# CHARACTER SHIFTS IN THE DEFENSIVE ARMOR OF SYMPATRIC STICKLEBACKS

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*Abstract.*—Natural enemies may contribute to the morphological divergence of sympatric species, yet their role has received little attention to date. We tested for character shifts in defensive armor of sympatric threespine sticklebacks (*Gasterosteus aculeatus* complex) previously shown to exhibit ecological character displacement in traits related to resource use. We scored five defensive armor traits in sympatric benthic and limnetic stickleback species from southwestern British Columbia and compared them with the same traits in nearby allopatric populations in the presence of the same predatory fish (*Oncorhynchus* sp.). This approach is analogous to tests of ecological character displacement that compare trophic traits of sympatric and allopatric species in the presence of the same community of resource types. Three patterns consistent with character displacement in defensive armor were found. First, limnetics in different lakes had consistently more armor than sympatric benthics. Second, the average amount of armor, averaged over both species, was reduced in sympatry compared to allopatric populations. This reduction was almost entirely the result of shifts by benthic species, whereas armor in limnetics was more similar to that in allopatric populations. Third, differences between sympatric benthics and limnetics in total armor were greater than expected from comparisons with allopatric populations. We interpret these patterns as the result of differences in habitat-specific predation regimes accompanying ecological character displacement and indirect interactions between sympatric stickleback species mediated by their top predators. These results suggest that predation may facilitate, rather than hinder, the process of divergence in sympatry.

*Key words.*—Adaptive radiation, antipredator traits, apparent competition, character shifts, divergence, *Gasterosteus aculeatus*, predation.

Closely related species are frequently more different in areas where they coexist than where they are found alone (Schluter 2000a). This pattern is most frequently attributed to ecological character displacement, evolutionary shifts of morphological traits in response to interspecific competition for resources between similar species (Brown and Wilson 1956; Grant 1972; Schluter 2000b). Numerous studies have demonstrated evidence consistent with morphological divergence as a result of interspecific competition (e.g., Fjeldså 1983; Dayan and Simberloff 1994; Chiba 1999; Adams and Rohlf 2000). For example, Adams and Rohlf (2000) reported a case of character displacement between two salamander species. Sympatric *Plethodon hoffmani* populations have evolved a faster closing, albeit weaker, jaw than allopatric populations, whereas sympatric *P. cinereus* populations have evolved a slower, stronger jaw. These differences in sympatry were correlated with differences in prey consumption, with *P. hoffmani* individuals consuming larger prey than did *P. cinereus* individuals.

Field studies of closely related, sympatric species have rarely considered how interactions with other trophic levels, notably natural enemies (e.g., parasites, parasitoids, predators), may contribute to the observed morphological differentiation. This is surprising, given that a number of theoretical models suggest that sympatric prey may experience character shifts as readily from interactions mediated via a higher trophic level, such as predators, as from resource competition (Holt 1977; Brown and Vincent 1992; Abrams 2000). Models typically focus on character shifts driven by (1) interactions between prey mediated through shared predators, or (2) each prey species responding differently to different predator environments.
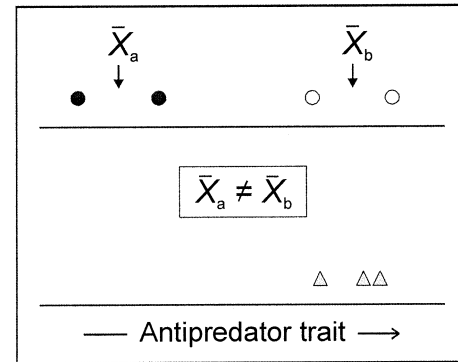
Predation by a single predator species may lead to shifts in antipredator traits of sympatric species to minimize risk of being preyed upon (Holt 1977; Jeffries and Lawton 1984; Brown and Vincent 1992; Holt and Lawton 1994; Abrams 2000). Divergence, convergence, parallel increases, and parallel decreases in antipredator traits are all possible outcomes in sympatry (Abrams 2000). Divergence is most likely when the presence of two prey species in sympatry leads to increased predation risk through a strong numerical response of predators and one species has a trait optimum lower than the most vulnerable trait value and the other species has a trait optimum greater. The mechanism is analogous to divergence of foraging traits in response to competition for shared resources except that the interactions between the species are mediated through a higher trophic level. Holt (1977) coined the term ''apparent competition'' to denote the difference between this interaction and resource competition. Alternatively, ''apparent mutualism'' may emerge in sympatry, leading to parallel decreases or convergence in sympatry (Abrams 2000). These shifts are most likely if the presence of two consumer species lowers the rate of predation experienced by both of them below that experienced when each occurs alone with the same predators. Predators may experience little to no numerical response to the presence of two prey species if their abundance is limited by other factors (e.g., nesting sites), resulting in reduced per capita predation risk. Predator interference and satiation may also lead to lowered rates of predation (Abrams 2000). Parallel decreases are expected when predation rate is reduced in sympatry and both species have trait optima less than the most vulnerable trait value, which may be most likely with energetically costly structures (e.g., protective shells).

