

FREQUENCY DEPENDENT NATURAL SELECTION DURING CHARACTER DISPLACEMENT IN STICKLEBACKS

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Abstract.—We know little about how natural selection on a species is altered when a closely related species consuming similar resources appears in its environment. In a pond experiment with threespine sticklebacks I tested the prediction that divergent natural selection between competitors is frequency-dependent, changing with the distribution of phenotypes in the environment. Differential growth and survival of phenotypes in a target stickleback population were contrasted between two treatments. In one treatment an offshore zooplankton feeder (the limnetic stickleback species) was added to the same pond as the target. In the other treatment I added the benthic stickleback instead, a species adapted to feeding on invertebrates from sediments and inshore vegetation. The target population was ecologically and morphologically intermediate with phenotypic variance artificially inflated by hybridization. Growth rates of phenotypes within the target population differed between treatments as predicted by character displacement. The impact of adding a second species always fell most heavily on those phenotypes in the target population resembling the added species most closely. However, those individuals in the target population that most resembled the added species did not experience reduced survival. Instead, consistent survival differences between populations suggested the presence of an inshore–offshore gradient in mortality risk. These results provide further support for the hypothesis of character displacement in sympatric sticklebacks. They suggest that displacement along the resource gradient also led to divergence in vulnerability to agents of mortality, probably including predation.

Key words.—Competition, character displacement, natural selection, frequency dependent selection, divergent selection, speciation, adaptive radiation, stickleback.

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The extent to which differences between closely related species are the evolved outcome of ecological interactions between them is not yet known (Thompson 1994; Schluter 2000a). The role of ecological interactions in the origin of species is even less well understood (Doebeli and Dieckmann 2000; Dieckmann et al. 2003). Most research on divergence has centered on resource competition, the antagonistic interaction that arises from depletion of shared resources. Evidence of its role comes from the large list of cases in which sympatric, closely related species show exaggerated differences in morphological traits affecting resource use (Schluter 2000a, b). This evidence is mainly observational, but the sheer number of cases suggests that “ecological character displacement,” phenotypic evolution driven or maintained by interspecific resource competition, is routinely involved in the evolution of species differences. The cases do not tell us much about the role of competition in the origin of species, but they provide indirect support for a central assumption of theories of competitive speciation: that disproportionately strong competition occurs between phenotypically similar individuals (Doebeli and Dieckmann 2000; Dieckmann et al. 2003).

Observational studies nevertheless have limitations that necessitate a complementary, experimental approach. First, the ecological interactions responsible for exaggerated differences of sympatric species have not been confirmed in the majority of cases (Schluter 2000a). Perhaps resource competition is not the only cause. Other candidate interactions include intraguild predation, apparent competition, and reproductive and aggressive interference. Second, deeper understanding of the processes underlying observed patterns and tests of the predictions of character displacement theory

in nature will be difficult to achieve by observational study alone. For example, the following predictions of character displacement theory for quantitative traits (Roughgarden 1976; Slatkin 1980; Abrams 1986; Taper and Case 1985, 1992; Doebeli 1996) have been tested rarely or not at all: (1) the strength of competition between species should decline with time as divergence proceeds (tested by Pacala and Roughgarden 1985; Pritchard and Schluter 2001; Gray and Robinson 2002; Pfennig and Murphy 2002); (2) resource competition should generate divergent natural selection between ecologically similar species (tested by Schluter 1994; Caruso 2000); (3) divergent natural selection generated via resource competition should be frequency dependent, and vary with the distribution of phenotypes in the environment (tested by Pfennig and Murphy 2002); and (4) the strength of divergent selection should first rise and then fall as divergence proceeds, in contrast to the predicted steady decline in the strength of competition (no tests). Abrams (1986) has also shown that under certain conditions competition may result in convergent character displacement, but there are no examples.

Here I present results of an experiment to test the expectation that divergent natural selection during character displacement is frequency-dependent (prediction (3)), changing with the frequency distribution of phenotypes present in the environment. I tested the prediction that selection is frequency dependent by using experimental ponds to contrast the influence of two morphologically different stickleback species on selection in a target, intermediate population. This is a test of frequency dependent selection in the broad sense, whereby alterations in selection occur in response to changes in the frequency distribution of all phenotypes in the envi-

ronment, not just phenotypes within the target population. This usage is consistent with that in the theoretical literature on character displacement, where divergence is predicted to depend on both interspecific and intraspecific frequency dependent selection (Taper and Case 1985; Abrams 1989; Doebeli 1996). Little distinction is made between the two because the source of frequency dependent selection is the same in both cases: it arises from the tendency of similar phenotypes to compete more strongly than different phenotypes regardless of species identity. The distinction between interspecific and intraspecific frequency dependence is further blurred when there is gene flow between competing populations (Dieckmann and Doebeli 1999), as evidence suggests took place during the process of character displacement of these sticklebacks (Rundle and Schluter 2003).

The possibility of ecological character displacement of threespine sticklebacks was first suggested by observational study of populations in small lakes of coastal British Columbia, Canada (Schluter and McPhail 1992). In all lakes containing two stickleback species, one species (the "limnetic") is small and slender, has many long gill rakers and a long narrow snout, and forages mainly on zooplankton in open water (McPhail 1984, 1992, 1994; Schluter and McPhail 1992). The second species, the "benthic", is always larger and more robust than the limnetic, has fewer, shorter gill rakers and a short, wide snout, and forages on invertebrates in the sediments or attached to vegetation. Solitary stickleback populations inhabiting similar lakes tend to be intermediate in morphology and consume both zooplankton and benthic invertebrates (Schluter and McPhail 1992). These differences are genetically based (McPhail 1984, 1992; Hatfield 1997; Peichel et al. 2001).

