

9

Natural Selection and Ecological Speciation in Sticklebacks

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9.1 Introduction

The idea that selection may be fundamental to the origin of species dates back at least to the synthesis of modern evolutionary theory, being present in the writings of Fisher (1930), Muller (1942), and Dobzhansky (1951). Nevertheless, despite almost three-quarters of a century since the idea was first proposed, little progress has been made in testing the role of selection in speciation (Coyne 1992; Schluter 1996a, 2001; Futuyma 1998). As the various chapters of this volume attest, however, the topic is enjoying a resurgence of interest (see also Schluter 2001). The purpose of this chapter is to summarize tests for the role of selection in speciation in a natural system: the sympatric populations of limnetic and benthic threespine sticklebacks that inhabit postglacial lakes in British Columbia, Canada (Plate 2). Our investigation specifically addresses the role of divergent selection between environments and niches in the origin of reproductive isolation, and the role of reinforcement in its completion. Throughout, we adopt Mayr's (1942) biological species concept, in which species are groups of actually or potentially interbreeding natural populations that are isolated reproductively from other such groups. We broaden this definition slightly to recognize that imperfect reproductive isolation can exist between species that nevertheless maintain their distinctiveness in nature (Rundle *et al.* 2001).

We focus on the question of “ecological speciation”, a term that encompasses various speciation scenarios in which divergent natural selection between niches or environments is ultimately responsible for the evolution of reproductive isolation (Box 9.1). Ecological speciation differs from adaptive speciation as defined in Chapter 1 in two ways (Boxes 1.1 and 19.1). First, ecological speciation can also occur in allopatry (Box 9.1) and, second, ecological speciation does not include cases in which disruptive selection and subsequent divergences result entirely from sexual selection. Ecological speciation includes cases in which reproductive isolation evolves wholly as an indirect by-product of adaptation to alternative resources and environments, as well as several mechanisms in which natural selection directly favors the evolution of reproductive isolation (Box 9.1). Later we attempt to determine whether, in the stickleback speciation process, direct natural selection has played a role. Speciation by sexual selection is also ecological speciation if divergent natural selection between environments drives divergence in mate preferences, leading to reproductive isolation (Schluter 2000).

Box 9.1 Defining ecological speciation

Ecological speciation occurs when reproductive isolation evolves ultimately as a consequence of divergent natural selection between niches and environments (Schluter 2000, 2001). Reproductive isolation may evolve indirectly as a by-product of adaptive divergence of other traits, or selection may directly favor the evolution of reproductive isolation. Ecological speciation is adaptive speciation (as defined in Chapter 1) if it is driven by frequency-dependent ecological interactions. Ecological speciation does not, however, encompass speciation by purely sexual selection, in the absence of divergent natural selection. Sexual selection is a part of ecological speciation only if divergent mate preferences are ultimately the outcome of divergent natural selection between environments, but not otherwise (summarized in Schluter 2000; Boughman 2002).

Indirect (by-product). In this form, reproductive isolation evolves indirectly as a by-product of adaptation to alternative environments or niches. Environmental differences lead to divergent natural selection on phenotypic traits (morphology, physiology, or behavior) and the resultant divergence in phenotype may bring about reproductive isolation (premating and/or postmating) as a side-effect, both in sympatry and allopatry (see Box 9.2). Divergent selection may arise from external differences between the two environments or niches occupied by the two populations (the “environment” in a narrow sense). Divergent selection may also arise from frequency-dependent interactions between individuals of the two populations, such as competition or predation (the “environment” in a wider sense). For instance, ecological character displacement caused by competition for shared resources in sympatry can cause ecological traits to diverge, which may produce some reproductive isolation as a by-product.

Direct. In this process, selection directly favors the evolution of reproductive isolation. It generally occurs when two populations are sympatric and individuals that mate heterospecifically have reduced fitness. Natural selection then directly favors individuals that discriminate against heterospecific mates, which leads to stronger premating isolation. Reduced fitness of individuals that mate heterospecifically can arise in two ways. First, if hybrid offspring have reduced fitness (i.e., partial postmating isolation exists), selection favors those individuals that hybridize less. This is the hypothesis of reinforcement (Dobzhansky 1940; Blair 1955). Second, heterospecific matings may impose fitness costs on the individuals (because of parasites, courtship costs, increased predation risk, etc.), and so those individuals that mate within their own population have a higher fitness. In contrast to reinforcement, here the fitness cost is borne by the mating individual and is not determined by the viability or fertility of its offspring.

Processes of speciation that do not involve divergent selection between environments are regarded as “nonecological”, because chance events dominate the initiation of the evolution of reproductive isolation. Mechanisms include genetic drift, founder events, and fixation of alternative advantageous alleles in allopatric populations that experience similar selection pressures. Speciation initiated by any of these alternative mechanisms may be completed by reinforcement in a second, sympatric

continued

Box 9.1 *continued*

phase. For this reason, demonstration of reinforcement does not by itself distinguish ecological from nonecological speciation. Tests of ecological speciation must focus instead on the role of selection at other stages of speciation, particularly early on in the process. Genetic drift, founder events, and alternative mutations may lead to divergent sexual selection independently of environment, which should also be regarded as a nonecological process of speciation.

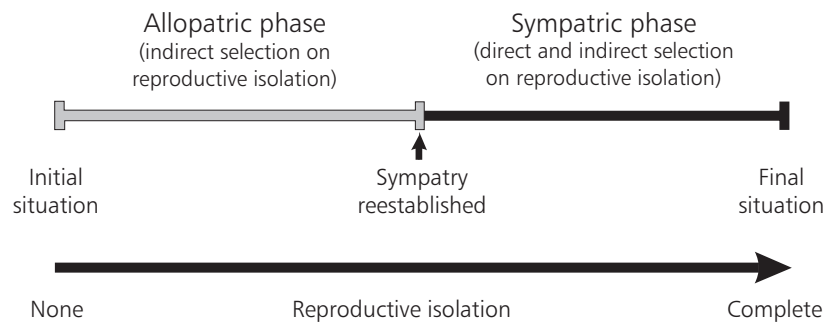
Ecological speciation may occur in a variety of geographic settings: allopatric, sympatric, and between. A useful general framework considers the process that leads to two coexisting species potentially to have two stages (Box 9.2). In the first stage, reproductive isolation begins to evolve between two allopatric populations as a by-product of adaptation to their unique ecological environments or niches. The second stage occurs after secondary contact: selection in sympatry strengthens reproductive isolation and completes the speciation process. Within this framework, completely allopatric and completely sympatric speciation are extremes in which one or the other stage is absent. Among the goals of speciation research is to determine how much reproductive isolation is built during each stage, and by what mechanisms.

