keyflower)	<i>M. lewisii</i> and <i>M. cardinalis</i> show considerable allopatric separation based primarily on differences in altitude inhabited. Reciprocal transplants demonstrate that each species is most fit in its native range.	Ramsey et al. 2003; Angert and Schemske 2005
Temporal isolation	<i>Inurois</i> (geometrid moth)	Populations of <i>I. punctigera</i> in colder climates show divergence for early or late flight periods conferring temporal reproductive isolation.	Yamamoto and Sota 2009
	<i>Mimulus</i>	Inland and coastal forms of <i>M. guttatus</i> experience selection for different growth and flowering times resulting in flowering phenology with little overlap.	Lowry et al. 2008b
Sexual and pollinator isolation	<i>Drosophila</i>	Divergent artificial selection in laboratory populations of <i>D. serrata</i> results in assortative mating.	Rundle et al. 2005
	<i>Gasterosteus</i> (stickleback fish)	Anadromous and freshwater <i>G. aculeatus</i> experience divergent selection for body size and assortative mating is based on this trait.	McKinnon et al. 2004
	<i>Mimulus</i>	In sympatry, <i>M. cardinalis</i> and <i>M. lewisii</i> experience almost complete reproductive isolation due to pollinator preference for floral traits.	Ramsey et al. 2003
Gametic isolation	<i>Echinometra</i> (sea urchin)	Lineage specific positive selection on <i>bindin</i> , a gamete recognition protein, was detected in <i>E. lucunter</i> , which experience a strong block to fertilization by sperm of its Neotropical congeners.	McCartney and Lessios 2004
	<i>Mimulus</i>	Divergent pollinator-selected style lengths in <i>Mimulus cardinalis</i> and <i>M. lewisii</i> lead to differentiated pollen tube lengths, reducing the amount of expected hybridization in mixed pollinations.	Ramsey et al. 2003
Intrinsic postzygotic isolation	<i>Drosophila</i>	Adaptive divergence in nuclear pore proteins causes lethality in hybrids of <i>D. melanogaster</i> and <i>D. simulans</i> .	Presgraves et al. 2003
	<i>Drosophila</i>	Hybrid incompatibility and sterility between <i>D. melanogaster</i> and sibling species <i>D. simulans</i> , <i>D. mauritiana</i> , and <i>D. sechellia</i> involves the <i>Hmr</i> gene that exhibits signature of positive selection.	Barbash et al. 2003; Maheshwari et al. 2008
	<i>Mimulus</i>	Hybrid inviability between <i>M. guttatus</i> populations on and off copper mine tailings is linked to two genes for copper tolerance.	Christie and Macnair 1987
Extrinsic postzygotic isolation	<i>Gasterosteus</i>	Divergent natural selection causes low fitness in <i>G. aculeatus</i> benthic–limnetic hybrids, despite the absence of intrinsic postzygotic isolation.	Hatfield and Schluter 1999; Rundle 2002
	<i>Sylvia</i> (warbler)	Hybrids between divergently migrating populations of <i>Sylvia atricapilla</i> exhibit unfit intermediate migration patterns.	Helbig 1991
	<i>Heliconius</i> (butterfly)	Mimicry in <i>H. cydno</i> and <i>H. melpomene</i> is disrupted in hybrids, causing increased predation on hybrids and reduced mating success.	Jiggins et al. 2001; Naisbit et al. 2001

divergent selection in *Drosophila*. Molecular genetic approaches also provide evidence of the role of natural selection in generating reproductive isolation, such as McCartney and Lessios' (2004) demonstration of direct selection on a protein involved in gametic isolation between sea urchins, and studies in *Drosophila* on loci involved in Dobzhansky–Muller incompatibilities (Barbash et al. 2003; Presgraves et al. 2003).

In the past decade, a number of papers have suggested that it is useful to distinguish ecological from nonecological mechanisms to elucidate the role of natural selection in speciation, for example, Schluter (2000, 2001, 2009), Rundle and Nosil (2005), and Nosil et al. (2009). What sets these efforts apart from previous discussion is the proposal of the term, “ecological speciation,” which is defined variously as

... when divergent selection on traits between populations or subpopulations in contrasting environments leads directly or indirectly to the evolution of reproductive isolation (Schluter 2001, p. 372);

... the process by which barriers to gene flow evolve between populations as a result of ecologically-based divergent selection (Rundle and Nosil 2005, p. 336);

A speciation process in which divergent natural selection drives the evolution of reproductive incompatibility (i.e., isolation) between taxa (Nosil et al. 2009, p. 145).

... the evolution of reproductive isolation between populations or subsets of a single population by adaptation to different environments or ecological niches (Schluter 2009, p. 737).

Schluter (2001) suggested that “. . . until recently, neither was there evidence to support ecological speciation, nor had tests been devised to distinguish ecological speciation from other mechanisms that might cause speciation in the wild, such as genetic drift” (Box 1 in Schluter 2001). Rundle and Nosil (2005, p. 336) suggest that the renewed focus on ecological speciation has developed alongside recent efforts for “. . . a reclassification of speciation models from a scheme of geography (i.e., sympatric vs. allopatric), to one that focuses on mechanisms for the evolution of reproductive isolation . . .”

The ecological speciation perspective has rekindled interest in the critical role of ecological factors in speciation, so in this sense it has been extremely valuable. However, natural selection and ecological factors have been at the center of discussions about speciation mechanisms for many decades. Consider the classic studies of Dobzhansky and colleagues on mechanisms of reproductive isolation in *Drosophila*. Dobzhansky (1951) concluded that gene flow between *Drosophila pseudoobscura* and *D. persimilis* was prevented by at least seven different isolating mechanisms, including such ecological factors as differences in habitat, preferred foods, and activity periods. Hiesey et al. (1971) conducted landmark studies on two closely related species of monkeyflowers, *Mimulus cardinalis* and *M. lewisii*, and through

extensive reciprocal transplant experiments, crossing studies, and physiological observations demonstrated unmistakably that ecology and natural selection were the major factors contributing to speciation. In birds, the extraordinary radiation of Hawaiian honeycreepers from a single common ancestor, with species differentially adapted for feeding on nectar, fruits, seeds, or insects (Amadon 1950), must surely represent an irrefutable example of divergent natural selection as a major cause of reproductive isolation.

Moreover, the suggestion that the ecological speciation perspective affords investigators a new opportunity to study the mechanisms of reproductive isolation fails to recognize that Dobzhansky (1937) and Mayr (1942) had already established a complete inventory of reproductive isolating barriers more than a half century ago. Given this historical appreciation of ecological factors in speciation, the new perspective serves to direct attention to how the presence and strength of divergent selection affects reproductive isolation, but provides little new insight into how ecological versus non-ecological mechanisms are involved.

We begin by examining the three proposed forms of nonecological speciation: speciation under uniform selection, polyploid speciation, and speciation by genetic drift (Table 1 in Schluter 2001). We show that ecology is involved in speciation by both uniform selection and polyploidy, and that genetic drift is unlikely to cause speciation unilaterally. Within the “ecological speciation” framework, speciation must sometimes be ecological and at other times, it must not be. Given our review of these cases, we ask the question: When is speciation nonecological?

SPECIATION UNDER UNIFORM SELECTION

Under uniform selection, allopatric populations experience the same selective environment, and may evolve similar phenotypes by fixing different adaptive mutations. If these alternate mutations interact negatively in hybrids, intrinsic postzygotic isolation and speciation may result. Because of historical contingency in the order in which mutations arise, this has been referred to as “mutation-order” speciation (Mani and Clarke 1990; Schluter 2009). While it is arguable whether selective environments between populations could ever truly be uniform, alternate mutations that would be universally beneficial could arise under similar selective regimes, potentially leading to this form of speciation.

The concept of differential response to uniform selection has been approached previously in studies of adaptation. Cohan and Hoffmann (1989) describe several lines of evidence including examples in which conspecific populations have diverged under seemingly similar selection in nature, and populations that have responded differently to equivalent artificial selection in the laboratory. However, under uniform selection, phenotypic divergence should be rare, and even distantly related taxa will often converge phenotypically when faced with similar selective

environments (Simpson 1953). Phenotypic convergence under uniform selection can be achieved by identical mutations at the same loci (i.e., parallelism), by different mutations within the same loci, or by mutations at different loci (i.e., convergence). The probability of reproductive isolation should increase along this “parallelism to convergence continuum,” but it is difficult to predict the adaptive trajectory populations will take (Arendt and Reznick 2008). For example, the gene *Mc1r* is known to be involved in light/dark coloration transitions in a wide diversity of taxa including lizards, birds, and several mammals, and Hoekstra et al. (2006) found this locus to be involved in adaptive coloration of beach mice along the Gulf Coast in Florida. Given the numerous examples of this locus regulating color over broad taxonomic categories, it is perhaps surprising that along the Atlantic coast, similarly colored populations of beach mice have reached the same phenotype by changes at different loci (Steiner et al. 2009). Different genetic responses to similar selective pressures have also been found among laboratory strains of microorganisms when the initial populations were genetically identical. Wichman et al. (1999) showed that replicate bacteriophage lines under strong uniform selection adapted using both parallel and unique mutations. Surprisingly, the mutations with the largest beneficial effects were often unique to each line. Similarly, Blount et al. (2008) found that replicate lines of *Escherichia coli* under uniform selection fixed different mutations, some of which allowed the utilization of a novel carbon source.

