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ECOLOGICAL SPECIATION IN STICKLEBACKS: ENVIRONMENT-DEPENDENT HYBRID FITNESS

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Abstract.—"Ecological" speciation occurs when reproductive isolation evolves as a consequence of divergent selection between populations exploiting different resources or environments. We tested this hypothesis of speciation in a young stickleback species pair by measuring the direct contribution of ecological selection pressures to hybrid fitness. The two species (limnetic and benthic) are strongly differentiated morphologically and ecologically, whereas hybrids are intermediate. Fitness of hybrids is high in the laboratory, especially F_1 and F_2 hybrids (backcrosses may show some breakdown). We transplanted F_1 hybrids to enclosures in the two main habitats in the wild to test whether the distribution of resources available in the environment generates a hybrid disadvantage not detectable in the laboratory. Hybrids grew more slowly than limnetics in the open water habitat and more slowly than benthics in the littoral zone. Growth of F_1 hybrids was inferior to the average of the parent species across both habitats, albeit not significantly. The contrast between laboratory and field results supports the hypothesis that mechanisms of F_1 hybrid fitness in the wild are primarily ecological and do not result from intrinsic genetic incompatibilities. Direct selection on hybrids contributes to the maintenance of sympatric stickleback species and may have played an important role in their origin.

Key words.—Adaptive radiation, hybrid fitness, natural selection, reproductive isolation, speciation, stickleback.

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The proximate mechanisms of reproductive isolation are well understood in a variety of organisms, but we remain ignorant of the ultimate causes of speciation (Endler 1989; Templeton 1989). One of the oldest hypotheses is that of "ecological" speciation, whereby divergent natural selection between populations exploiting distinct resources or environments drives the evolution of differences leading ultimately to reproductive isolation (Dobzhansky 1937, 1951; Mayr 1942, 1963; Maynard Smith 1966; Endler 1977; Rice and Hostert 1993; Schluter 1996, 1998). The ecological speciation hypothesis is general and applies whether reproductive isolation evolves in sympatry, parapatry, or allopatry and whether speciation occurs solely as a by-product of selection or additionally involves reinforcement (Schluter 1996, 1998). Its core principle is that pre- and postzygotic isolation build between populations climbing separate adaptive peaks that correspond to distinct ecological niches. Hybrids, if they occur and are intermediate in phenotype, fall between the peaks and are removed by selection. This selection constitutes the environmental component of hybrid fitness and is manifested only in the wild (Rice and Hostert 1993; Waser 1993; Schluter 1998). An environment-dependent reduction in hybrid fitness is a prediction of ecological speciation that has rarely been tested (Grant and Grant 1992; Craig et al. 1997; Feder 1998).

Studies of hybrid fitness are typically carried out in a laboratory setting. Consequently they measure only the genetic component of postzygotic isolation (Dobzhansky 1937, 1951). (By "genetic" we mean reduced hybrid fitness stemming from the breakup of favorable allele combinations [Lynch 1991; Waser 1993].) This genetic component of postzygotic isolation is easier to measure than the ecological component because it requires no special environmental context for its detection. However, measurements of the genetic component reveal little about the forces that gave rise to it (i.e., natural selection or genetic drift). In contrast, measurements of the ecological component of hybrid fitness yield direct evidence of the role of divergent selection between environments in the origin of reproductive isolation. Such a test of divergent selection is most straightforward when species are still young, before a substantial genetic component of postzygotic isolation has accumulated.

In this paper we test for an ecological component of reduced hybrid fitness in ayoung species pair of sticklebacks (*Gasterosteus aculeatus* complex) inhabiting Paxton Lake, British Columbia, Canada (McPhail 1992, 1994; Schluter and McPhail 1992). The species are ecologically divergent. One of them (the limnetic) mainly exploits plankton in open water and has a fusiform body, narrow gape, and many, long gill rakers (gill rakers are protuberances along the gill arches that seive ingested prey and direct fluid movement within the buccal cavity; Sanderson et al. 1991). The other species (the benthic) exploits littoral invertebrates in sediments or attached to vegetation and has a robust body, wide gape, and few, short gill rakers. The two species exhibit strong premating isolation (Hatfield and Schluter 1996; Nagel and Schluter 1998; see also Ridgway and McPhail's [1984] study of a second stickleback species pair), but molecular data suggest a history of gene flow between them (Taylor et al. 1997). Presumed hybrids, as judged from morphological characteristics, are still occasionally detected in the wild (McPhail 1992). F_1 (and F_2) hybrids are intermediate between the parental species in these morphological traits (McPhail 1984, 1992; Hatfield 1997), and we hypothesized that this would confer a reduced fitness in the wild: hybrids would do worse than limnetics in open water and worse than benthics in the littoral zone.