At present there are two kinds of evidence for ecological speciation. First, laboratory experiments using *Drosophila* demonstrated the feasibility of the model, both in allopatry (Kilias *et al.* 1980; Dodd 1989) and in sympatry (Rice and Salt 1988, 1990). For example, premating isolation evolved between laboratory populations that adapted to different environments, but not between populations that independently adapted to the same environment (Figure 9.1). Such results indicate that the model is feasible, but they do not indicate the importance of ecological speciation in nature. Second, a few results from nature are consistent with the hypothesis of ecological speciation. At least three examples are known in which traits with adaptive significance also form the basis of the reproductive isolation between species: Darwin's finches in the Galapagos Islands (Boag and Grant 1981; Ratcliffe and Grant 1983; Price *et al.* 1984), yellow monkey flower (*Mimulus guttatus*) living on copper-contaminated and uncontaminated soils (Macnair and Christie 1983; Christie and Macnair 1984), and sympatric threespine sticklebacks (Nagel and Schluter 1998). This implies that reproductive isolation somehow followed adaptive divergence in traits.

Here we review further evidence for ecological speciation in wild sticklebacks. Measurements of the strength of premating isolation among populations are consistent with a dual role of selection during two phases of ecological speciation. Initial premating barriers probably arose between allopatric populations as a by-product of adaptation to different environments, and later these were strengthened in sympatry by direct selection against heterospecific mating. Our results

Box 9.2 The geographic context of ecological speciation

Much attention has been given to the geographic context of speciation; the majority of this work focused on the possibility of fully sympatric speciation versus the necessity of an allopatric phase. The hypothesis of ecological speciation is general and makes no claim as to the geographic context of speciation. An all-purpose geographic scenario for the ecological process that leads to two coexisting species is for reproductive isolation to begin during an allopatric phase and to be completed in sympatry, as illustrated below.



Reproductive isolation is initially absent on the left of the figure above, at the start of the speciation process, and evolves to completion on the right. The vertical arrow indicates the point at which sympatry between the two populations is reestablished, and may occur anywhere along the time line (shown here at the center). Allopatric and fully sympatric speciation represent the two extremes of this general scenario, whereby one or the other of the phases is missing and reproductive isolation evolves completely in either allopatry (complete reproductive isolation achieved before sympatry is established) or sympatry (no allopatric phase precedes the evolution of reproductive isolation). The mechanisms of divergent selection depend to some extent on geography. Premating and postmating isolation may evolve as by-products of adaptation to different environments or niches in either phase, but frequency-dependent interactions are added as an agent of divergent selection in the sympatric phase. Direct selection on premating isolation occurs in the sympatric phase, either via reduced hybrid fitness (i.e., reinforcement) or fitness costs incurred during heterospecific encounters. The sympatric phase corresponds to adaptive speciation processes as defined in Chapter 1 (also see Box 19.1).

Evidence suggests that both phases were present in the origin of sympatric benthic and limnetic sticklebacks (Box 9.3). The amount of reproductive isolation that evolved during each phase remains uncertain, however. The placement of the division that separates allopatric and sympatric phases, and the precise mechanisms of selection involved, are the subjects of continuing research.

clearly demonstrate ecological speciation and reveal that at least part of the isolation evolved as a result of direct selection (adaptive speciation, *sensu* Dieckmann *et al.*, Chapter 1). However, the precise amount of premating isolation that evolved during each phase is uncertain and remains to be determined.

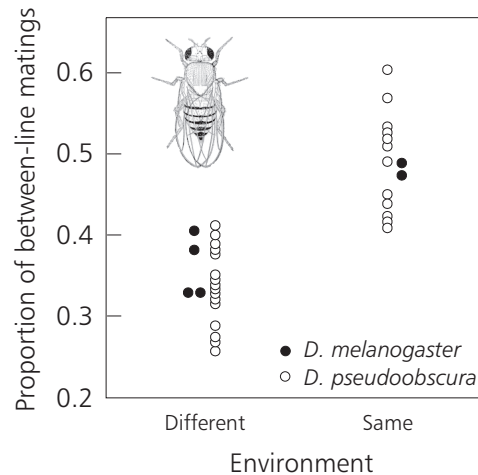


Figure 9.1 Proportion of matings that occur between independently evolved lines of *Drosophila*, derived from a common ancestor, as a function of the similarity of their environments. Sources: Open circles *D. pseudoobscura*, Dodd (1989); filled circles *D. melanogaster*, Kiliias *et al.* (1980).

9.2 Natural History of the Sympatric Sticklebacks

Marine threespine sticklebacks (*Gasterosteus aculeatus*) colonized freshwater lakes and rivers along the coast of British Columbia after the retreat of the Pleistocene glaciers 10 000–12 000 years ago, and gave rise to permanent freshwater populations (McPhail 1993). While most lakes or rivers contain a single population, species pairs evolved in a few small, low-elevation lakes (Figure 9.2). In every case, one species of each pair, the benthic, is a larger, deeper bodied fish (Plate 2), with fewer, shorter gill rakers, that feeds on invertebrates in the littoral zone of the lake. The other species, the limnetic, is a smaller, more fusiform fish (Plate 2), with longer, more numerous gill rakers, that feeds primarily on zooplankton in the open water of the lake (McPhail 1984, 1992, 1994; Schluter and McPhail 1992). Phenotypic differences between limnetics and benthics are genetic and persist over multiple generations in a common laboratory environment (McPhail 1984, 1992; Hatfield 1997). Both comparative (Schluter and McPhail 1992) and direct experimental evidence (Schluter 1994) indicate that these differences are the result of divergent natural selection caused, in part, by frequency-dependent resource competition. Within each lake, limnetics and benthics constitute biological species. They are isolated reproductively and behaviorally (Ridgway and McPhail 1984; Nagel and Schluter 1998), and hybrids, which are intermediate in phenotype, suffer a reduced fitness as a result of ecological mechanisms (Schluter 1995; Hatfield and Schluter 1999). Sexual selection is also likely to act against hybrid males (Vamosi and Schluter 1999).