The present experiment follows an earlier test of prediction (2) in which natural selection on a target intermediate stickleback was contrasted between two treatments, one in which the target was present alone, and another in which the limnetic species was added (Schluter 1994). The density of the target was held constant between treatments to control for intrapopulation competition, with the consequence that adding the limnetic increased total density of fish in one treatment. As predicted, the impact of the addition fell most heavily on those phenotypes within the target population most resembling the added competitor in morphology and habitat use. In contrast, the target phenotypes most different from the limnetic competitor were unaffected by its presence. Strong competition therefore occurred only between neighboring phenotypes, but frequency dependent selection was not tested directly by varying the frequency distribution of competitor phenotypes. The present experiment carries out the crucial test. As in the previous selection experiment I used an intermediate population with inflated levels of phenotypic variance, created using a mixture of hybrids, to increase the sensitivity of measurements of changing natural selection.

MATERIALS AND METHODS

Experimental Ponds

The experiment was carried out in the summer of 1996 in experimental ponds on the campus of the University of Brit-

ish Columbia, Vancouver, BC. Each pond is 23×23 m² with a bottom that slopes gradually from 0 m at the edges to 3 m deep in the center. The ponds were constructed in 1991 and then seeded with plants and invertebrates from Paxton Lake, Texada Island, British Columbia, an 11-ha lake that was also the source of the limnetic and benthic species used in the experiment. The ponds are lined with polyethylene overlaid with 0.25 m of sand, and are bordered with limestone extracted from surface mines near Paxton Lake. Apart from their construction, initialization, and use in prior experiments, the ponds are unmanipulated environments. In other experiments, ponds have sustained thousands of sticklebacks over multiple generations, their life-cycles similar to those of wild fish (unpubl. obs.). All invertebrates found in the diets of experimental fish were characteristic of their wild source populations, including copepods (calanoids, open water cyclopoids, benthic cyclopoids, and harpacticoids), cladocera (chiefly chydorids), ostracods, gammarids, chironomid larvae, and mayfly larvae (unpubl. obs.). Measured growth rates of individual fish in the ponds are not artificially low compared to wild fish (Pritchard and Schluter 2001). Nevertheless, the ponds are younger and smaller than natural lakes, and littoral and open water habitats are in closer proximity. Insect predators (dragonfly nymphs, *Aeshna*, and backswimmers, *Notonecta*) were common in the ponds but the dominant fish predator, cutthroat trout, was absent. Predation by wild birds (kingfishers, herons) was present but sporadic.

Each of three ponds used was divided with an impermeable plastic sheet of 4-mil UV-resistant polyethylene a few days prior to beginning the experiment. Different experimental treatments were then applied to each side. The top edge of each barrier was held above the water surface using styrofoam. The lower edge was fixed to the bottom with a 30 m steel chain and concrete flagstones placed at 1 m intervals by a scuba diver. Barriers were still intact at the end of the experiment, and we detected no contamination of treatments by movement of fish between pond sides.

Fish Populations

The experiment utilized three fish populations: benthics, limnetics, and the target intermediate population. Benthic and limnetic sticklebacks came from Paxton Lake on Texada Island. The ecology and morphology of these populations is similar to that of benthic and limnetic populations from other lakes of the region (Schluter and McPhail 1992), except that the Paxton Lake benthic has exceptionally reduced body armor (McPhail 1992; S. M. Vamosi and D. Schluter unpubl. obs.).

Intermediate sticklebacks came from Cranby Lake, which is located a few hundred meters from Paxton Lake but occurs in a different drainage. Cranby Lake was chosen because it is similar to Paxton Lake in size, vegetation characteristics, and prey types available, and it is likewise productive, spring-fed, and occurs on the same limestone substrate. Phylogenetically, the Cranby Lake population is closest to the benthic species in Priest Lake, also on Texada Island, and is roughly equidistant from the Paxton benthic and Paxton limnetic species (Taylor and McPhail 2000).

I used hybridization between populations to inflate levels

of phenotypic variance in feeding-related traits in the target intermediate population, to increase the sensitivity of measurements of natural selection (Schluter 1994; for a review of the pros and cons of manipulating phenotypes in studies of natural selection, see Sinervo and Basolo 1996). Hybridization is a reasonable tactic because F_1 offspring of crosses between local stickleback populations have fitness similar to those of parental populations when all are raised in the laboratory (Hatfield and Schluter 1999). F_1 -hybrid offspring between stickleback species may have reduced fitness in the wild, but this appears to stem from their intermediate morphology rather than from intrinsic incompatibilities between parental genomes (Hatfield and Schluter 1999; Rundle 2002). Finally, unanticipated effects of hybridization per se on growth and survival are controlled in the experimental design because they are common to both treatments. F_1 -hybrid offspring were made by artificially crossing females from Cranby Lake with males from either the limnetic ($C \times L$ cross) or the benthic species ($C \times B$) from Paxton Lake. The target intermediate population consisted of a mixture of equal numbers of $C \times L$, $C \times B$, and offspring from crosses between Cranby individuals ($C \times C$). By crossing males to the same female population, egg size (Kassen et al. 1995), and larval size differences between crosses were minimized.

Crosses were made in early May by stripping eggs from wild-caught females and fertilizing them with the minced testes of euthanized males. Females were returned to their native lake. Clutches of up to six females were fertilized by a single male to minimize the number of males euthanized. Eggs were transported to the University of British Columbia and raised as described in Hatfield and Schluter (1999). The fish were added to experimental ponds about one month after hatching.

Design of Experiment

A split-pond design with two treatments was used. The target intermediate population was introduced to both sides of three divided ponds. The benthic species was added to one side of each pond, randomly chosen, and the limnetic species was added to the other side (designated hereafter as the +benthic and +limnetic treatments). The starting density of target fish was the same on both sides, ensuring that any treatment effects detected could be attributed solely to the added competitors. Because equal numbers of competitors were added under both treatments, total fish density was also the same on both sides of each pond at the start of the experiment. The design thus tested directly whether the frequencies of different phenotypes of fish present in the ponds made a difference to selection. Experimental fish were added on June 19–21, 1996, about one month after hatching. One thousand five hundred individuals of the target intermediate population, plus 1000 individuals of the putative competitor (benthic or limnetic), were added to every half-pond.

