

REPORT

Experimental evidence that competition between stickleback species favours adaptive character divergence

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Abstract

The ecological character displacement hypothesis assumes that the effects of interspecific resource competition cause divergent selection to favour phenotypes that exploit non-shared resources. This model predicts that interspecific competition declines with increased divergence. Direct tests of this decline are rare despite much comparative evidence for character displacement. We tested this prediction using a pair of divergent brook sticklebacks. Brook sticklebacks sympatric with ninespine sticklebacks have diverged from local allopatric brook populations, and so the two types of brook sticklebacks potentially represent pre- and post-displacement forms. We used enclosures placed in a lake to compare short-term fitness (growth) of sympatric (post-displacement) and allopatric (pre-displacement) brook forms in the presence and absence of ninespine sticklebacks. Brook sticklebacks grew less in the presence vs. absence of ninespine sticklebacks, indicating that interspecific competition occurred. As expected, allopatric brook forms had lower growth than sympatric forms when ninespine sticklebacks were present. This result suggests that ecological character displacement has occurred.

Keywords

Adaptive divergence, *Culaea inconstans*, ecological character displacement, field experiment, frequency-dependent selection, interspecific competition, *Pungitius pungitius*.

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INTRODUCTION

Ecological character displacement (ECD) is the evolutionary divergence of traits related to resource use caused by competition for those resources with an ecologically similar species (Brown & Wilson 1956; Grant 1972; Schluter & McPhail 1992). ECD theory predicts that the impact of competition (the depletion of shared resources) will vary among the different phenotypes of a population, causing divergent selection that disfavors those phenotypes most closely resembling the competitor. Only under this condition of frequency-dependent selection are traits related to resource use expected to diverge between competing species. Relatively few direct tests of interspecific competition have been performed in putative ECD systems, and only three have examined the assumption that frequency-dependent selection has led to divergence under natural conditions (Schluter 2000a).

If divergence has ultimately been caused by competition, then we expect that the effects of competition will

decline with the divergence of the species. This prediction can potentially be tested using populations that represent pre-displacement and post-displacement phenotypes (i.e. summoning Connell's 1980 "ghost of competition past"). However, such a test faces two difficulties. The first is finding suitable divergent populations, and the second is an appropriate experimental design. Previous studies have either used field enclosures (Pacala & Roughgarden 1985), or large constructed semi-natural ponds (Pritchard & Schluter 2001) to test for differential competitive effects on species-pairs that vary with respect to divergence. The former study was imperfect because comparisons were made between groups enclosed on different islands and so levels of divergence are potentially confounded with island effects. The latter study is imperfect because resources or some other relevant factor in the semi-natural environments may not reflect natural conditions. We attempt to address both of these problems by returning to field enclosures placed in a single natural lake environment.

Although competition is not necessarily ubiquitous, evidence that competition occurs under natural conditions has become less controversial (Connell 1983; Schoener 1983; Gurevitch *et al.* 1992). The same cannot be said for the evolutionary consequences of competition (Schluter 2000b). For example, interspecific competition that causes divergent frequency-dependent selection is a rarely tested assumption of ECD. The addition of an ecologically similar or closely related species increases the density of phenotypes that utilize similar limited resources. This changes the relative frequency of phenotype classes using a spectrum of resources, which is expected to result in the fitness of less frequent phenotypes (that can exploit alternate resources) to be favoured as the per capita energy gain of more frequent phenotypes declines. Density-dependent competitive effects alone are not expected to play a role in the evolution of divergence because all phenotypes are expected to be equally negatively affected (Calow 1999), which does not necessarily translate into less frequent phenotypes being selected over more frequent phenotypes. Therefore, competitive species interactions can cause the evolutionary divergence of ecologically similar species only when they result in divergent frequency-dependent selection (Schluter 2000a,b).

Morphological divergence along a littoral–benthic vs. pelagic gradient is increasingly recognized as a hallmark of northern fishes that inhabit postglacial lakes, such as threespine stickleback species-pairs (Lavin & McPhail 1985; Schluter & McPhail 1992, 1993), brook charr (Bourke *et al.* 1997) and sunfish (Ehlinger & Wilson 1988; Robinson *et al.* 1996), with further examples in Schluter (1996a) and Robinson & Schluter (2000). Benthic-type fish regularly have deeper bodies, larger subterminal mouths and fewer, shorter gill rakers and a variety of other traits that differ from pelagic-type fish; these traits are expected to improve habitat-specific foraging performance (Schluter 1996a,b; Robinson & Schluter 2000).