Sympatric species may also diverge in antipredator traits in the complete absence of interactions between them via shared predators. Instead, consumer species may evolve to exploit different habitats under the influence of resource competition only (i.e., ecological character displacement) that then secondarily favors divergence in antipredator traits if different habitats contain different predators or favor different modes of defence (Abrams 2000). A number of empirical studies have documented differences in predator communities associated with different habitats (e.g., Mittelbach 1984; Reimchen 1994; Pitt 1999). For example, a predator × habitat interaction appears to characterize the risk of predation for desert gerbils (Kotler et al. 1991, 1993): snakes are more dangerous in the bush microhabitat, whereas owls are the main threat in the open microhabitat. In this scenario, populations living in the bush microhabitat would be expected to evolve antipredator defenses that reduce their consumption by snakes. Divergence appears to be the most likely outcome of habitat-specific predation risk (Abrams 2000).
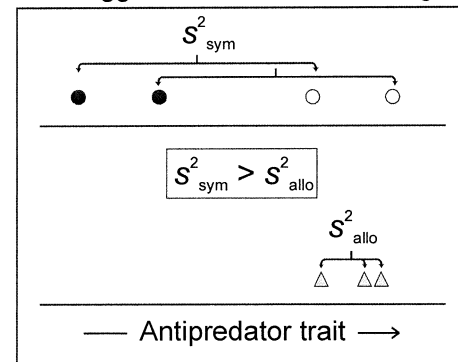
The first step to evaluating alternative hypotheses for the role of interactions between consumer species in their divergence of antipredator traits is to compare the antipredator traits of sympatric consumer species with those of allopatric populations in the presence of the same predators. This procedure is analogous to the standard test of ecological character displacement involving comparison of resource-gathering traits of sympatric species with those of allopatric populations in the presence of the same resources (reviewed in Schluter 2000a,b). Antipredator traits of sympatric species may be expected to exhibit one or more of three patterns compared to allopatric populations: consistent direction divergence, exaggerated difference divergence, and midpoint shift (see Fig. 1). Consistent direction divergence occurs when sympatric species that differ in their use of habitats or resources also exhibit a regular difference in antipredator traits (Fig. 1A). In this example, populations of species B always have higher values of the antipredator trait (e.g., shell thickness) than sympatric populations of species A. Exaggerated difference divergence occurs when character states of sympatric species differ more greatly from one another than do randomly paired allopatric populations (Fig. 1B). Consistent divergence in antipredator traits is not a prerequisite for exaggerated divergence, nor does the presence of exaggerated divergence imply a consistent divergence. For this reason we treat them separately. Finally, the mean of sympatric species may be shifted relative to the mean of allopatric populations (Fig. 1C). Such a shift, when it occurs in combination with a pattern of consistent direction divergence, implies that different ecological types in sympatry are displaced in unequal amounts relative to the mean of allopatric populations. All else being equal, any one of the three patterns alone or in combination would imply that the interactions between sympatric consumer species, mediated either by competition for shared resources or via shared predators, influence the evolution of antipredator traits.

Here we test for these three patterns in antipredator traits of sympatric threespine sticklebacks that exhibit ecological character displacement in trophic morphology and habitat use (Schluter and McPhail 1992). Benthic and limnetic sticklebacks have divergent habitat and resource use preferences,

### A. Consistent Direction Divergence

$$\bar{X}_a \qquad \bar{X}_b$$

$$\boxed{\bar{X}_a \neq \bar{X}_b}$$

—— Antipredator trait ——→

### B. Exaggerated Difference Divergence

$$s^2_{sym}$$

$$\boxed{s^2_{sym} > s^2_{allo}}$$

$$s^2_{allo}$$

—— Antipredator trait ——→

### C. Midpoint Shift

$$\bar{X}_{sym}$$

$$\boxed{\bar{X}_{sym} \neq \bar{X}_{allo}}$$
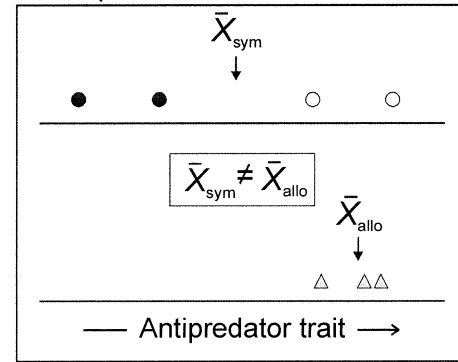
$$\bar{X}_{allo}$$

—— Antipredator trait ——→

FIG. 1. Values of an antipredator trait (e.g., shell thickness) in hypothetical sympatric (open and filled) and allopatric (shaded triangles) populations. Open and filled circles indicate different ecological types in sympatry (e.g., populations occupying either of two microhabitats). (A) consistent direction divergence between species in sympatry (mean character value for ecotype a [$\bar{x}_a$] tends to be less than the mean value for ecotype b [$\bar{x}_b$], across all pairs of populations). (B) exaggerated difference divergence in sympatry (variance among sympatric species [$s^2_{sym}$] within lakes is greater than the variance among allopatric populations [$s^2_{allo}$] in different lakes). (C) midpoint shifts in sympatry (mean value of sympatric populations [$\bar{x}_{sym}$] is not equal to the mean value of allopatric populations [$\bar{x}_{allo}$]). The example illustrates a midpoint reduction in sympatry; traits may shift upward in sympatry instead.

feeding on benthic invertebrates in the littoral zone and zooplankton in the open water zone, respectively (Larson 1976; Schluter and McPhail 1992; Vamosi et al. 2000). To the best of our knowledge, sympatric populations are restricted to the Strait of Georgia region of British Columbia. Molecular and geological evidence indicates that each pair formed independently via separate invasions of fresh water by the marine species around the end of the last ice age (Schluter and McPhail 1992; McPhail 1993, 1994). Within each lake, benthics are descended from an earlier invasion than that giving rise to limnetics (Withler and McPhail 1985; McPhail 1994; Kassen et al. 1995; Taylor and McPhail 2000). An important distinction between this system and most other cases of ecological character displacement is that benthics and limnetics are not found in allopatry. Rather, allopatric stickleback populations tend to have intermediate trophic morphology and habitat use (Schluter and McPhail 1992). Each allopatric population is the result either of a single marine invasion or a failed double invasion in which one species went extinct or the two species fused via hybridization (Vamosi 2003). Thus, allopatric populations serve as the reference point for both sympatric populations: all else being equal, in the absence of limnetics, the null expectation is that benthics should have evolved to be indistinguishable from other allopatric populations, and vice versa (as in Fig. 1B, C).