Given these examples, it is clear that allopatric populations adapting under uniform selection may fix different mutations. When alternate mutations are fixed in separated populations of sexual organisms, postzygotic isolation can evolve by Dobzhansky–Muller incompatibilities (Orr 1995). However, we are not aware of empirical studies specifically linking mutation-order processes in adaptation to reproductive isolation. Recent theoretical work suggests that it is more difficult to fix alternate mutations involved in postzygotic isolation when there is high variation in selection coefficients among beneficial alleles (Unckless and Orr 2009); however, the empirical work in adaptation certainly suggests it is possible. The question remains as to how often postzygotic isolation will result when the genetic response to selection differs among populations.

Despite a lack of concrete examples, mutation-order speciation is plausible, yet we question whether this form of speciation should be considered “nonecological.” Presenting this as an alternative to ecological speciation gives the false impression that ecology is not involved in the process. In fact, Schluter (2009) suggests, “selection can be ecologically based under mutation-order speciation, but ecology does not favor divergence as such.” Where selection generates reproductive isolation, it is usually as a byproduct (with the exception of reinforcement), so selection does not typically “favor” the evolution of barriers regardless of

whether it is divergent or uniform. Making distinctions between these two forms of selection may indeed be useful (as when predicting which form of selection would generate reproductive isolation most rapidly), but it seems erroneous to consider one more ecological than the other. It is only when reproductive isolation evolves in the absence of selection that we can eliminate ecology from consideration as an important contribution to speciation.

Schluter (2009) describes the evolution of meiotic drive as a possible nonecological example of mutation-order speciation, where the fixation of an adaptive mutation (the repressor) could be contingent upon the nature of an initial mutation (the driver). However, once a driving allele arises in one population and not the other, selection is no longer uniform. In one population, the evolution of a responder is now favored whereas in the other it is not. Selection is therefore divergent, but is it ecological? Although the outcome of genetic conflict can be thought of as, “a form of adaptation to the internal genomic environment” (Phadnis and Orr 2009), few would label this kind of interaction ecological. Therefore, it is difficult to place these examples into the ecological speciation framework despite satisfying its one requirement.

POLYPLOID SPECIATION

Speciation initiated by polyploidy, that is, whole-genome duplication, is observed in a wide variety of organisms, including fish, amphibians, yeast, and plants. Two main mechanisms of polyploid speciation have been recognized; autopolyploids arise from within populations of a single biological species whereas allopolyploids are formed following hybridization between different species (Ramsey and Schemske 1998). In each case, polyploidy is typically initiated by the production of unreduced gametes, that is, gametes with the somatic chromosome number.

There is abundant evidence in plants that crosses between ploidy levels are less successful than crosses within ploidy levels, due in large part to a mismatch between the ploidy of the developing embryo and the ploidy of the endosperm (Ramsey and Schemske 1998). The fertility of progeny produced from between-ploidy crosses is also much reduced due to a high frequency of chromosomal duplications and deficiencies that render gametes inviable (Ramsey and Schemske 1998; Husband and Sabara 2004). Furthermore, these reproductive barriers are present immediately following hybrid formation; hence, it is reasonable to conclude that such postzygotic isolation might cause speciation without ecological divergence.

Indeed, Schluter (2000, 2001), and Rundle and Nosil (2005) regard polyploidy as nonecological because they assume that reproductive isolation between polyploids and their progenitors does not evolve by divergent natural selection. We suggest that polyploid speciation can only be considered nonecological if each polyploid is viewed as a new species at the instant it is formed, regardless of whether it establishes a self-sustaining population

that is reproductively isolated from its progenitor. This criterion for species status in polyploids is consistent with the BSC, in that it requires the evolution of reproductive isolation between populations and not simply between individuals.

Assuming that population establishment is a prerequisite for recognizing species, does ecology play a role in polyploid speciation? Consider the situation in which autotetraploids arise within a single population of diploids. Given the low frequency of unreduced gamete formation, these neotetraploids are rare (Ramsey and Schemske 2002). Assuming sexual reproduction and an outcrossing mating system, the neotetraploid will mate mainly with its more abundant diploid progenitor, and will produce few offspring, most of which are sterile triploids. In contrast, diploids will mate mainly with other diploids, and will produce fertile, diploid progeny. The converse is expected if diploids are in the minority, as they are then more likely to mate with tetraploids. All other things being equal, once one of the cytotypes has a frequency >50%, it will rapidly sweep to fixation.

Levin (1975) described this frequency-dependent mating success in polyploid systems as the “minority cytotype disadvantage.” The minority disadvantage experienced by neopolyploids can be overcome in a number of ways, including (1) demographic stochasticity that drives neopolyploids from the minority to the majority cytotype, (2) reduced gene flow via increased self-fertilization or asexual reproduction, (3) migration to a new geographic region so as to eliminate gene flow with progenitors, and (4) the expression of ecological attributes in neopolyploids allowing them to coexist with their progenitors, or to replace them (Ramsey and Schemske 2002). Unless one or more of these conditions are realized, a neopolyploid will become extinct despite the presence of strong postzygotic barriers.

It is unknown which outcome of neopolyploid formation is most common. Because minority cytotype exclusion is likely to be rapid, it would seem that the persistence of neopolyploids requires some initial ecological differentiation. In this regard, it is well established that chromosome duplication can imbue neopolyploids with distinct ecological, morphological, and physiological characteristics (Levin 2002; Ramsey and Schemske 2002). Furthermore, rapid genome reorganization following polyploidy, particularly in allopolyploids, can generate new genetic and phenotypic variation, providing an increased opportunity for divergent natural selection between polyploids and their progenitors (Song et al. 1995; Soltis and Soltis 2000). Figure 2 illustrates two different outcomes of polyploid formation. First, the new polyploid may possess insufficient ecological differentiation from its progenitor to allow coexistence in the face of minority cytotype disadvantage, and will fail to establish (Fig. 2A). Alternatively, the new polyploid may display substantial ecological novelty that allows it to persist and to continue to adapt as it establishes in a new ecological niche (Fig. 2B). In this latter case polyploidy may allow the

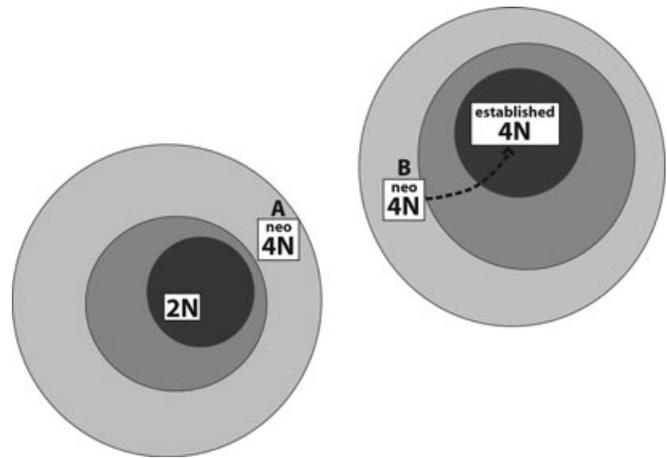


Figure 2. The role of ecology in the establishment of polyploids. Autopolyploidy is presented for simplicity, but equivalent processes function with allopolyploidy. Circles represent topology of a simple two-trait adaptive landscape with darker circles representing trait combinations of higher fitness. A diploid progenitor (2N) sits upon an adaptive peak. There are two potential outcomes of neopolyploid formation: (A) The neopolyploid (neo 4N) could reside at a lower elevation of the same adaptive peak occupied by the progenitor. In this case, the neopolyploid faces both competitive disadvantage and minority cytotype exclusion, and will likely not establish. (B) The neopolyploid could initially reside at the base of a new adaptive peak, and adapt to this new niche (dashed line, neo 4N → established 4N). In this case, speciation can be considered ecological because polyploidy causes an initial change in ecology followed by subsequent adaptation as the neopolyploid climbs the new adaptive peak.

traversing of an adaptive valley, placing the neopolyploid near a new adaptive peak. Because it is highly unlikely that the neopolyploid will immediately reach the new adaptive peak, it will begin to adapt toward this alternate optimum. As a result, the two cytotypes experience divergent selection, and therefore the process is consistent with the mechanism of ecological speciation. Such ecological differentiation could provide substantial reproductive isolation, and thus overcome the frequency-dependent minority disadvantage inherent in neopolyploids.

