
Behavioral Ecology and Speciation

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Much of the diversity of life exists among species. Understanding the mechanisms by which new species arise is therefore a fundamental goal in evolutionary biology. Central to this endeavor is a species definition. Here, as with the vast majority of speciation research, we utilize the biological species concept. According to the biological species concept, “species are groups of interbreeding natural populations, which are reproductively isolated from other such groups” (Mayr 1942, p. 120). Reproductive isolation is the result of isolating barriers, defined as those biological features of organisms that reduce or prevent gene exchange with members of other populations (Coyne & Orr 2004). Such barriers are usually based on genetic differences between populations, although not always. Increased attention of late, for example, has focused on whether learning may play a role (Irwin & Price 1999). By focusing speciation research on understanding how such barriers arise, the biological species concept has provided a highly successful framework for the investigation of Darwin’s “mystery of mysteries,” the origin of species.

Numerous types of reproductive barriers exist, and these are often divided into two main categories, distinguished by whether they occur before or after mating (pre- and postmating, respectively). The resulting reproductive isolation they generate can be thought of as a quantitative trait, capable of taking on intermediate values, that accumulates between populations during the speciation

process. This can occur when populations are geographically separated (allopatric), when they occur together (sympatric), or may involve intermediate scenarios of partial overlap (e.g., parapatry) or secondary contact following a period of allopatry. The geographic context of speciation is of interest not only because it affects the possibility of gene flow (which tends to abolish reproductive barriers), but also because it affects the mechanisms that can contribute to the evolution of reproductive isolation (Rundle & Nosil 2005). For example, premating isolation (including behaviors such as mate preferences; see below) may be strengthened by selection in sympatry in response to reduced hybrid fitness (i.e., postmating isolation) in a process known as reinforcement (Servedio & Noor 2003).

The various types of reproductive barriers have been recently reviewed (Coyne & Orr 2004); our interest here is with those that are behavioral in nature. At least five can be distinguished, all involving differences in preferences of some sort. One of the best studied, *sexual isolation*, is caused when divergent mate preferences cause individuals to be less attracted to, and hence less likely to mate with, individuals from another population. This is termed *behavioral isolation* by Coyne and Orr (2004), but we avoid this usage here because all forms of reproductive isolation with which we are concerned involve behavior. Chapter 24 provides a detailed treatment of mate choice, including how mate preferences are defined and methods for their

quantification. Demonstrating sexual isolation is straightforward, and numerous examples exist, although in many of these the specific trait(s) on which the preferences act have not been identified (Coyne & Orr 2004). A classic case comes from Darwin's finches (*Geospiza*) in the Galapagos, in which field mate choice experiments using model birds have shown that males preferentially court conspecific over heterospecific females (Ratcliffe & Grant 1983). Sexual isolation depends on body size, beak shape, and most importantly on male song (Grant & Grant 1997a). Males learn their song from their

fathers, and females prefer males who sing songs similar to their father, suggesting that they imprint on their father's song. Remarkably, preference for song appears to override species discrimination in cases in which males sing heterospecific songs (i.e., because they imprinted incorrectly), indicating that hybridization depends, at least in part, on learning in both sexes (figure 27.1).

Mate preferences can also contribute to a second type of behavioral isolation if they reduce the mating success of hybrid offspring. Termed *sexual selection against hybrids* (Schluter 2000), this reproductive

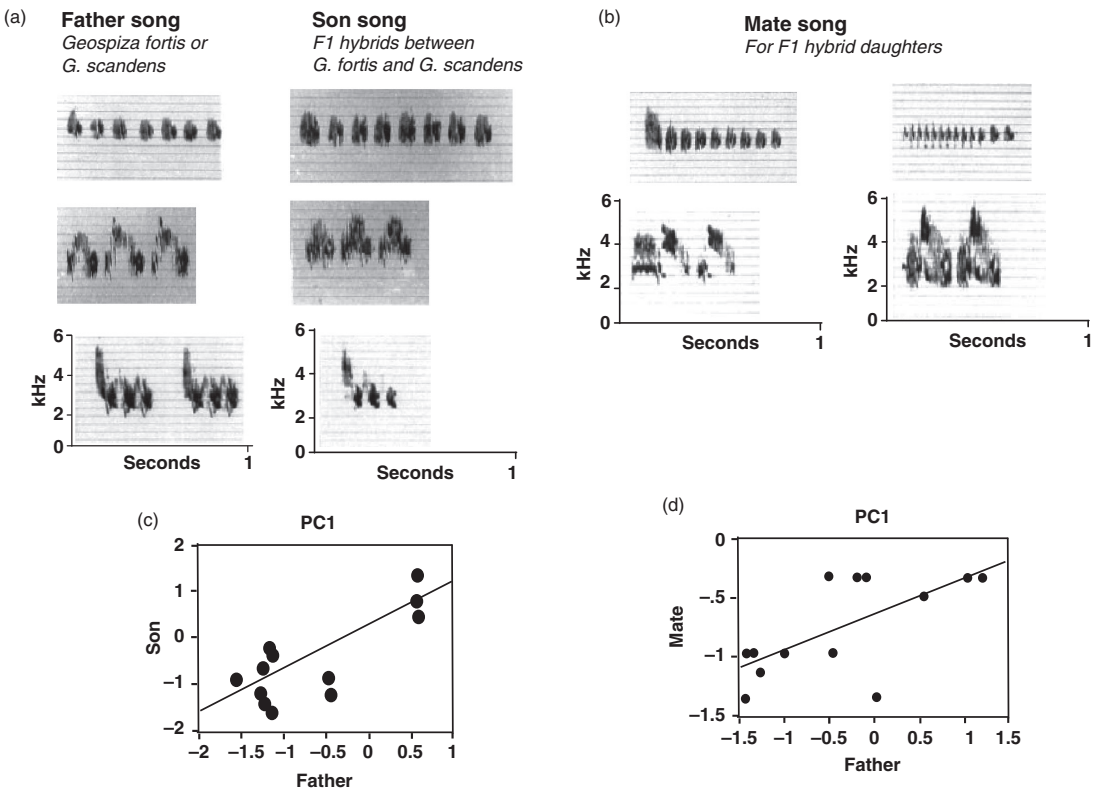


FIGURE 27.1 Sexual isolation depends on song learning and imprinting in Darwin's finches. (a) Males learn the song of their fathers. Sonograms show the song of male *Geospiza scandens* (top) and *G. fortis* (bottom) next to their F1 hybrid sons. The middle male is *G. scandens* but sings a *G. fortis* song, indicating this male is misimprinted and that song is not genetically based. (b) F1 hybrid females mate with males whose song resembles their fathers. Sonograms show songs sung by mates of two daughters of the fathers shown in the corresponding rows in panel (a). (c) Regression of song principal component 1 for sons and their fathers (slope = 1.008, $P = 0.0004$) indicating the close correspondence of song. (d) Regression of song principal component 1 for the mates of these daughters and their fathers, again indicating their close correspondence (slope = 0.29, $P = 0.014$). Panels (a–c) reprinted from Grant and Grant (1997b) with permission, and panel (d) reprinted from Grant and Grant (1997a) with permission.

barrier arises if the sexual displays of hybrids are intermediate and fail to stimulate the preferences of either parent species, or if hybrids themselves prefer rare or incompatible phenotypes (such that hybrids have reduced mating success). Reduced mating success of hybrids has been shown in a number of cases (Schluter 2000). For example, in stickleback fish inhabiting postglacial lakes of British Columbia, Canada, hybrid males are less successful at acquiring mates relative to the parent species (limnetics) alongside which they nest (Vamosi & Schluter 1999). In two species of tree frogs inhabiting the southeastern United States, first-generation hybrid males produce a sexual advertisement call that is intermediate, and hence unattractive, to females of either parent species (Hobel & Gerhardt 2003).