We tested for character shifts in antipredator traits of sympatric species (benthics and limnetics) by comparing their defensive armor with that of allopatric populations. The defensive armor consists of a number of bony structures (Fig. 2) that in concert increase the probability of postcapture escape from vertebrate predators (trout and diving birds) (Reimchen 1983, 1994). When locked and erect, dorsal and pelvic spines increase the effective diameter of the stickleback, which, in addition to having sharp points, decreases the handling success of gape-limited predators such as cutthroat trout (Reimchen 1991). Lateral plates have a number of functions, including buttressing the dorsal and pelvic spines (Reimchen 1983), protecting the epidermis from lacerations by toothed predators (Reimchen 1992), and possibly interfering with the pharyngeal jaw mechanism of predatory fish (Reimchen 2000). The pelvic girdle buttresses the pelvic spines and helps create a protective bony ''ring'' around the stickleback midsection (Reimchen 1983).

## MATERIALS AND METHODS

### Populations

The study was carried out on stickleback populations of small lakes in the Strait of Georgia region of British Columbia, Canada (Fig. 3). Two categories of lakes were sampled. First, we sampled sympatric benthic and limnetic species from the five lakes previously studied by Schluter and McPhail (1992; Table 1). These five lakes occur in four unconnected watersheds. Two of the lakes, Priest and Emily, occur in the same watershed and are connected by a stream, but we treat them separately because there is little evidence of mixing between them (e.g., the benthic species in the two lakes have almost no mtDNA haplotypes in common; Taylor and McPhail 1999). The remaining three lakes occur in drain-

ages connected separately to the sea. Second, we sampled sticklebacks from six lakes containing only a single species of stickleback (hereafter ''allopatric populations''; Table 1). All six allopatric populations occur in separate drainages. The number of replicate allopatric populations reflects the rarity of lakes in the Strait of Georgia region that contain fish communities composed of sticklebacks and only a single piscivorous trout species (Vamosi 2003). Five of the allopatric populations sampled coexist only with cutthroat trout (*Oncorhynchus clarki*), and the sixth (Kwassin) coexists only with rainbow trout (*O. mykiss*). We included Kwassin Lake as an additional control after determining that its morphological characteristics were similar to those of stickleback populations from lakes containing cutthroat trout, a congener of rainbow trout. Although we lack quantitative estimates of predator densities, visual surveys and trapping suggest that many of the common avian (e.g., belted kingfisher, common loon, great blue heron) and invertebrate (e.g., backswimmer, dragonfly larva) predators of sticklebacks are present at both categories of lakes. We also sampled a marine (anadromous) population of sticklebacks from a stream (Fig. 3); only a single marine population was used because casual observation indicates that anadromous populations have uniformly robust armor. Because marine sticklebacks do not experience the same predation regime as the lake populations, we do not include this population in formal statistical analyses but rather for reference to the ancestral state.

### Measurements

We measured sticklebacks from the collections of Schluter and McPhail (1992) and the University of British Columbia Fish Museum. In total, we measured 181 benthic, 142 limnetic, 221 allopatric, and 40 marine sticklebacks (see Appendix for sample sizes of lake populations). Sticklebacks from lakes containing sympatric populations were classified as either benthic or limnetic according to body shape (Schluter and McPhail 1992); defensive armor itself was not used to classify specimens.

Eight external traits were measured on the left side of individual fish: standard length, body depth, gape width, lengths of the first and second dorsal spines, the pelvic spine, and the pelvic girdle, and number of lateral plates (Fig. 2). The first three measurements were collected for size adjustment, and the latter five are measures of armor. Individuals lacking a trait (e.g., no first dorsal spine) were assigned a value of zero. All traits were ln-transformed ($X + 1$) prior to analysis. Because of the variation in overall body size among individuals within populations and between populations, we corrected for size before comparing defensive armor traits among population types by calculating a composite ''size'' variable (i.e., first principal component, PC1). All three traits used contributed equally, and significantly, to PC1: body depth (component coefficient = 0.58), standard length (0.57), and gape width (0.57). The first principal component accounted for 94% of the variance among individuals. To correct for size we regressed each armor trait separately against PC1, using separate univariate regressions for each trait and population, and calculated for each population a mean value of $Y$ corresponding to $X = 5.0$, the overall mean
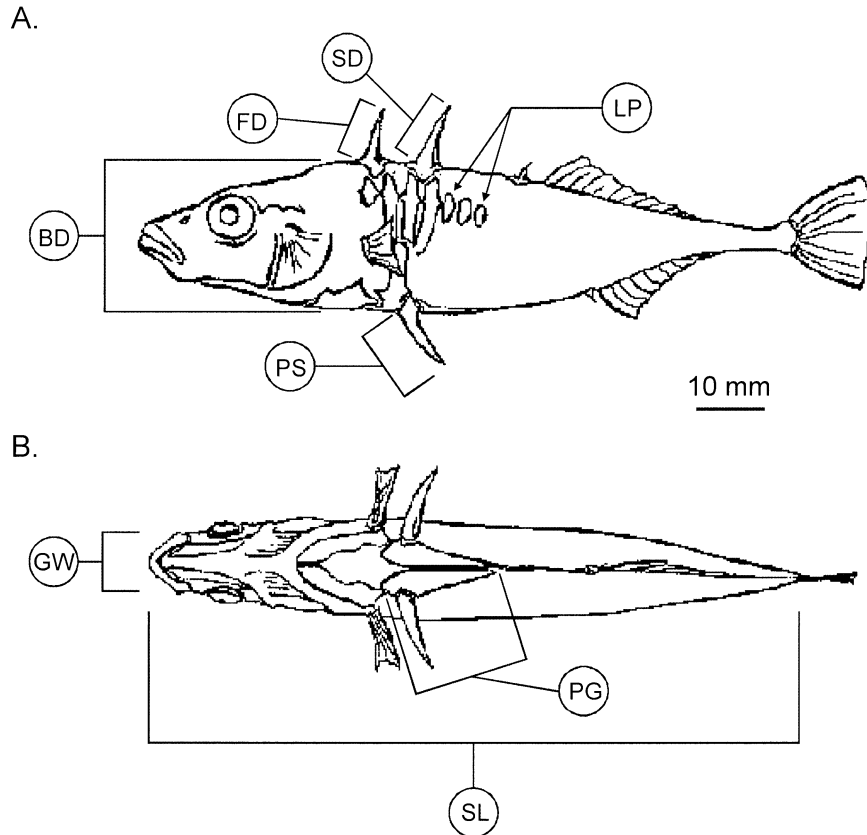
FIG. 2. Defensive armor traits considered in this study. Traits shown in the lateral view (A) are: body depth (BD, measured just anterior of the first dorsal spine); length of first dorsal spine (FD, measured at the front from base to tip); length of second dorsal spine (SD, measured at front from base to tip); number of lateral plates (LP, total number of any staining plates regardless of size); and length of pelvic spine (PS, measured at the front from base to tip). Traits shown in the ventral view (B) are: gape width (GW, measured at its widest point); length of pelvic girdle (PG, measured along longest axis); and standard length (SL, tip of the upper jaw to the end of the last vertebra).