If polyploid speciation is nonecological, then postzygotic barriers should be of primary importance. If polyploid speciation is ecological, we expect to find a mix of prezygotic and postzygotic barriers, the latter caused by both extrinsic and intrinsic factors. Testing these alternatives requires estimates of the magnitude of reproductive isolation between neopolyploids and their progenitors for pre- and postzygotic factors. To our knowledge, few such studies are available. Husband and Sabara (2004) examined multiple barriers contributing to reproductive isolation between diploid and tetraploid fireweed (*Chamerion angustifolium*, Onagraceae), and found that total isolation between cytotypes was 99.7%. Despite poor seed set in intercytotype crosses and

low triploid fertility, prezygotic barriers such as geographic and pollinator isolation accounted for 97.6% of the total reproductive isolation. Thus, in this system, postzygotic factors presently contribute very little to reproductive isolation, but it is not known if postzygotic isolation was of primary importance in the early stages of polyploid establishment. If so, the prezygotic barriers now in place might have evolved because of selection to reduce hybrid formation, that is, reinforcement. This too is essentially unexplored.

Whether we view polyploid speciation as ecological or nonecological is largely a semantic argument. If we recognize a polyploid as a new species at the time of its origin, regardless of its ability to persist, then polyploid speciation is nonecological. If instead, polyploids are regarded as new species only if they can establish a self-sustaining population that is reproductively isolated from its progenitor, then polyploid speciation is often ecological.

SPECIATION BY GENETIC DRIFT

We consider speciation to be ecological when externally imposed selection results in reproductive isolation; therefore, speciation by drift is nonecological. Although factors that influence the magnitude of genetic drift, such as variation in population size or mating success, may often have an ecological basis, this does not imply that speciation mediated by genetic drift is also “ecological.”

In a verbal model, speciation by genetic drift alone is simple and plausible. Alternate mutations are fixed in allopatric populations by stochastic processes, and eventually cause complete isolation. In actuality, genetic drift is unlikely to result in substantial reproductive isolation for a variety of reasons (reviewed in Turelli et al. 2001; Coyne and Orr 2004). One argument against speciation by genetic drift is that most of the traits that have the potential to be involved in reproductive isolation are likely subject to natural selection, and it will be difficult for drift to alter such adaptive traits unless drastic population bottlenecks are involved. For example, following an event that separates two plant populations, the average flowering time in the two populations could be different due solely to drift. The temporal isolation that results would most likely be minor because it would only result from the random assortment of preexisting genetic variation. Selection may favor divergence in flowering time because of some environmental condition (e.g., a difference in growing season), in which case temporal isolation will likely arise. If no conditions favor divergence, flowering time will likely be subject to stabilizing selection because any individual that differs significantly from peak flowering will have reduced mating opportunities; however, this could be theoretically overcome for short-lived, self-compatible species (Devaux and Lande 2008).

A similar argument can be made for other forms of isolation, including intrinsic postzygotic isolation. In order for a mutation

to potentiate a Dobzhansky–Muller incompatibility, it must be integrally involved in some developmental or physiological process in the organism. The negative epistatic interactions in hybrids must disrupt some important process or structure to result in incompatibility or sterility. Therefore, mutations that occur at these loci will rarely be neutral, and are unlikely to be fixed by genetic drift alone. Alternatively, if mutations within these loci are neutral (synonymous substitutions for example), they are more likely to be fixed by drift, but are probably less likely to disrupt hybrid performance in most cases.

An additional argument against genetic drift as a driver of speciation is the time required for it to cause complete reproductive isolation. Theoretical treatments show that isolation by genetic drift alone is slow, and would be overshadowed by even extremely weak selection (Nei 1976; Nei et al. 1983). A related problem is that if speciation is incomplete, gene flow upon secondary contact will erase any isolation that has arisen by drift alone (e.g., Payne and Krakauer 1997). Consider the above example of two plant species with flowering times that diverged through genetic drift. If temporal isolation does not become complete in allopatry, gene flow upon secondary contact will eliminate reproductive isolation. Therefore, reproductive isolation by drift takes much longer to evolve than by selection, and it must evolve to completion before it can result in new species. These conditions create considerable doubt that genetic drift alone is a significant mechanism of speciation.

Given the difficulty in generating significant reproductive isolation by genetic drift in large populations, much of the work on the role of drift in speciation has centered on founder effects. Mayr (1963) and Carson (1968) envisioned situations in which small populations involved in founder events could shift to new adaptive peaks through the reorganization of the genome following population fluctuations. Although many of the assumptions in these models have been successfully challenged (Coyne and Orr 2004), it has been shown that speciation by founder effect is theoretically possible (Gavrilets and Hastings 1996). However, the assumptions required of these models are restrictive (Turelli et al. 2001), and laboratory attempts to recreate founder effect speciation (which should theoretically evolve quickly) result in failure of reproductive isolation to arise (Rundle et al. 1998; Rundle 2003).

Purported examples of speciation by genetic drift on islands exist based on data from neutral genetic markers (e.g., Comes et al. 2008); however, reproductive isolation has not been measured in any of these systems, providing little insight into whether there is a link between genetic drift and speciation. In fact, very few direct empirical studies of reproductive isolation evolving via drift exist. In one example in which drift has been proposed as the cause of reproductive isolation, shell chirality (direction of coiling) in the snail genus *Euhadra* may produce reproductive isolation via a single mutation inherited through a maternal effect

(Ueshima and Asami 2003). Snails of opposite chirality cannot mate with one another, providing a complete prezygotic barrier in a single nonadaptive step. The presence of snail species of both chiralities within the genus suggests that this transition has indeed resulted in new lineages; however, the unique mode of inheritance in this example raises doubts as to whether this is a common phenomenon.

While drift may not commonly result in speciation unilaterally, Templeton (2008) argues that it is erroneous to consider speciation a binary drift/selection process. Rather, drift and selection could work simultaneously and/or interact during divergence. A potential example involves the role of chromosomal rearrangements in reproductive isolation. Chromosomal rearrangements, such as inversions, can have an impact on divergence by creating linkage groups of loci involved in multiple forms of reproductive isolation that cannot be disrupted by recombination (Noor et al. 2001; Rieseberg 2001). Empirical evidence suggests that such chromosomal inversions may contribute to the maintenance of species boundaries despite interspecific gene flow (e.g., Brown et al. 2004). Chromosomal inversions may sometimes be fixed by genetic drift, but the loci within the inversions may be subject to selection. Reproductive isolation could therefore be a product of both the adaptive loci within the inversion and the inversion itself, resulting in speciation that cannot be unambiguously defined as either ecological or nonecological.

SEXUAL SELECTION AND SEXUAL CONFLICT

Sexual selection has received increasing recent attention as a potential factor in speciation (West-Eberhard 1983; Ritchie 2007). Closely related species often differ conspicuously in traits that affect mating success, leading to speculation that sexual selection contributes to diversification. However, secondary sexual traits and preferences must coevolve in order for sexual behavioral isolation to arise, and there are few systems in which the link has been made (Panhuis et al. 2001). Comparative studies of the effect of sexual selection on speciation have shown mixed results, with a positive association between sexual selection and speciation rates in some groups (e.g., Armqvist et al. 2000; Owens et al. 1999), but not in others (e.g., Gage et al. 2002; Katzourakis et al. 2001). Although speciation solely by sexual selection may occur, it is difficult to categorize within the ecological speciation framework because it can theoretically operate in either the presence or absence of divergent natural selection (Schluter 2001).

The link between sexual selection and sexual isolation presents situations in which genetic drift could conceivably lead to reproductive isolation. However, because female choice and male traits must both drift in concert to produce isolation, it is difficult to imagine conditions under which drift could work alone (Coyne and Orr 2004). In his influential model of sexual selection, Lande (1981) proposed that female choice for arbitrary male traits can

drift along a line of neutral equilibrium. This and subsequent theory has shown that it is possible for sexual isolation to evolve as female preference and male traits drift along this line (Wu 1985; Uyeda et al. 2009). However, the potential for sexual isolation by drift is drastically reduced if there is a cost associated with female choosiness (Turelli et al. 2001).