We begin by presenting measures of the fitnesses of parent species and their F_1, F_2, and backcross hybrids in the laboratory. This part of our study extends earlier work by McPhail (1992; see also McPhail 1984), who examined hatching success of F_1 and F_2 hybrids between the same two species. Here

TABLE 1. Crossing design for the second generation (1993). Ten families of each cross type were made in the first generation (1992). First initial of a male or female (i.e., L in LB) refers to the species of its mother (limnetic or benthic), and the second refers to the species of its father. Numbers in table refer to the number of families.

	Male			
Female	LL	LB	BL	BB
LL	6	4	4	2
LB		8	9	
BL		8	8	
BB	6	4	4	6

we add both backcrosses, include a larger number of families, and measure a greater number of fitness components. Second, we report growth rates of laboratory-reared F_1 hybrids and parent species transplanted to field enclosures. We use the difference between fitness of hybrid sticklebacks in the wild and in the laboratory as a measure of the ecological contribution to hybrid inferiority. This part of our study builds on an earlier transplant experiment that used 10th-generation hybrids (individuals from a wild population established nine generations previously from F_1 hybrids). These hybrids experienced a foraging and growth disadvantage in both the habitats of the parental species (Schluter 1993, 1995). However, fitness of 10th generation hybrids does not give a measure of postzygotic isolation between species. The present study is the first to use the contrast between field and laboratory fitnesses to test an ecological mechanism of F_1 hybrid inferiority.

METHODS

Experimental Crosses and Rearing Conditions

We made all crosses by extracting eggs from gravid females using gentle abdominal pressure and combining the clutch in a petri dish with macerated testes dissected from a male. We then immediately selected a random subset of 30 eggs from the clutch to equalize egg densities. Each clutch was raised separately in half of a divided 100-L aquarium held at a constant temperature and a midsummer light regime (18°C; 16:8 L:D). Assignment of families to aquaria was random. We placed clutches in mesh-bottomed 250-ml containers aerated from below. Eggs that exhibited arrested or abnormal development were removed daily, using Swarup (1958) as a guide. As fish hatched they were counted and placed in the tank. Hatchlings were fed for several days on infusoria and were then switched to a diet of live *Artemia* nauplii and later of frozen crustaceans and insect larvae. Fish were fed twice daily to satiation by apportioning roughly the same amount of food to each aquarium. After six months of growth, fish were brought into reproductive condition by taking them through a three-month period of low temperature and short day length (4–10°C; 8:16 L:D) followed by a gradual shift to the original temperature and light regime.

We made crosses of benthic, limnetic, F_1 hybrid, F_2 hybrid, limnetic backcross, and benthic backcross using the design and sample sizes shown in Table 1. The first generation was made in 1992 using wild-caught fish from Paxton Lake. We used only laboratory-reared individuals to make the second generation in 1993. No sibs were ever crossed, and families were not sampled more than once for each type of cross.

We used only male F_1 hybrids when making backcrosses with parent species because of space limitations. A karyotype study by Chen and Reisman (1970) suggested that males are heterogametic in *G. wheatlandii*, the closest relative of *G. aculeatus*. We therefore assumed that a heterogametic sex, if present in the Paxton Lake species, was more likely to be the male. If males are heterogametic, then hybrid males are more likely than females to show reduced fertility and viability (an effect known as Haldane's rule; Turelli and Orr 1995; Wu et al. 1996; Orr 1997). If neither sex is heterogametic (a possibility in *G. aculeatus*; Chen and Reisman 1970) then our crossing design will not bias the results.

Laboratory Measures of Hybrid Fitness

Egg Fertilization Success.—We checked for egg fertilization 12–24 h after making a cross. Fertilization was scored under a dissecting microscope when the egg plasma membrane had separated from the shell and cell division was clearly visible. Fertilization success is the percent (of 30 eggs) fertilized. Results include all crosses made in both 1992 and 1993.

Egg Hatch Success.—Hatch success is the fraction of fertilized eggs hatched. Results include all crosses made in both 1992 and 1993.