A third behavioral isolating barrier involves preferences not for mates, but for habitats (i.e., habitat choice). Divergent habitat preferences may create a form of premating isolation, termed *habitat isolation*, if the likelihood of between-population

matings is reduced, generally because individuals mate in their preferred habitat (box 27.1). Habitat choice can produce microhabitat isolation between geographically sympatric populations and has therefore received attention as a possible route to sympatric speciation. The majority of this work has focused on phytophagous insects that feed and mate on their host plants, although rigorous demonstrations are few. Sympatric host races of the apple maggot fly (*Rhagoletis pomonella*), which utilize either apples or hawthorns, are partially isolated by differences in host preferences (Feder et al. 1994). Habitat isolation is also implicated between this species and a close relative, *R. mendax*, that is found exclusively on blueberry plants. Although hybrids are absent between these species in nature, they are readily produced when individuals are confined to the same field cage (Feder & Bush 1989).

The fourth potential form of behavioral isolation is the temporal equivalent of habitat isolation, involving preferences to mate at different times.

BOX 27.1 Habitat Preferences and the Formation of New Species

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Divergent habitat preferences can result in reproductive isolation (i.e., assortative mating) between populations in different habitats. Such habitat preference can play a unique role in speciation by overcoming some of the major theoretical criticisms of the controversial process of sympatric speciation (i.e., speciation without geographical barriers to gene flow between populations; Bush 1969; Coyne & Orr 2004). Specifically, mate preferences might often diverge between populations only because genes that affect mate preference are associated with genes conferring adaptation to different environments (i.e., divergent selection on fitness genes spills over to mate preference genes). One of the main criticisms of sympatric speciation is that gene flow between populations and subsequent recombination will destroy genetic associations between the two types of genes (preference and fitness genes), thereby preventing the evolution of preference genes. In the case of habitat preference, these two types of genes can be one and the same (i.e., habitat choice affects fitness such that habitat preference genes are also fitness genes) so that selection automatically affects habitat preference genes. When this is the case, sympatric speciation is strongly facilitated. This point aside, even when genes affecting habitat preference are different from genes affecting fitness, divergent habitat preferences can play a role in speciation similar to mating preferences, thereby contributing to speciation in organisms that do not diverge in mating preferences.

Here I describe two main ways by which divergent habitat preferences might promote speciation: (1) directly, by acting as a form of reproductive isolation, and (2) indirectly, by reducing gene flow, thereby facilitating the adaptive divergence that can incidentally drive the evolution of other forms of reproductive isolation during ecological speciation (figure 1). I then discuss two distinct mechanisms by which divergent habitat preferences

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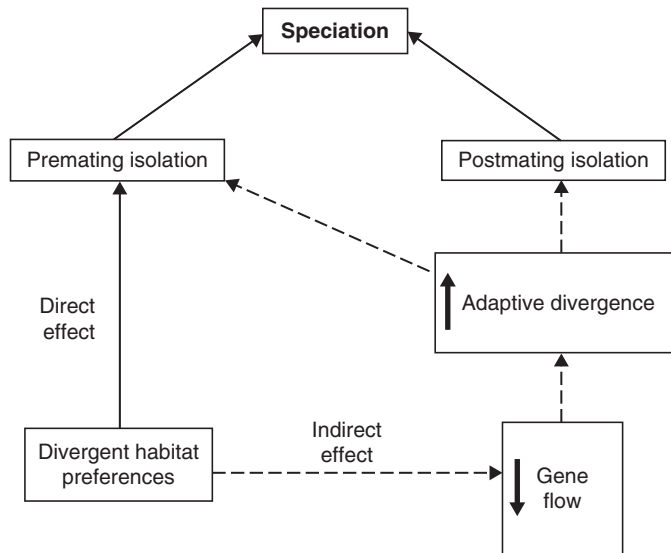


Figure 1 Schematic representation of how divergent host-plant preferences can promote speciation either (1) directly (solid lines), by causing premating isolation, or (2) indirectly (dashed lines), by reducing gene flow, thereby facilitating the adaptive divergence that drives the evolution of other forms of reproductive isolation during ecological speciation. This indirect role applies to any form of reproductive isolation.

themselves might evolve, and conclude with some outstanding questions concerning the role of habitat preferences in speciation. I focus on the host-plant preferences of herbivorous insects (see Funk et al. 2002 and Tilmon 2007 for thorough reviews), but the arguments apply to other forms of habitat preference.

How Do Divergent Habitat Preferences Contribute to Speciation?

When mating occurs on the host plant, divergent host-plant preferences act as a form of premating isolation on insect populations living on different plant species (Bush 1969). Specifically, premating isolation occurs because divergent host preferences reduce between-host movement, and thus reduce contact and interbreeding between individuals from different hosts (figure 1). Divergent host preferences have been documented in a wide range of insect taxa, including Lepidoptera (moths and butterflies), Coleoptera (beetles), Diptera (flies) and Phasmids (walking-stick insects; Funk et al. 2002; Tilmon 2007 for reviews). An example of divergent host preferences in *Timema* walking-stick insects is depicted in figure II (Nosil 2007). Divergent host preferences can contribute to speciation in two ways, directly by causing assortative mating, and indirectly by reducing gene flow and thus facilitating overall adaptive divergence.

Directly, by Acting as a Form of Reproductive Isolation

There are only a few cases in which host preferences have been shown to actually result in assortative mating (i.e., truly result in reproductive isolation). Some examples of such studies include cage experiments showing increased assortative mating between host races

of *Eurosta solidaginis* goldenrod flies when host plants are present relative to when they are absent (Craig et al. 1993), mark-recapture studies of apple and hawthorn host races of *Rhagoletis* flies, suggesting that the tendency of flies to reproduce on the same host species that they used in earlier life history stages strongly reduces gene flow between the races (Feder et al. 1994), and a combination of field and molecular data indicating that host choice reduces gene flow between alfalfa and clover races of *Acyrtosiphon pisum* pea aphids (Via 1999). Thus, in at least some cases, the degree of assortative mating caused by divergent host preferences can be adequate for subpopulations using different hosts to evolve genetic differences. However, further demonstrations that divergent host preferences cause substantial reproductive isolation are needed.

Indirectly, by Reducing the Constraining Effects of Gene Flow

Adaptive divergence is often constrained by gene flow (Nosil 2007). Thus, when divergent host preferences act as a barrier to gene flow, as described above, they can facilitate adaptive divergence: in other words, they facilitate adaptation to different habitats by different subsets of the population. In turn, adaptive divergence can cause the incidental evolution of any form of reproductive isolation (e.g., sexual isolation, hybrid sterility), via by-product models of ecological speciation: in other words, reproductive isolation evolves as a secondary (indirect) consequence of local adaptation, rather than as a direct result of selection (Funk et al. 2002; Coyne & Orr 2004). Thus, by reducing gene flow, divergent host preferences can indirectly promote speciation. However, this indirect role for host preference in promoting speciation is likely to be of importance only for cases of divergence in the face of gene flow (i.e., nonallopatric speciation). Moreover, this type of indirect role of behavior in speciation is not unique to the particular reproductive barrier of host preference, because (by definition) any form of reproductive isolation reduces gene flow, thereby potentially facilitating adaptive divergence (figure I).