size. Number of lateral plates was uncorrelated with size within populations, and we did not adjust this trait. Population means for size-adjusted armor traits and ln-transformed number of lateral plates are provided in the Appendix.

*Analyses*

We first analyzed ''total armor'' and subsequently individual armor elements. Total armor was calculated as the first principal component extracted from the covariance matrix of size-adjusted armor traits and ln-transformed number of lateral plates. The first principal component accounted for 79% of the variation among populations in their size-adjusted armor traits. Length of pelvic spine had the highest loading (component coefficient = 0.52), followed by length of first dorsal spine (0.51), length of pelvic girdle (0.47), number of lateral plates (0.45), and length of second dorsal spine (0.19). The major axis of variation in armor (hereafter, total armor) separated populations with short spines, a short pelvic girdle, and few lateral plates at one extreme from those with long spines, a long pelvic girdle, and many plates at the other extreme (Fig. 4). The population of benthics from Paxton Lake represented the low extreme, with many individuals having no lateral plates and (or) no pelvic girdle and spines. Limnetic and allopatric populations appeared to have similar distributions for total armor, with both being more heavily armored than benthic populations. As has been found for a number of other freshwater populations of threespine sticklebacks (e.g., Hagen and Gilbertson 1972; Reimchen 1994; Vamosi 2003), our limnetic, benthic, and allopatric populations have substantially reduced armor compared to marine sticklebacks. Marine sticklebacks, for example, had 31–34 lateral plates (on the left side of the body), whereas individuals from freshwater populations were never observed with more than nine lateral plates.

Nonparametric and robust methods were used to test for consistent direction divergence, exaggerated difference divergence, and midpoint shift because armor trait distributions were often conspicuously nonnormal. We tested for consistent direction divergence in armor traits by comparing the mean trait values of benthics with the mean trait value of limnetics. Two-sample tests rather than matched pairs tests were used because there was no significant lake effect on the mean value of sympatric limnetic and benthic species for any trait (Kruskal-Wallis tests, all $P > 0.30$; one way ANOVAs, all $P > 0.50$). We tested for exaggerated difference divergence in armor traits by comparing the mean absolute deviation of sympatric species with the mean absolute deviation of allopatric populations. The mean absolute deviation of a
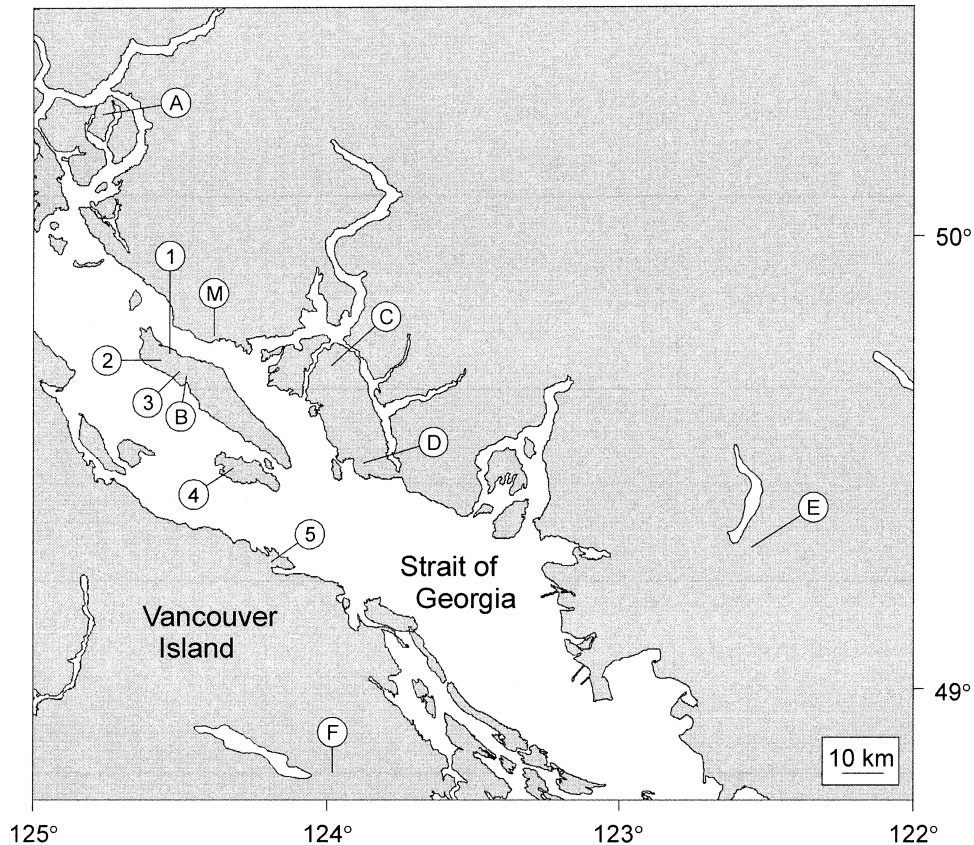
Fɪɢ. 3. Map of the study area. Numbered lakes contain two species of threespine sticklebacks, and lettered lakes contain allopatric populations. Lakes are (1) Emily, (2) Priest, (3) Paxton, (4) Hadley, (5) Enos, (A) Tom, (B) Cranby, (C) Klein, (D) Trout, (E) Mike, and (F) Kwassin. Marine sticklebacks were sampled at Lang Creek (M).

sample is the sum of the absolute values of the deviations from the mean, resulting in a measure of dispersion about the mean (e.g., Zar 1996). This is a robust analogue of the *F*-test used by Schluter and McPhail (1992), one not requiring normal distributions of population means. The number of sympatric and allopatric populations determines the degrees

of freedom for the test. We tested for midpoint shifts in each trait by comparing the means of the sympatric pairs of species with the mean trait values of allopatric populations.