Juvenile Growth Rate.—Growth was measured at 18 weeks after fertilization, by dividing total family mass by the number of individuals. These data were available for only the second-generation crosses (1993). Because size at hatching is negligible, mass at 18 weeks is an estimate of total growth to that date. Estimates were corrected for variation in final density using nonparametric regression, as described in Hatfield (1997), yielding data in the form of residuals. Residuals were then rescaled by adding to them the grand mean mass per individual. This density correction is imperfect because density changed gradually over the course of the experiment, but there is no reason to think that comparisons between crosses are biased.

To assess growth of hybrid juveniles we carried out a linear regression of family mean mass on a cross score representing the mixture of genes inherited from each parent species: -1 for limnetics, 1 for benthics, 0 for F_1 and F_2 hybrids, $-½$ for limnetic backcrosses, and ½ for benthic backcrosses. A linear relationship is expected if genes distinguishing the species act in a purely additive fashion when combined in the hybrids (Lynch 1991; Hatfield 1997). We also carried out a full analysis of variance in which each of the six cross types was entered as a separate factor level. A significantly better fit of the data to the full model than to the linear regression model is taken to indicate hybrid inferiority (or superiority) in growth. Fits were compared statistically using the general linear test approach (Neter et al. 1990); *ln*-transforming the data did not influence the results, and we present only analyses of untransformed data.

Female Fecundity.—We tested the hypothesis that F_1 hybrid females are equivalent to parent species in relationships of fecundity (number of eggs produced in a clutch) to body

TABLE 2. Means (± SD) of initial body mass and seven other morphological traits from fish used in the field experiments. All traits except mass, gill raker number, and plate number were *ln*-transformed and size-corrected using regression on a general size variable (cf. Schluter and McPhail 1992). Original units in millimeters.

Trait	Limnetics	F_1 hybrids	Benthics
Mass (g)	1.07 (0.21)	1.23 (0.21)	1.39 (0.19)
Standard length	3.93 (0.02)	3.90 (0.03)	3.87 (0.03)
Body depth	2.34 (0.03)	2.36 (0.04)	2.38 (0.03)
Mouth width	1.43 (0.04)	1.47 (0.05)	1.52 (0.04)
Gill raker number[1]	23.9 (0.99)	21.3 (1.38)	18.0 (1.54)
Gill raker length[2]	0.41 (0.08)	0.25 (0.13)	0.05 (0.09)
Pelvic spine length	1.84 (0.06)	1.60 (0.13)	−0.05 (0.16)
Plate number[3]	12.8 (1.15)	8.89 (1.83)	2.19 (2.29)

[1] Total number on first gill arch.
[2] Length of longest raker on first gill arch.
[3] Includes any staining plate (regardless of size) from both sides of an individual.

size (standard length). Measurements were made on the first or second clutch of one-year-old first-generation females. Females from different families were combined into a single dataset.

Combined Fitness Measure.—We took the product of fertilization success, hatch success, and growth to yield a combined fitness measure. We included only the 1993 crosses because data on all three fitness components were available. We tested hybrid performance using the same approach described above for growth. A significantly better fit of the data to the full model than to the linear regression model indicates hybrid inferiority (or superiority) in the combined fitness measure; *ln*-transforming the data did not influence the results, and we present only analyses of untransformed data.

Growth Rate in the Field

We compared growth rates of limnetics and benthics to that of their morphologically intermediate F_1 hybrids (Table 2) in the native habitats in Paxton Lake. Fish were confined to the open water or littoral zone using enclosures. The field experiment was carried out in April and May 1993. Experimental studies with enclosures suffer the fewest logistical constraints at this time of year: enclosures in the open water have the fewest benthic organisms settling on the mesh, and in both habitats diets of fish inside enclosures are similar to those of wild fish outside the enclosures (Schluter 1995).

We used 24 benthics (B) taken from 10 families, 24 limnetics (L) from seven families, and 48 F_1 hybrids from 13 families (six L × B crosses [female × male] and seven from B × L crosses). All fish were approximately 10 months old. This ensured that fish were large enough to be retained in mesh enclosures, but small enough to have potential for significant further growth. None of the fish was in reproductive condition at the start of the experiment, although a few males were in breeding color and a few females were gravid by its end. Deletion of gravid females from the dataset did not alter the results and we retain them in our analyses.

