

Experimental test of predation's effect on divergent selection during character displacement in sticklebacks

Table 2. Selection on trophic and armor phenotypes in target populations arising from differences in survival

		Trophic selection		Armor selection				
Pond	Competition treatment	Mean gill raker count (\pm SE)	Survival differential		Mean plate count, all (\pm SE)	N	Mean plate count, 13 (\pm SE)	N
Predator addition								
5	+benthic	13.91 (0.10)	-0.17		-	-	-	-
5	+marine	14.08 (0.07)						
6	+benthic	13.61 (0.17)	-0.68*		5.56 (0.08)	157	4.85 (0.14)	47
6	+marine	14.29 (0.09)						
7	+benthic	14.07 (0.09)	-0.04		5.35 (0.07)	279	4.81 (0.14)	90
7	+marine	14.11 (0.10)						
Predator reduction								
1	+benthic	14.08 (0.10)	0.13		5.40 (0.06)	278	4.96 (0.09)	95
1	+marine	13.95 (0.08)						
2	+benthic	13.98 (0.09)	-0.18		5.16 (0.07)	335	4.47 (0.12)	122
2	+marine	14.16 (0.09)						
3	+benthic	14.23 (0.08)	-0.16		5.54 (0.05)	375	4.80 (0.10)	105
3	+marine	14.39 (0.09)						
4	+benthic	13.91 (0.11)	0.12		5.16 (0.06)	262	4.68 (0.09)	105
4	+marine	13.79 (0.08)						

Mean gill raker count is the average number of gill rakers on the first gill arch of surviving target individuals. Survival differential is the difference in this trait between competition treatments within ponds (i.e., +benthic mean minus +marine mean). Larger negative values indicate stronger selection. Mean plate count is the average number of lateral plates (on the left side of the body) for surviving target individuals from each pond, with sample sizes (N) as indicated. Mean plate count was calculated by using all surviving target individuals (mean plate count, all) and by using only those surviving target individuals that had 13 or fewer gill rakers (mean plate count, 13). *, $P < 0.001$.

Table 3. Pond conditions at the end of the experiment

Pond	Competition treatment	Survival			Mean growth (mm ± SE)		
		Target	Benthic or marine	Total	Target	Benthic or marine	Total
Predator addition							
5	+benthic	0.21	0.19	0.2	44.9 (0.2)	46.2 (0.4)	45.1
5	+marine	0.24	0.14		45.6 (0.2)	41.8 (0.2)	
6	+benthic	0.07	0.26	0.17	45.5 (0.4)	47.7 (0.3)	45
6	+marine	0.16	0.17		45.9 (0.2)	41.1 (0.1)	
7	+benthic	0.21	0.16	0.19	43.7 (0.3)	45.1 (0.5)	44.6
7	+marine	0.19	0.17		47.0 (0.2)	42.4 (0.1)	
Predator reduction							
1	+benthic	0.21	0.18	0.25	39.9 (0.2)	41.7 (0.4)	39.9
1	+marine	0.36	0.23		40.5 (0.1)	37.2 (0.1)	
2	+benthic	0.19	0.23	0.2	42.0 (0.3)	45.7 (0.4)	42.8
2	+marine	0.23	0.13		42.3 (0.2)	39.8 (0.1)	
3	+benthic	0.25	0.22	0.23	42.6 (0.2)	46.8 (0.8)	41.8
3	+marine	0.22	0.22		41.3 (0.2)	38.8 (0.1)	
4	+benthic	0.43	0.46	0.36	41.6 (0.3)	42.0 (0.5)	41.2
4	+marine	0.29	0.29		41.5 (0.2)	38.1 (0.1)	

Survival is the number of sticklebacks retrieved divided by the number introduced (± 0.01 SE in all cases except benthics in pond 4 where $SE = 0.02$). Total survival (± 0.01 SE) combines all sticklebacks in a pond (target + benthic + marine). Mean growth is the average standard length of stickleback retrieved. SE for survival estimates incorporate sampling error in the estimate of relative survival only. Total growth combines all sticklebacks in a pond (± 0.1 SE). In all cases, data from the +marine treatment derive from a complete enumeration of all fish retrieved, while data from the +benthic treatment are estimates based on the classification of a random sample of 175–260 individuals from each pond side.

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Supporting Text

Data Analysis

Discriminant Function. To discriminate benthics from target individuals at the end of the experiment, we employed a linear discriminant function derived from the measurement of 154 lab- and pond-raised individuals of known type (64 Paxton benthic, 17 Cranby ' Priest benthic hybrids, 32 Cranby ' Paxton benthic hybrids, and 41 Cranby ' Paxton limnetic hybrids). The following three traits were measured on each of the known individuals: the number of lateral plates, the number of gill rakers, and pelvic spine ratio. These traits were used because they are not affected by diet of the fish, unlike some soft-bodied traits (1, 2). The number of lateral plates was the average of the total count of any staining plate, regardless of its size, on both sides of the individual. The number of gill rakers was the total number counted on the first gill arch on the left side of individuals. The pelvic spine ratio was the length of the pelvic spine, measured on the left side of an individual, divided by standard length of the fish. The frequency distributions of all traits were similar in the mixture of known and experimental fish. The resulting discriminant function classified the individuals of known cross types with 97% accuracy. Three of the 64 benthics were misclassified as nonbenthics, and two of the 90 target fish were misclassified as benthics. The use of a quadratic discriminant function, which does not assume a common variance-covariance matrix for the traits in the groups being distinguished, did little to improve the classification (unpublished observations) so all subsequent analyses employ the linear function.