The geographic context of limnetic–benthic speciation has been contentious, but the present evidence favors a “double-invasion” scenario rather than fully sympatric speciation (Box 9.3). Under this scenario, originally proposed by McPhail (1993), the limnetic–benthic pair in each lake is the result of two separate invasions

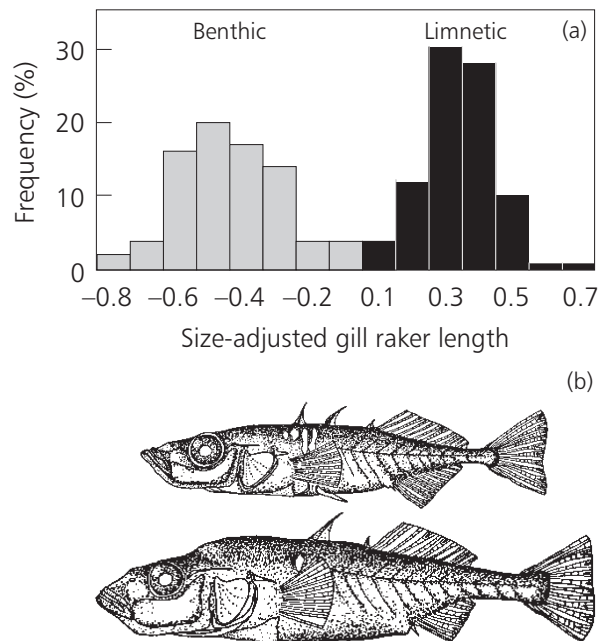


Figure 9.2 (a) Frequency distribution of size-adjusted gill raker lengths for Paxton Lake benthic (gray) and limnetic (black) sticklebacks. Gill rakers are protuberances from the gill arch that are thought to function during feeding to sieve particles of food or to direct the movement of water through the oral cavity. Plankton-feeding fish tend to have more numerous, longer gill rakers (see Schluter and McPhail 1993). *Source:* Schluter and McPhail (1992). (b) Limnetic (above) and benthic (below) sticklebacks from Paxton Lake, British Columbia. *Source:* Schluter (1993).

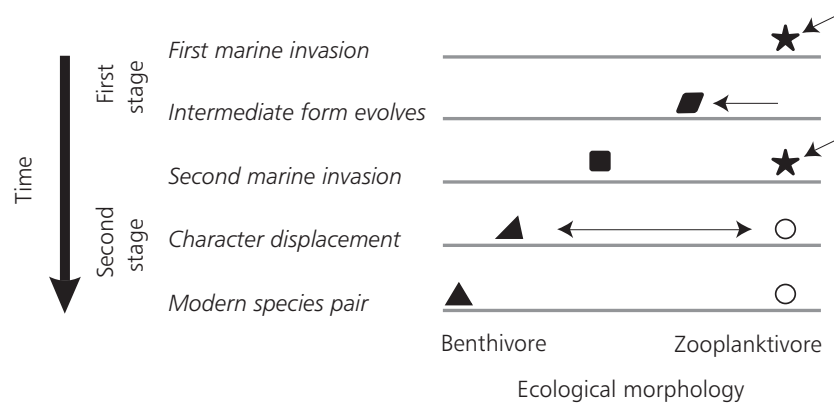
by the marine stickleback into freshwater, in which the second, more recent, invader evolved into the modern limnetic. Speciation thus involved an initial stage of allopatry during which the first invader was adapting to freshwater prior to the second invasion of the marine form. The species have persisted subsequently, despite at least some gene flow between them.

While the geographic context of speciation has been debated, genetic evidence indicates that the phenotypic similarity of benthics and of limnetics from three different lakes (Paxton, Priest, and Enos) is the result of parallel evolution and not shared ancestry. The evidence includes unique assemblages of mitochondrial DNA (mtDNA) that characterize the species pairs from each of these lakes (Taylor *et al.* 1997; Taylor and McPhail 1999) and a phylogenetic and genetic distance analysis of six nuclear microsatellite loci (Box 9.4; Taylor and McPhail 2000). For example, each lake is dominated by mtDNA haplotypes not found in any other lakes, each of which is a small number of base-pair transitions away from common marine haplotypes (Taylor and McPhail 1999).

In Section 9.3 we turn our attention to mechanisms of speciation, and describe two separate tests of ecological speciation in our attempt to understand the evolution of reproductive isolation from beginning to end. The first takes advantage of the independent evolution of the limnetics and benthics from these three lakes.

Box 9.3 Double invasion and stickleback speciation

The geographic context of speciation of the limnetic–benthic pairs, whether they arose within each lake via sympatric speciation or instead had an allopatric phase, is unclear and not all the evidence points to one conclusion. The weight of evidence, however, favors the double-invasion scenario of McPhail (Schluter and McPhail 1992; McPhail 1993) as depicted below (figure modified from Taylor *et al.* 1997). In this scenario each coexisting benthic and limnetic pair results from two separate invasions by the marine threespine stickleback (*Gasterosteus aculeatus*) into freshwater after the retreat of the glaciers at the end of the Pleistocene period (< 13 000 years ago).