Sexual selection most likely facilitates speciation by interacting with natural selection during ecological divergence (Ritchie 2007). The spectacular instances of sexual selection such as Hawaiian *Drosophila* (Templeton 1979) or African cichlids (Seehausen et al. 1999) exhibit similarly remarkable ecological differentiation, and it seems likely that sexual selection would have little opportunity to cause speciation without some niche divergence. Ecological factors can affect traits and preferences directly or by mediating the outcome of sexual signals. In an example of the latter, Boughman (2001) describes an instance of sensory drive in sticklebacks in which male nuptial color and female preference differ with light environment. In this case, sexual selection may be driving reproductive isolation, but it is dependent on the ecological context in which the signals are expressed. In such cases, it is possible for sexual isolation to arise in the absence of divergent natural selection, but the process seems better described as ecological than as nonecological speciation.

Sexual conflict occurs when the evolutionary interests of males and females differ (Parker 1979). This form of antagonistic coevolution is thought by some to be a ubiquitous part of sexual interactions (Ritchie 2007), and empirical evidence shows that it may be a common intraspecific phenomenon (Stockley 1997). Theoretical work reveals that sexual conflict can either promote or inhibit speciation depending on the outcome of the interaction (Parker and Partridge 1998). Because sexual conflict can arise in the absence of external selection pressures, it may rarely result in speciation without the contribution of ecological factors.

THE PERVASIVE EFFECT OF SELECTION ON REPRODUCTIVE ISOLATION

Because we contend that speciation via uniform selection, polyploidy, and sexual selection has important ecological components, speciation by drift alone is the only purported example remaining that can be considered nonecological. However, given the capacity of selection to have rippling effects throughout the genome, it can be a much more powerful source of reproductive isolation than genetic drift. For example, the fixation of a single adaptive mutation could have effects on reproductive isolation by a variety of mechanisms and at multiple stages.

Coyne and Orr (2004) defined reproductive isolation that is favored by selection (i.e., reinforcement) as “direct,” whereas all other forms of isolation arise as “indirect” outcomes of selection. We prefer to use “reinforcement” instead of “direct” and to use “byproduct” when selection does not favor reproductive isolation

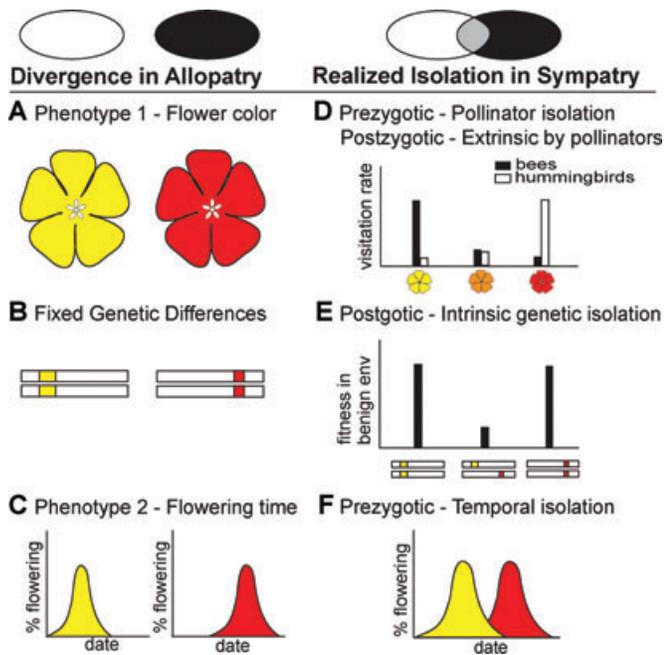


Figure 3. A hypothetical example of how natural selection on a single trait may confer reproductive isolation at multiple life-history stages. Two allopatric populations of plants experience divergent selection for flower color (A), resulting in the fixation of a single adaptive mutation in each population (B) conferring yellow- and red-flowered phenotypes. Development time is affected by this mutation due to pleiotropy, leading to divergence in flowering time in the two populations (C). If these incipient species come back into contact, this single fixed difference could lead to the expression of multiple forms of reproductive isolation. Direct prezygotic isolation arises due to pollinator preferences, as the red-flowered morph is favored by hummingbirds and the yellow-flowered morph is favored by bees (D). Neither pollinator visits hybrids often, resulting in extrinsic postzygotic isolation as well (D). The fixation of alternate mutations in the two populations also leads to the realization of indirect intrinsic postzygotic isolation through a Dobzhansky–Muller incompatibility (E). Indirect prezygotic isolation also arises as the red and yellow morphs show significant divergence in flowering time due to pleiotropy (F).

per se. Within byproduct, direct isolation arises when the trait conferring reproductive isolation is the target of selection, and indirect isolation arises by pleiotropy or linkage. Although the biological mechanisms described by our system are equivalent to the terminology proposed by Coyne and Orr (2004), we feel that our usage of direct and indirect is more intuitive as it is analogous to the way these terms are used in traditional selection experiments.

Figure 3 illustrates a hypothetical example of the pervasive nature of selection on reproductive isolation. The figure depicts two plant populations diverging in allopatry. Geographic differences in pollinator assemblages result in divergent selection for

color—a yellow morph and a red morph (Fig. 3A), which leads to fixation of alternate alleles (Fig. 3B). The flower color locus could have pleiotropic effects on other traits such as flowering time (Fig. 3C). If these two incipient species come back into contact, several forms of isolation are realized from these two adaptive mutations. Because divergence occurred in allopatry, the isolation that results is a byproduct of selection. Bees are the primary pollinator of the yellow morph whereas hummingbirds are the primary pollinators of the red morph. This specificity confers prezygotic pollinator isolation, limiting heterospecific pollination (Fig. 3D). If hybrids are formed there may also be extrinsic postzygotic isolation if the intermediate phenotype of the hybrid is less attractive to pollinators (Fig. 3D). Both of these forms of isolation are “direct byproducts” of selection because flower color was the target of selection. Intrinsic postzygotic isolation may arise if these two mutations interact negatively in hybrids (Fig. 3E), and, temporal isolation may arise as a pleiotropic consequence of a genetic correlation between flower color and flowering time (Fig. 3F). Both of these would be considered “indirect byproducts” of selection.

This example illustrates how even a single adaptive mutation can produce reproductive isolation at multiple stages in the life history. In this case, selection on flower color may cause pollinator isolation, temporal isolation, and both extrinsic and intrinsic postzygotic isolation. The importance of such a “multiplier effect” for the evolution of reproductive isolation is unknown and warrants further investigation.

ECOLOGY AND SPECIATION: OVERVIEW

A serious drawback of the ecological speciation framework is the implication that any given speciation event is either ecological or nonecological. Although early advocates of ecological speciation may not have intended to portray this as a dichotomy (D. Schluter and H. Rundle, pers. comm.), usage in the literature suggests otherwise. For example, the title of a recent paper by Schluter (2009), “Evidence for ecological speciation and its alternative” connotes a system in which speciation can be classified as either ecological or nonecological. Many forms of reproductive isolation can be involved in speciation, and although we contend that drift rarely acts unilaterally, a combination of selective and stochastic forces may contribute to the evolution of reproductive isolation in any given speciation event. For a pair of incipient species, some traits involved in reproductive isolation may be under divergent selection (ranging in intensity), some traits under uniform selection, and some under no selection at all. Therefore, unless complete reproductive isolation results from a single trait, it will often be impossible to unambiguously classify a given speciation event. In addition, the pervasive effect of selection suggests that adaptive evolution and speciation are inseparable, casting doubt on whether speciation is ever nonecological.

We propose that a more fruitful approach is to first identify the isolating barriers that contribute to speciation regardless of their mode of origin, and to then investigate how they evolved. We see two major opportunities. First, despite the historical focus on the geography of speciation, there is presently little appreciation of how a genetically based difference in geographic distribution can itself be a form of reproductive isolation. Second, although many workers recognize the need to study the forms of reproductive isolation that are most “important” to speciation, there are many ways to define importance. To evaluate importance, a method is needed for evaluating the contributions of individual barriers to total reproductive isolation.

Geographic Isolation: A Neglected Isolating Barrier

Adaptation to different habitats can influence the geographic distribution of species, potentially affecting encounter rates between populations, and therefore imparting reproductive isolation. In his classic book “Variation and Evolution in Plants,” Stebbins (1950) describes the importance of ecological differentiation for species formation, and in a section titled “Spatial and Ecological Isolation in Relation to Species Formation,” identifies an issue that remains controversial today. He notes (p. 197) “. . .geographically isolated races, as well as allopatric species, are usually separated from their relatives in adjacent regions by ecological barriers as well as by geographic ones.” Here, contrary to the oft-stated view that speciation mechanisms are best studied in regions of sympatry (Coyne and Orr 2004), he portends recent proposals to consider genetically based geographic differences as legitimate components of reproductive isolation (Schemske 2000; Ramsey et al. 2003).