After three months, fish were retrieved from the ponds for counting and measurement. Twelve minnow traps were set overnight in each half-pond to retrieve as many as a third of the fish present. The following day, 0.5 kg of 5% rotenone (Syndel Laboratories, Vancouver, BC) was stirred into each half pond using an electric outboard motor. We used dip nets

to collect fish as they swam to the surface. Fish that sank to the bottom were retrieved later the same day. Surveys of the pond on the following days uncovered few additional specimens, and we are confident that our method recovered the large majority of fish from the ponds. To minimize variability in capture success between replicates, the same capture effort was applied to both sides of a given pond by the same individual workers on the same day. Captured fish were anesthetized with MS-222 and then fixed in 10% formalin. Fish were removed from formalin after two weeks and rinsed overnight with tap water. They were then stained in a 2% potassium hydroxide and alizarin red solution for 24 h to stain bone tissue and facilitate measurement. Fish were finally preserved in 38% isopropyl alcohol.

Fish Identification

My analyses were carried out on a random sample of 100 individuals from each pond side, 600 fish in total. These fish were identified as benthics, limnetics, and target fish (the intermediate population) according to discriminant functions generated from measurements of 240 fish of known identity (50 limnetics, 63 benthics, and 127 target intermediates) preserved and stained in the same way as experimental fish. Known limnetics and benthics were from single-species pond populations established in a separate experiment. Known target intermediate fish were laboratory raised. Fish are identified at this stage because their small size prevents us from marking juveniles prior to pond introduction.

Using a microscope and digital calipers, a single researcher measured standard length of every fish along with the number of lateral plates on both sides of the body, the number of gill rakers on the first branchial arch of the left side, and the length of the first dorsal spine. Fish were then photographed using a Nikon D1 color digital camera. Each fish was placed on its left side on a flat surface between two mirrors angled outward at 45 degrees, such that a photograph taken from above obtained dorsal, ventral, and lateral views simultaneously.

Separate discriminant functions were generated to distinguish benthics from target fish in the +benthic treatment and limnetics from target fish in the +limnetic treatment, because different suites of traits are informative in the two cases. In each analysis the fish of known identity provided the “training” dataset used to create a discriminant function that was subsequently applied to the fish retrieved from ponds. Because the known target fish were raised in the laboratory instead of the ponds, it is conceivable that the effects of developmental environment on morphological traits (Day et al. 1994) would lead to errors in the identification of pond-raised target fish. I address this possibility later in this section. I used cross-validation to assess the accuracy of the discriminant functions (Venables and Ripley 1998). The method involves leaving out each observation one at a time and predicting the class of the “new” observation using the discriminant function calculated with the remaining observations. In the training dataset the classifications successfully removed limnetics from the mixture of known limnetics and target fish, and benthics from the set of known benthics and

target fish, with 100 percent accuracy. All analyses were carried out using S-Plus 6.1 (Insightful Corp. 2002).

Only two armor traits discriminated benthics from target fish: presence/absence of a pelvic girdle and presence/absence of the first dorsal spine. All 63 known benthics entirely lacked a pelvic girdle and/or they lacked the first dorsal spine. In contrast, all 127 laboratory-raised target fish possessed both a pelvic girdle and the first dorsal spine. Benthics also had few or no lateral plates compared to target fish.

Discrimination of limnetics and target fish was based on body shape. Limnetics have unusually narrow and slender bodies, large eyes, a long snout, and many gill rakers compared with other stickleback populations, including the target forms. Shape of individuals was quantified using the program tpsDig (vers. 1.31; Rohlf 2001) to record X and Y coordinates of thirteen landmarks on the dorsal and lateral images of specimens. All landmarks were located at margins of bone tissue. These coordinates were used to calculate a total of 15 Euclidean distances between pairs of landmarks, yielding several dimensions of body depth, snout length, and orbit diameter. These distances (hereafter, traits) were corrected for body size before use in the discriminant function, as follows. First, each trait was regressed against standard length using only the known limnetic fish. Next, this regression was used to predict the trait values of all limnetics and target fish from their standard lengths. Finally, the size-corrected measurement for every individual was calculated as the difference between its observed and predicted values of the trait. The number of gill rakers (uncorrected for body size) was included in the discriminant function.

A possible concern with this method to identify fish is that the known crosses and experimental fish were not raised in identical environments, which could lead to errors in identification if phenotypic plasticity of traits is present (Day et al. 1994). Limnetics and benthics were raised in ponds without competing species; known target fish were laboratory raised (known pond-raised target fish were unavailable). This concern is most relevant when separating limnetics from target fish. The separation of benthics from target fish was based on armor traits that are not known to be greatly sensitive to growth environment (Day et al. 1994). For example, almost all benthics from Paxton Lake lack a pelvic girdle whether they are raised in the native lake, the ponds, or the laboratory (D. Schluter, unpubl. obs.). Development of gill raker number is also resilient to diet, but the other shape traits used to discriminate limnetics from target fish might be less so (e.g., snout length; Day et al. 1994).