The sequence began with the first invasion of lakes by the marine species, which then evolved into an intermediate phenotype characteristic of most small, single-species lakes today (Schluter and McPhail 1992). The marine form invaded a second time after a secondary rise in sea level a few thousand years later. Ecological character displacement, driven by frequency-dependent resource competition, increased the ecological and phenotypic divergence in sympatry (Schluter 1994). The first invader gave rise to the present day benthic species, while the second invader remained a plankton specialist like its marine ancestor, and has evolved into the present-day limnetic species. The double-invasion scenario can be accommodated within the framework of adaptive dynamics, as outlined in Box 9.5.

The evidence in favor of double invasion is as follows:

- Allozyme frequencies from two lakes indicate that the limnetic species is similar to the present-day marine species (Nei's $D_N \approx 0.02$; McPhail 1984, 1992), whereas benthics are more distant (Nei's $D_N \approx 0.07$). However, the distances in all cases are small.
- Like the marine species, limnetics from the two lakes tested could successfully complete development in 28 parts per thousand (p.p.t.) of sea water, from egg to hatchling, whereas the success rate of benthics from the same lakes was poor (Kassen *et al.* 1995). This supports the idea of two invasions spaced apart in time, with the most recent invasion leading to the modern limnetic species (assuming that salinity tolerance has decayed with time since the colonization of freshwater).

continued

Box 9.3 *continued*

- Analysis of six microsatellite loci showed that allele frequencies in the limnetics are more similar to those of the present-day marine species than to allele frequencies of the benthics (Taylor and McPhail 2000), although the differences are again small. In addition, the microsatellite allele frequencies fit the unconstrained maximum-likelihood phylogeny (Box 9.4) significantly better than a tree in which the limnetic and benthic species from each lake are constrained to be sister species, as would be the case if they had resulted from sympatric speciation (Taylor and McPhail 2000).
- There are morphological indications of recent hybridization between the limnetic species in Emily Lake (the lake of lowest elevation in the drainage that includes the Priest Lake species pair) and the marine species that breeds in the stream that drains Emily Lake to the ocean (McPhail, personal communication). This marine population is indicated as Marine 4 in Box 9.4.

In contrast to these indications of double invasion, restriction fragment length polymorphism (RFLP) analysis of mtDNA is more consistent with sympatric speciation: dominant haplotypes within each lake occur in both species, which suggests they are sister species (Taylor and McPhail 1999). However, we suggest that these results show a recent mtDNA gene flow between sympatric species (Taylor *et al.* 1997; Taylor and McPhail 2000).

9.3 Parallel Speciation of Limnetics and Benthics

One mechanism of ecological speciation occurs when reproductive isolation evolves as a by-product of adaptation to different environments (Box 9.1). When replicate, closely related populations independently adapt to their environments, a remarkable pattern termed parallel speciation may result (Schluter and Nagel 1995). Parallel speciation is a special case of the phenomenon of parallel evolution, a form of homoplasy in which a similar trait evolves repeatedly in closely related, independently evolving lineages. Parallel evolution provides strong evidence for natural selection in the evolution of the trait, because genetic drift is unlikely to produce predictable changes in independent lineages in correlation with the environment (Clarke 1975; Endler 1986; Schluter and Nagel 1995).

Parallel speciation is a form of parallel evolution in which the traits that evolve predictably in correlation with the environment also affect reproductive isolation. When reproductive isolation evolves as a by-product of adaptation to the environment in multiple, independent populations, the predicted outcomes are twofold:

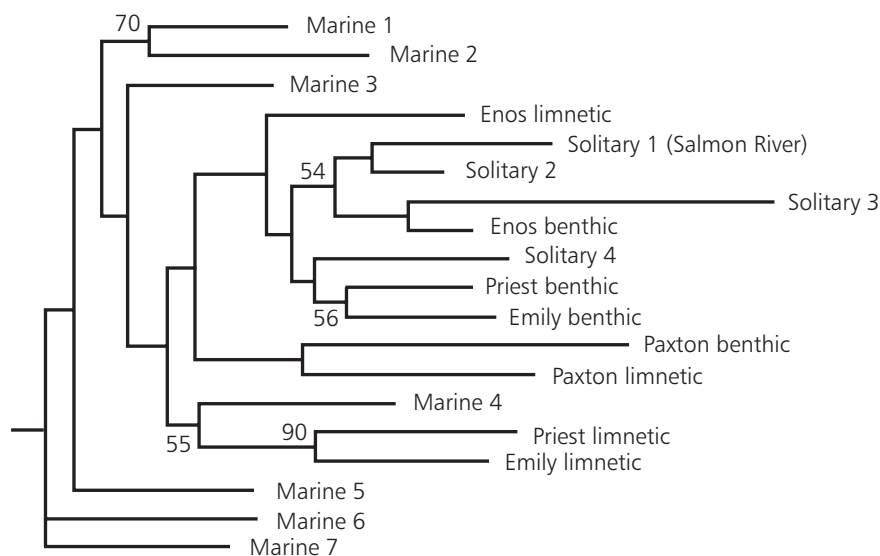
- Reproductive compatibility between populations that evolved independently in similar environments.
- Reproductive isolation between populations that evolved independently in different environments.

Box 9.4 Independent parallel evolution

Currently, three drainages in the region (Paxton Lake, Priest Lake, and Enos Lake) contain a pair of limnetic and benthic species (a fourth pair in Hadley Lake drainage was recently extirpated by introduced catfish). How many origins of limnetic and benthic populations have led to this pattern? One possibility is that each species arose exactly once, and that the pair subsequently colonized other drainages together. The other possibility is that each species pair arose independently from the others, and acquired their similarities by parallel evolution under common natural selection pressures. The weight of evidence favors the latter possibility.

The first line of evidence comes from mtDNA. Each of the drainages is dominated by haplotypes that are not found in any other drainage nor the sea (Taylor *et al.* 1997; Taylor and McPhail 1999). The majority of these are distinguished from common marine haplotypes by a single restriction site; most of the remaining unique haplotypes are a single site away from other haplotypes in the same drainage. In no case are unique haplotypes from two different drainages distinguished by as little as one restriction site. In other words, haplotypes in each drainage trace their origins to the sea, not to other lakes. Unfortunately, mtDNA gene flow between limnetics and benthics within each drainage (Box 9.3) prevents us from teasing apart their separate histories in this way.