Two Different Causes of Habitat Preference Evolution

For habitat preferences to lead to eventual speciation, habitat preferences must evolve to differ between populations. How can selection favor the evolution of behavioral differences within or between populations that are initially monomorphic for a single strategy (e.g., a generalist behavior, or preference for a single host plant)? Host preferences can diverge both with and without selection against switching between different, utilized hosts (the term *utilized* refers to host species that an insect species uses; other host species that the insect species cannot or does not use may exist in the environment as well). I review two hypotheses for the causes of preference evolution, but note that whenever preference evolution is driven by divergent selection, pairs of populations feeding on different host plant species will exhibit greater divergence in preference than pairs of populations feeding on the same host (Funk et al. 2002; Nosil 2007; figure II). This pattern of greater preference divergence between different-host pairs has been observed, for example, in *Timema* walking-stick insects and *Neochlamisus* leaf beetles.

Selection against Host-Switching (Fitness Trade-Offs Hypothesis)

When switching between utilized hosts is maladaptive (i.e., when local adaptation results in fitness trade-offs between hosts), host preferences can diverge via selection against individuals that switch between hosts. Under this scenario, preference for the native host is favored because individuals choosing another host suffer reduced fitness. Although selection actively favors reduced host switching, it may act indirectly on host preference loci via their genetic association with other loci conferring host-specific fitness (e.g., loci affecting color pattern or physiology). In this hypothesis, selection acts only in populations in which there is the opportunity for switching between utilized hosts (i.e., when more than one utilized host is available in the environment). One possible outcome is greater preference

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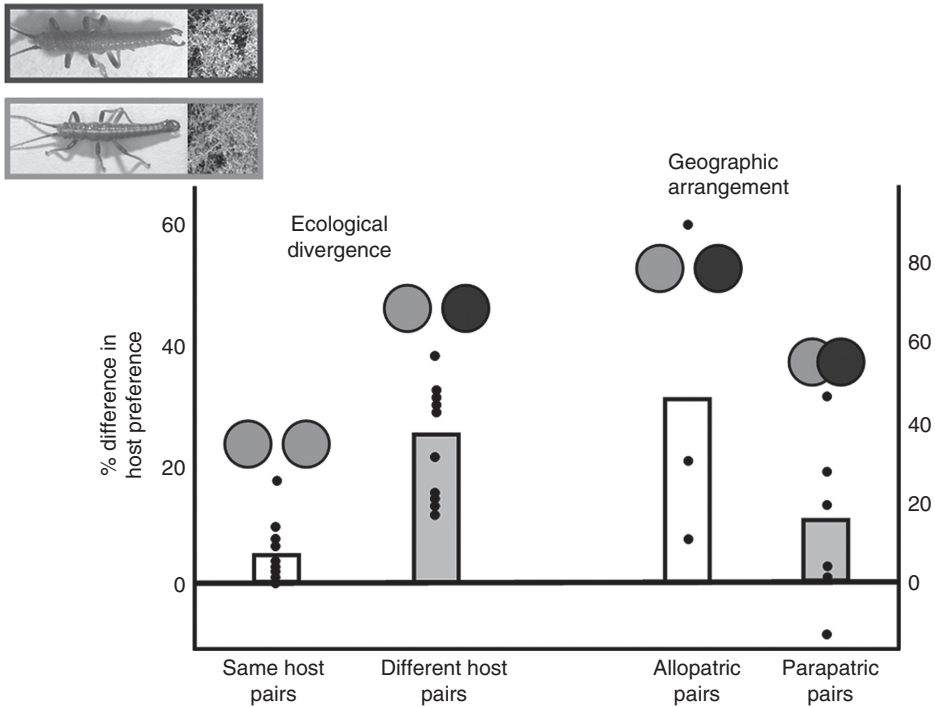


Figure II Divergent host-plant preferences between *Ceanothus* (darker circles) and *Adenostoma* (lighter circles) host ecotypes of *Timema cristinae* walking-stick insects. The y-axis refers to the mean percent difference between population pairs in individuals choosing *Ceanothus* in host choice trials (each small black dot represents the mean difference of a population pair, and the bars represent the average of the population pairs). *Ecological divergence* shows how different-host population pairs are more divergent in host preference than same-host population pairs (here, all pairs are allopatric), indicating that host preferences have differentiated due to divergent natural selection. *Geographic arrangement* shows how allopatric population pairs differ more in host preference than parapatric pairs, suggesting that gene flow constrains divergence. Thus, habitat preferences could contribute to speciation both by acting as a form of reproductive isolation and by reducing gene flow to indirectly facilitate adaptive divergence and the incidental evolution of other forms of reproductive isolation. The strong divergence in host preference observed in allopatry could arise via the *information processing* hypothesis discussed in the text. Modified from Nosil (2007) with permission.

divergence in geographic regions where multiple hosts are utilized (in sympatry or parapatry) than between geographically isolated populations that use a single, yet different, host (allopatry). This pattern can be thought of as character displacement of host preference, with preferences evolving in a reinforcement-like process.

Selection for Efficiency (Information Processing/Cognitive Constraints Hypotheses)

There is no selection against switching between hosts when only one host is utilized in the local environment. Under this scenario, search and efficiency costs can favor increased

preference for the single, utilized host because individuals without strong preferences accrue lower fitness, but for reasons other than switching to an alternate host (Bernays & Wcislo 1994). For example, due to cognitive constraints associated with information processing, generalized individuals without strong preferences might take longer to locate or to decide whether to feed on the utilized host (e.g., as observed in *Neochlamisus* leaf beetles; Egan & Funk 2006), thereby wasting time and energy while increasing predation risk. Alternatively, such individuals may suffer low fitness because they attempt to use a nonutilizable host. When preference evolution is driven by such selection, populations in habitats in which only a single host is utilized still evolve preference for that host. Thus, unlike the host-switching scenario above, the cognitive constraints hypothesis can drive preference evolution within allopatric populations, and may contribute to allopatric speciation (and it does not predict character displacement). In fact, under this hypothesis, if gene flow constrains divergence, then preferences may actually be more divergent in allopatry than in parapatry/sympatry (figure II).

Summary and Outstanding Questions

Although divergent habitat preferences appear common, direct evidence that they actually cause premating isolation in nature is lacking (Funk et al. 2002). Likewise, further data on the causes of preference evolution would be useful, particularly given the implications for the geographic mode of speciation in which they are involved. In particular, the prevalence and ecological causes of fitness trade-offs between hosts remains in debate. Data on the genetic basis of habitat preferences is also lacking, and has implications for both the likelihood and rate of speciation (Coyne & Orr 2004). Finally, a major outstanding question from a behavioral perspective is whether habitat choice involves preference for the native host, avoidance of alternative hosts (e.g., Forbes et al. 2005), or both.