However, it is unlikely that effects of phenotypic plasticity were large enough to create many errors in identification of limnetics and target fish in the experimental ponds. Although limnetics raised on a benthic diet show greater resemblance to benthics than otherwise, the effect is small in traits that greatly distinguish the species (Day et al. 1994). Additionally, body shape of limnetic and intermediate species raised in the laboratory are readily distinguishable by eye even though all fish are raised on the same standard diet (D. Schluter, unpubl. obs.) Finally, the potential consequences of phenotypic plasticity were assessed with a further discriminant analysis that used only experimental pond fish. First, I identified and removed the benthics from the +benthic treatment

samples on the basis of armor traits, as described above. Second, I carried out a discriminant analysis on gill raker number and the shape traits to distinguish remaining fish between the two pond treatments. I used a quadratic discriminant function because the dispersion of observations was expected to be greater among fish from the +limnetic treatment (because limnetics as well as target fish were present) than fish from the +benthic treatment (which now included only target fish). As anticipated, the resulting posterior probabilities of assignment to the +limnetic treatment had a sharply bimodal distribution in the +limnetic treatment, with a clear gap separating the modes, but a unimodal distribution in the +benthic treatment. Most fish in both treatments, those resembling known target fish in morphology, could not be confidently assigned a posteriori to one or the other treatment (modal posterior probability = 0.62; this number departs from 0.5 because of unequal numbers of target fish in the samples from the two treatments). However, fish resembling known limnetics in the +limnetic treatment were confidently assigned to the +limnetic treatment (modal posterior probability = 0.96). Identifying fish as limnetic or target according to these posterior probabilities produced virtually the same classification as the previous analysis that was based on the training data set of known fish raised outside the experiment. Only seven of 300 fish were reassigned in the present analysis (all seven were target fish now classified as limnetics). Elimination of these seven observations altered none of the experimental results described below. I therefore concluded that effects of phenotypic plasticity were too small to complicate identification of target and nontarget fish from ponds.

Measuring Selection on Target Phenotypes

The prediction of frequency dependent natural selection was tested by comparing growth rate and survival of target phenotypes between treatments. The target fish from each pond side were analyzed as a single population with high variability (Schluter 1994). To compare growth and survival, a composite phenotype axis was created that put the most benthic-like individuals within the target population at one extreme (relatively deep bodies, short, wide snouts, and few, short gill rakers), the most limnetic-like individuals within the target population at the other extreme (relatively slender bodies, long, narrow snouts, long numerous gill rakers), and intermediate phenotypes in the middle. This was accomplished using two traits known to differ between the three cross types comprising the target population but, unlike many of the traits of interest, are unaffected by developmental shifts in response to diet (Day et al. 1994): gill raker number and number of armor plates (Schluter 1994). Relative to other phenotypes, $C \times L$ individuals have many gill rakers and many plates, $C \times B$ individuals have few of both, and $C \times C$ individuals have few gill rakers but many plates. The composite phenotype axis was computed as the first discriminant function separating laboratory-raised $C \times B$ and $C \times L$ individuals in these two traits. Function scores were then also computed for $C \times C$ individuals, confirming that they had an intermediate range of phenotypes. Finally, scores were computed for all target fish retrieved from the ponds at the

end of the experiment. All analyses of growth and survival of target phenotypes were carried out on this composite phenotype index.

Analysis of Growth and Survival

Measures of growth and survival were taken on the random sample of 100 fish from each pond side. I used final size (standard length) of individual fish as the measure of growth rate, since starting size was small and similar between crosses. Growth rate in stickleback is highly correlated with food intake (Schluter 1995), and in fish in general growth rate is closely linked to fitness through its effects on overwinter survival, fecundity, mating success, and breeding date (Shuter and Post 1990; Schultz et al. 1991; Conover 1992; T. Hatfield and D. Schluter, unpubl. obs.).

Relative survival of limnetic, benthic, and target fish was estimated as the proportion of individuals in the random samples of 100 fish identified from each pond side. Absolute survival was estimated by multiplying these proportions by the total number of fish retrieved from each pond side at the end of the experiment, divided by the number introduced at the start of the experiment. This measure is an underestimate because an unknown (but relatively small) number of fish in each pond escaped capture when the experiment was terminated. However, the same effort was applied to each pond side when retrieving fish, so bias should be similar between treatments.

I tested for treatment effects using paired *t*-tests having two degrees of freedom, using the three ponds as replicates. Growth was ln-transformed prior to analysis. Survival proportions were arcsine square root transformed prior to paired *t*-tests. The prediction of frequency-dependent selection is one sided and therefore I used one-tailed statistical tests; all other tests are two-tailed. All analyses were carried out using S-Plus 6.1 (Insightful Corp. 2002).

RESULTS

I tested treatment effects on *mean* growth and survival ("fitness") of the target population and on *relative fitness of different phenotypes* within the target population. Differences between treatments in mean fitness would indicate that the net effect of an interaction between target and nontarget fish was frequency dependent. Differences between treatments in relative fitness of different phenotypes would indicate that natural selection is frequency dependent, that is, that selection changed with the phenotype of the added competitor (benthic vs. limnetic). The present experiment was designed to test the prediction of frequency dependent selection, but effects on mean fitness are also tested because they might be informative about the ecological interactions generating selection. For example, there should be no difference in mean fitness of target fish between treatments if the net strength of resource competition experienced by the target population is the same regardless of which competitor is added. Detection of a difference in mean fitness would thus indicate more complex interactions between the target and different nontarget populations.

Growth Rate

There was a tendency for mean growth rate (ln-transformed final standard length) of target fish to be higher in the +limnetic sides of ponds than in the +benthic sides (Table 1, Fig. 1). The tendency was variable between ponds and the overall treatment effect on mean growth was small and not significant (paired $t = 2.48$, $df = 2$, $P = 0.13$). Therefore, the average growth reduction experienced by target individuals was roughly similar regardless of whether the limnetic or the benthic competitor species was added to ponds.

However, the relative growth rate of different phenotypes within the target population was strongly affected by whether the limnetic or the benthic competitor species was added (Table 1, Fig. 1). Linear regressions of ln-transformed growth rate on composite phenotype differed consistently between treatments in the direction predicted by character displacement. Relative to the +benthic treatment, adding limnetics to a pond side depressed the growth rate of the most limnetic-like phenotypes within the target population. Conversely, relative to the +limnetic treatment, adding benthics reduced growth of the most benthic-like phenotypes in the target population. A paired *t*-test on regression slopes confirms a highly significant treatment effect on the direction of selection ($t = 6.31$, $df = 2$, $P = 0.006$, one-tailed test). Selection on phenotypes was therefore frequency dependent, as predicted by character displacement, changing with the phenotype of the added competitor.