Brook sticklebacks (*Culaea inconstans*) and ninespine sticklebacks (*Pungitius pungitius*) are found in a series of postglacial kettle lakes near Kirkland Lake, Ontario, Canada. The two species are ecologically similar throughout their North American ranges, and appear to prefer resources found in the littoral zone (Wootton 1976; Scott & Crossman 1998). The similar resource use and coexistence of both species in single lakes raises the possibility of current or historic resource competition. We found several isolated lakes where both brook and ninespine sticklebacks coexist (hereafter referred to as sympatric populations or sites) and a greater number of lakes containing only brook sticklebacks (hereafter referred to as allopatric populations or sites). No allopatric populations of ninespine sticklebacks (hereafter referred to as ninespines) have been found locally to date. The study lakes are located on a 240-km sandy, glacial esker that runs north–south near the Arctic–Great Lakes watershed divide in Northern

Ontario, and were formed within the last 9500 years (Connelly 1972; Crossman & McAllister 1986; Pielou 1991). The lakes appear ecologically similar in that they are all deep, spring-fed, and embedded in a sandy substrate providing each lake with a small catchment dominated by boreal forest. They also share similar aquatic macrophytes and relatively depauperate vertebrate assemblages with the brook charr (*Salvelinus fontinalis*) as a potential fish predator.

Morphometric analyses of 15 resource-related traits have revealed that sympatric brook stickleback and allopatric brook stickleback populations are morphologically divergent (Gray 2001). Brook sticklebacks sympatric with ninespines have significantly shorter gill rakers, longer jaws and somewhat deeper bodies than allopatric brook stickleback populations, suggestive of a more specialized benthic phenotype. This pattern of morphological divergence is repeated across three sympatric and four allopatric populations and parallels the benthic–pelagic divergence of other stickleback systems (Schluter & McPhail 1992) and of northern freshwater fish in general (Robinson & Wilson 1994).

If ECD has played a role in the divergence of brook sticklebacks that coexist with ninespines, then we predict that: (1) there should be evidence of interspecific resource competition in the presence of a competitor, and (2) competitive effects on sympatric brook sticklebacks (representing the post-displacement phenotype) will be reduced compared to allopatric brook sticklebacks (representing the pre-displacement phenotype). Reciprocal predictions about the effects of competition on ninespine sticklebacks are also expected, but are more speculative because of the absence of local allopatric ninespine populations and any evidence of divergence. For this reason, we focus on brook sticklebacks as the target species in this experiment. First, we predict that target allopatric brook sticklebacks will have reduced growth (lower short-term fitness) in the presence vs. the absence of ninespine sticklebacks. We justify testing for such competitive effects using the allopatric brook sticklebacks by invoking Connell's (1980) "ghost of competition past", which assumes that allopatric brooks represent the pre-displacement phenotype and so should potentially experience higher levels of competition. Second, we expected sympatric brook sticklebacks (post-displacement) to have higher growth than allopatric brook sticklebacks (pre-displacement) in the presence of the ninespine competitor.

MATERIALS AND METHODS

Two target brook stickleback populations were used: sympatric brooks native to the lake where the experiment took place (the post-displacement phenotype), and allopatric brooks transplanted from a nearby lake that does not contain ninespine sticklebacks (the pre-displacement phenotype).

Ninespines were residents of the lake where the experiment took place.

Field experiments were conducted in August 2000 in Rozon Lake (48°15'36" N, 79°52'43" W, near Kirkland Lake, Ontario), a kettle lake containing natural sympatric populations of brook and ninespine sticklebacks. The ambient density of brook and ninespine sticklebacks was determined for the littoral zone using a 5.0-m long seine sampling a 6.0-m swath perpendicular to the shore at six locations in the lake. Mean littoral zone density was approximately 23 brook and 23 ninespine sticklebacks per 1.0 m² area. We limited total starting fish density in our enclosures (0.44 m² bottom area) to 20 fish. Enclosures were 0.75 m diameter open-ended cylinders, 1.2 m high, made of 0.4 cm wire mesh, placed upright in less than 0.75 m of water. Approximately 25 cm below the water surface was a 50-cm thick mat of submerged vegetation (*Chara* sp.).