A number of cases of such temporal (or *allochronic*) isolation exist in the literature, including different species of corals or green algae that release their gametes in brief mass-spawning events at different times of the day (Coyne & Orr 2004). However, we are not aware of any case in which temporal isolation has been shown to involve divergent preferences for when to mate, as opposed to innate responses to external cues. An intriguing possibility is provided by an evolution experiment in melon flies (*Bactrocera cucurbitae*) in which replicate populations were selected for either fast or slow development time (Miyatake & Shimizu 1999). Time of mating diverged in response to this selection, with fast developers mating earlier in the evening than slow developers, and this generated premating isolation in mate choice trials. The degree to which divergent preferences were involved is unclear, however, and the authors suggest that it may have been a side effect (i.e., pleiotropy) of changes in genes affecting the circadian clock.

Finally, although behavioral isolation is generally restricted to animals, one form can also occur

in flowering plants (angiosperms) when gene flow is reduced between species because of differential visitation by animal (usually insect) pollinators. Termed *ethological pollinator isolation*, differences in preference occur in the pollinators, usually with reference to flower characteristics such as color, odor, shape, position, and nectar content (Grant 1994). Although it is relatively straightforward to demonstrate, there are only a few rigorous examples (Coyne & Orr 2004). One of the most striking occurs in two species of North American monkeyflower. The bee-pollinated *Mimulus lewisii* has broad, pink flowers with recessed anthers and produces little nectar, whereas the hummingbird-pollinated *M. cardinalis* has red, tubular flowers with exerted anthers and high nectar volume. Despite the fact that these species can be easily crossed in the greenhouse, hybrids are extremely rare when they are sympatric in nature. Direct observation indicates that pollinator isolation between these sister species is almost complete, and genetic manipulations implicate differences in flower color and nectar volume as key traits the

pollinators are targeting (Schemske & Bradshaw 1999; Ramsey et al. 2003).

FROM PATTERN TO PROCESS: THE EVOLUTION OF SEXUAL ISOLATION

A comprehensive understanding of the role of behavior in speciation goes beyond simply tallying the prevalence of the various forms of reproductive isolation involving behavior; we'd also like to know how preferences diverged to produce these barriers. Much less work has been done in this regard. In the remainder of this chapter we address this topic by focusing specifically on the evolution of sexual isolation. We chose this focus for two reasons. First, sexual isolation arises from differences in mate preferences; understanding mating behaviors and their evolution has long been the province of sexual selection, a subject that has been much studied by behavioral ecologists.

The second reason for our focus on sexual isolation is that it is often recognized as the primary source of reproductive isolation between existing species in nature (Mayr 1963). Although this may be due in part to the fact that it is easier to detect than many other barriers, and it will also have a larger effect on total isolation relative to those occurring later (e.g., postmating forms), a number of lines of evidence suggest that sexual isolation may also be key in initiating speciation (Coyne & Orr 2004). These include data from *Drosophila* and cichlids that sexual isolation is required for species to coexist in nature (Coyne & Orr 1997; Seehausen et al. 1997). In addition, fine-scale local mate preferences within species, in which individuals prefer mates from their own population over those from other populations (Ryan & Wilczynski 1988), also suggest that preference divergence may occur early during population differentiation. Finally, there is a long history of laboratory studies of speciation, largely in *Drosophila*, that have demonstrated that partial sexual isolation can evolve over relatively short timescales (Rice & Hostert 1993), although direct comparisons with the evolution of other forms of reproductive isolation have received little attention.

In the following section, we provide a conceptual overview of the various mechanisms by which mate preferences may diverge to generate sexual isolation, including the relevant models of sexual

selection. We then present two case studies that have used rather different approaches to the empirical investigation of some of these mechanisms. Finally, because this is an active area of research and there are many directions for future work, we close by outlining a selection of key outstanding questions.

CONCEPTS

Sexual Selection and Mate Preference Divergence

The evolution of mate preferences is not a simple phenomenon, in part because it involves an interaction between different traits in the two sexes: generally one or more sexual display traits in one sex and a preference for these trait(s) in the other sex. Here we address the classic scenario of female choice for male display traits; although the reverse scenario of male choice also exists in nature, it has received less attention and its evolution may differ in important ways from that of female mate choice (see chapter 20). As pointed out by Fisher (1930), the existence of mate preferences in females will generate sexual selection on the preferred male display trait, and the resulting assortative mating (females with the preference mate with males with the preferred display) generates a statistical association (i.e., a positive genetic correlation) between the traits. This causes indirect selection for the preference in a self-reinforcing coevolutionary process that has come to be known as *Fisher's runaway* (see chapter 24).

This coevolutionary process can rapidly amplify small initial differences among populations in display traits and/or preferences, generating sexual isolation (Lande 1981; West-Eberhard 1983; Schluter 2000). How these differences first arise has been the topic of much theory, and models of speciation by sexual selection can be divided into two main classes depending on the mechanism by which divergence is initiated (Schluter 2000; Coyne & Orr 2004). In the first, although selection is involved, the ultimate reason that preferences diverge is chance events, including arbitrary differences in starting conditions, unique mutations, and/or the order in which they occur. It is these chance events that cause selection to follow unique evolutionary trajectories in different populations. In the second, selection differs between environments and it is this divergent selection that is the ultimate cause of divergence in mating traits.

Fisher's runaway is the original example of a chance-based mechanism. In the classic case in which preferences are neutral (i.e., females with and without the preference do not differ in survival or fecundity), the coevolutionary exaggeration of display trait and preference is eventually halted at one of many possible positions along a *line of equilibria* on which natural selection opposing the male trait precisely balances sexual selection favoring it (Kirkpatrick & Ryan 1991; see also figure 24.3). Sexual isolation can arise because chance events cause separate populations to arrive at alternate positions along this line (Lande 1981). A runaway process cannot, however, maintain a costly female preference at equilibrium. Such costs are generally considered to be inevitable (although insufficient attention has been given to quantifying them), and their presence generates direct selection on the preference that, in most cases, causes the line of equilibria to collapse to a single point at which female fitness is maximized and the preference is absent (Arnqvist & Rowe 2005). It is therefore unlikely that this process alone is responsible for mate preference divergence among populations.

Interlocus sexual conflict is another example of a chance-based mechanism by which preferences may diverge. As explained in detail in chapter 23, sexual conflict arises from differences in the evolutionary interests of males and females with respect to reproduction (Arnqvist & Rowe 2005). Interlocus sexual conflict occurs when different loci in males and females affect the value of a shared trait arising from male-female interactions. Because the trait can take on only a single value, males and females cannot simultaneously achieve their sex-specific optimum. The result is an evolutionary tug-of-war that can generate cycles of adaptation and counteradaptation in a process of sexually antagonistic coevolution. This process occurs independently in separate populations, following potentially unique evolutionary trajectories due to chance events, thereby promoting population divergence and hence reproductive isolation.