Survival

Treatment effects on survival of target fish did not mirror those on growth, suggesting different causes. There was no significant treatment effect on selection arising from differences in survival (Table 2; one-tailed paired *t*-test on mean phenotypes of survivors, $t = 0.16$, $df = 2$, $P = 0.89$). Directional selection was not constant everywhere, but the major differences were between ponds not between treatment sides within ponds (Table 2). Survival selection was therefore not frequency dependent.

Mean relative survival in the target population nevertheless depended strongly on the phenotype of the added competitor (Table 3). Target individuals made up fewer than 60 percent of the fish retrieved from the +benthic sides of ponds, their percentage at the start of the experiment, whereas in the +limnetic pond sides the proportion of target individuals at the end of the experiment was always higher than 60%. This difference in relative survival of target fish represented a significant treatment effect (paired $t = 4.84$, $df = 2$, $P = 0.040$).

Changes in absolute survival of the competitor species was probably the major determinant of change in relative survival of target fish, although changes in absolute survival of target fish may also have contributed (Table 4). Survival of limnetics was significantly worse than survival of benthics (paired $t = 5.21$, $df = 2$, $P = 0.035$). Whereas 40–50% of benthics survived the experiment, fewer than 30% of the limnetics did so (Table 4). Absolute survival of target fish tended to be higher in the +limnetic treatment than in the +benthic treatment in all three ponds, but the tendency was

TABLE 1. Growth measurements of target fish in different treatment sides of ponds. Mean growth is the mean of ln-transformed standard length, originally in mm (\pm SE). Slopes are from separate linear regressions of ln-transformed standard length against composite phenotype (\pm SE). Sample sizes (n) are the numbers of target fish in random samples of 100 fish from each pond side. \bar{X} is the sample mean of the three pond values.

| Pond | Mean growth | | | Slope | | | n | |
|-----------|-------------|-------------|----------------|----------------|------------|------------|-----------|------------|
| | + benthic | + limnetic | + benthic | + benthic | + limnetic | + limnetic | + benthic | + limnetic |
| 1 | 3.71 (0.01) | 3.75 (0.01) | -0.005 (0.008) | -0.020 (0.008) | 56 | 78 | | |
| 2 | 3.61 (0.01) | 3.62 (0.01) | -0.001 (0.011) | -0.016 (0.009) | 44 | 66 | | |
| 3 | 3.70 (0.01) | 3.74 (0.01) | -0.016 (0.009) | -0.025 (0.009) | 54 | 65 | | |
| \bar{X} | 3.69 (0.03) | 3.70 (0.04) | -0.007 (0.005) | -0.020 (0.003) | | | | |

variable and there was no treatment effect overall (paired $t = 2.31$, $df = 2$, $P = 0.15$).

DISCUSSION

Each pair of threespine stickleback species found in small coastal lakes of British Columbia apparently formed as a consequence of two invasions of freshwater by the zooplanktivorous marine species near the end of the last ice age (Schluter and McPhail 1992; McPhail 1994; Taylor and McPhail 2000; Rundle and Schluter 2003). One explanation for the phenotypic changes that ensued, developed from observational data, invokes both adaptation to distinct freshwater habitats and competition for resources (Schluter and McPhail 1992; McPhail 1994). In this scenario, adaptation to the new lake environment produced an intermediate phenotype in the first colonist, allowing exploitation of both offshore zooplankton and benthic invertebrates. In lakes colonized twice, competition between the intermediate resident and the newly added marine zooplanktivore displaced the resident toward a benthic phenotype (the progenitor of the benthic species) and caused the second invader (the progenitor of the limnetic species) to remain specialized on zooplankton. Assortative mating between the sympatric species was probably incomplete but strengthened over time (Rundle and Schluter 1998), perhaps aided by competition which reduced hybrid fitness. Such scenarios involving competition have been suggested for phenotypic divergence and speciation in many adaptive radiations (Schluter 2000a), yet have rarely been tested. The stickleback system provides a model for experimental tests of the consequences of species addition on interactions, natural selection, and divergence.

In the present experiment, the growth impacts of species addition on the target population fell most heavily on those phenotypes resembling the added species most closely, as predicted by character displacement theory. The result was that patterns of selection on the target population differed consistently between treatments. Starting densities of the target fish, as well as total fish density per replicate, were identical between treatments, and therefore changes in relative growth rates of different phenotypes in the target population are attributable to differences between treatments in the frequency distribution of phenotypes present. Competition was consistently strongest between neighboring phenotypes and weakest between distant phenotypes. These results support the hypothesis developed from observational data that competition for resources promoted ecological and phenotypic divergence between sympatric species.

Without a third treatment in which the target species was present alone, I cannot identify which of the two added species, limnetic or benthic, generated stronger selection on the target intermediate population. However, the mean growth rate was roughly similar between treatments, in contrast to a previous experiment in which total density also varied between treatments (Schluter 1994). This suggests that both benthic and limnetic species affected mean growth in the target population, and therefore probably selection too.