## Rᴇsᴜʟᴛs

Sympatric limnetics and benthics exhibited consistent direction divergence and midpoint shift in most defensive armor traits, with exaggerated difference divergence also detected in a smaller subset of traits (cf. Fig. 1). Total armor was consistently lower in benthics than limnetics with no overlap between them in their distributions of population means (Fig. 4, Table 2). This pattern was repeated for four of five armor elements contributing to total armor: lengths of the second dorsal spine, pelvic spine, and pelvic girdle, and number of lateral plates. Length of first dorsal spine was also lower in benthics, albeit not significantly ($P = 0.06$). In the case of pelvic spine length and number of lateral plates there was no overlap in the distributions of population means (Table 2). Thus, differentiation in defensive armor between sympatric benthics and limnetics has paralleled their character displacement in trophic morphology and use of habitats and food resources (Larson 1976; Schluter and McPhail 1992).

Divergence in total armor of sympatric benthics and limnetics was significantly exaggerated compared with randomly paired allopatric populations (Table 3). A pattern of signif-

TABLE 1. Physical characteristics of lakes containing sympatric benthics and limnetics and those containing allopatric populations of sticklebacks.

| Lake | Elevation (m) | Surface area (ha) | Mean depth (m) |
|---|---|---|---|
| Lakes with species pair | | | |
| Emily | 23 | 7.2 | 3.0 |
| Enos | 55 | 17.6 | 4.0 |
| Hadley* | 50 | 6.7 | 3.5 |
| Paxton | 88 | 17.0 | 6.2 |
| Priest | 75 | 44.3 | 5.4 |
| Lakes with allopatric population | | | |
| Cranby | 69 | 44.6 | 3.2 |
| Klein | 121 | 13.5 | 12.0 |
| Kwassin | 175 | 2.2 | 1.9 |
| Mike** | 229 | 4.2 | 3.4 |
| Tom | 195 | 17.0 | 1.7 |
| Trout | 145 | 7.6 | 5.8 |

* Both species extinct as a consequence of catfish introduction in the early 1990s. ** Rainbow trout introduced subsequent to collection of samples.
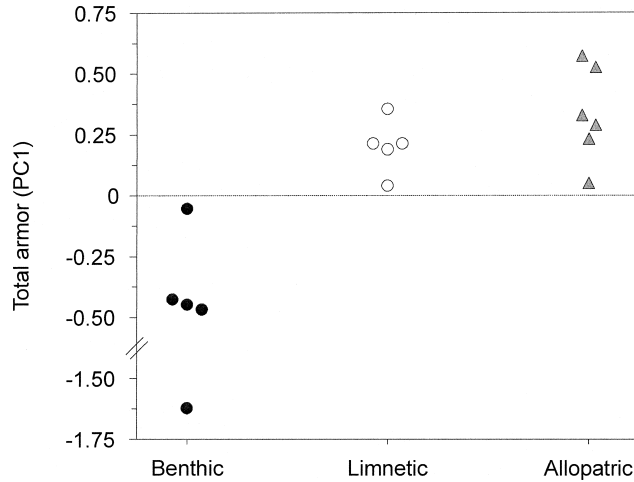
FIG. 4. Total armor of benthic (filled), limnetic (open), and allopatric (triangle) populations. This variable accounted for 79% of the total variance among population means in armor traits. There was no overlap in the distribution of total armor of limnetic and allopatric populations with that of benthic populations.

TABLE 3. Tests of exaggerated difference divergence in sympatry. Sympatric values are the mean absolute deviations between sympatric benthics and limnetics, averaged across five lakes. Allopatric values are the average of mean absolute deviations of six allopatric populations. $P$-values are from Wilcoxon rank sum tests. Significant differences are indicated in bold.

| Trait | Sympatric | Allopatric | $P$ |
|---|---|---|---|
| Total armor | 0.40 | 0.14 | **0.04** |
| Length of first dorsal spine | 0.23 | 0.11 | 0.36 |
| Length of second dorsal spine | 0.06 | 0.11 | 0.27 |
| Length of pelvic spine | 0.18 | 0.10 | 0.72 |
| Length of pelvic girdle | 0.19 | 0.06 | **0.04** |
| Number of lateral plates | 0.23 | 0.08 | 0.10 |

icant exaggerated difference divergence was observed for length of pelvic girdle, marginal exaggerated difference divergence for number of lateral plates, but not for lengths of the first and second dorsal spines or length of pelvic spine. Absence in some traits of significant exaggerated difference divergence despite the presence of consistent direction divergence is associated with high variation among populations within ecological types (Table 3, Appendix). The results from individual armor traits contrast with that for trophic characters, the majority of which exhibit exaggerated divergence in sympatric species (Schluter and McPhail 1992).

Finally, sympatry has been accompanied by a net reduction of defensive armor, averaged over the two species, compared with allopatric populations (Fig. 4, Table 4). This pattern was seen in total armor as well as in length of the first dorsal spine and lengths of both pelvic elements. Length of the second dorsal spine and number of lateral plates were the exceptions to the pattern (Table 4). Mean levels of armor in the latter two traits were also lower than the means of allopatric populations, but not significantly lower. These midpoint shifts in armor traits in sympatry were the outcome primarily of reductions in armor in benthic populations,

whereas limnetic populations were more similar to allopatric populations (Fig. 4). Benthics had significantly reduced total armor compared with allopatric populations (Wilcoxon rank sum test, $n = 5,6$, $P = 0.008$) as well as significant reductions in all individual armor traits (all $P < 0.05$). There was no overlap between benthic and allopatric populations in the means for total armor and lengths of the pelvic girdle elements. In contrast, mean total armor in limnetics was similar to that in allopatric populations (Fig. 4; Wilcoxon rank sum test, $n = 5,6$, $P = 0.12$). The same was true of all individual armor elements (all $P > 0.12$).