Enclosures for the open water habitat were cylindrical in shape, 1 m in diameter, 6 m in depth, and made of 6-mm knotless nylon mesh. They were designed to allow plankton to drift through the mesh, and allow diel vertical migration of prey. Twenty-four of these enclosures were suspended from two rafts anchored in a deep section (7–8 m) of the lake. Twenty-four enclosures placed in the littoral zone represented the benthic environment. The littoral zone enclosures were 1 × 1 m square, had open bottoms, and the sides were made of the same nylon mesh. These were placed along the edge of the lake in undisturbed littoral substrate at a depth of 1–1.5 m and were emptied of wild fish prior to the experiment. Enclosures were the same as those used by Schluter (1995).

A pair of fish was introduced to each enclosure. Twelve open-water enclosures received F_1 hybrids, whereas 12 received limnetics. In the littoral zone 12 enclosures received F_1 hybrids and 12 received benthics. This design compared growth of hybrids in each habitat to the single parental species that predominantly forages there. Fish were weighed and individually marked by clipping dorsal spines before being placed in the enclosures. Three weeks later they were removed and weighed again, then anaesthetized, preserved in formalin, stained with alizarin red, and stored in isopropyl alcohol for morphological and diet analysis (Schluter and McPhail 1992). The average growth rate (mg day^{-1}) of the two fish in each enclosure was used as the independent observation. We deleted enclosures from the analysis if one or both the fish had died or appeared moribund. This gave us a total of 20 littoral enclosures and 19 enclosures from the open-water habitat.

We examined stomach contents of experimental fish to confirm that enclosures replicated the appropriate habitats (22 benthics and 18 hybrids from the littoral zone, and a random sample of 12 limnetics and 12 hybrids from the open water; subsamples were taken because of the more numerous stomach contents of fish from open water). Littoral-zone prey consisted mainly of insect larvae (mean percent in diet was 32% and 18% for hybrids and benthics, respectively), gammarids (22%, 34%), and benthic copepods (32%, 27%). Diets in the open water consisted mainly of pelagic copepods (70%; 52%) and cladocera (21%; 23%). The enclosures thus succeeded in limiting hybrids to a diet characteristic of the local habitat and similar to that of the parent species occupying the same habitat. This suggests that no intermediate niche was available for hybrids within either littoral zone or open water.

RESULTS

No fitness differences were detected in any measure between F_1 hybrid offspring of the two parent combinations

TABLE 3. Proportion of eggs fertilized in laboratory crosses, and the proportion of fertilized eggs that hatched. *N*, number of families.

Offspring cross	*N*	Proportion fertilized	SE	*N*	Proportion hatched	SE
Limnetic	17	0.994	0.006	16	0.93	0.02
Limnetic backcross	10	0.997	0.003	8	0.90	0.03
F_1 hybrid	24	1		24	0.94	0.02
F_2 hybrid	35	0.998	0.001	35	0.94	0.01
Benthic backcross	8	0.988	0.009	8	0.80	0.03
Benthic	16	1		16	0.91	0.02

(LB vs. BL crosses; cf. Table 1). The two kinds of F_1 hybrids are therefore combined in all our analyses.

Laboratory Fitness Measures

Egg Fertilization Success.—Egg fertilization rate was high and not significantly different among cross types (Table 3; ANOVA on arcsine square root of proportions, $F_{5,104} = 1.72$, $P = 0.14$).

Egg Hatch Success.—Hatch success was high for all types of eggs except benthic backcrosses (Table 3). Differences among cross type were highly significant (ANOVA on arcsine square-root transformed proportions, $F_{5,101} = 4.10$, $P = 0.002$). Tukey's post hoc comparisons of means (Zar 1996) suggested that hatching success of the benthic backcrosses was significantly lower than that of the limnetic, F_1, and F_2 crosses; no other comparisons were significant.

Juvenile Growth Rate.—Results on juvenile growth rate were inconclusive. Significant differences among crosses were detected in mean growth rate (ANOVA, $F_{5,60} = 2.43$, $P = 0.045$). The linear regression of juvenile growth rate on cross score was not statistically significant (Fig. 1; $F_{1,64} = 3.02$, $P = 0.087$), possibly because growth of F_1 hybrids is elevated or growth of backcrosses depressed relative to the linear expectation (Fig. 1). However, the fit of the data to the full ANOVA model, in which each cross type is included as a separate factor level, was not significantly better ($F_{4,60} = 2.29$, $P = 0.076$). For this reason the source of significant variation among crosses is uncertain and cannot unambiguously be attributed to hybrid inferiority/superiority. In any case, none of the means differed from that expected under the linear model by more than 10% (Fig. 1), indicating that nonadditive effects on growth, if present, were not large.