A conceivable alternative mechanism to explain the results is that the added species were mutualists of the target population, rather than competitors, and that the beneficial effects

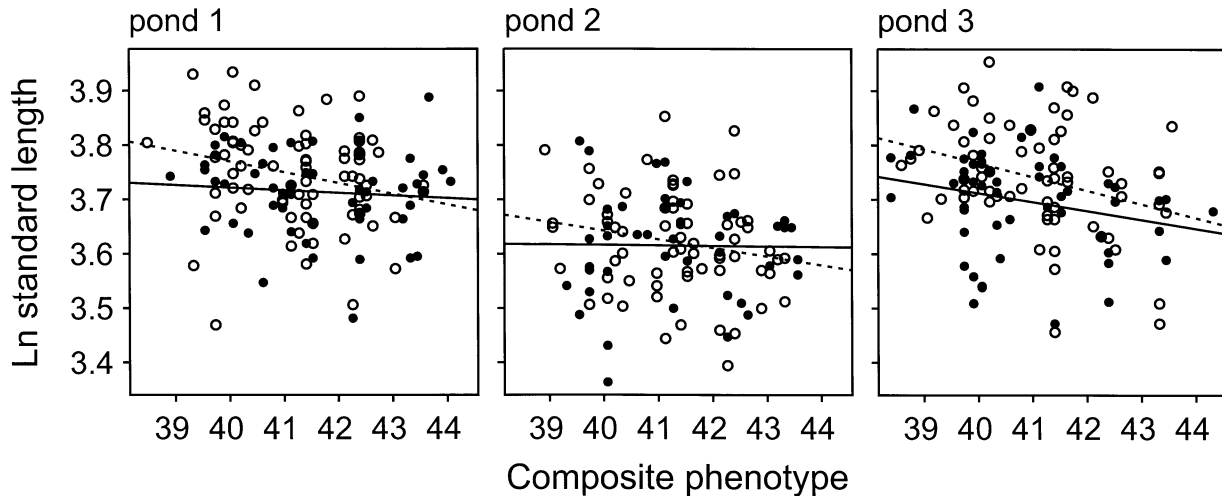


FIG. 1. Relationship between ln-transformed final size and composite phenotype in the target intermediate population in the two treatments. In each panel (pond), the most benthic-like phenotypes are on the left and the most limnetic-like phenotypes are on the right. Points are individual fish. Lines are linear regressions of size on phenotype for each treatment: +benthic (filled circles and solid line) and +limnetic (open circles and dashed line).

of species addition were felt most strongly by those phenotypes within the target population most distant from the added species. Besides lacking an obvious mechanism, this alternative is inconsistent with previous experiments which show that adding a species to a pond always decreases mean growth rate of other populations present, implying competition (Schluter 1994; Pritchard 1998; Pritchard and Schluter 2001; Rundle 2002).

The growth results corroborate those of a previous experiment that contrasted selection on the same target intermediate population in the presence and absence of the limnetic species only (Schluter 1994). Adding the limnetic depressed the growth rates of those individuals in the target population resembling it most closely, but did not affect the most benthic-like phenotypes in the target population. Because the starting density of the target fish was held constant between treatments, to control for intraspecific competition, adding the limnetic species increased total fish density in that treatment. It could always be argued that the changes in selection accompanying species addition might have been the same regardless of which species was added (i.e., that selection was only density dependent not frequency dependent; Bernardo et al. 1995). Results of the present experiment confirm that adding a different competitor having a different phenotype changes the pattern of selection. In the context of the double invasion scenario outlined above, the results imply that the phenotype of the second colonist to the lakes was

TABLE 2. Mean composite phenotype of surviving target fish. Sample sizes are in Table 1. \bar{X} is the sample mean of three pond values.

| Pond | Treatment | |
|-----------|-------------|-------------|
| | +benthic | +limnetic |
| 1 | 41.6 (0.18) | 41.2 (0.13) |
| 2 | 41.3 (0.19) | 41.3 (0.13) |
| 3 | 40.8 (0.19) | 41.1 (0.16) |
| \bar{X} | 41.2 (0.23) | 41.2 (0.18) |

crucial to generating the shift from an intermediate phenotype to a benthic phenotype in the prior resident.

The demonstration of frequency-dependent selection also verifies an important assumption of models of competitive speciation: that strongest competition occurs between phenotypically most similar individuals. According to competitive speciation theory, disproportionately strong competition against intermediate phenotypes generates the disruptive selection against intermediate phenotypes that might favor the evolution of premating isolation in sympatry (Dieckmann and Doebeli 1999; Doebeli and Dieckmann 2000). Sympatric sticklebacks do not strictly conform to this conceptual model of speciation, because most molecular, geological, and physiological data suggest a process of species origination that included an allopatric phase (summarized in Rundle and Schluter 2003). Nevertheless, it is likely that premating isolation was incomplete at the time of secondary contact (Rundle and Schluter 1998, 2003), leaving open the possibility that persistence and further divergence of species were aided by competition against intermediate phenotypes (including hybrids).

Three predictions of ecological character displacement have now been tested experimentally in these sticklebacks. Pritchard and Schluter (2001) showed that competition be-

TABLE 3. Relative survival of target fish, estimated as the proportion of target fish in random samples of 100 fish retrieved from each pond side at the end of the experiment (\pm SE). The proportion of target fish at the start of the experiment was 0.60 (1500 target fish of 2500 total individuals introduced to each pond side). \bar{X} is the sample mean of three pond values.

| Pond | Treatment | |
|-----------|-------------|-------------|
| | +benthic | +limnetic |
| 1 | 0.56 (0.05) | 0.78 (0.04) |
| 2 | 0.44 (0.05) | 0.66 (0.05) |
| 3 | 0.54 (0.05) | 0.65 (0.05) |
| \bar{X} | 0.51 (0.04) | 0.70 (0.04) |

TABLE 4. Absolute survival of each cross type (\pm SE) in the two treatments. Absolute survival was estimated from relative survival (Table 3) and the total number of fish retrieved from each pond side at the end of the experiment. Standard errors incorporate sampling error in the estimate of relative survival only. \bar{X} is the sample mean of three pond values.

| Pond | +benthic treatment | | +limnetic treatment | |
|-----------|--------------------|-------------|---------------------|-------------|
| | Benthic | Target | Target | Limnetic |
| 1 | 0.49 (0.06) | 0.50 (0.04) | 0.58 (0.03) | 0.20 (0.04) |
| 2 | 0.46 (0.04) | 0.24 (0.03) | 0.33 (0.02) | 0.26 (0.04) |
| 3 | 0.43 (0.05) | 0.34 (0.03) | 0.35 (0.03) | 0.28 (0.04) |
| \bar{X} | 0.46 (0.02) | 0.36 (0.08) | 0.42 (0.08) | 0.25 (0.02) |

tween the marine species and the target intermediate population was stronger than that between the marine species and the benthic species, in accord with the prediction that competition strength declines during character displacement. Second, Schluter (1994) showed that natural selection on the intermediate species is altered when the limnetic species is added to its environment, favoring divergence. Finally, the present experiment confirmed that divergent selection is frequency dependent. In combination with observational evidence, the results from these experiments provide critical evidence in support of the hypothesis of character displacement in sticklebacks.