The above analyses revealed that individual armor elements tended to behave differently from one another in the various tests of character shifts. Length of the pelvic girdle, for example, was the only trait to show significant patterns for all three tests. This variability from trait to trait in the pattern of character shift implies that sympatry was accompanied not merely by changes in total armor, but also by changes in allocation to different armor elements. Indeed, while limnetics had similar amounts of total armor to allopatric populations (Fig. 4), they tended to have more lateral plates but shorter dorsal spines (Fig. 5). Furthermore, dorsal spine lengths were negatively associated with the number of lateral plates among both limnetic and allopatric populations (Fig. 5). Shifts in allocation to different armor elements suggests in turn that sympatry changed more than total predation intensity, but it also changed the form of selection imposed by predators on different armor elements.

## DISCUSSION

We tested for character shifts in antipredator traits of sympatric benthic and limnetic threespine stickleback species by

TABLE 2. Tests of consistent direction divergence in sympatry. Values are means of benthics and of limnetics from the five two-species lakes. Traits other than number of lateral plates are size adjusted, whereas number of lateral plates is ln-transformed ($X +$ 1). $P$-values are from Wilcoxon rank sum tests. Significant differences are indicated in bold. An asterisk indicates that there was no overlap between benthic and limnetic populations in mean armor distributions.

| Trait | Benthic | Limnetic | $P$ |
|---|---|---|---|
| Total armor* | $-0.60$ | 0.20 | **0.01** |
| Length of first dorsal spine | 0.73 | 1.15 | 0.06 |
| Length of second dorsal spine | 1.25 | 1.38 | **0.04** |
| Length of pelvic spine | 1.35 | 1.71 | **0.02** |
| Length of pelvic girdle* | 1.87 | 2.25 | **0.01** |
| Number of lateral plates* | 1.50 | 1.95 | **0.01** |

TABLE 4. Tests of midpoint shifts in sympatry. Sympatric values are means of sympatric benthics and limnetics from five lakes. Allopatric values are means of allopatric populations. $P$-values are from Wilcoxon rank sum tests. Significant differences are indicated in bold.

| Trait | Sympatric | Allopatric | $P$ |
|---|---|---|---|
| Total armor | $-0.20$ | 0.33 | **0.01** |
| Length of first dorsal spine | 0.94 | 1.33 | **0.01** |
| Length of second dorsal spine | 1.32 | 1.49 | 0.08 |
| Length of pelvic spine | 1.53 | 1.84 | **0.01** |
| Length of pelvic girdle | 2.06 | 2.26 | **0.01** |
| Number of lateral plates | 1.72 | 1.83 | 0.32 |

comparing their defensive armor traits with those of allopatric populations that coexist with only a single piscivorous trout species. Our procedure is analogous to previous tests of ecological character displacement that compared trophic traits of sympatric benthic and limnetic stickleback species with those of allopatric populations in the presence of the same community of resource types (Schluter and McPhail 1992). Sympatric species show exaggerated divergence in trophic traits that, together with later experimental studies of competition and selection (Schluter 1994, 2003; Pritchard and Schluter 2001), point to ecological character displacement as an important process in the evolution of species differences in trophic traits. Here we have shown that the same sympatric species also exhibit patterns of character shifts in defensive armor traits.

The simplest explanation for our findings is that armor differences between sympatric species reflect differences in habitat-specific selection pressures, either because of differences between habitats in the types of predators encountered or in the best modes of defense there (Abrams 2000). Under this hypothesis, divergence between limnetics and benthics in defensive armor is solely a by-product of ecological character displacement: competition has favored divergence in habitat, resource use and body morphology, which has in turn altered the interaction between stickleback populations and their predators, leading to divergence of defensive traits. Here, competition is the only interaction between sympatric stickleback species.

Several further observations are in accord with this explanation. Armor is chiefly a defense against vertebrate predators (Reimchen 1983, 1994), and vertebrates probably prey more heavily upon limnetics, which inhabit the open water zone, than benthics, which are found in the vegetated littoral zone (Vamosi 2002; Vamosi and Schluter 2002). In the open water, limnetics are more likely than benthics to encounter diving birds (e.g., loons, cormorants), which have been shown to be significant predators of subadult and adult sticklebacks ($>$ 40 mm standard length; Reimchen 1994). Limnetics may also have higher encounter rates than benthics with cutthroat trout, which also forage in open water (Nilsson and Northcote 1981; Reimchen 1994; Vamosi and Schluter 2002). In the face of higher predation rates by vertebrate predators in open water, we expect limnetics to invest more in plates and spines that deter vertebrate predators and increase the chances of post-capture escape and recovery.

In contrast, benthics may experience increased risk of predation, especially as juveniles, by large invertebrate predators (e.g., backswimmers, diving beetles, dragonfly naiads) that chiefly inhabit the littoral zone (Foster et al. 1988; Reimchen 1994; Vamosi 2002). Armor is no defense against invertebrate predators and, indeed, may be a liability. Invertebrate predators may be better able to grasp threespine sticklebacks having many plates and long spines (Reimchen 1980; Ziuganov and Zotin 1995; Vamosi 2002). Experiments with juvenile benthics and limnetics in littoral wading pools indicated that limnetics had significantly lower relative survival than benthics when exposed to predatory backswimmers (*Notonecta* sp.), which were frequently observed grasping limnetics by their erect spines (Vamosi 2002). Reduced encounter rates with vertebrate predators should relax selection

for defensive armor in benthics (Reimchen and Nosil 2002). Although we know of no good studies measuring the energetic costs of building armor, Andraso and Barron (1995) demonstrated a trade-off between defensive armor and escape-response performance in brook stickleback (*Culaea inconstans*), implying that the cost of elaborated armor may be worth paying only in the presence of vertebrate predators. Thus, the combination of release from predation by vertebrates and an increase in mortality by invertebrate predators should favor reduction in defensive armor elements in benthics.