Coyne and Orr (2004) refer to this as macrospatial habitat isolation and acknowledge that it can be a potent source of reproductive isolation between taxa. Given that this form of reproductive isolation acts first in the sequence of possible barriers (Ramsey et al. 2003), it has considerable potential to reduce gene flow (Schemske 2000; Coyne and Orr 2004). However, despite numerous examples in which habitat isolation appears to play an important role in speciation (e.g., Clausen et al. 1940; Werner and Platt 1976; Lynch 1978; Feder and Bush 1989; Rand and Harrison 1989; Richman and Price 1992; Wang et al. 1997; Ramsey et al. 2003; Nosil 2007), it is approached haphazardly, with few estimates of the strength of isolation imparted by this barrier. In a recent review of 19 studies in which multiple forms of isolation have been measured (Lowry et al. 2008a), only two (Ramsey et al. 2003; Kay 2006) include an estimate of geographical isolation, although more studies examined geographic differences in fitness that contribute to habitat isolation.

The primary difficulty lies in the potentially complex relationship between the niche occupied by species, the geographic

landscape over which the appropriate ecological conditions exist, and dispersal limitation (Crispo et al. 2006; Nosil 2008; Price 2008). Geographic ranges are the product of both ecological and historical factors (Endler 1982; Coyne and Orr 2004; Thorpe et al. 2008), and most speciation events probably begin with an allopatric phase (Coyne and Orr 2004). Therefore, one cannot assume that all geographic isolation is based upon biological differences between taxa. Dobzhansky (1937, p. 231) recognized this problem in discussing the role of geography in speciation, saying, “. . . Geographical isolation is therefore on a different plane from any kind of physiological one. This consideration has to be qualified, because the occupation of separate areas by two species may be due not only to the fact that they have developed there, but also to the presence of physiological characteristics that make each species attached to the environment. . . .” Clearly Dobzhansky appreciated that historical processes leading to allopatry will often give way to ecological processes as populations adapt to different habitats.

The challenge presented by the interplay between ecology and history in determining species distributions is illustrated by the wide range of approaches for treating allopatric taxa in studies of speciation. As espoused by Wiens (2004, p. 916), because gene flow ceases, completely allopatric populations are species, and “. . . there is no requirement that these lineages evolve additional reproductive isolating mechanisms.” Similarly, it has been proposed that geographic separation (whether complete or not) can be treated simply as a form of assortative mating (Kirkpatrick and Ravigne 2002). At the other extreme, some authors suggest that allopatric taxa can never be considered true species until secondary contact tests their integrity in sympatry. For example, Coyne and Orr (2004) refer to the “species problem” as “. . . why do sympatric, sexually reproducing organisms fall into distinct clusters?” In referring to the definition of ‘non-adaptive’ radiation, Rundell and Price 2009 defines relevant ecological differences among species as those “ecological differences that are associated with coexistence in sympatry.” Similarly, Mallet (2008) refers to the requirement of sympatry as “the real advance made by the biological species concept.” Secondary contact does indeed help diagnose the completion of speciation, to quote Grant (1957), “sympatry is the final test of species status.” However, sympatry is not inevitable. In fact, the traits involved in habitat isolation could conceivably prevent secondary contact from ever occurring (Coyne and Orr 2004).

By these contrasting perspectives, allopatric taxa that differ in habitat may or may not be different species under the BSC. Mayr’s “single most important event in evolution” has either undoubtedly happened, or is yet to occur. As originally proposed, the BSC defines species as “. . . groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups” (Mayr 1942). The word “potentially” accounts

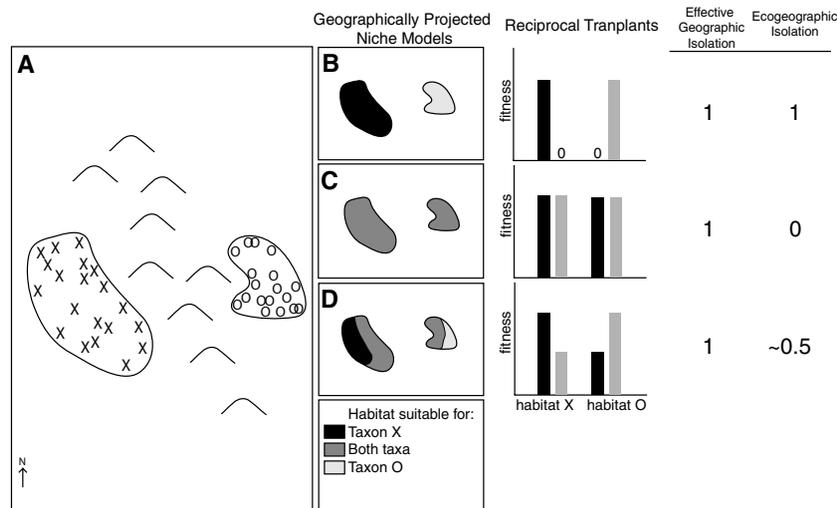


Figure 4. Assessing effective and ecogeographic components of geographic isolation. Populations of two taxa represented by X's and O's are separated by a mountain range. Effective geographic isolation is complete, such that taxa X and O experience no gene flow (A). Panels B–D show the outcome of estimating ecogeographic isolation by ecological niche modeling and reciprocal transplants. In (B) ecogeographic isolation is complete; each taxon is adapted to its own environment such that ecological niche modeling and reciprocal transplants show that the taxa would not survive in each other's geographic range. (C) Ecogeographic isolation is absent; the taxa are equally fit in the alternate range. (D) Ecogeographic isolation is incomplete. On average, each species survives and reproduces better in its own environment, but portions of the alternate range are also suitable.

for species whose geographic distribution prevents complete assessment of isolation, such as the case of allopatric populations. Mayr himself clearly struggled with the problem of allopatry, removing the word “potentially” from later versions of the definition (Mayr 1984). We suggest that Mayr's original formulation of the BSC is the correct one, and that the word “potential” allows for the assessment of reproductive isolation even in allopatric taxa.

SEPARATING ECOLOGICAL FROM HISTORICAL FACTORS

To resolve this paradox, we propose that differences in geographic distribution caused by adaptation to different habitats be treated like any other form of reproductive isolation, as a quantitative estimate of how much gene flow is reduced by intrinsic differences between taxa. To distinguish the independent contributions of genetic and historical processes, we separate geographic factors into ecogeographic isolation and effective geographic isolation. We define “ecogeographic isolation” as the degree to which differences in geographic distribution are based on genetic differences between taxa. “Effective geographic isolation” is the actual spatial separation experienced by populations, and includes both ecogeographic isolation and differences in distribution based solely on historical factors. Therefore, effective geographic isolation will sometimes be greater than ecogeographic isolation between populations such as the case of populations separated solely by geologic features. However, only ecogeographic isolation is relevant to speciation under the BSC, because it represents the portion of effective geographic isolation due to genetic differ-

ences between populations. This distinction sets up the possibility of discordance between status assigned under the BSC and other species concepts, as populations may diverge morphologically and/or phylogenetically in allopatry without complete reproductive isolation.

An illustration of the difference between ecogeographic and effective geographic isolation is presented in Figure 4. For taxa X and O, effective geographic isolation is simply the degree to which current geographic ranges are allopatric. This has been measured previously by either overlaying published range maps (e.g., Barraclough and Vogler 2000; Fitzpatrick and Turelli 2006) or resampling within virtual quadrats (Ramsey et al. 2003; Kay 2006). Using the standard convention these two taxa would have complete effective geographic isolation (Fig. 4A), yet further investigation is required to determine the degree of ecogeographic isolation.

Reciprocal transplants have been used historically to examine genetically based local adaptation (Turesson 1922; Clausen et al. 1940), and more recently to assess reproductive isolation resulting from fitness variation across habitats, both within (e.g., Lowry et al. 2008b) and between species (e.g., Wang et al. 1997; Angert and Schemske 2005). Reciprocal transplants address the question: Do the current geographic distributions of taxa reflect intrinsic biological differences between them (Schemske 2000)? The two extremes are the easiest to interpret. For example, for taxa X and O in Figure 4, if reciprocal transplants show that the fitness of taxon X is high in its own habitat and zero in the habitat of taxon O, then ecogeographic isolation is complete (Fig. 4B). On the

other hand, if reciprocal transplants show that the fitness of X and O are equivalent in each other's range, the effective geographic isolation they experience is completely due to historical processes, and there is no ecogeographic isolation (Fig. 4C).