Medium-sized (0.80–1.40 g wet mass) sympatric target brook and ninespine sticklebacks were collected from Rozon Lake on August 10, 2000, using baited minnow traps and dip nets. Allopatric (transplanted) target brooks were collected from Bea Lake (48°20'43" N, 79°52'04" W), a kettle lake approximately 10 km north of Rozon Lake. All fish were kept in coolers with water from Rozon Lake while individual fish were weighed (live wet mass) with an electronic balance (0.001 g) and placed systematically into buckets that were randomly assigned to one of the 36 enclosures as detailed below. Fish were removed from the enclosures on September 11, 2000 (after approximately 1 month of growth). Recovery was accomplished by surrounding each enclosure with a plastic sheet and infusing compressed carbon dioxide into the water through large air stones. Surfacing fish were caught with a dip net, and the vegetation was rigorously prodded to ensure that all remaining sticklebacks were recovered. Fish were then weighed (live wet mass), killed in clove oil, placed in 10% formalin for 1 month, and after rinsing in water were stored in 70% ethanol.

Growth calculations

Competition was assessed by its effect on the per capita growth of all fish within an enclosure over the 30-day experiment. The growth of individual fish within enclosures was not expected to be independent, and so we treat the enclosure as the experimental unit. Median size-specific growth was estimated for each enclosure as the natural logarithm of the final median mass of the group divided by the initial median mass of the group.

Transplant effect

We used natural field enclosures located in a single lake to address the problems faced in other studies of competitive

effects (see Introduction). However, we potentially included a new confounding effect, because allopatric brooks were transplanted from a nearby lake whereas sympatric brooks were not. We tested the null hypothesis of no transplant effect experienced by allopatric brooks compared to native sympatric brooks by comparing the growth of each in the absence of ninespines.

Twelve pairs of enclosures were arranged haphazardly in the littoral zone along two shores of the lake (east = five pairs, west = seven pairs). Paired cages always included one enclosure with target sympatric brooks and one enclosure with target allopatric (transplanted) brooks. Six pairs of enclosures were stocked at low density (10 brooks) and the remaining six pairs of enclosures at high density (20 brooks). We used ANCOVA to test for a transplant effect on the median growth of allopatric brooks vs. sympatric brooks with the number of fish recovered from each enclosure as a covariate in order to adjust for the realized intensity of density-dependent competition (two-tailed significance reported).

Competition and divergence

We used a paired design to test the prediction that target sympatric brooks (post-displacement) had higher growth than target allopatric brooks (pre-displacement) in the presence of ninespines. Six pairs of enclosures were arrayed haphazardly along the littoral zone on the east side of the lake. One enclosure in each pair contained sympatric brooks ($n = 10$ per cage) and the other contained allopatric brooks ($n = 10$). Ten ninespines were added to each enclosure. We performed a paired t -test to compare the median growth of sympatric vs. allopatric brooks (one-tailed significance is reported).

We also performed a paired t -test on the growth of the ninespine sticklebacks present in these enclosures, to test the prediction that ninespine growth would be higher in the presence of sympatric vs. allopatric brooks.

Interspecific competition

We used data from half of each experiment above to test if the addition of ninespines reduced the growth of target brooks. We compared the median growth of three treatments of target allopatric brooks: at low density alone, high density alone, and in the presence of ninespine sticklebacks (respective starting ratios of brooks to ninespines: 10B : 0N, 20B : 0N, 10B : 10N). Only enclosures with allopatric brooks were used in this test. Differences in growth among the three treatments were tested using a two-factor ANOVA (factors: treatment, three levels; and lake shore, east vs. west, treated as a block). Post hoc multiple comparisons among treatments were carried out using Tukey's HSD test ($\alpha = 0.05$).