With respect to behavior, conflict can arise over such shared traits as mating rate, with selection generally favoring higher rates in males than in females. The resulting sexually antagonistic adaptations can involve behavioral traits in both sexes, including those in males that stimulate, harass, or otherwise cause females to mate at a greater rate (e.g., male persistence or exploitation of preexisting sensory biases in females), and those in females that

increase their resistance to these male traits (e.g., changes in female perception). (The evolution of *preferences for* and *resistance to* male sexual traits are equivalent.) Whether sexual isolation is likely to result is not straightforward, however, and contrasting views exist in the literature (Ritchie 2007). If females evolve to resist the specific charms of conspecific males, this could conceivably cause them to prefer (i.e., be less resistant to) heterospecific males, inhibiting speciation (Coyne & Orr 2004). Likewise, males that are more persistent during courtship could increase the likelihood of heterospecific matings. On the other hand, it has been suggested that the evolutionary options available to females to decrease their response to manipulative male traits are almost infinite, including diverse routes involving changes in perception. Thus, sexually antagonistic coevolution may be particularly likely to favor a novel female perceptive trait, promoting sexual isolation (Arnqvist & Rowe 2005). Successive rounds of sexually antagonistic coevolution could also leave males in possession of a suite of ineffective traits, to which conspecific females have evolved partial resistance. If the presence of such traits is necessary to achieve matings, heterospecific males may fail to stimulate females sufficiently (Holland & Rice 1998).

Determining the contribution of interlocus sexual conflict to the evolution of sexual isolation will require empirical data, but unfortunately the few data that exist are equivocal. In the laboratory, Martin and Hosken (2003) used experimental evolution in dung flies (*Sepsis cynipsea*) to show that partial sexual isolation evolved between replicate populations in which sexual selection was permitted, but not between populations in which sexual selection was prevented (by enforcing lifelong monogamy). This is consistent with sexual conflict because in the sexual selection present treatment, behavioral isolation was stronger between larger, more dense populations in which sexual conflict was greater (as confirmed by direct measures of sexual activity), than it was between smaller, less dense populations with relaxed sexual conflict. However, it is difficult to rule out, or even determine, the potential contribution of other processes of sexual selection. In addition, similar experiments in two species of *Drosophila* have provided contrasting results, with no sexual isolation evolving between populations experiencing greater sexual conflict (Wigby & Chapman 2006; Bacigalupe et al. 2007).

In the second class of models, the divergence of mate preferences is initiated ultimately as a by-product of divergent selection adapting populations to their different niches or environments (Schluter 2000). This may occur via spatial variation in natural selection on the display trait, with preferences evolving as a correlated response to the resulting changes in the display trait (Lande 1982). Alternatively, natural selection may act on sensory or communication systems, favoring signals, sensory systems, and behaviors that function well within their specific environment, even outside of the mating context (e.g., to facilitate prey capture or predator avoidance). Adaptation of communication systems to different environments may thereby cause mate preference to diverge in a process known as sensory drive (Endler 1992; Boughman 2002). Given the great variation in habitats that exists, and the fact that subtle differences can affect sensory processes and signal transmission, divergence through sensory drive may be widespread. Several recent examples have been found of either mating trait, sensory, or preference divergence correlated with habitat. This has been well studied in *Anolis* lizards, in which the color of male dewlaps evolves to be conspicuous in different light environments. For example, background light is rich in ultraviolet in the habitat of *A. cooki*, but not *A. cristatellus*. The dewlaps of each species contrast highly against their different backgrounds, and the species differ in how well they see ultraviolet light (Leal & Fleishman 2002). Similar patterns have even been found among populations of *A. cristatellus* from different habitats (Leal & Fleishman 2004).

In all of the above models, sexual selection can amplify initial differences between populations (Kirkpatrick & Ryan 1991), causing the rapid coevolutionary diversification of display traits and preferences and thereby generating sexual isolation. Consistent with this, comparative evidence suggests that sexual selection has been central to speciation in nature (Coyne & Orr 2004). For example, closely related species, especially members of some of the most famous adaptive radiations such as the Hawaiian *Drosophila*, often exhibit spectacular diversity in traits that appear to be under sexual selection (e.g., male sexual displays) and are often characterized by striking patterns of sexual dimorphism. More compellingly, formal sister group comparisons have shown a correlation between species richness and various surrogate measures of the strength of sexual selection in a number of

taxonomic groups (Coyne & Orr 2004), although not in others (Ritchie 2007). Nevertheless, although the above studies implicate sexual selection in speciation, they are unable to provide direct tests of the evolutionary mechanisms involved in preference divergence. Experimental tests of these mechanisms, however, have received limited attention. In the following section we provide an overview of the work from two systems that is beginning to address these issues.

CASE STUDIES

Species Pairs in the Threespine Stickleback

A number of ecologically and genetically differentiated, sympatric, and reproductively isolated pairs of threespine stickleback species (*Gasterosteus* species complex) occur in different regions of the globe (McKinnon & Rundle 2002; Boughman 2007a). The recent, post-Pleistocene origin of most of these species pairs allows insight into the mechanisms operating relatively early in the speciation process, minimizing the complication of trait differences that may accumulate subsequent to speciation. Moreover, the same pairs have evolved repeatedly and independently, providing evolutionary replication. These factors make them a powerful model for understanding the evolutionary mechanisms that cause new species to form. We focus here on the best studied limnetic and benthic species pairs that coexist in several lakes in coastal British Columbia, Canada. Discovered by McPhail and colleagues (McPhail 1994), the species are specialized to exploit different ecological niches within a lake (Schluter & McPhail 1992): limnetics feed primarily on zooplankton in the open water, whereas benthics feed on invertebrates in the benthic zone. They also mate in different habitats: limnetics in shallow, open areas at high density, and benthics in deeper, vegetated areas at low density, although nesting habitats overlap substantially. Although reproductive isolation arises from a number of barriers (McPhail 1994; Boughman 2007a), the fact that hybrids are viable and fertile yet extremely rare in nature implicates sexual isolation as a primary reproductive barrier.

Sexual isolation arises because limnetics and benthics strongly discriminate against heterospecific mates based on a combination of body size,

male nuptial color, and odor; surprisingly, although courtship behaviors differ, they do not appear to have a strong role in causing sexual isolation (Boughman et al. 2005). Limnetics recognize conspecifics using differences in body size and color, whereas benthics use body size and odor (Boughman et al. 2005; Boughman 2007a; Rafferty & Boughman 2006). Body shape may also play a role, although this possibility requires additional work. Because there is mutual mate choice in threespine sticklebacks (Kozak et al. 2009), mate preferences in both sexes may contribute to sexual isolation. We focus largely on female preferences, however, because the majority of work has been done on them.

*How Preferences Diverge:
Divergent Selection between
Environments*

The majority of evidence implicates divergent selection between environments as the ultimate cause of preference divergence in sticklebacks (Boughman et al. 2005). Chance-based mechanisms have received less attention, largely because the biology of sticklebacks suggests that these are less likely. Sexual conflict over mating rate appears doubtful, for example, because males cannot force females to mate (females mate only when they have a clutch of eggs to spawn). Fertilization is also external, reducing the likelihood of postcopulatory manipulation via seminal fluids. However, competition for mates does occur and conflict over other aspects of mating is plausible. The role of sexual conflict in preference divergence in sticklebacks therefore warrants further study.