The second line of evidence is from analyses of allelic variation at six microsatellite loci (Taylor and McPhail 2000). When the limnetics and benthics are considered apart from other populations, almost none of the total genetic variation among individuals is partitioned between the classes “limnetic” and “benthic” (2.4–4.4%, which is not significantly different from zero). This variance component would be larger if limnetics and benthics arose exactly once. In addition, the maximum-likelihood phylogeny of all populations tested, shown below (unrooted tree modified from Taylor and McPhail 2000), suggests a polyphyletic origin of both limnetics and benthics. Despite uncertainty in most of the groupings (numbers below indicate bootstrap support levels greater than 50%), this tree fits the data significantly better than those that constrain either limnetic or benthic populations to be monophyletic (Taylor and McPhail 2000).

*continued*

Box 9.4 *continued*

The phylogeny illustrates the greater overall similarity of marines to limnetics than to benthics (Box 9.3). The four solitary populations tested, from different lakes and streams, tend to cluster with the benthics, which suggests that most were formed at the time of the first invasion rather than the second. Measurements of salinity tolerance bear this out for the one solitary population tested (Kassen *et al.* 1995). The marine samples are very similar to one another in microsatellite allele frequencies, as would be expected from high levels of gene flow among sites. Monophyly (with the exception of Marine 4) of the freshwater populations is also consistent with high levels of gene flow among marine populations and does not require a single marine population to be the common ancestor of all freshwater populations.

As in parallel evolution generally, the repeated evolution of similar mechanisms of premating isolation in independent populations that inhabit similar environments strongly implies that natural selection was the cause (Schluter and Nagel 1995). Reproductive compatibility is predicted by selection regime rather than by geographic proximity or phylogenetic history. Despite its significance to our understanding of ecological speciation in nature, prior to our work no conclusive tests of parallel speciation were found in the literature.

We took advantage of the independent evolution of limnetics and benthics in Paxton, Priest, and Enos Lakes to test two specific predictions of parallel speciation (Rundle *et al.* 2000):

- Populations of the same “ecomorph” from different lakes (e.g., limnetics from Paxton, Priest, and Enos Lakes) should be compatible reproductively, despite the known isolation between limnetics and benthics within a lake (Nagel and Schluter 1998).
- Limnetics and benthics from different lakes (e.g., Enos limnetics and Paxton benthics) should be isolated reproductively, even though they have not encountered one another before.

These predictions were tested by measuring the strength of premating isolation between various combinations of populations. The strength of premating isolation was measured in no-choice mating trials conducted in the laboratory using wild-caught individuals. 753 mating trials were performed. Details of the methodology, population combinations, and sample sizes can be found in Rundle *et al.* (2000). Statistical analyses treated populations of females (not individual females) as replicates and corrected for phylogeny. Strong reproductive isolation between limnetics and benthics within each lake was used as a benchmark for other comparisons (Figure 9.3, comparison A). Females spawned significantly more often with males of their own population (38%) than with males of the other species within the same lake (15%); note that this mating frequency between sympatric species is artificially higher in our no-choice laboratory setting than in the wild, where hybridization rarely if ever occurs.

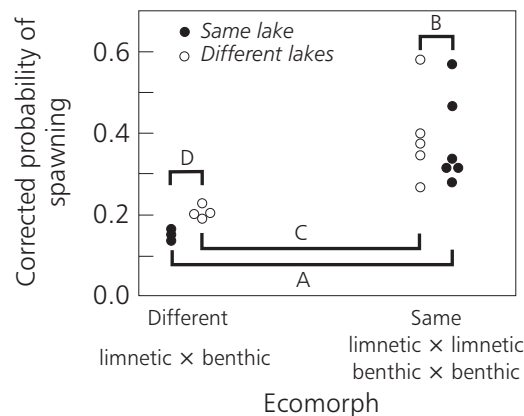


Figure 9.3 Population mean probabilities of spawning between pairs of populations as a function of ecomorph. Comparison A represents the test for reproductive isolation between limnetics and benthics within a lake. Comparisons B and C represent the two tests of parallel speciation. The strength of reproductive isolation between limnetics and benthics from the same or different lakes is compared in D. Our statistical tests employed a conservative paired *t*-test that treated each population of females as a replicate and corrected for phylogeny, and the comparisons shown here represent the nature of the tests, but do not depict the actual analyses performed. *Source*: Rundle *et al.* (2000).

Both predictions of parallel speciation were met. First, reproductive isolation was lacking among populations of the same ecomorph from different lakes (Figure 9.3, comparison B). Females mated just as readily with males of the same ecomorph from a different lake (39%) as with males from their own population (38%). Second, reproductive isolation was present between limnetics and benthics from different lakes (Figure 9.3, comparison C). Females mated significantly more often with males of their own ecomorph from a different lake (39%) than with males of the other ecomorph from a different lake (20%). Interestingly, the probability of spawning between limnetics and benthics from different lakes (20%) was slightly higher than that between limnetics and benthics from the same lake (15%), a difference that approached statistical significance ($t_5 = 2.36$, $P = 0.065$; Figure 9.3, comparison D). This last result is the first hint that premating isolation between limnetics and benthics was strengthened in sympatry.

The parallel evolution of premating isolation in correlation with the environment provides strong evidence for ecological speciation in nature. In a little over 10 000 years populations of sticklebacks descended from the same marine ancestor, but that inhabited different environments, have evolved strong premating isolation. Remarkably, during this same period of time no reproductive isolation has arisen between populations that adapted independently to similar environments. Similar results have been observed in a number of other systems (Funk 1998; McPeck and Wellborn 1998; McKinnon *et al.*, unpublished; see Morrell 1999), which suggests that parallel speciation may not be a rare occurrence. Our results strongly implicate natural selection in speciation and suggest that reproductive isolation between limnetics and benthics has evolved as a by-product of adaptation to

different habitats. The by-product mechanism can occur both in allopatry and in sympatry (Box 9.2), but the degree to which this isolation evolved during each of the two phases remains to be determined.