Cage effects

In order to test for cage effects in these experiments we compared resource use of experimental fish inside to wild-caught fish outside the enclosures. We expected that if cage effects were important, then we should observe (1) an overall difference in the total numbers of prey items eaten per fish inside compared to outside the cages, and/or (2) differences in the numbers of prey items per fish in any of three functional prey categories (benthic type prey taxa, pelagic type prey taxa, and prey taxa using both benthic and pelagic habitats) in the stomachs of fish inside vs. outside the enclosures (prey categories identified using Peckarsky *et al.* 1990; see Gray 2001 for a complete list). We identified stomach contents to Order and counted all prey items in the stomachs from random samples of experimental fish and wild fish collected from outside the cages at the end of the experiment. We used separate ANOVAS to test for differences in the total abundance and the three prey category abundances of prey eaten inside vs. outside the enclosures for brooks and ninespines separately. Count data were transformed as the square root of the count plus 0.5, so that means and variances were uncorrelated, and two-tailed significance is reported.

RESULTS

Recovery results

Fewer ninespines than brooks were recovered from all enclosures at the end of the experiment (Table 1; Fisher's exact test: two-tailed $P < 0.001$). This result indicates species-specific experimental effects and probably lower levels of interspecific competition than we had intended, but otherwise does not interfere with our analyses of the potential effects of competition on the target brook sticklebacks. Recovery was approximately equal for the target sympatric and allopatric (transplanted) brook sticklebacks (Table 1; Fisher's exact test: two-tailed $P = 0.66$), suggesting that possible transplant effects on allopatric brooks were not so extreme that they caused different levels

of mortality. Significantly more sympatric and allopatric brooks died in the presence of ninespines compared to their absence in the high density brook treatment, suggesting that interactions between species may be more severe than interactions among conspecifics (Table 1; Fisher's exact test: two-tailed $P = 0.056$ and $P = 0.005$ for sympatric and allopatric brooks, respectively). There was no difference in the number of ninespines recovered from enclosures with sympatric brooks compared to allopatric brooks (Table 1; Fisher's exact test: two-tailed $P = 0.75$).

Cage effects

There were no differences in either the total number of all prey items or of all prey items in the benthic and both habitat categories found in the stomachs of experimental brooks inside compared to wild brooks caught outside the cages (all $P > 0.3$, Table 2). However, four times the number of pelagic prey items were present in the stomachs of experimental (inside) compared to wild (outside) brooks. The diets of ninespines were more strongly influenced by cage effects (Table 2). Experimental (inside) ninespines had significantly fewer benthic, pelagic and total prey items in their stomachs compared to wild (outside) ninespines.

Transplant effect

There was weak statistical evidence of a possible transplant effect on the growth of allopatric (transplanted) brooks compared to sympatric (native) brooks (ANCOVA: population source $F_{1,22} = 3.88$, two-tailed $P = 0.063$; final density covariate $F_{1,22} = 2.67$, two-tailed $P = 0.12$; see Table 3). Slopes were homogeneous, and the adjusted mean growth of sympatric target brooks was 0.059 units greater than that of allopatric brooks.

Interspecific competition

The growth of allopatric (transplanted) brook sticklebacks varied among the three competition treatments (ANOVA:

Table 1 The proportion of target brook sticklebacks and ninespine sticklebacks recovered from field enclosures after 1 month of growth. Treatment indicates the number and species of fish in each of six enclosures per treatment. The total category reflects proportions of brooks or ninespines combined over all treatment and population combinations

Treatment	Target		Competitor	
	Sympatric brooks	Allopatric brooks (transplanted)	Ninespines (with sympatric brooks)	Ninespines (with allopatric brooks)
Low density (10 Brooks)	0.94	0.93	–	–
High density (20 Brooks)	0.91	0.92	–	–
Competition (10 Brooks + 10 Ninespines)	0.80	0.75	0.55	0.48
Total	0.88		0.52	

Table 2 Mean (\pm standard error) of the number of prey items found in the stomachs of random samples of brook and ninespine sticklebacks that were used in the competition experiment (experimental fish) and wild fish caught outside the enclosures at the end of the experiment. Prey were categorized into three functional groups: benthic, pelagic or both-type prey (found in both benthic and pelagic habitats). Separate ANOVAs were used to test for differences in prey numbers per stomach for each prey category and the total prey between experimental vs. wild-caught fish for each species