Selection on Trophic Traits Arising from Differences in Target Survival Methods. We estimated selection on the target populations arising from differences in their survival in the following way. A survival differential was calculated for each pond as the mean gill raker count among survivors in the +benthic competition treatment minus the mean count in the +marine treatment. Divergent natural selection on the target population between competition treatments would be indicated by a positive survival differential, because, under divergent selection, we expect the survival of more limnetic-like phenotypes to be highest in +benthic treatment, whereas benthic-like phenotypes should survive best in the +marine treatment. A negative differential would indicate selection on the target-favoring convergence in trophic morphology with the competitor (benthic and/or marine). Survival differentials were compared between competition treatments by using a paired *t* test between pond-sides, treating ponds as replicates. Sample sizes for these tests are as shown in Table 1 in the main text. Survival differentials were compared between predation treatments by using two-sample *t* tests that treated ponds as replicates.

Results. Little selection on trophic morphology was generated by differences in the survival of target individuals. Within ponds, the average gill raker count in target populations at the end of the experiment varied little between the +benthic and +marine competition treatments (Table 2). The exception was pond 6 in which the survival differential was significantly negative, indicating that target individuals that resembled the added population (benthic or marine) in trophic morphology tend to survive best. Nevertheless, survival differentials did not differ consistently between competition treatments (paired *t* test: $t_6 = 1.36, P = 0.22$) or predation treatments ($t_5 = 1.42, P = 0.21$). This result suggests that predators did not generate selection via differences in target survival.

Selection on Target Armor Traits

Measuring Selection. Armor traits are hypothesized to affect the vulnerability of sticklebacks to vertebrate predators, so selection on armor is expected to arise from differences in target survival among ponds. We tested for selection on armor by comparing the mean lateral plate count of target individuals between predation treatments, treating whole ponds as replicates. Selection by predators favoring more heavily armored target individuals would be indicated by a higher mean plate count in the predator addition than in the predator reduction ponds, whereas the reverse pattern would be indicative of selection for reduced armor. Significance of the differences in mean plate count between predation treatments was tested using a two-sample *t*-test. In addition, because stickleback mortality varied widely among ponds and there was overlap between predation treatments, we also tested the effect of mortality (a continuous variable) on mean plate count. Selection by predators and other agents of mortality favoring more heavily armored target individuals would be indicated by a significant positive relationship between mortality and mean plate count, whereas the reverse pattern would be indicative of selection for reduced armor.

The hybrid nature of the target population means that any relationship between mean plate count and survival is not necessarily indicative of selection acting directly on this trait. Rather, it could be the result of selection acting on other traits that covary with plate count, such as trophic traits. For this reason, the preceding analyses were conducted on two separate datasets: first, by using the mean plate count of all individuals within each pond classified as target individuals, and, second, by using a subset of this data composed primarily of Cranby ' benthic hybrids. These hybrids were composed of an equal proportion of individuals made by crossing Cranby females with benthic males from Paxton lake and Priest lake. Benthics from these two lakes are similar in their trophic phenotypes but differ in their armor phenotypes (3-5), allowing us to estimate selection on armor traits while controlling for

trophic morphology. We restricted our analysis to Cranby ' benthic hybrids by using the subset of all target individuals having 13 or fewer gill rakers. This subset likely excludes nearly all of the Cranby ' limnetic hybrids (only two of 72 individuals had 13 or fewer gill rakers within known laboratory-raised Cranby ' limnetic hybrids; H.D.R., unpublished observations), but likely also excludes a significant number of Cranby ' benthic hybrids (31 of 78 known laboratory-raised Cranby ' benthic crosses had more than 13 gill rakers). The two types of Cranby ' benthic hybrids differ little in their mean number of gill rakers (Cranby ' Priest benthic = 13.1 ± 0.1 SE, Cranby ' Paxton benthic 13.2 ± 0.2 SE) and the exclusion of fish having more than 13 gill rakers is not expected to alter the proportion of these types in our sample fish. Pond 5 was excluded from all armor analyses because the Cranby ' benthic hybrids in this pond were composed of unequal numbers of these two types of crosses (see *Methods* in the main text).

Results. Among target populations, mean number of plates did not differ consistently between predation treatments (Table 2). This result held whether we measured mean plate count using all target individuals ($t_4 = 0.89, P = 0.42$) or the subset of target individuals composed primarily of Cranby ' benthic hybrids ($t_4 = 0.65, P = 0.55$). There was also no significant relationship between mean plate count and mortality among ponds. Again, this result held whether we measured mean plate count using all target individuals ($r = 0.52, df = 4, P = 0.29$) or the subset of target individuals composed primarily of Cranby ' benthic hybrids ($r = 0.31, df = 4, P = 0.55$). None of these results change if the +benthic and +marine sides of each pond are analyzed separately (unpublished observations). These results all suggest that predators and other agents of mortality did not generate selection on armor phenotypes of target individuals.

1. Schluter, D. (1994) *Science* **266**, 798-800.
2. Day, T., Pritchard, J. & Schluter, D. (1994) *Evolution (Lawrence, Kans.)* **48**, 1723-1734.
3. McPhail, J. D. (1992) *Can. J. Zool.* **70**, 361-369.
4. McPhail, J. D. (1994) in *Evolutionary Biology of the Threespine Stickleback*, eds. Bell, M. A. & Foster, S. A. (Oxford Univ. Press, Oxford), pp. 399-437.
5. Schluter, D. & McPhail, J. D. (1992) *Am. Nat.* **140**, 85-108.