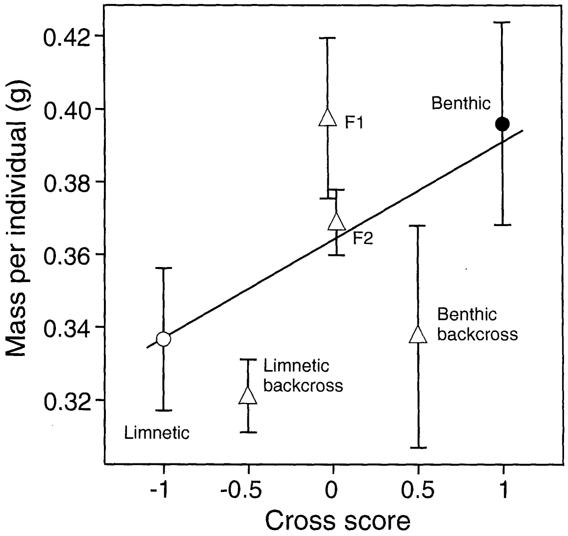

FIG. 1. Mean growth of six cross types reared in the laboratory (± SE), based on family means. Sample sizes are given in Table 1. The line is the regression of family means on cross score; it is the predicted line for all cross types if mean growth is the additive effects of genes inherited from each parent species.

Female Fecundity.—Fecundity was strongly related to body size (Fig. 2) (analysis of covariance of the three cross types, $F_{1,65} = 30.00$, $P < 0.001$). Slopes were not different among limnetics, benthics, and F_1 hybrids ($F_{2,65} = 1.39$, $P = 0.26$) nor were the intercepts ($F_{2,65} = 0.30$, $P = 0.74$). Thus, by this measure the data do not indicate a fecundity disadvantage in F_1 hybrids.

Combined Fitness Measure.—We obtained a combined fitness measure as the product of fertilization rate, hatch success and growth rate (Table 4). This measure differed significantly among crosses (ANOVA, $F_{5,56} = 4.24$, $P = 0.002$). As in the case of growth rate alone, a linear regression of combined fitness on cross score was not significant ($F_{1,60} = 0.10$, $P = 0.75$). However, the data fit the full ANOVA model significantly better than the linear regression ($F_{4,56} = 5.28$, $P = 0.001$), suggesting the presence of hybrid inferiority or superiority. Tukey post hoc tests indicated that benthic backcrosses were significantly lower than benthics, F_1, and F_2 hybrids. The latter three groups were not significantly different from each other or from any of the other crosses. We interpret this as support for hybrid breakdown in the benthic backcrosses. Mean fitness of benthic backcrosses was 77% (± 6% SE) that expected under the linear regression model.

Growth Rate in the Field

In contrast to the laboratory results, F_1 hybrids had a lower mean growth rate than the corresponding parent species in both habitats in the wild (Fig. 3). Hybrids grew at 73% the rate of benthics in the littoral zone, and at 76% the rate of limnetics in the open water. Significant hybrid inferiority was confirmed by the significant main effect of species (F_1 hybrid vs. parent species) on *ln*-transformed growth rates ($F_{1,36} = 8.081$, $P = 0.007$) in a two-way ANOVA. The second main effect, habitat (littoral zone vs. open water), was also significant ($F_{1,36} = 45.92$, $P < 0.001$), reflecting higher overall growth in the littoral zone. The interaction between habitat and species was not significant, indicating that the magnitude of hybrid disadvantage (on a *ln* scale) is roughly equal in the two environments.

Hybrids showed this disadvantage despite conditions that

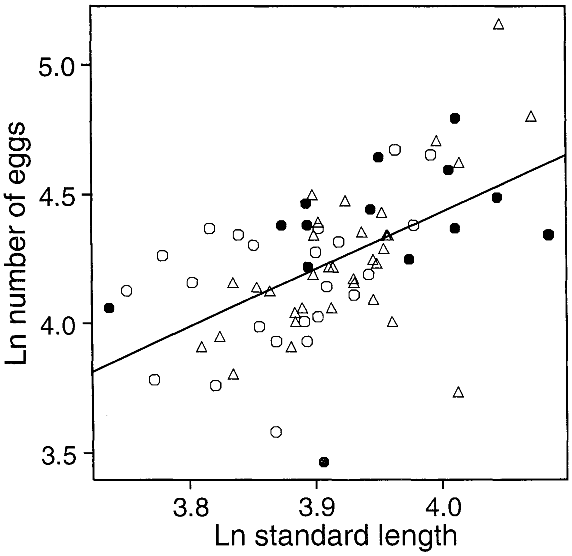

FIG. 2. Fecundity versus length relationship for laboratory-reared female limnetics (○), benthics (●), and F_1 hybrids (△). The line represents a regression through all points.