Yet, although it is likely that sympatric sticklebacks indeed face different habitat-specific selection pressures from predators, there are indications that such differences are not sufficient to fully explain observed differences in armor between limnetics and benthics. As explained below, there are further hints that divergence of limnetics and benthics was also driven in part by interactions between sympatric stickleback species mediated via their shared vertebrate predators. Under this hypothesis, competition for shared resources is not the only interaction driving divergence.

The main finding supporting this interpretation is that sympatric species exhibit a downward shift in the amount of defensive armor, averaged over both species, compared with allopatric populations. This pattern is contrary to the prediction of the by-product of ecological character displacement argument, that is, allopatric populations should have levels of defensive armor intermediate to that of sympatric species. This prediction is based on the observation that allopatric populations tend to exploit both habitats (Schluter and McPhail 1992): encounter rates with invertebrate predators in the littoral zone should be higher than for limnetics, whereas encounter rates with vertebrate predators in open water should be higher than for benthics. Not only would allopatric populations be predicted to have less armor than limnetics according to hypothesized differences in habitat-specific predation pressures, those that forage predominantly in the littoral zone should have defensive armor levels comparable to that of benthics. Sticklebacks from Kwassin Lake, for example, have previously been shown to be indistinguishable from benthics in their diet and habitat use during the non-breeding season (table 2 in Schluter and McPhail 1992), yet did not overlap with benthics in any defensive armor traits (see Appendix).

A related observation suggesting this interpretation is that if divergence in armor of limnetics and benthics were driven solely by competition followed by habitat-specific predation, then armor of allopatric populations should also vary as a function of habitat use. Allopatric populations that make greater use of the benthic environment (i.e., littoral zone) should have reduced armor compared to those that make greater use of the limnetic environment (i.e., open water zone). Yet, we find that variation in armor traits among allopatric populations is small and population mean values for armor traits were not correlated with mean gill raker number ($r = -0.08 - -0.51$, $n = 5$, all $P > 0.29$), which is an index of foraging in open water environments (Schluter and McPhail 1992). Thus, allopatric populations maintain comparatively large amounts of armor, regardless of their relative use of the two habitats.
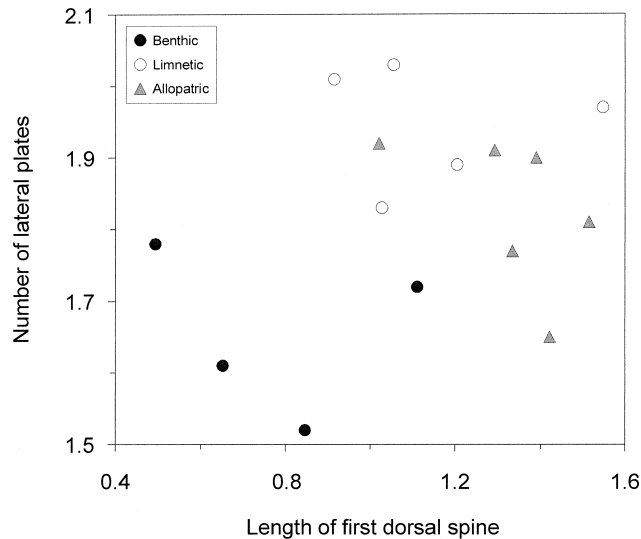
FIG. 5. Mean length of first dorsal spine and number of lateral plates of benthic (filled), limnetic (open), and allopatric (triangle) populations. Spine lengths are ln-transformed ($X + 1$) and size adjusted and number of lateral plates is ln-transformed ($X + 1$). Values for benthics from Paxton Lake are excluded.

The observation of differential allocation to armor elements suggests that, although selection for total armor by vertebrate predators is roughly equal for limnetics and allopatric sticklebacks, their encounter rates with specific vertebrate predators are different. Reimchen (1995) has shown that in a solitary stickleback population subject to predation by both trout and diving birds, periods of higher trout predation were associated with selection for higher plate number, whereas the opposite was true when bird predation was stronger. Reimchen and Nosil (2002) also found that in a trout-free lake, selection for more spines coincided with the presence of diving birds. A shift toward shorter spines and more plates in limnetics, compared with allopatric populations, suggests that limnetics suffer relatively more predation by trout and relatively less by diving birds. Thus, the presence of two prey species in sympatry may have subtle effects on predation risk and the form of natural selection, which may provide a partial explanation for the lack of reported cases of predator-driven character shifts (Abrams 2000).

The downward shift in armor observed in sympatry suggests that when two stickleback species are present in a lake the overall impact of vertebrate predators is reduced compared with their impacts when only one stickleback species is present. A reduction in per capita predation risk might be the outcome of a number of mechanisms. First, predators such as cutthroat trout may exhibit only a weak numerical response to the presence of two stickleback species if they are not limited solely by densities of their stickleback prey but also by other factors, such as breeding sites or overwinter survival. Second, the presence of stickleback prey in both habitats may result in increased predator interference and (or) satiation (Abrams 2000). Third, predators may become less selective in sympatry, with each species experiencing a release from predation compared to that experienced by a single allopatric population. One possible scenario that would produce asymmetric reductions in the armor of sympatric populations would involve a weak numerical response by trout to the presence of two stickleback species (i.e., increased number of stickleback prey). With a slight increase in the total number of sticklebacks eaten and a moderate bias towards limnetics (Vamosi and Schluter 2002), moderate reductions in benthics and little to no reduction in limnetics would be expected. Here, the proportion of individuals consumed in each population is lower than in allopatry, despite the greater numbers of sticklebacks consumed in total. Which scenario(s) are acting will depend on stickleback densities and numerical and functional responses of predators in allopatry and sympatry. We currently lack knowledge about population numbers and the nature of the relationship between sticklebacks and their predators, as in many systems (Abrams and Ginzburg 2000). If any of these mechanisms are acting, then ''apparent mutualism'' sensu Abrams (2000) occurs between sympatric benthics and limnetics, an indirect interaction mediated by shared predators. The apparent consequences of this interaction are highly asymmetric, producing a large shift in the benthic but only a small shift in the limnetic, with sympatric divergence in total armor the result.