Next we consider the possibility that some portion of the isolation results from selection that directly strengthens premating isolation in sympatry (e.g., adaptive speciation via reinforcement), and that this occurred in parallel in each lake. As noted in Figure 9.3 (comparison D), premating isolation between limnetics and benthics may be slightly stronger when the two populations are from the same lake than when the two populations are from different lakes, suggesting a role for selection in sympatry. We now turn our attention to this second stage of ecological speciation.

9.4 Premating Isolation Strengthened in Sympatry

To test whether selection strengthened premating isolation between sympatric limnetics and benthics during a second stage of ecological speciation, we tested for reproductive character displacement. Reproductive character displacement is the pattern of greater premating isolation between two taxa in areas of sympatry rather than allopatry (Brown and Wilson 1956; Howard 1993). Its presence suggests that a greater degree of reproductive isolation may have evolved in sympatry. Indeed, if the second stage of ecological speciation commonly involves the strengthening of premating isolation in sympatry, reproductive character displacement is the predicted outcome. Comparison D in the previous section (Figure 9.3) is not a sufficient test of reproductive character displacement because all populations were sympatric with some other population. Reproductive character displacement is a pattern that involves a comparison of populations from areas of sympatry versus allopatry, so a stronger test requires that solitary (i.e., allopatric) populations be included.

A number of mechanisms of ecological speciation can produce reproductive character displacement, two of which also fall under the definition of “adaptive speciation”. Most attention has been paid to reinforcement, defined as the process whereby premating isolation is strengthened in sympatry as an adaptive response to reduced hybrid fitness (Dobzhansky 1940; Blair 1955). Other processes include direct selection in sympatry for premating isolation that results from fitness costs borne by individuals that mate heterospecifically, and a nonadaptive process termed “biased extinction”. These alternative mechanisms are described below. While reproductive character displacement was once considered a rare phenomenon (e.g., Littlejohn 1981; Phelan and Baker 1987), more recent work suggests otherwise (Coyne and Orr 1989, 1997; Howard 1993; Noor 1995, 1997; Sætre *et al.* 1997; Higgie *et al.* 2000).

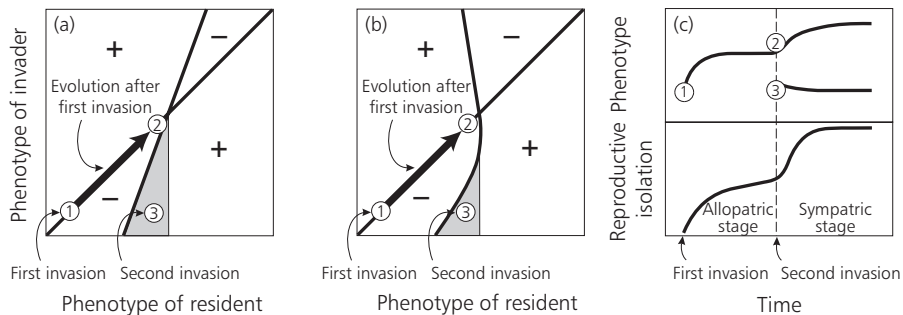
We focused on reproductive character displacement of benthic female mate preferences (Rundle and Schluter 1998). Our study used females from three populations: the benthic from Priest Lake (a two-species lake) and two solitary populations, one from Beaver Lake and the other from the Salmon River. The Salmon River population is related closely to the Priest Lake benthic (Box 9.4), whereas

Box 9.5 Double invasion and frequency-dependent selection

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The double-invasion scenario described in Box 9.3, which includes an initial allopatric phase and a later sympatric phase, requires that selection be frequency dependent. That is, the fitness landscape of the second invader is dependent on the presence and the phenotype of the first invader. Without frequency dependence, the two forms (depicted by a square and a star in Box 9.3) would not have persisted as competitors in sympatry following the second invasion. Instead, one of the two morphs would have been driven to extinction by the other. Moreover, ecological character displacement can only occur under frequency-dependent selection: in its absence, the second invader is forced to pursue the same evolutionary course as did the first one initially. Below we use the adaptive dynamics framework to depict the possible role of frequency dependence in a simple scenario. We consider a single trait axis along which the two stickleback phenotypes are differentiated. A low value along this axis represents the zooplanktivore phenotype. Greater values indicate more benthic-feeding phenotypes.

The linear pairwise invasibility plot in panel (a) below represents one scenario of events that might have led from a single marine stickleback ancestor to two sympatric species within a lake. The signs (+, -) indicate, for each value of the resident phenotype, the invader phenotypes that can (+) and cannot (-) invade when there is competition for resources. The crossed lines delimit regions of different sign.



The hypothetical sequence of events begins at (1) when the marine form, a zooplanktivore, first invades the lake. Subsequently, adaptation to the new environment [arrow from (1) to (2)] causes this first invader to evolve toward an intermediate phenotype that represents an evolutionary branching point (intersection of the two thick lines). A lake with a single intermediate population at the branching point can be invaded by nearby phenotypes larger or smaller (i.e., the population experiences disruptive selection). The area shaded in gray indicates the trait values of zooplanktivore-like phenotypes permitted to invade once the resident population is close to the branching point. In the scenario in panel (a), the marine form invades a second time (3) before the first population has reached the branching point, which

continued

Box 9.5 *continued*

results in two phenotypes within the lake. Although the new marine invader and the evolved lake form now reside on the same side of the branching point, they can coexist and continue to evolve. Ecological character displacement, driven by frequency-dependent competition for resources, causes further divergence between the two populations until they eventually reach an evolutionarily stable combination of phenotypes [top section of panel (c)].