	Experimental fish	Wild-caught fish	P-value
Brooks:	$n = 39$	$n = 27$	
Benthic	10.5 (3.40)	10.9 (2.33)	0.51
Pelagic	8.5 (1.65)	1.9 (0.82)	< 0.001
Both	1.03 (0.33)	1.4 (0.37)	0.38
Total	20.0 (3.96)	14.2 (2.55)	0.3
Ninespines:	$n = 16$	$n = 30$	
Benthic	0.88 (0.44)	5.5 (0.61)	< 0.001
Pelagic	1.3 (0.90)	14.9 (7.29)	0.07
Both	0.31 (0.12)	4.8 (3.98)	0.32
Total	2.5 (1.16)	25.1 (7.97)	< 0.001

Table 3 Means (standard error in parentheses) of group-median growth in target brook sticklebacks and ninespine sticklebacks. Means are averaged over six replicate enclosures in each treatment. Growth over the 31-day experiment was estimated as the natural logarithm of the final median mass divided by the initial median mass in each enclosure. Treatment indicates the initial number and species of fish in each of six enclosures per treatment

Treatment	Target		Competitor	
	Sympatric brooks	Allopatric brooks (transplanted)	Ninespines (with sympatric brooks)	Ninespines (with allopatric brooks)
Low density (10 Brooks)	0.082 (0.045)	0.035 (0.032)	–	–
High density (20 Brooks)	0.024 (0.016)	–0.045 (0.018)	–	–
Competition (10 Brooks + 10 Ninespines)	0.104 (0.040)	–0.012 (0.022)	–0.105 (0.061)	–0.062 (0.056)

$F_{2,17} = 6.04$, $P = 0.013$; see Table 3). Allopatric brooks reared alone (low density, $n = 10$ brooks/enclosure) had significantly higher growth compared to both the higher density treatment ($n = 20$ brooks/enclosure), and in the presence of 10 ninespines (Fig. 1; Tukey HSD, $\alpha = 0.05$, $Q = 2.62$). Both the high density brook and the ninespine addition treatments resulted in a net loss in wet body mass in the target allopatric brooks that were not significantly different from each other.

Competition and divergence

In the presence of ninespines, the target allopatric brooks had significantly lower growth than sympatric brooks (Table 3; Fig. 2; paired $t = -3.23$, $P = 0.012$). Adding a transplant effect of 0.059 (estimated using ANCOVA of transplant and density effects above) to the growth of allopatric brooks weakened the statistical evidence of a difference between the growth of sympatric compared to allopatric brooks (paired $t = -1.57$, $P = 0.088$). There was no significant difference in the median growth of ninespine

sticklebacks in the presence of sympatric compared to those reared with allopatric brooks (Table 3: paired $t = -0.73$, $P = 0.75$). Target allopatric brooks, representing the putative pre-displacement phenotype, were more adversely affected by ninespines than the post-displacement sympatric brook phenotype, although the magnitude of the effect may have been amplified by transplant effects here. This suggests that the effect of competition on brooks declines as phenotypes diverge between species.

DISCUSSION

Experimental evidence of competition between two closely related species where they coexist is not particularly controversial (Connell 1983; Schoener 1983; Gurevitch *et al.* 1992), however, field tests of the differential effects of competition on different phenotypes are novel (Schluter 2000a). Using native phenotypes enclosed in a natural field environment we demonstrated a short-term, negative effect of ninespine sticklebacks on the growth of brook sticklebacks. We interpret this as direct evidence of competition

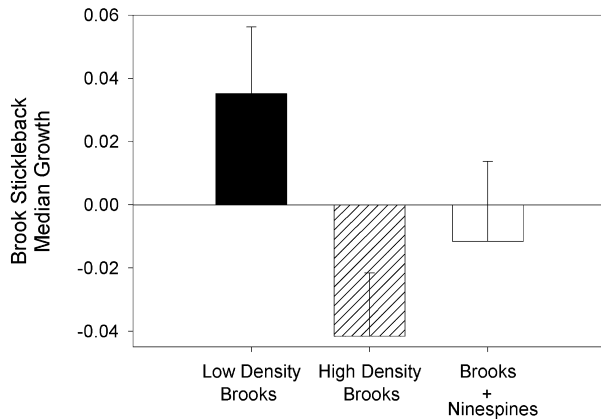


Figure 1 Means (with standard error bar) of group-median growth in allopatric target brook sticklebacks alone at low (dark bar) and high (hatched bar) density (initial $n = 10$ and 20 brooks per enclosure, respectively), and in the presence of ninespines (white bar; 10 brooks + 10 ninespine sticklebacks initially). Means are averaged over six replicate enclosures in each treatment. Tukey's HSD multiple comparisons approach ($\alpha = 0.05$) found significant evidence of differences in growth between the low density and the other two treatments, but not between the high density and the ninespine treatment. Growth over the 31-day experiment was estimated as the natural logarithm of the final median mass divided by the initial median mass per enclosure.