If the above interpretation invoking apparent mutualism and its asymmetric effects on divergence is correct, then the sticklebacks would represent the first such example from nature. No good examples are yet known in which interactions between prey species mediated via shared predators have contributed to their divergence (Abrams 2000; Schluter 2000a). Observed patterns of divergence in sympatric sticklebacks therefore warrant further attention, including experimental tests of its causes.

Alternative mechanisms may also account for reduced armor mainly in benthics. Lakes with allopatric and sympatric populations may differ systematically in environmental features that affect the phenotype but are not readily apparent from our field observations or surveys of lake characteristics (Vamosi 2003). For example, lakes with sympatric sticklebacks may have lower levels of dissolved calcium than other lakes, a feature that has been correlated with armor reduction in some stickleback populations (Giles 1983; Bell et al. 1993). The observation that the total armor of limnetics is similar to that of allopatric populations makes this explanation unlikely. The presence of calcium-rich marl deposits in Paxton Lake (Larson 1976), where benthics showed the greatest reduction in armor, further discounts this possibility. Nevertheless, the possibility of unknown systematic differences between lakes is a serious problem, one common to all comparisons of populations in sympatry and allopatry, including those used to test ecological character displacement (Schluter 2000a), and one that is difficult to rule out. This is another reason why experimental tests of mechanisms should be attempted.

A less satisfactory explanation is that the reduced defensive armor of benthics is merely the result of benthic populations having been in fresh water for a longer period than limnetics, as suggested by physiological and molecular data (Withler and McPhail 1985; Kassen et al. 1995; Taylor and McPhail 2000). However, observations that the defensive armor of marine stickleback populations can shift dramatically in a relatively short amount of time ($\leq 30$ generations; Klepaker

1993; Bell 2001; Kristjánsson et al. 2002; Vamosi, unpubl. obs.) after isolation in fresh water, argues that there has been ample time for selection to mold armor to current predation regimes.

One other study, besides our own, of land snails (*Mandarina* spp.) on the Bonín Islands (Chiba 1999), has documented character shifts of putative defensive traits between sympatric species in association with ecological character displacement. Habitat segregation in sympatric snails was accompanied by divergence of shell coloration, such that species in wet and sheltered sites had dark shells and species in dry and exposed sites were brighter and often polymorphic. The shell color differences between habitats may reflect divergent selection for protective coloration in the face of contrasting background colors between habitats similar to that observed for *Cepaea nemoralis* (e.g., Shepard 1951; Cain and Sheppard 1954) although, to the best of our knowledge, this hypothesis has not yet been tested for *Mandarina* snails.

Because competition often leads to habitat differences and character displacement between sympatric species (Schluter 2000a, 2000b) and these different habitats may have divergent predation pressures (Terry 1981; Kruuk and Gilchrist 1997; Chiba 1999), it is likely that many sympatric species may show character shifts in defensive traits, yet there are few examples. This lack of evidence regarding the role of predation, especially the significance of shared predation, in morphological divergence of sympatric species partly reflects the secondary importance that predation has historically been given to competition in studies of divergence (Schluter 2000a). Future studies of closely related species that have sympatric and allopatric populations should attempt to evaluate the relative contributions of shared predation and habitat-specific predation pressures to evolutionary shifts of antipredator traits.

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Corresponding Editor: T. Smith

APPENDIX

Population mean values of total armor and individual armor elements. Traits other than number of lateral plates are ln-transformed ($X + 1$) and size-adjusted, whereas number of lateral plates is ln-transformed ($X + 1$). Population ecological types are benthic (B), limnetic (L), and allopatric (A).

| Lake | Eco. type | N | Total armor (PC1) | First dorsal spine | Second dorsal spine | Pelvic spine | Pelvic girdle | No. lateral plates |
|------|-----------|---|-------------------|--------------------|---------------------|--------------|---------------|--------------------|
| Emily | B | 39 | −0.45 | 0.85 | 1.21 | 1.43 | 1.99 | 1.52 |
| Enos | B | 30 | −0.05 | 1.11 | 1.35 | 1.61 | 2.08 | 1.72 |
| Hadley | B | 43 | −0.47 | 0.49 | 1.25 | 1.46 | 2.03 | 1.78 |
| Paxton | B | 30 | −1.62 | 0.54 | 1.20 | 0.74 | 1.24 | 0.85 |
| Priest | B | 39 | −0.43 | 0.65 | 1.24 | 1.53 | 2.04 | 1.61 |
| Emily | L | 39 | 0.04 | 0.92 | 1.28 | 1.62 | 2.23 | 2.01 |
| Enos | L | 40 | 0.21 | 1.03 | 1.50 | 1.84 | 2.31 | 1.83 |
| Hadley | L | 10 | 0.36 | 1.55 | 1.31 | 1.59 | 2.27 | 1.97 |
| Paxton | L | 30 | 0.19 | 1.20 | 1.43 | 1.72 | 2.18 | 1.89 |
| Priest | L | 23 | 0.21 | 1.06 | 1.29 | 1.75 | 2.25 | 2.03 |
| Cranby | A | 68 | 0.05 | 1.02 | 1.27 | 1.67 | 2.17 | 1.92 |
| Klein | A | 40 | 0.33 | 1.29 | 1.43 | 1.79 | 2.28 | 1.91 |
| Kwassin | A | 40 | 0.57 | 1.39 | 1.60 | 2.02 | 2.38 | 1.90 |
| Mike | A | 30 | 0.53 | 1.51 | 1.67 | 1.96 | 2.27 | 1.81 |
| Tom | A | 13 | 0.23 | 1.33 | 1.42 | 1.75 | 2.21 | 1.77 |
| Trout | A | 30 | 0.29 | 1.42 | 1.54 | 1.84 | 2.20 | 1.65 |