Under this simple scenario, competitive exclusion precludes a second invasion until the phenotype of the first invader approaches the branching point. Insufficient reproductive isolation may also preclude a second invasion that is too soon after the first. Thus, the timing of the second invasion is dependent not only on favorable geologic events that allow the movement of individuals from the sea into the lake, but also on the evolution of the resident within the lake. As shown in the bottom section of panel (c), partial premating isolation is assumed to build prior to the second invasion (the allopatric phase) as a by-product of phenotypic divergence. After the second invasion (the sympatric phase), natural selection directly on premating isolation (i.e., reinforcement) or on correlated traits might further strengthen premating isolation.

Alternative invasion scenarios that involve more complicated forms of interaction between phenotypes are possible, and lead to nonlinear pairwise invasibilities as in panel (b) above. For example, the first invader might evolve not to a branching point, but instead to an evolutionarily stable attractor that permits no invasion by nearby phenotypes [intersection of the two thick lines in panel (b)]. In this case the intermediate population is under stabilizing selection, not disruptive selection. Such stabilizing selection might occur if zooplankton and benthic resources in the lake peak at different times in the season, such that a generalist intermediate phenotype can exploit both of them in sequence when more specialized zooplanktivore or benthic phenotypes cannot.

In this scenario, the line that delimits (+) and (−) regions in the lower part of the plot is curved to the left. In this case a second invasion is possible if the invading zooplanktivore morph is sufficiently different in phenotype from the resident morph, as the latter population evolves toward an intermediate phenotype. This might happen if a portion of the zooplankton resource base cannot be exploited by an intermediate phenotype. Following a successful invasion, ecological character displacement and reinforcement might cause further divergence between the two populations, as in the previous scenario.

We do not know which of the two above scenarios, intermediate branching point or intermediate stable attractor, best describes the double invasion process in sticklebacks. Distinguishing them would require determining whether the intermediate form in single-species lakes most often experiences stabilizing or disruptive selection. A series of future experiments could also compare the fitnesses of different invading phenotypes in the presence of alternative resident phenotypes.

the phylogenetic affinities of the Beaver Lake population are unknown. We compared the probability of spawning of these females with limnetic and benthic males from Paxton Lake, another two-species lake. Reproductive character displacement of benthic female mate preferences would be indicated if Priest benthic females show a more marked difference in their propensities to mate with limnetic and benthic males than do females from solitary populations. To minimize lake effects on mating behavior, none of the female populations came from the same lake as the males.

Reproductive character displacement is mainly of interest if inferences can be made as to the process that caused it. With this in mind, our test for reproductive character displacement accounted for ecological character displacement that may strengthen premating isolation in sympatry as a by-product (Box 9.1). Divergent selection created by competition for resources may cause ecological characters to diverge and, if these characters also affect mate choice, reproductive character displacement may evolve as a side-effect. Indeed, both comparative (Schluter and McPhail 1992) and direct experimental evidence (Schluter 1994, 1996a) indicate that ecological character displacement has occurred between limnetics and benthics. We were especially concerned about displacement of body size, because this trait affects the probability of interspecific mating (Nagel and Schluter 1998). For this reason we compared the mate preferences of benthic females only with solitary populations of females that closely resembled true benthics in morphology. As the resemblance was not perfect, we also corrected statistically for body-size differences in the analysis. A total of 239 no-choice mating trials were conducted in the laboratory. Details of the methodology, populations used, and sample sizes are given in Rundle and Schluter (1998).

Reproductive character displacement of benthic female mate preference was clear (Figure 9.4). Priest benthic females spawned much more readily with benthic males than with limnetic males, whereas this difference was small in females from both solitary populations. This pattern remained after statistically correcting for differences in the body size of the females from the three populations.

To distinguish the various processes that can produce reproductive character displacement is difficult. The first task is to rule out mechanisms in which selection plays no part, such as biased extinction. Under biased extinction, incipient species come together in multiple, independent localities, but the populations persist as separate species only in the sites in which the initial level of premating isolation is sufficiently strong. Cases in which isolation is weak result in a fusion of the populations or the extinction of one by reproductive interference (Butlin 1987, 1989). Sympatric pairs that survive are thus a biased subset of all possible combinations; they include only the pairs in which premating isolation was strong enough to permit coexistence. Under this mechanism, premating isolation does not evolve in sympatry. However, speciation may still be regarded as ecological (but not adaptive) if selection causes the initial isolation to evolve in allopatry. In this case, only the first stage of ecological speciation occurs and speciation is entirely allopatric.

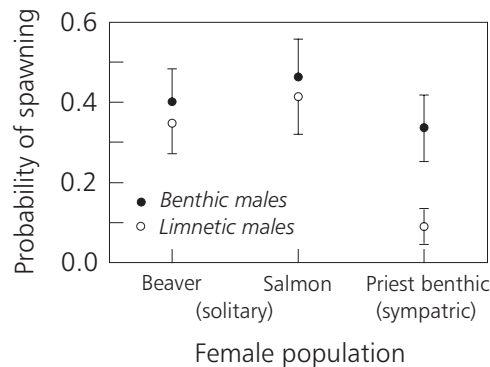


Figure 9.4 Mean probability of spawning (± 1 SE) for various combinations of male species and female population. The pattern of reproductive character displacement is indicated by the lower probability of spawning with heterospecific males exhibited by the populations of sympatric as opposed to solitary females. In some instances, error bars are shown in only one direction for clarity. *Source*: Rundle and Schluter (1998).

Biased extinction predicts that a range of levels of premating isolation should exist among randomly paired solitary populations, and the most extreme solitary pairs should exhibit virtually complete isolation, such as that found between existing sympatric species. With only two solitary populations investigated thus far, we are unable to test rigorously this prediction. However, it is striking that relatively little isolation occurs in both of these populations, chosen because they are very benthic-like in morphology, and at least one (Salmon River) is phylogenetically close to the benthic population tested. For this reason, we view biased extinction as insufficient on its own. Nevertheless, our results imply that some variation in reproductive isolation exists among solitary populations, because the isolation detected in the two solitary populations tested seems too weak to prevent fusion with limnetics. However, no-choice mating trials may be too insensitive to detect the variation present, and may underestimate levels of premating isolation (e.g., isolation between sympatric limnetics and benthics is weaker in our trials than in the wild).