between the two species. Furthermore, we demonstrated that the negative competitive effect on the post-displacement sympatric brook phenotype was reduced (resulting in higher growth) compared to the pre-displacement allopatric brook phenotype. We interpret this as evidence that competition declines as divergence between competing species increases. This is consistent with the expectation that frequency-dependent selection imposed by competition with ninespines favoured more benthic over more pelagic phenotypes of brook sticklebacks, leading to increased morphological specialization and the divergence of brook sticklebacks where they coexist with ninespines in this system.

An alternative explanation for the difference in growth observed between sympatric and allopatric brooks could be that water temperature, which has a positive influence on fish growth, differed between sympatric and allopatric lakes. The average water temperatures measured at 1.0 m in late August 2001 of three sympatric (including Rozon Lake) and four allopatric lakes (including Bea Lake) were not significantly different (t -test: $P = 0.17$). Because the enclosures were placed in less than 1.0 m of water, we conclude that there is little evidence that differences in water temperature between sympatric and allopatric lakes were responsible for the difference in growth observed between sympatric and allopatric target brooks.

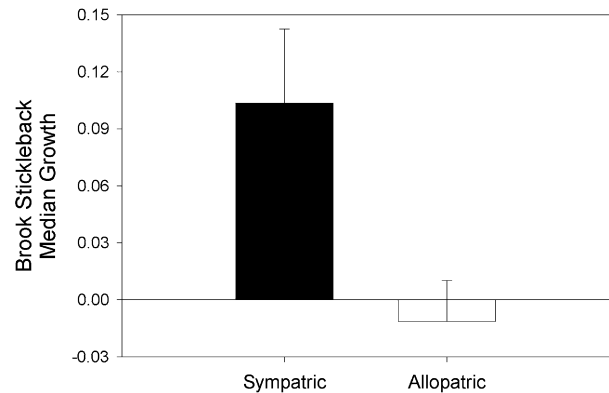


Figure 2 Means (with standard error bar) of group-median growth for sympatric brook sticklebacks representing post-displacement phenotypes (black bar) and allopatric brooks representing pre-displacement phenotypes (white bar), both reared in the presence of ninespine sticklebacks. The growth of pre-displacement target brooks was significantly reduced compared to the post-displacement brooks ($P = 0.012$). The magnitude of the difference between treatments is reduced, however, if an estimated transplant effect (0.059 units of growth) is applied to allopatric brooks (see Results). Growth was estimated as in Fig. 1.

We detected no difference in either the mortality or growth of ninespine sticklebacks placed with sympatric compared to allopatric brooks. We expected that ninespines reared with allopatric (pre-displacement) brooks would also suffer more severely from competition than those placed with sympatric (post-displacement) brooks. This suggests that competition and perhaps character displacement may be asymmetric in this system as in other fish systems (Schluter 2000a). However, this conclusion is preliminary because of the absence of local allopatric ninespine populations for comparison, and because of apparently greater experimental effects on ninespines compared to brooks as discussed next.

Species-specific cage effects occurred in our experiment. Minor cage effects were observed on the diets of experimental brooks, which ate slightly more pelagic prey than wild-caught brooks. More obvious cage effects influenced the ninespines, with experimental fish eating much less of almost all prey types inside compared to outside the enclosures. We suspect that the cages may have caused high direct behavioural interactions between the more aggressive and territorial brooks and the less aggressive and gregarious ninespines that may be less common in the natural lake environment. We have frequently observed such dominance in mixed species groups of sticklebacks held in laboratory aquaria. This may have caused the ninespines in our enclosures to forage less and resulted in their higher mortality compared to brooks. Although such cage effects

are obviously important, we expect that they had the net effect of reducing the level of interspecific resource competition in enclosures because they resulted in reduced densities (higher mortality) of subordinate ninespines that ate less. Thus, we may have actually applied much lower levels of interspecific resource competition in the experiment than occur in nature, making our test potentially more conservative.