were excellent for growth. Mean growth rate of benthics was 31.5 mg/day, which is 50% higher than mean benthic growth rates observed in field experiments carried out in two previous years (Schluter 1995). Mean growth rate of limnetics was slightly higher than in these previous experiments (Schluter 1995). The better habitat for hybrid growth was the littoral zone, where they grew at more than twice as fast as in open water (Fig. 3).

DISCUSSION

We tested the hypothesis of ecological speciation in a young stickleback species pair by measuring the contribution of ecological selection pressures to hybrid fitness. By several measures fitness of F_1 and F_2 hybrids is high in the laboratory, but benthic backcrosses may show some breakdown. In contrast to the laboratory results, F_1 hybrids transplanted to field enclosures grew more slowly than the parent species in the habitat of that parent. This difference between laboratory and field results suggests that reduced F_1 hybrid fitness in the wild represents an ecological rather than a genetic component of postzygotic isolation. The result underscores the importance of measuring hybrid fitness in natural environments because postzygotic isolation between closely related species may otherwise be underestimated. The environmental component of hybrid fitness also implicates ecological selection pressures in the origin of reproductive isolation.

Laboratory Fitness Measures

Fitness measures of F_1 and F_2 hybrids were high in the laboratory setting. A lower egg hatch success and lower combined fitness measure in benthic backcrosses (offspring of benthic female × F_1 male crosses) were the main indications of reduced laboratory fitness in a hybrid cross in the laboratory. This result indicates that some genetic incompatibilities have accumulated between the parent species. The role of environment in the origin of these incompatibilities cannot be tested with current data.

TABLE 4. Combined fitness measures for laboratory-reared crosses from 1993. Combined fitness is the product of fertilization, hatch success, and growth in families in which all three were measured. *N*, the number of families.

Offspring cross	*N*	Combined fitness	SD
Limnetic	5	0.33	0.03
Limnetic backcross	7	0.30	0.01
F_1 hybrid	5	0.35	0.02
F_2 hybrid	32	0.34	0.01
Benthic backcross	7	0.25	0.02
Benthic	6	0.34	0.03

The contrast between laboratory F_2 and backcross results is puzzling because reductions in fitness resulting from dominance and epistasis between alleles from different parent species should affect both cross types (cf. Lynch 1991). Therefore, no genetic model comprised of terms for dominance, additive × additive epistasis, additive × dominance epistasis, and higher-order epistatic effects predicts our results for hatch success or combined fitness. The backcross results might be an artifact of the small number of families used (Table 1). However, they may suggest instead the presence of maternal effects (e.g., Dobzhansky 1937): all mothers of backcrosses were limnetic or benthic (i.e., parent species), whereas every F_2 fish had an F_1 mother.

Three additional aspects of fitness of hybrids between limnetics and benthics have been explored in the laboratory. Hatfield (1996) used the same crosses to measure levels of fluctuating asymmetry in morphological traits. No differences were detected between parent species, F_1, F_2, and backcross hybrids. Second, Hatfield and Schluter (1996) tested mating success of F_1 hybrid males with female benthics and

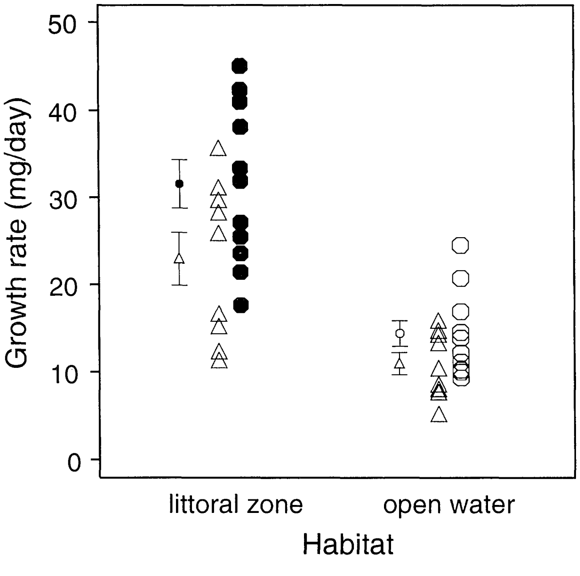

FIG. 3. Growth rates of fish transplanted to the littoral zone and open water of Paxton Lake. Symbols indicate limnetics (○), benthics (●), and F_1 hybrids (△). Smaller symbols and vertical intervals indicate means (± SE).