The next task is to identify which ecological mechanism is responsible for reproductive character displacement. These include direct and indirect selection for increased premating isolation in sympatry (Box 9.1). We examine each of these in turn.

Reproductive character displacement can evolve indirectly when unique selective pressures created by interactions between the two species in sympatry (e.g., competition, predation) strengthen premating isolation as a by-product (Box 9.1). For instance, as noted above, competition for resources may cause ecological character displacement, which in turn may cause reproductive character displacement as a by-product. By choosing solitary females that closely resembled benthic females in morphology, we minimized this possibility in our study and thus view this mechanism as unlikely. However, it is conceivable that additional unmeasured ecological characters that diverged in sympatry are responsible for the increased

reproductive isolation, and that our sampling of solitary populations did not control for such confounding factors.

Alternatively, reproductive character displacement is the outcome of direct natural selection on premating isolation (Box 9.1). This occurs if individuals that avoid heterospecific mating have higher fitness for one of two reasons. First, if partial or complete postmating isolation exists, individuals that avoid heterospecific encounters tend to have more fit (i.e., nonhybrid) offspring. This is the hypothesis of *reinforcement*. Reinforcement requires a number of preconditions, which include that hybridization has occurred in the past and that hybrids are selected against (Butlin 1989; Howard 1993). These preconditions appear to be met in the sticklebacks (Rundle and Schluter 1998; Hatfield and Schluter 1999; Taylor and McPhail 1999, 2000). The second mechanism that results in higher fitness occurs when individuals that avoid heterospecific matings avoid the costs incurred as a result of these encounters. In this “reinforcement-like” process (Servedio 2001), the cost to heterospecific encounters is not manifest through a reduced fitness of the individual’s offspring, but rather by a reduced fitness of the individuals themselves. For instance, a benthic female that spawns with a limnetic male may be exposed to new and harmful parasites, while the limnetic male exposes the eggs already in his nest to egg predation by the benthic female (Foster 1994). As yet no attempts have been made to measure such costs of heterospecific encounters in these sticklebacks.

In conclusion, the combined data are consistent with the hypothesis that premating isolation is strengthened directly by selection in sympatry. This occurs as an adaptive response to either reduced hybrid fitness (reinforcement) or to costs imposed by heterospecific encounters.

9.5 Concluding Comments

Selection has long been thought to play a fundamental role in the formation of new species, but only recently has evidence from nature begun to accumulate in support of this (Schluter 1996a, 2000, 2001). The hypothesis of ecological speciation, under which reproductive isolation ultimately evolves as a result of divergent selection between environments or niches, gains strong support from the species pairs of threespine sticklebacks that inhabit postglacial lakes in British Columbia, Canada. Our results show that reproductive isolation has evolved in correlation with the environment, and that reproductive character displacement of the mate preferences of benthic females has occurred. This suggests that selection has had a dual role that corresponds to two stages in the evolution of premating isolation:

- Premating isolation evolved initially as a by-product of adaptation to different environments during the first, allopatric stage.
- Later, premating isolation was strengthened directly by selection during a second, sympatric stage.

Nevertheless, we do not yet fully understand the roles of these two mechanisms in the evolution of the premating isolation. The results shown in Figure 9.3 indicate that most premating isolation is explained by a common environment, with only a

small component explained by a shared lake (i.e., sympatry; comparison D). It is tempting to conclude that the majority of the reproductive isolation thus evolved as a by-product of adaptation to alternative environments, with only a minor role for direct selection in sympatry. An alternative hypothesis is that these same results represent parallel responses to direct selection on premating isolation in sympatry. For instance, if reinforcement occurs by strengthening the dependence of mate choice on some key trait (e.g., body size), and this trait evolves in parallel in different lakes, parallel reinforcement may result. Such a process is consistent with the lack of premating isolation observed between limnetic males and solitary, benthic-like females (Figure 9.4). However, it seems unlikely that reinforcement could occur in parallel in independent lakes, unless it acted to strengthen mate preferences that had already evolved in parallel as a by-product of adaptation to the environment. Additionally, some premating isolation must have been present at secondary contact to prevent fusion of the two populations because postzygotic isolation, while present, is too weak to prevent fusion on its own (Schluter 1995; Hatfield and Schluter 1999). To determine the relative roles of by-product and direct selection in sympatry requires further studies of the mate preferences of solitary populations that differ in phenotype to establish how much isolation evolved during the allopatric phase. Conclusions as to the relative contribution of mechanisms of adaptive speciation await this data.

We need also to consider that mechanisms other than reinforcement may have caused the enhanced divergence of mate preferences in sympatry. Reproductive character displacement may also occur as a result of fitness costs to individuals that court or spawn with members of the other species. To distinguish between these alternatives remains an interesting possibility that is yet to be explored.

Finally, we have not yet identified all the traits that underlie the parallel mate preferences, although body size is a strong candidate (Borland 1986; Nagel and Schluter 1998). The probability of spawning between ecomorphs within a lake is strongly size dependent in no-choice mating trials, in which hybridization occurs only between the largest individuals of the smallest species (limnetics) and the smallest individuals of the larger species (benthics; Nagel and Schluter 1998). Body size is probably an adaptation to foraging in one or other of the two lake environments (Schluter 1993). However, body size is correlated with a number of other characters that need to be separated to understand their individual roles in causing the isolation.

Regardless of the precise mechanism or the exact traits, we have shown that reproductive isolation evolved in parallel under a common selective regime; this resulted in the repeated evolution of the “same” species in different lakes. Selection leading to premating reproductive isolation probably occurred during two phases, the first in allopatry and the second in sympatry. This combination of roles, in which premating isolation evolves both as a by-product of adaptation and later as a direct adaptive response, may be general and help to explain the high rates of phenotypic divergence and speciation that characterize some of the spectacular adaptive radiations discussed elsewhere in this volume.

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