Tests of the significance of competitive effects under natural conditions using manipulative field experiments are crucial to our understanding of how competition can influence adaptive divergence. Schluter (1994) performed a test in experimental ponds using artificially propagated threespine stickleback (*Gasterosteus aculeatus*) phenotypes in order to amplify phenotypic variation and to test for frequency-dependent selection imposed by competition with a closely related threespine stickleback species. Although his experiment added significantly to our understanding of how natural selection can differentially affect phenotypes, it did not necessarily represent natural phenotypic nor morphological variation presently or historically available in that system. Our use of natural allopatric brook sticklebacks, that are morphologically less specialized than sympatric conspecifics, may better represent an historical pre-displacement form, and potentially an historic level of competition (Connell 1980). One potential benefit of artificially induced variation is the opportunity to study the quantitative genetics of traits related to resource use, which may permit better predictions about responses to selection.

A single optimal experimental design that tests predictions about the strength of interspecific competition in relation to phenotypic divergence is not obvious. Pacala & Roughgarden (1985) tested the degree to which competitive effects varied between pairs of *Anolis* lizard species that differed in their degree of body size in two different guilds, using field enclosures on two different islands. Each pair of species was tested for interspecific competitive effects on the native island but not reciprocally on the other island (instead the authors made considerable efforts to choose ecologically similar sites on each island). Nonetheless, this method potentially confounds divergence-related competitive effects with unknown ecological differences between the two islands. Pritchard & Schluter (2001) attempted to correct for the confounding effect of environment by testing for competition among naturally derived threespine stickleback phenotypes in large replicated artificial ponds. Although this eliminates the experimental problem faced by Pacala and Roughgarden (and potentially problems with propagating phenotypes in earlier studies), it introduces a potentially new problem if the diversity of prey in the ponds does not accurately mimic that found in natural lake environments. Our experiment returns to the natural system and the use of field enclosures. We attempted to address the

problems present in these methods by locating all enclosures in one lake. However, while solving one set of problems it raises another. Moving allopatric brook sticklebacks into the non-native sympatric lake could have imposed a transplant effect if the allopatric brook sticklebacks were locally adapted to their native lake. This possibility could have been ruled out had we simultaneously conducted the entire experiment in the allopatric source lake; however, we chose not to risk introduction of ninespines into the allopatric lake.

We attempted to design our experiments in order to test for an effect of transplanting allopatric brook sticklebacks into the sympatric lake, by comparing the difference in growth between sympatric and allopatric brooks at varying densities (in the absence of ninespines). We detected only weak evidence of a transplant effect, justifying our comparison of the unadjusted growth between sympatric and allopatric brooks in the presence of ninespines. However, the weak evidence of a transplant effect prompted us to retest the growth difference between sympatric brooks and allopatric brooks after adjusting the growth of transplanted allopatric brooks. The result of this analysis showed the same trend as in the unadjusted test, with sympatric (post-displacement) brooks still having higher growth than allopatric (pre-displacement) brooks but with a somewhat reduced level of significance. Although it seems likely that allopatric brooks experienced some form of transplant effect here, we also conclude that there was a reduction in the competitive effect imposed by ninespine sticklebacks on sympatric compared to allopatric brooks.

Controversy exists around the appropriate experimental design to test for an effect of interspecific competition in ECD studies. Bernardo (1995) suggested that the appropriate design is a replacement experiment that holds density-dependent competitive effects constant among treatments. The null hypothesis tested is that if interspecific competition occurs, then the replacement of half of the conspecifics by heterospecifics should result in the same negative effect on growth of the target (conspecific) species. The replacement method adds interspecific competitive effects, holds overall density effects constant, but also reduces intraspecific competitive effects. Schluter (1995) argued that this replacement design is flawed because the method confounds intraspecific and interspecific competitive effects, because both are allowed to change among treatments. Instead, he proposed an additive design where the density of the target species is held constant between treatments. The null hypothesis tested in the additive design is that, if there is no interspecific competition between two species, then adding heterospecifics should result in no change in the growth of the target species. Here interspecific effects are added, intraspecific effects are held constant, but density increases.