As will often be the case, taxa X and O could also show some amount of genetically based adaptation among populations within their geographic range, in which case assessing the amount of isolation becomes more challenging. The magnitude of fitness differences provides the best estimate of isolation, but several considerations must be taken into account. Because the niche of a species is a multidimensional range of appropriate conditions i.e., a volume rather than a single point in hyper-dimensional space, the results of reciprocal transplants may differ across the geographic range. For example, if two allopatric species are distributed along a continuous environmental gradient, transplant experiments conducted on a small scale may show weaker selection against the "away" population than if the experiments were carried out from the opposite ends of the respective geographic ranges. Therefore, to estimate ecogeographic isolation it is necessary to compare the average fitness from multiple populations that span the geographic range of each taxon. Partially sympatric taxa illustrate the problem particularly well, as there are clearly portions of the range in which both taxa survive, but other portions that can only support one of the taxa. These cases seem very likely to show fitness variation across geography because dispersal limitation is probably not the predominant factor limiting overlap. In fact, in reciprocal transplants between species with abutting or partially overlapping ranges, it is common to see strong fitness differences in alternate habitats (e.g., Wang et al. 1997; Angert and Schemske 2005; Geber and Eckhart 2005).

Recently, Nosil et al. (2005, p. 705) proposed the term "immigrant inviability" to represent a new form of isolation, "... the reduced survival of immigrants upon reaching foreign habitats that are ecologically divergent from their native habitat." Although Nosil et al. (2005) acknowledge that others have recognized this as a part of prezygotic habitat isolation, they argue that immigrant inviability is biologically distinct from other factors such as habitat preference. However, assessing the viability of immigrants is what a reciprocal transplant study achieves; members of one species or population are moved from their home habitat into the habitat of another. The viability of these immigrants is not a separate form of isolation from habitat isolation; it is a measure of the genetic basis of habitat differences. To treat immigrant inviability as a novel form of isolation would require first estimating immigration rates. While this would be worthwhile when combined with data on the fitness of immigrants, it was not part of the original proposal by Nosil et al. (2005).

Treating immigrant inviability as a new form of isolation can be problematic, as illustrated by a recent paper in which Lowry et al. (2008a) compiled studies of various forms of reproductive

isolation to assess the relative importance of different barriers. In doing so, they treated geography and immigrant inviability as independent isolating barriers. As discussed above, differential fitness in reciprocal environments (immigrant inviability) is a measure of the genetic basis of ecogeographic isolation. Without a genetic basis, geographic isolation is not a form of isolation based upon intrinsic differences between populations. It is therefore inappropriate to treat geographic isolation and immigrant inviability as independent phenomena when developing a composite metric, as it is essentially counting the same form of isolation twice.

Despite their intuitive appeal, reciprocal transplant experiments are laborious in the best systems and impossible in most, so alternative approaches for estimating ecogeographic isolation are needed. A potential solution is the application of ecological niche models. This approach uses GIS technology to build a predictive map of suitable environmental conditions from spatial data on abiotic variables and species occurrences (Peterson and Vieglais 2001; Kozak and Wiens 2006; Kozak et al. 2008; Nakazato et al. 2008; Warren et al. 2008). Niche similarity can be calculated using recently developed methods for comparing ecological niche models (Warren et al. 2008); however, to assess how differences in niche translate into ecogeographic isolation, it is useful to project niche models onto the geographic range over which the two taxa occur. Niche similarity and overlap in geographically projected niche models will commonly yield different results. For example, two species of plants may be identical in most niche parameters such as precipitation, temperature, seasonality, etc., but grow on different soil types. A niche similarity index would show these two species as highly similar, but if the two soils to which they are adapted occur in disjunct areas, the geographically projected niche model would predict that this small niche difference translates into substantial ecogeographic isolation.

To assess the strength of ecogeographic isolation, the projected niche maps for two taxa can be overlaid and the degree of allopatry in projected niche models estimated. This can be done separately for the two taxa under consideration, as there will often be asymmetries in how much isolation each experiences. If a projected niche model shows that suitable habitat for taxa X and O is found only in their current ranges (Fig. 4B), we would conclude that the current geographic separation between them, i.e., the effective geographic isolation, is a direct consequence of genetically based, ecological differences, and therefore ecogeographic isolation is 1. Alternatively, if historical processes were solely responsible for the effective geographic separation, niche models would show that equally suitable habitat for both species is found throughout each other's geographic range (Fig. 4C). In this case, there is no ecogeographic isolation. In the third scenario, niche modeling shows that half of the geographic range of taxon X is suitable for taxon O, and vice versa. Therefore, the zone of

overlap in projected niche models represents the region in which both taxa could live if not limited by dispersal, and ecogeographic isolation is estimated as 0.5 (Fig. 4D).

Niche modeling can be employed equally well with taxa exhibiting some degree of sympatry. While sympatry indicates that a portion of the geographic range is suitable for both species, an ecological niche model represents an average niche across all samples used to generate the model. Therefore, despite coexistence in some regions, two taxa can show significant niche differences, and achieve some ecogeographic isolation as a result. As mentioned above, in cases with partial sympatry, we would predict a higher correlation between effective and ecogeographic isolation because dispersal limitation is unlikely to be the cause of differences in geographic range.

Ecological niche modeling has some critical limitations, and must be interpreted with caution (Guisan and Thuiller 2005). The most important issue for the study of ecogeographic isolation is that a difference in niche-models between two taxa only truly shows that the two taxa are found in areas where environmental conditions are different. Although suggestive, there is no guarantee that these differences in habitat occupied are due to genetically based differences between the taxa, or that they would necessarily occupy different habitats in sympatry. In addition, ecological niche models can only reliably measure the geographic effect of differences in niche given current geologic and climatological conditions. Through evolutionary time-scales, it is quite possible that geologic/climate change could bring habitats that are currently separated into closer proximity. This is of course also possible through human-induced disturbance (e.g., Lamont et al. 2003). However, it also seems very possible that as climate changes, favorable ecological conditions will remain spatially distinct.

Ecological niche modeling and reciprocal transplant data could be combined to take advantage of the strengths of both approaches. If reciprocal transplants were performed across a wide range of climatic conditions, it would be possible to build an ecological niche model using fitness of the transplants in place of presence/absence data. Projecting this “transplant niche model” onto the geographic landscape could then be an excellent tool for measuring the overlap in ecogeographic isolation. Because these models would be based upon the fitness of organisms, they would be a more reliable predictor of adaptation to habitat. Future studies that compare results from the two methods would help determine if niche modeling is an appropriate proxy for transplant studies.

Assessing the “Importance” of Reproductive Barriers

Because speciation may often require the evolution of several forms of reproductive isolation (Mayr 1947, 1963; Ramsey et al.

2003; Lowry et al. 2008a), it is necessary to consider the strength of each barrier in light of its relative contribution to total reproductive isolation. Reproductive isolation that acts early in the life-cycle of an organism has the potential to greatly impact gene flow, because barriers that act later can only stop gene flow that remains after earlier barriers. Coyne and Orr (1989) proposed a method for estimating total reproductive isolation in which each barrier is first assigned a chronological position within an organism’s life cycle, and then the contribution of each isolating barrier to the total is discounted by the net effect of all previously acting barriers. They applied this approach to study mating isolation and intrinsic postzygotic isolation in *Drosophila*, and Ramsey et al. (2003) expanded the method to include multiple barriers in a study of *Mimulus*. It is critical to note that this approach can only assess the contribution of the current barrier strength to total isolation, and may not necessarily represent the barriers in place at the time of speciation because reproductive isolation continues to evolve after speciation is complete. Hence, studies of recently diverged taxa provide the most accurate picture of the barriers involved in speciation, and comparative work of multiple, recently diverged species may be the only way to assess the order in which barriers typically arise.

Coyne and Orr’s method for estimating total reproductive isolation and the contribution of individual barriers has been adopted in only a few studies of speciation (Ramsey et al. 2003; Nosil et al. 2005; Matsubayashi and Katakura 2009). This is due in part to questions about its validity. For example, Martin and Willis (2007) suggest that the potential for a lack of independence between barriers could confound the composite metric of isolation proposed by Coyne and Orr. For this reason, in a summary of isolating barriers in plants, Lowry et al. (2008a) calculated the mean isolation for prezygotic barriers and compared this to the mean for postzygotic barriers, concluding that prezygotic barriers were stronger (0.838 vs. 0.407). Although they acknowledge that the magnitude of this difference is probably underestimated due to the sequential nature of barriers, we stress that the difference between their summary measures of barrier strength and those based upon Coyne and Orr’s method is probably substantial. Consider two hypothetical, partially sympatric populations for which we have estimated barrier strengths for two prezygotic barriers (ecogeographic and temporal isolation) and two postzygotic barriers (extrinsic postzygotic isolation and hybrid sterility) and found that each individual barrier has an equal strength of 0.80. Using the approach of Lowry et al. (2008a), both prezygotic barriers would be combined (ecogeographic isolation + ((1 – ecogeographic isolation) × temporal isolation)) to give a mean strength of 0.96 and the two postzygotic terms would be combined to give an equivalent result. By contrast, using the method of Coyne and Orr (1989), the total isolation is estimated as 0.9984, and the contributions for the four barriers are, respectively, 0.80 (geographic), 0.16 (temporal),

0.032 (extrinsic postzygotic) and 0.0064 (hybrid sterility), and the difference between the contribution from prezygotic factors (0.96) is markedly greater than that from postzygotic factors (0.0384). These two approaches lead to very different interpretations. Following Lowry et al. (2008a), we would conclude that pre- and postzygotic barriers provide the same isolation but we cannot determine the total isolation whereas the approach of Coyne and Orr would suggest that the two populations are isolated primarily by prezygotic barriers and that the total isolation is nearly complete (>99%).