limnetics and found it to be as high as that of males of the two parent species. Finally, Hatfield (1995) compared parental care of limnetic and F_1 hybrid males. Male sticklebacks ventilate the eggs with frequent fanning of their pectoral fins, and eggs without proper ventilation fail to develop (van den Assem 1967; Sargent and Gebler 1980). Nesting males in halved 100-L aquaria were presented with single limnetic females, all of whom spawned. Eggs were collected after seven days, preserved in 2% formalin, and later examined for signs of abnormal embryo development. An embryo was scored as abnormal if the optical cup was not fully formed in one or both eyes (Swarup 1958). Embryo development success was high (based on $n = 17$ males) and no differences between male F_1 and limnetic males were detected (Hatfield 1995). Only one male (a limnetic) had fewer than 80% normal embryos. By this index, F_1 hybrid males are not deficient.

F_1 Growth Rate in the Field

Growth rate of F_1 hybrids in the laboratory is not inferior, and may even be slightly superior, to growth of the parent species (Fig. 1). Nevertheless F_1 hybrids have a morphology that is intermediate between that of the parent species (Table 2), and this is expected to compromise their foraging efficiency in the two main habitats in the wild (Schluter 1993). We transplanted limnetics, benthics, and F_1 hybrids to enclosures to test this ecological mechanism of hybrid fitness. In contrast to the laboratory results, growth rate of F_1 hybrids was reduced by about 25% in field transplants. In an earlier enclosure experiment, Schluter (1995) found that growth of individuals from a wild 10th-generation hybrid population (also morphologically intermediate) was lower than that of the parent species in the habitat of that parent. He also observed a correlation between growth rate of a cross in a given habitat and its foraging success there. We suggest that reduction in F_1 hybrid growth in the current experiment is also linked to reduced foraging efficiency, but further tests are needed to confirm this.

An alternate hypothesis not yet considered is that lower F_1 growth in the field represents an interaction between environment and the genetic determinants of postzygotic isolation. For example, it is possible that the physiological consequences of a breakup of favorable allele combinations in hybrids may only be expressed in stressful environments. A repetition of the field transplant with backcrosses might distinguish this hypothesis from the purely ecological model. Genetic breakdown is usually more evident in backcrosses than F_1 hybrids, whereas the ecological model predicts that backcrosses should have higher fitness in the wild than F_1 hybrids because they are more similar morphologically to the parent species.

A fitness set (Levins 1968) summarizes the relative fitnesses of limnetic, benthic, and hybrid species over the two habitats and the three years in which transplant experiments have been conducted (Fig. 4). The figure pools the current field results with those of Schluter (1995), grouping laboratory-raised F_1 hybrids with wild 10th-generation hybrid individuals from the previous study. The overall negative relationship indicates that improved growth in one environment is at the cost of reduced growth in the other. This pattern is confirmed by a strong interaction between habitat and species in a three-way ANOVA of growth rate on year, habitat, species, and habitat × species ($F_{2,100} = 8.70$, $P = 0.0003$).

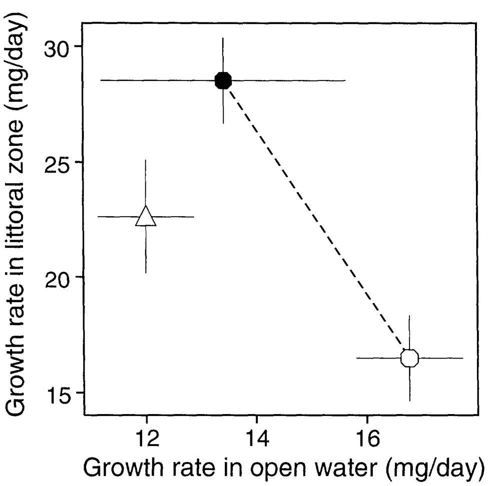

FIG. 4. Fitness set combining data herein with data from transplants in 1991 and 1992 involving both species and 10th-generation hybrids from a wild population (Schluter 1995). Differences in mean growth rate among years were substracted before combining. Overall means (± SE) are shown for limnetics (○), F_1 hybrids (△), and benthics (●). The dotted line connects the means of the two parent species.