These findings match the results of the few tests of predictions of character displacement from other systems. The prediction that character displacement should yield lower competition between species has been confirmed for Caribbean *Anolis* lizards (Pacala and Roughgarden 1985); brook and ninespine sticklebacks (*Culaea* and *Pungitius*, respectively, Gray and Robinson 2002); and spadefoot toads, *Spea* (Pfennig and Murphy 2002). Evidence that competition should generate divergent natural selection between competing species has been found in an observational study by Caruso (2000), who detected divergent selection on floral traits in populations of *Ipomopsis aggregata* sympatric with *Castilleja linariaefolia* but not in allopatric *I. aggregata* populations. Pfennig and Murphy (2002) found that the magnitude of the difference in percent carnivory between tadpole populations of two species of spadefoot toad, *Spea bombifrons* and *S. multiplicata*, correlated with the frequency of *S. bombifrons*, indirectly suggesting that strength of divergent selection is frequency dependent. Differences between populations in percent carnivory are partly genetic, and thus represent evolutionary divergence not just phenotypic plasticity (Pfennig and Murphy 2002). The *Spea* work and the present stickleback study are the only studies addressing frequency-dependent divergent selection on quantitative traits in character displacement. Nevertheless, studies of fitnesses of alternative discrete morphs within species provide additional examples of frequency-dependent selection arising from resource competition (Pfennig 1992; Hori 1993; Benkman 1996). In every case, individuals of the same morph compete more strongly than individuals of different morphs, favoring the rarer morph and promoting the maintenance of the intraspecific diversity.

Results on differential survival of target individuals in the present experiment did not match results for growth. The

earlier experiment of Schluter (1994) saw a similar outcome. Apparently, differential growth rate of phenotypes in a treatment does not directly lead to a similar pattern in survival, at least over the short time frame of these pond experiments (three months within a summer). The implication is that patterns of short-term survival are not strongly influenced by competition for resources, but have other causes instead. Predation on sticklebacks is a leading candidate. Although natural vertebrate predators of these stickleback populations are infrequent (diving birds) or absent (cutthroat trout) from the ponds, natural insect predators (mainly notonectids and odonates) are abundant and probably represent the major source of mortality of juvenile stickleback (Reimchen 1980, 1994; Foster et al. 1988; Vamosi 2001). Notonectids (backswimmers) have frequently been observed to prey upon juvenile sticklebacks in the ponds (D. Schluter, pers. obs.).

Whatever the cause, variation between cross types was the most noticeable source of variation in mean survival. Survival rates in the phenotypically intermediate target population were intermediate between the high survival of benthic individuals and the low survival of limnetics (Table 4). This suggests a gradient in mortality risk that is correlated with phenotype and habitat use. Limnetics, which use the open water habitat, are the most vulnerable, whereas benthics, which use mainly the vegetated littoral habitat, are least vulnerable. A pond experiment with the native fish predator, cutthroat trout, has previously shown limnetics to be more vulnerable to predation than benthics (Vamosi and Schluter 2002). If such a gradient is verified in nature, then it would imply that character displacement in sticklebacks led to more than simply a partitioning of resources, but also to a contrast in vulnerability to different agents of mortality. The role of predation and other agents of mortality on divergence between sympatric stickleback species deserves direct study, because such factors may assist or ameliorate resource competition and thereby influence character displacement.

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LITERATURE CITED

- Abrams, P. A. 1986. Character displacement and niche shift analysed using consumer-resource models of competition. *Theor. Popul. Biol.* 29:107–160.
- . 1989. The importance of intraspecific frequency-dependent selection in modeling competitive coevolution. *Evol. Ecol.* 3: 215–220.
- Benkman, C. W. 1996. Are the ratios of bill crossing morphs in crossbills the result of frequency-dependent selection? *Evol. Ecol.* 10:119–126.
- Bernardo, J., W. J. Resetarits, Jr., and A. E. Dunham. 1995. Criteria for testing character displacement. *Science* 268:1065–1066.
- Caruso, C. M. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. *Evolution* 54: 1546–1557.