In contrast to the unlikely role of chance, a number of lines of evidence strongly implicate divergent selection between environments in the evolution of sexual isolation between these species (Schluter & McPhail 1992; Rundle et al. 2000). The first involves the parallel evolution of mating traits (i.e., mate preferences and the traits on which they act) among independent populations such that those adapted to different environments are sexually isolated, whereas those that are independently adapted to similar environments are reproductively compatible (Schluter 2000). Referred to as parallel speciation, such parallel evolution of mating traits strongly implicates divergent selection because chance-based mechanisms are unlikely to cause repeated trait evolution in correlation with

environment. Using over 750 mating trials involving three independently evolved species pairs, Rundle et al. (2000) demonstrated that limnetics and benthics were reproductively isolated from one another no matter what combination of lakes were involved. In contrast, limnetics from the separate lakes all mated freely with one another, as did benthics, despite having independently evolved from a common ancestor. In this case, parallel speciation involved the parallel evolution of both female mate preferences and male mating traits including body size and color (Boughman et al. 2005).

The importance of selection in the divergence of mate preferences is also highlighted by two studies demonstrating reproductive character displacement of stickleback mate preferences. Reproductive character displacement is the pattern in which pre-mating isolation between two species is stronger in areas of sympatry as compared to allopatry (Servidio & Noor 2003). This pattern has been demonstrated for both benthic females (Rundle & Schluter 1998) and limnetic males (Albert & Schluter 2004). In each case, true benthics and limnetics (i.e., the species pairs occurring sympatrically within a lake) discriminate against members of the other species, whereas morphologically benthic- or limnetic-like individuals from single-species lakes (i.e., allopatric populations) do not. Such results are consistent with a reinforcement process in which selection has caused mate preferences to diverge in response to reduced hybrid fitness, although it is difficult to rule out other potential mechanisms from correlative data alone. In the sticklebacks, however, the case appears reasonably strong (Rundle & Schluter 1998; Albert & Schluter 2004; Kozak et al. 2009).

Divergent sexual selection has also been implicated in preference divergence between limnetics and benthics and appears to arise from several sources. Differences in mating habitat reduce encounter rates between the species, and can also affect how sexual selection acts within each species, including male-male competition and female choice, and may thus contribute to divergent sexual selection between them. Studies in other stickleback populations have shown that differences in ecology can alter the intensity and targets of sexual selection (Candolin et al. 2007). Moreover, the benefits that females of other species derive from their choice of mates may also depend on ecological conditions (Welch 2003). Use of different mating habitats may therefore not only generate habitat isolation directly, but it may also contribute to divergent selection on female

preferences and male traits. To date, most of the work on divergent sexual selection has focused on mechanisms of evolution in color preference and expression, focusing particularly on the hypothesis of sensory drive. More work is needed, however, concerning mechanisms of divergence in size and odor, as well as preferences for these traits. Parasite-mediated selection is a strong candidate that could easily influence both odor and color preferences.

Tests of sensory drive implicate differences in the light environment of limnetic and benthic mating habitats as the primary factor in the divergence of color preferences. In the shallow, open areas where limnetics mate, the light spectrum is broad. This contrasts with the red-shifted light characteristic of the deeper, vegetated areas where benthics mate (Boughman 2001). Sensory drive posits that differences in light environment should affect the conspicuousness of male nuptial color and the evolution of color perception (Endler 1992; Boughman 2002). These in turn are predicted to cause male color and female preference to evolve in correlation with lighting conditions in the signaling environment. Consistent with these predictions, both perception and preference for red are correlated with light environment (figure 27.2a–b; Boughman 2001). Likely because differences in habitat choice expose fish to distinct light regimes, females differ in their sensitivity to red light, with limnetic females being more sensitive than benthic females. Limnetic females have correspondingly stronger preferences for red nuptial color during mating.

The precise differences in male nuptial color are congruent with the predictions of sensory drive. Male color matches the perceptual tuning of females and the light environment (Boughman 2001) and both species appear to have conspicuous nuptial colors, although they achieve this in different ways. The large, bright red throats of limnetic males stand out by being brighter than the background in broad spectrum light, but blend into the background in red-shifted light. The reduced red or black of benthic males stands out by being darker than the background in both light environments. Consistent with this, black nuptial color and red-shifted water are correlated geographically in solitary populations of sticklebacks (Reimchen 1989), suggesting sensory drive influences color evolution broadly.

Divergence in female color preference contributes to sexual isolation in the species pairs, both because it causes sexual isolation and because it generates divergent sexual selection on male color, which has led to its evolutionary diversification (Boughman 2001). Indeed, sexual isolation increases with the extent of between species differences in both male color and female color preference (figure 27.2c; Boughman 2001) and with the strength of preference for red (Boughman 2007b). Preference evolution therefore generates sexual isolation because females with strong red preferences are more likely to mate with males expressing high red color and to reject males expressing dull or black color.

The stickleback species pairs also give us some insight into the role of learning in mate preference

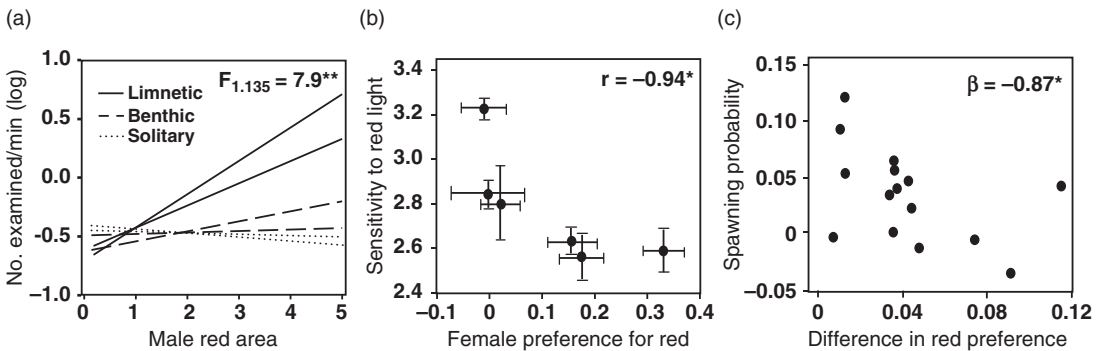


FIGURE 27.2 Preference divergence and sexual isolation in limnetic-benthic pairs of sticklebacks. (a) Color preference functions for six populations. Lines are from linear regression of preference on male color. (b) Correlation of spectral sensitivity to the strength of female preference for red in six populations. (c) Spawning probability between pairs of populations as a function of divergence in the strength of color preference. Panels (b) and (c) redrawn from Boughman (2001).

divergence. Preferences for both social partner and mates appear to depend on social environment, and the more social limnetic species shows a stronger effect. In an experiment manipulating social environment during rearing, adults of both species preferred the species they had been raised with as social partners, even if these were heterospecific individuals (Kozak & Boughman 2008). In addition, although mate preferences in benthics showed little effect of social environment, sexual isolation was undermined in limnetics raised with benthics, resulting in mate preferences for heterospecifics (Kozak & Boughman 2009). Finally, in a separate test of imprinting in first generation limnetic-benthic hybrids, males showed no effect (Albert 2005). However, the lack of experience with pure species may have contributed to the lack of preference by hybrid males. Imprinting in pure species juveniles has not yet been examined, nor have the effects of these manipulations on female species recognition and mate preference been evaluated. Both are needed for a comprehensive understanding of the role of learning in sexual isolation.