Schluter (1995) argued that the additive design is superior in tests of competition related to ECD because although density effects are confounded with interspecific effects, density effects alone are not expected to result in differential competitive effects that favour some phenotypes and not others in the target population.

We incorporated both methods in our test of competition between allopatric brooks and ninespines (Fig. 1). We were able to reject the null hypotheses both of the replacement design and of the additive design, both results being consistent with our conclusion that interspecific competition occurred between brooks and ninespines in our experiment.

Although this study provides evidence for resource competition between brooks and ninespines, and evidence that the effects of competition decline with divergence, we cannot yet definitively conclude that ECD has caused the divergence of brooks where they coexist with ninespines. There are at least five other criteria that must be met in order to demonstrate ECD (Schluter & McPhail 1992; Robinson & Wilson 1994). The first is that the divergence of sympatric from allopatric brook sticklebacks represents genetic changes and not just environmentally induced shifts in plastic traits. Although similar morphological differences have persisted in wild populations over 2 years of observation (Gray 2001), this is not conclusive evidence that additive genetic variation that could respond to selection is present in these traits. However, all previous quantitative genetic tests in other stickleback systems (e.g. McPhail 1994; Hatfield 1997) have revealed considerable genetic variation in gill raker architecture, mouth size and body form, the same morphological traits that we focus on here. This leads us to provisionally conclude that there is probably sufficient heritable variation in the traits of these brook sticklebacks to respond to selection. A second criterion is that traits are functionally related to resource use. Failure to detect such a relationship could mean that traits may have diverged for reasons other than natural selection (such as drift). Laboratory foraging experiments have demonstrated that sympatric brooks were more efficient foragers on benthic prey than ninespines, and vice versa for pelagic prey (Gray 2001). These differences were associated with gill raker architecture and body size variation as expected, implying such a functional relationship.

The third and fourth criteria of ECD are that the character shifts are not due to chance, and that sites of sympatry and allopatry are ecologically similar, particularly with respect to resource availability. We have evidence consistent with both of these. The pattern of divergence is replicated in several isolated sympatric and allopatric lakes on the postglacial esker. All of the study lakes are deep, spring-fed kettle lakes that are not connected by surface streams. The lakes containing sympatric and allopatric brook

populations appear to be ecologically similar with respect to a variety of biotic and abiotic factors, including benthic and pelagic prey availability (Gray 2001). Our provisional conclusion is that the main difference among these lakes appears to be the presence vs. absence of ninespines, respectively. The replication of the divergence under similar ecological conditions is also not consistent with an important role played by chance in creating the pattern. The final criterion is that the observed shifts are caused by evolutionary changes and not other processes such as biased colonization. Gray (2001) provides evidence that trait means of four allopatric brook populations lie within the range of trait means bounded by brooks and ninespines in the three sympatric sites. The repeated pattern of trait increases in sympatry suggests that evolutionary, as opposed to non-evolutionary, processes are responsible for the observed character shifts (Schluter 2000b).

The evolutionary consequences of species interactions are relatively easy to study in postglacial northern lakes compared to more species-rich tropical lakes, because they are ecologically less complex, having relatively low species diversity, and they afford enormous replication at the whole lake level. The lower diversity and complexity of postglacial lakes is a result of their geological youth (often less than 10 000 years), having formed after the retreat of the Pleistocene glaciers (Connelly 1972; Crossman & McAllister 1986; Pielou 1991). As a result, the evolution of divergence is recent and consequently rapid. Repeated or parallel patterns of divergence involving many northern freshwater fish taxa have been documented both among taxa (Robinson & Wilson 1994; Schluter 1996a), and within taxa in the case of trophic polymorphisms (Robinson & Schluter 2000). Rapid and parallel patterns of divergence are now accepted as strong evidence for a role played by natural selection. Northern freshwater fish assemblages are therefore attractive for studies of natural selection, and for tests of the role played by ecological factors, such as species interactions, in adaptive divergence. The evidence that competition plays an important role in adaptive divergence and radiation in the fishes of postglacial northern lakes is primarily based on comparative studies (e.g. Schluter 1996a; Robinson & Schluter 2000), and very few studies have used experimental manipulations (e.g. Schluter 1994). Our study adds to a growing body of experimental evidence that suggests that competition and species interactions are important mediators of evolution.

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