- Conover, D. O. 1992. Seasonality and the scheduling of life history at different latitudes. *J. Fish Biol.* 41 Suppl. B:161–178.
- Day, T., J. Pritchard, and D. Schluter. 1994. Ecology and genetics of phenotypic plasticity: a comparison of two sticklebacks. *Evolution* 48:1723–1734.
- Dieckmann, U., and M. Doebeli, 1999. On the origin of species by sympatric speciation. *Nature (London)* 400:354–357.
- Dieckman, U., A. J. Metz, M. Doebeli, and D. Tautz. Eds. 2003. Adaptive speciation. Cambridge Univ. Press, Cambridge, U.K.
- Doebeli, M. 1996. An explicit genetic model for ecological character displacement. *Ecology* 77:510–520.
- Doebeli, M. and U. Dieckmann. 2000. Evolutionary branching and sympatric speciation caused by different types of ecological interactions. *Am. Nat.* 156(Suppl.):S77–S101.
- Foster, S. A., V. B. Garcia, and M. Y. Town. 1988. Cannibalism as the cause of an ontogenetic shift in habitat use by fry of the threespine stickleback. *Oecologia* 74:577–585.
- Gray, S. M., and B. W. Robinson 2002. Experimental evidence that competition between stickleback species favours adaptive character divergence. *Ecol. Lett.* 5:264–272.
- Hatfield, T. 1997. Genetic divergence in adaptive characters between sympatric species of sticklebacks. *Am. Nat.* 149:1009–1029.
- Hatfield, T., and D. Schluter. 1999. Ecological speciation in sticklebacks: environment-dependent hybrid fitness. *Evolution* 53:866–873.
- Hori, M. 1993. Frequency dependent natural selection in the handedness of scale-eating cichlid fish. *Science* 260:216–219.
- Kassen, R., D. Schluter, and J. D. McPhail. 1995. Evolutionary history of threespine sticklebacks (*Gasterosteus* spp.) in British Columbia: insights from a physiological clock. *Can. J. Zool.* 73:2154–2158.
- Insightful Corp. 2002. S-Plus 6.1 for Windows, professional ed. release 1. Insightful Corp. Seattle, WA.
- McPhail, J. D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. *Can. J. Zool.* 62:1402–1408.
- . 1992. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): evidence for genetically divergent populations in Paxton Lake, Texada Island, British Columbia. *Can. J. Zool.* 70:361–369.
- . 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of southwestern British Columbia. Pp. 399–437 in M. A. Bell and S. A. Foster, eds. *Evolutionary biology of the threespine stickleback*. Oxford Univ. Press, Oxford, U.K.
- Pacala, S. W., and J. Roughgarden. 1985. Population experiments with the *Anolis* lizards of St. Maarten and St. Eustacius. *Ecology* 66:129–141.
- Peichel, C. L., K. S. Nereng, K. A. Ohgi, B. L. E. Cole, P. F. Colosimo, C. A. Buerkle, D. Schluter, and D. M. Kingsley. 2001. The genetic architecture of divergence between threespine stickleback species. *Nature* 414:901–905.
- Pfennig, D. W. 1992. Polyphenism in spadefoot toad tadpoles as a locally adjusted evolutionarily stable strategy. *Evolution* 46:1408–1420.
- Pfennig, D. W., and P. J. Murphy. 2002. How fluctuating competition and phenotypic plasticity mediate species divergence. *Evolution* 56:1217–1228.
- Pritchard, J. 1998. Competition and character displacement in sticklebacks. Ph.D. diss., University of British Columbia, Vancouver, BC.
- Pritchard, J. R., and D. Schluter. 2001. Declining competition during character displacement: summoning the ghost of competition past. *Evol. Ecol. Research* 3:209–220.
- Reimchen, T. E. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaptation to predation? *Can. J. Zool.* 58:1232–1244.
- . 1994. Predators and morphological evolution in threespine stickleback. Pp. 240–276 in M. A. Bell and S. A. Foster, eds. *The evolutionary biology of the threespine stickleback*. Oxford Univ. Press, Oxford, U.K.
- Rohlf, F. J. 2001. tpsDig.vers. 1.31. Distributed by author; <http://life.bio.sunysb.edu/morph/soft-dataacq.html>.
- Roughgarden, J. 1976. Resource partitioning among competing species: a coevolutionary approach. *Theor. Popul. Biol.* 9:388–424.
- Rundle, H. D. 2002. A test of ecologically dependent postmating isolation between sympatric sticklebacks. *Evolution* 56:322–329.
- Rundle, H. D., and D. Schluter. 1998. Reinforcement of stickleback mate preferences: sympatry breeds contempt. *Evolution* 52:200–208.
- . 2003. Natural selection and speciation in sticklebacks from beginning to end. In U. Dieckmann, A. J. Metz, M. Doebeli, and D. Tautz, eds. *Adaptive speciation*. Cambridge Univ. Press, Cambridge, U.K. *In press*.
- Schluter, D. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. *Science* 266:798–801.
- . 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. *Ecology* 76:82–90.
- . 2000a. The ecology of adaptive radiation. Oxford Univ. Press, Oxford, U.K.
- . 2000b. Ecological character displacement in adaptive radiation. *Am. Nat.* 156(Suppl.):S4–S16.
- Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. *Am. Nat.* 140:85–108.
- Schultz, E. T., L. M. Clifton, and R. R. Warner. 1991. Energetic constraints and size-based tactics: the adaptive significance of breeding-schedule variation in a marine fish (Embiotocidae: *Micrometrus minimus*). *Am. Nat.* 138:1408–1430.
- Shuter, B. J., and J. R. Post. 1990. Climate, population viability, and the zoogeography of temperate fishes. *Trans. Am. Fish. Soc.* 119:314–336.
- Sinervo, B., and A. L. Basolo. 1996. Testing adaptation using phenotypic manipulations. Pp. 149–185 in M. R. Rose and G. V. Lauder, eds. *Adaptation*. Academic Press, San Diego, CA.
- Slatkin, M. 1980. Ecological character displacement. *Ecology* 61:163–177.
- Taper, M. L., and T. J. Case. 1985. Quantitative genetic models for the coevolution of character displacement. *Ecology* 66:355–371.
- . 1992. Coevolution among competitors. *Oxford Surv. Evol. Biol.* 8:63–109.
- Taylor, E. B., and J. D. McPhail. 2000. Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus*. *Proc. R. Soc. Lond. B Biol. Sci.* 267:2375–2384.
- Thompson, J. N. 1994. *The coevolutionary process*. Chicago Univ. Press, Chicago, IL.
- Vamosi, S. M. 2001. The role of predation in the evolution of sympatric stickleback species. Ph.D. diss., University of British Columbia, Vancouver, BC.
- Vamosi, S. M., and D. Schluter. 2002. Impacts of trout predation on fitness of sympatric sticklebacks and their hybrids. *Proc. R. Soc. Lond. B Biol. Sci.* 269:923–930.
- Venables, W. N., and B. D. Ripley. 1998. *Modern applied statistics with S-Plus*. Springer, Berlin.

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