Sexual Isolation and Mate Preferences in *Drosophila serrata*

Drosophila serrata is a forest-dwelling generalist native to the eastern and northern coastal areas of Australia and extending into Papua New Guinea and surrounding northern islands (Jenkins & Hoffmann 2001). Mate preferences within *D. serrata*, which have been well characterized via a series of quantitative genetic, behavioral, and evolutionary experiments, are based, at least in part, on a suite of long-chain, nonvolatile contact pheromones composed of cuticular hydrocarbons (CHCs; figure 27.3; Blows & Allan 1998; Howard et al. 2003). Males and females express the same suite of CHCs, although they are sexually dimorphic in relative concentrations, and individuals of both sexes discriminate among potential mates using variation in the relative concentrations of these CHCs (Chenoweth & Blows 2003, 2005).

Mate preferences for CHCs are also important for sexual isolation between *D. serrata* and the closely related *D. birchii*. These species are almost identical morphologically (Schiffer & McEvey 2006), and interspecific hybrids between them are viable and fertile (Blows 1998). Nevertheless, hybridization appears to be extremely rare (Blows 1998; Higgie et al. 2000), suggesting strong sexual isolation

arising from distinct mate preferences. Consistent with this, CHC profiles of the two species differ greatly and include chemically unique hydrocarbons in each (Blows & Allan 1998; Howard et al. 2003). Perfuming experiments in both sexes, in which the chemical cues of one species were physically transferred to individuals of the other, caused an increase in the frequency of hybridization from essentially zero to over 30%, strongly implicating divergent mate preferences in both sexes as a cause of sexual isolation (Blows & Allan 1998).

As a case study for the divergence of mate preferences, empirical studies with *D. serrata* have proceeded on two fronts. One uses this species as a laboratory model system to test the feasibility of the various models by which mate preferences may diverge. The other seeks to understand the specific mechanisms responsible for a naturally occurring pattern of CHC and mate preference divergence between populations that are sympatric versus allopatric with *D. birchii*.

How Preferences Diverge: Manipulative Tests of Divergent Selection between Environments

Laboratory evolution experiments have a long history in speciation research, and the majority of this work addresses the evolution of sexual isolation via various processes (Rice & Hostert 1993; Coyne & Orr 2004). These experiments generally track the evolution of assortative mating between pairs of populations and, when found, the implication is that display traits and mate preferences for them have diverged. Direct tests of the mechanisms responsible for mate preference divergence are limited, however.

Laboratory evolution with *D. serrata* has begun to address mechanisms, focusing on the second class of models by which preference divergence may be initiated: as a by-product of divergent selection between environment. Using experimental evolution, 12 replicate populations were derived from a common ancestor and allowed to independently evolve in one of three treatment environments, yielding four replicate populations in each (Rundle et al. 2005). These environments varied the food provided to the larvae, and consisted of rice, corn, or yeast, the latter being the laboratory environment to which the ancestor was already well adapted. CHCs evolved in the different treatment environments, with adaptation inferred from the

parallel evolution of replicate populations within each treatment. Female mate preferences for male CHCs also diverged among populations, with a component of the divergence (at least 17%) occurring among treatments. Divergence among treatment environments indicates that preferences can evolve ultimately as a result of divergent natural selection.

The relative importance of the two classes of models (i.e., chance-based versus divergent selection) is unclear, however. Although a component of preference divergence occurred among environments, the majority was present among replicate populations within treatments, suggesting that it was not initiated by divergent selection between environments. Although this suggests a key role for the chance-based mechanisms, this within-treatment variance is confounded with measurement error because only a single preference estimate was obtained for each population; at least some of the differences among populations represent error in estimating preferences. The relative roles of these two classes of mechanisms will therefore require an experimental design that independently manipulates the opportunities for both processes within a single experiment, and that includes repeated preference measurements in each population.

*How Preferences Diverge: Reinforcement in Natural Populations of *D. serrata**

In nature, both CHCs and mate preferences for them vary among geographic populations of *D. serrata* that span a large part of the species's range along the east coast of Australia. This variation includes a pattern of reproductive character displacement in which the CHCs of northern populations, which are sympatric with *D. birchii*, differ from those of the allopatric populations to the south (Higgie et al. 2000; Chenoweth & Blows 2008). This character displacement has been meticulously mapped in the contact zone on the Byfield Peninsula in central Queensland and is maintained over a fine spatial scale of a few kilometers (Higgie & Blows 2007). This is remarkable given the high levels of gene flow among populations estimated from microsatellite markers (Magiafoglou et al. 2002).

A similar pattern exists for female mate preferences for CHCs in males, with much of the among-population variation associated with the presence versus absence of *D. birchii* (Rundle et al. 2008). In

mate choice trials in which females from four separate populations, two sympatric and two allopatric to *D. birchii*, were all presented with males from a single laboratory stock of *D. serrata*, allopatric females discriminated against males with sympatric-like CHC phenotypes, preferring allopatric-like males instead (Higgie & Blows 2007). Sympatric females, in contrast, showed no such discrimination (figure 27.3). Because the male phenotypes among which the females were choosing was held constant, this demonstrates an evolved difference in female preferences. Estimates of sexual selection generated by these different preferences correspond with the existing pattern of reproductive character displacement in male CHCs, suggesting that the different preferences may have been responsible for CHC divergence (Higgie & Blows 2007).

How did CHCs and preferences diverge between sympatry and allopatry? Manipulative tests, again exploiting the technique of experimental evolution, have focused on CHC divergence, although some insight into preference evolution can be gained. In an elegant experiment, Higgie et al. (2000) used six populations of *D. serrata* that were collected from the wild, three from locations sympatric with *D. birchii* (*field sympatric* populations) and three from allopatry (*field allopatric* populations). All six populations were brought into the laboratory and independently exposed for nine generations to experimental sympatry with *D. birchii*. CHCs evolved in all of the field allopatric populations as a consequence of experimental sympatry, and for seven of the eight CHCs measured, the direction of change in males matched the pattern of reproductive character displacement in the contact zone in nature (Higgie & Blows 2007). In contrast, experimental sympatry had little effect on CHC evolution in the field sympatric populations: changes were less pronounced, and those that did occur were inconsistent among the replicate populations, resulting in little net change overall (Higgie et al. 2000).

Heterospecific matings were extremely rare throughout this experiment, suggesting that reinforcing selection was not arising from the production of low fitness hybrids (i.e., postmating isolation). Rather, measurements of mating efficiency in *D. serrata* demonstrated that field allopatric males achieved almost 50% fewer matings with *D. serrata* females when *D. birchii* was present as compared to absent; field sympatric males were unaffected (Higgie et al. 2000). This suggests that sexual selection acted directly on males in the