An empirical example of the effect of the sequential nature of barriers is illustrated by measures of isolation between *Mimus cardinalis* and *M. lewisii* (Ramsey et al. 2003). In this study, geographic isolation, pollinator isolation, gametic isolation, and intrinsic postzygotic isolation had strengths of 0.59, 0.99, 0.83, and 0.41 respectively (averaged across the potentially asymmetrical isolation between species for simplification). Although each of these barriers may be considered strong on an individual basis, placing them in the linear sequence reveals that geographic isolation has the highest contribution to total isolation at 0.59 whereas pollinator isolation (0.40), gametic isolation (0.0083), and intrinsic postzygotic (0.00070) exhibit declining relative contributions to the total. In this example, the overwhelming strength of pollinator isolation virtually guarantees that gene flow cannot proceed beyond this barrier, in that very little potential gene flow remains for later acting barriers to prevent. This result has been validated by an extremely low incidence of hybrid seeds found in nature (Ramsey et al. 2003).

The approach of Lowry et al. (2008a) does not eliminate the issue of nonindependence because any correlation in barrier strength for different barriers will still affect the mean. Is it necessary to consider nonindependence when estimating the relative importance of different barriers? When one trait causes multiple forms of isolation, statistical nonindependence does not occur unless the expression of one form of isolation affects the outcome of other forms. Therefore, multiplicative combination of barriers may often be suitable. There may be cases in which the strength of one barrier is moderated by the presence of another. For example, two taxa that reside at different altitudes may be primarily isolated by ecogeography. The timing of reproduction may also differ, but this could be due only to differences in the growing season between the two habitats, and is thus an indirect pleiotropic effect of the genes that contribute to ecogeographic isolation. In these cases, including temporal isolation in the linear sequence of independent barriers may be inappropriate, but it is not known how often this type of nonindependence occurs in nature.

Other issues have been raised in regards to estimating total isolation and the contributions from individual barriers. Martin and Willis (2007) contend that the manner in which prezygotic isolation is calculated is not equivalent to postzygotic measures.

Part of the issue involves developing appropriate null expectations for isolation. For example, when two taxa encounter each other in nature, their relative abundances determine the null expectation for heterospecific matings. While this is undoubtedly true, data on relative abundances at contact zones are rarely available or easily obtained. Independent zones of contact are likely to differ in species relative abundances, and it would be necessary to average over large expanses of geography to develop an appropriate null. An additional issue is how to construct a null expectation when two taxa are allopatric. If allopatry is a purely historical process (effective geographic isolation occurs, but none of it is ecogeographic), then other forms of isolation are important despite not being manifested in nature. How do we calculate a null expectation if taxa never meet? We suggest that estimating total isolation based upon an assumption of equal relative abundances will be more useful than not making the calculation.

Regardless of how individual barriers are calculated, there is wide agreement that estimating the relative contribution of each to reductions in gene flow is one of the primary problems in speciation research. For example, Martin and Willis (p. 68, 2007) state “a major challenge to evolutionary biologists involves identifying the degree to which individual barriers contribute to total isolation observed between species,” but conclude that this may be unattainable given the difficulties discussed above. Although we agree that it is important to develop the correct metrics for achieving this goal, we contend that ignoring the sequential nature of barriers and/or omitting potentially important barriers (such as ecogeographic isolation) is bound to create far worse misconceptions about how speciation works.

ARE ALL ISOLATING BARRIERS RELEVANT TO SPECIATION?

This framework for investigating reproductive isolation has serious consequences for how we view the importance of different forms of isolation. For example, if ecogeographic isolation between two taxa is complete at the time of speciation, there is no opportunity for barriers that operate only in sympatry to contribute to the total isolation. Even if later acting barriers are strong, the potential isolation is not realized unless it prevents gene flow in nature. It therefore seems irrelevant to assess the contribution of later acting barriers if hybrids are never formed. Using the multiplicative approach of Coyne and Orr to assess reproductive isolation solves this problem by scaling the strength of isolation by how much gene flow remains.

Given that most speciation events start with an allopatric phase (Coyne and Orr 2004), the first reproductive barrier that probably arises in many systems involves adaptation to different habitats. Therefore, without some estimate of ecogeographic isolation, estimates of reproductive isolation will be at best incomplete, and at worst misleading. For example, consider the

influential papers on *Drosophila* by Coyne and Orr (1989, 1997). They collected data from the literature for two components of reproductive isolation, sexual isolation and intrinsic postzygotic isolation. While these barriers are amenable to laboratory measurement, are they relevant to speciation? For the approximately 50% of species pairs in their study that are sympatric, these barriers might contribute to reproductive isolation but they cannot now be regarded as contributing to reproductive isolation in the remaining allopatric species pairs, although this potential isolation could be realized if species become sympatric in the future. Estimating the level of ecogeographic isolation and calculating its relative strength to the forms already measured might change our view of how speciation actually occurs in this important system.

Along these lines, it is perhaps surprising to consider that some of the genes in *Drosophila* associated with postzygotic genetic incompatibilities and identified as “speciation genes” (reviewed in Noor and Feder 2006) occur in species pairs that are completely allopatric. Although there is no reason to believe that the hybrid incompatibility and sterility genes studied thus far would differ substantially from those that actually operate in nature, referring to these as speciation genes seems dubious. For example, the gene *Odysseus* (*Ods*) is believed to play an important role in hybrid sterility between *Drosophila simulans* and *D. mauritiana* (Ting et al. 1998). However, these two species are completely allopatric, so it is unlikely that the *Ods* gene has actually prevented gene flow in nature (Noor and Feder 2006). Thus, it would seem that *Ods* is best viewed as a gene that might contribute to reproductive isolation if the species were to come back together in secondary contact, but as far as we are aware, there is no evidence that it was involved in the initial speciation process.

To illustrate this point, consider two allopatric populations experiencing divergent selection. If divergent selection based on habitat differences is strong, habitat isolation will begin to evolve first as effective geographic isolation becomes ecogeographic isolation. Other forms of reproductive isolation, such as mating isolation or intrinsic postzygotic isolation will eventually evolve, and the potential strength of these components will increase with time. It is important to note that the relative contribution of these forms of isolation depends largely on the geographical arrangement of populations in the future. If populations remain allopatric, most barriers (with the exception of ecogeographic isolation) will not be realized in nature. If populations become sympatric in the future, some portion of the potential strength of other barriers could be realized, and their contribution to total reproductive isolation would increase.

The strength of divergent selection experienced by the allopatric populations could affect which forms of isolation arise and at what rate. For example, if habitats in allopatry are very different, divergent selection is likely to be strong, and ecogeographic isolation will likely arise first as presented above. However, if

selection is only weakly divergent or uniform, ecogeographic isolation may be much slower to arise, and other forms of isolation may outpace its evolution.

The dynamics governing which forms of isolation arise first are unlikely to be drastically different under sympatric or parapatric speciation. Models of sympatric speciation require the simultaneous evolution of at least two forms of reproductive isolation, assortative mating and isolation due to adaptation (such as extrinsic postzygotic that would result from adaptation to a novel food source) (Bolnick and Fitzpatrick 2007). Through time, these two forms of isolation would arise at the same rate, and must reach completion relatively quickly. Intrinsic postzygotic isolation cannot begin to evolve until total isolation is complete by these other forms. Parapatric speciation presents a similar situation in which ecological forms of isolation arise before intrinsic postzygotic isolation can evolve to an appreciable level.

HOW DO WE DEFINE “IMPORTANCE” OF REPRODUCTIVE BARRIERS?