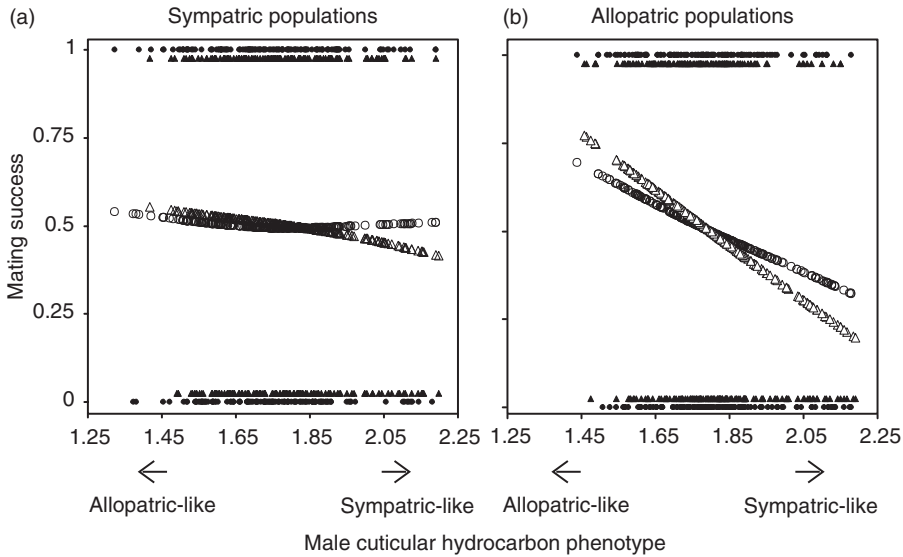


FIGURE 27.3 Mate preferences in female *D. serrata* for CHCs in males. Each panel shows females from two separate populations that are either sympatric or allopatric with *D. birchii* in nature. In each case, females chose among males from a single lab population created by hybridizing a sympatric (a) and allopatric (b) population to create males with a wide range of CHC phenotypes. Males are arrayed along an axis of reproductive character displacement for multiple CHCs in which lower scores (to the left) are characteristic of allopatric-like combinations and higher values (to the right) are characteristic of sympatric-like combinations. The actual mating success of individual males are given by filled symbols (with the two populations displaced slightly for clarity), whereas the open symbols in the middle represent the predicted mating success at the population level.

presence of *D. birchii*, although the precise mechanism is not known. Unfortunately, female mate preferences were not measured in these experimental populations, so the precise mechanism responsible is not yet known. Likely possibilities are direct selection on preferences in sympatry caused by differences in mating efficiency, or as a correlated response to altered selection on male CHCs. Plasticity of preferences in field allopatric females due to the presence of *D. birchii* is also of interest, as this could initiate divergence in a nongenetic manner.

FUTURE DIRECTIONS

As both case studies above attest, divergent selection between environments may be a powerful mechanism of mate preference divergence, driving the evolution of sexual isolation during the early stages of speciation. In addition, selection arising in sympatry from the presence of the other (incipient) species may also be an important source of

divergent selection. In these systems and in many others as well, other mechanisms of preference divergence, such as sexual conflict, have received limited attention and manipulative tests are badly needed. Experimental evolution is a powerful tool in this regard that has yet to be fully exploited. By manipulating the presence of divergent natural selection, and having control over population sizes and mating systems, the full range of mechanisms of preference divergence can be explored, and interactions among them evaluated. To date, evolution experiments have provided equivocal results concerning the role of sexual conflict in the evolution of sexual isolation. Although Martin and Hosken (2003) demonstrated stronger sexual isolation among populations experiencing greater sexual conflict in dung flies, similar experiments in two species of *Drosophila* found no evidence of any reproductive isolation (Wigby & Chapman 2006; Bacigalupe et al. 2007). Additional work is clearly needed to develop a comprehensive understanding of the role of sexual conflict in the evolution

of sexual isolation. As Coyne and Orr (2004) note, if chance-based mechanisms such as sexual conflict turn out to be important in preference divergence, it could fundamentally alter the common perception that divergent natural selection is the primary mechanism of speciation.

More work on natural systems that goes beyond simply asking whether sexual selection is associated with speciation is needed to address the mechanisms involved in preference divergence. There are a few examples in which this is occurring, including African cichlids (Maan et al. 2006) and Anolis lizards (Leal & Fleishman 2002), in which sensory drive (see box 24.1) has been implicated in color divergence and differences in color preference confer sexual isolation. In addition, many lacewing species (*Chrysoperla* spp) show almost no morphological or ecological divergence, yet are isolated by differences in preference and production of their vibrational songs (Wells & Henry 1998). These songs diverge in correlation with characteristics of the substrate upon which mating occurs, again implicating sensory drive. Other intriguing examples are the mimetic *Heliconius* butterflies that are isolated by differences in color pattern and pattern preference (Jiggins et al. 2004). Color pattern is aposematic, and many species form Mullerian complexes, implicating predation as the ultimate cause. Additional examples are needed before generalization will become feasible.

Comparative methods have also been underutilized in this regard, despite being well suited to addressing questions concerning behavior's influence on speciation. A number of studies have shown that sexual selection is associated with greater diversification rates (reviewed in Coyne & Orr 2004; Ritchie 2007). It may be possible, however, to extend such studies to address specific forms of sexual selection and other mechanisms of divergence, and to explore their association with pre- and postmating reproductive isolation.

Although we have focused here on mechanisms for the initial divergence of mate preferences, there are other important areas of speciation research in need of behavioral data. For example, whether reproductive isolation can evolve entirely in sympatry is a topic that has long occupied students of speciation. It is now generally accepted that sympatric speciation can occur, and interest has shifted instead to determining its prevalence in nature. Establishing individual cases is a difficult prospect, however, and insight may instead be gained by the empirical

examination of parameters and assumptions identified by theory as having strong influences on its likelihood (Bolnick & Fitzpatrick 2007). Key ingredients in the models include a source of disruptive selection and a mechanism of assortative mating. Assortative mating can arise in a number of different ways, but behavior is often involved; habitat preferences, preferences for social partners, and mate preferences are commonly assumed mechanisms. How assortative mating arises is important because two aspects of it—its cost and whether or not it generates sexual selection—are central to the ease of speciation (Otto et al. 2008). If individuals that mate assortatively incur a fitness cost, sympatric speciation becomes much more difficult because these costs can overwhelm the indirect benefits of assortative mating that come through the reduced production of heterozygotes. Obstacles to sympatric speciation can also arise when the mechanism of assortative mating generates sexual selection on the locus under disruptive selection. This is because sexual selection can eliminate polymorphisms at key loci and can oppose disruptive selection, if the most common types of females prefer the most common types of males. Determining how conducive conditions are for sympatric speciation in nature will therefore require detailed behavioral data concerning mechanisms of assortative mating within populations, any costs associated with this, and whether or not sexual selection is generated.

Finally, behavioral ecology and speciation also intersect in the study of alternate mating tactics. There is growing interest in examining how behaviors such as sneaking may overcome sexual isolation, generating hybrids that impede the speciation process (Magurran 1998; Groning & Hochkirch 2008). Behavioral and paternity data from sympatric species for a few well-studied systems are needed to evaluate the extent of gene flow that can result from such interactions. Determining whether this is a rare or pervasive impediment to speciation will require data from a wide range of taxa. Such studies can have conservation implications, given the preponderance of hybridization between native and exotic species (Groning & Hochkirch 2008).

SUGGESTIONS FOR FURTHER READING

Comprehensive treatments of speciation are provided by Schluter (2000) and Coyne and Orr (2004);

- Price (2007) tackles the topic with a focus on birds. Our understanding of how mate preferences diverge was shaped by to a large degree by a review of their evolution within populations by Kirkpatrick and Ryan (1991). Those wishing to study mate preferences should also familiarize themselves with the intricacies surrounding their estimation (e.g., Wagner 1998), statistical analysis (McGuigan et al. 2008), and genetic basis (Chenoweth & Blows 2006). A recent review of sexual selection and speciation is provided by Ritchie (2007).
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