One issue that deserves attention is that “importance” of reproductive barriers can be defined in multiple ways. We suggest that the most important barriers are those that block the most gene flow at the time of speciation. However, some barriers may be ephemeral whereas others may be essentially irreversible over the course of divergence. Prezygotic barriers such as ecogeographic, temporal, or mating isolation may fluctuate with changing ecological conditions. Speciation may certainly occur by these forces alone, but it is conceivable that evolutionary trajectories may then reticulate in the future. However, once two taxa achieve complete reproductive isolation by intrinsic postzygotic means, their permanence as independent lineages is essentially guaranteed. Such a distinction may be useful in comparing reproductively isolated taxa that differ markedly in their absolute levels of intrinsic postzygotic isolation despite equivalent levels of total isolation. In this sense, although the magnitude of total isolation estimates the current effective barriers, a measure that is based on those barriers that are unlikely to dissipate after secondary contact could provide a meaningful estimate of permanence. Thus, two species with strong prezygotic isolation but without intrinsic postzygotic isolation may be considered less “permanent” than those exhibiting strong intrinsic postzygotic isolation even if the species pairs being compared have the same total isolation. It is worth noting that the sequential ordering of barriers is unnecessary when estimating just the total isolation (H. A. Orr, personal communication). While we submit that sequential ordering is necessary when the goal is to assess how much isolation is contributed by each barrier, this too is the subject of disagreement (H. A. Orr, personal communication). Nevertheless, many barriers are experienced sequentially in nature, resulting in inevitable consequences for which forms of reproductive isolation will prevent the most gene flow during

divergence. Further study is clearly needed to identify the most appropriate methods for assessing the importance of different isolating barriers.

Summary

Since its inception, the field of speciation research has been marked by spirited debate. Even fundamental problems such as how reproductive barriers evolve and which barriers contribute to speciation have not yet been resolved. There is continued interest in settling these persistent controversies. For example, recent proposals to distinguish ecological from nonecological speciation (Schluter 2001; Rundle and Nosil 2005; Schluter 2009) have brought greater attention to the role of ecology and natural selection in the evolution of reproductive isolating barriers. Yet, since Darwin, ecology has always been acknowledged as a major factor in speciation (Dobzhansky 1937; Stebbins 1950; Mayr 1947; Dobzhansky 1951; Mayr 1963; Schemske 2000; Coyne and Orr 2004). Of the three purported categories of nonecological speciation (Schluter 2001), only genetic drift is unambiguously nonecological, but it is probably too slow to cause speciation without some ecological adaptation occurring along the way. Hence, speciation purely by genetic drift is probably rare. Of the remaining categories of putative nonecological speciation, speciation by uniform selection and by polyploidy virtually always involve ecological factors. Ecology is so intertwined with the evolution of reproductive isolation during adaptive divergence that we question whether “pure” nonecological speciation ever occurs in nature. Hence, we suggest that the recently proposed dichotomy between ecological and nonecological speciation is both flawed and unnecessary.

The longstanding view that speciation mechanisms can be studied only in sympatric populations has led to the virtual neglect of geographic isolation as a legitimate isolating barrier. We argue that a genetically based difference in the geographic ranges of populations and species due to local adaptation is an important and overlooked isolating barrier. This ecogeographic isolation can be distinguished from the effective geographic isolation, which can include contributions from historical and genetic factors, through reciprocal transplant experiments and/or ecological niche modeling. Although these approaches are often difficult to implement, they are necessary to evaluate how genetically based differences in the spatial distributions of populations and species may have contributed to speciation, and how current and future distributions might affect gene flow.

One of the major goals of speciation research is to identify the relative contributions of relevant isolating barriers between recently diverged species. This requires (1) estimating the strength of all potential isolating barriers, (2) evaluating the time course for the evolution of barrier strengths, and (3) determining how

each potential barrier contributes to the total isolation. In terms of the order of appearance in the life history, the isolating barriers that first come into play are of particular significance as subsequent barriers can only stop gene flow that remains after the effects of earlier-acting barriers. In this regard, ecogeographic isolation is often the first potential barrier to operate, and thus must be considered in any comprehensive study. Failure to include early-acting barriers, may lead to an overemphasis on the importance ascribed to barriers such as gametic isolation and intrinsic genetic incompatibilities that can only operate in sympatry.

We therefore suggest that it is useful to distinguish barriers that actually reduce gene flow under current conditions from those for which a reduction in gene flow is expected only if the geographic distribution of species or populations changes. For example, local adaptation leading to habitat isolation should generally be regarded as realized isolation whereas hybrid sterility is a potential barrier for allopatric populations but can become realized if populations become sympatric. Making the distinction between realized and potential isolating barriers and identifying their rates of evolution is critical for distinguishing barriers that contribute to speciation from those that evolve after speciation is complete.

We see the following as fertile opportunities for improving our understanding of speciation:

(1) Estimate the strength of ecogeographic isolation. Although some studies have examined effective geographic isolation between taxa, few have added information that allows for the assessment of ecogeographic isolation (e.g., Angert and Schemske 2005). In many systems, potentially important barriers such as mating discrimination and intrinsic postzygotic isolation have been measured without parallel information on ecogeographic isolation. Adding this component of isolation will allow a more accurate assessment of realized barrier strengths.

(2) Estimate the components of reproductive isolation between sister species and assess the rate of evolution of different reproductive barriers. Because reproductive isolation accumulates after speciation is complete, studies of taxa that have only recently become species are particularly informative (e.g., Ramsey et al. 2003; Kay 2006; Martin and Willis 2007). Furthermore, comparative studies of multiple pairs of sister species are needed to determine if there are general patterns, e.g., which barriers evolve first? This question can be addressed in part by estimating the relationship between genetic distance and the magnitude of reproductive isolation for individual barriers, such as the classic studies of Coyne and Orr (1989, 1997). When such data are compared to estimates of contemporary barrier strength, it will be possible to assess which barriers are most “important” in speciation.

(3) Consider the distinction between ephemeral and permanent reproductive barriers. Although the linear sequential model provides guidance for evaluating the importance of contemporary barriers, later acting barriers may deserve attention due to their increased potential for permanence.

(4) Identify the traits involved in speciation. Few studies have investigated both the isolating barriers important in speciation and the traits responsible for those barriers (e.g., McKinnon et al. 2004; Bradshaw and Schemske 2003).

(5) Assess how adaptation contributed to the evolution of traits conferring reproductive isolation. Although it seems clear that adaptation is a key feature of speciation, few studies have identified the link between natural selection and adaptive traits that directly or indirectly cause reproductive isolation. The use of segregating hybrid populations (Schemske and Bradshaw 1999) and/or allele substitution lines (Bradshaw and Schemske 2003) can help reconstruct the selective scenarios that resulted in speciation.

(6) Elucidate the genetic architecture of reproductive isolating barriers. This will require methods such as Quantitative Trait Locus (QTL) mapping for estimating the number, location, magnitude of effect and mode of action of genes and genomic regions that contribute to reproductive isolation (e.g., Bradshaw et al. 1998; Bouck et al. 2007). In addition, reverse genetic approaches such as comparative outlier scans applied to hybridizing taxa could be used to distinguish regions of the genome where gene flow is restricted due to local selection (e.g., Egan et al. 2008; Nosil et al. 2008). These two approaches can be combined to provide additional insight into the interaction between adaptation and the genome (Via and West 2008). We expect that most isolating barriers will result directly or indirectly from the evolution of adaptive traits, but it is also important to characterize neutral or deleterious genes that confer reproductive isolation, as might be expected in some cases of intrinsic postzygotic incompatibilities. Furthermore, although it is well established that through pleiotropy, adaptive mutations can affect multiple ecological traits (McKay et al. 2003), the role of pleiotropy in speciation is largely unstudied. Thus, genetic approaches for comparing direct and indirect contributions to reproductive isolation are of particular interest.

(7) Evaluate the role of chromosomal rearrangements in speciation. Stochastic forces may sometimes interact with adaptive processes to affect the resulting reproductive isolation. Chromosomal inversions, for example, may enhance isolation by building complexes of genes that are protected from recombination (e.g., Brown et al. 2004).

We advocate an approach to the study of speciation first envisioned by Dobzhansky and Mayr in which reproductive isolation is the primary focus. Although these early leaders of our field

would have almost certainly embraced new molecular and computational approaches for the study of speciation, the conceptual framework they established is still applicable today. We continue to seek answers to fundamental questions such as: Which forms of reproductive isolation are responsible for speciation? What traits and selective forces are involved? and What is the genetic basis of reproductive isolation?

The answers will come from comprehensive studies of populations and species living in sympatry or allopatry, for which we estimate all relevant isolating barriers, whether they are ecological or nonecological or act prior to or after fertilization or hybrid formation. This is consistent with Mayr's view (Mayr 1947, p. 278) that "... most isolating mechanisms between closely related species that have been studied thoroughly were found to be multiple. There always seem to be involved (1) differences in the ecological requirements, (2) reduction of the mutual sexual stimulation, and (3) reduction in the number and the viability of the offspring." We suggest that future progress will be best achieved by embracing this inclusive approach towards understanding the "biology of speciation."

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