Hybrid inferiority is indicated if growth rate of F_1 hybrids is lower than that of a parent species in the single preferred habitat of that parent. Such a reduction was confirmed in the present study (Fig. 3). Hybrid fitness is comparatively worse if mean growth falls below the dashed line in Figure 4, for here hybrid growth is no better than the growth of both parent species each averaged over *both* habitats. The hybrid mean indeed falls below this line, but not significantly ($F_{1,100} = 1.32$, $P = 0.25$; tested using a ANOVA model in which the means of all three "species" were constrained to lie on a single line).

Although these results are highly informative, our understanding of the ecological component of hybrid fitness remains incomplete. For practical reasons our transplant experiment confined individuals to one or other of the two main habitats in the lake. It did not allow individuals to find other habitats that may have yielded higher growth. Our experiment also lasted only three weeks, a comparatively short period even for a short-lived species (maximum life span 1–3 yr). It examined growth rate, not fecundity, survival under threat of predation, or disease. It was carried out on adult fish only. More recent experiments in an enclosure in the littoral zone of the lake and in experimental ponds suggest that selection on juvenile hybrids may be weaker than detected on adults (Vamosi, Hatfield, and Schluter, unpubl. data). How fitness of hybrids accumulates over the life span obviously deserves further attention.

Ecological Speciation in Sticklebacks

Our results suggest that divergent selection still acts against intermediate phenotypes in the wild and directly contributes to postmating isolation between the sympatric species. They constitute the third test supporting the hypothesis that sympatric sticklebacks are the result of ecological speciation, that is, that they formed as a consequence of divergent natural selection between distinct environments. Two earlier tests asked whether premating isolation is associated with morphological adaptation to alternative environments. First, the probability of hybridization strongly depends on the phenotypes of paired individuals: cross-mating occurs only between the smallest individuals of the larger species (the benthic) and the largest individuals of the smaller species (Nagel and Schluter 1998). Second, premating isolation (like morphology) has evolved in parallel in independently evolved species pairs inhabiting similar environments (Nagel 1994; Schluter and Nagel 1995; Rundle, Nagel and Schluter, unpubl. data). Both results may be accounted for if premating isolation evolves, at least in part, as a simple by-product of morphological divergence. However, some evidence suggests that assortative mating between limnetics and benthics has become strengthened in sympatry, possibly via "reinforcement" (Rundle and Schluter 1998).

Hybrid Fitness and Ecological Speciation

Hybrids between closely related species often exhibit high viability and fertility in a laboratory setting. Examples include Hawaiian and many other *Drosophila* (Coyne and Orr 1989; Templeton 1989), Galápagos finches (Grant and Grant 1992), Hawaiian silverswords (Carr and Kyhos 1981) and indeed many perennial flowering plants (Grant 1981; Gill 1989; Rieseberg and Wendel 1993; Macnair and Gardner 1998), some East African cichlid fishes (Fryer and Iles 1972), as well as other postglacial fishes (Wood and Foote 1990). The long-term persistence of such species in sympatry, where they often hybridize, seems inconsistent with the finding of little or no hybrid inferiority. Our data suggest that hybrids may fare more poorly than parental species in a wild setting, where an intermediate phenotype may experience ecological disadvantages.

Few other studies have explored the ecological basis of hybrid fitness. Grant and Grant (1992, 1996) showed that the changing availability of large and small seeds is the primary factor controlling fitness of hybrids between medium- and small-beaked ground finches (*Geospiza fortis* and *G. fuliginosa*) on a Galápagos island. Life histories of host races of the apple maggot *Rhagoletis pomonella*, particularly the timing of diapause, appear to be adaptations to the divergent phenologies of their different host plants (Feder 1998). Hybrid offspring of individuals that switch hosts are heavily disadvantaged as a consequence. Craig et al. (1997) showed that F_1 and F_2 hybrids between two host races of the fly *Eurosta solidaginis* survived poorly on the host plants of their parents, although the pattern of fitnesses was complex. Expanded leaf phenology of hybrids between *Populus* species may explain the far greater levels of insect herbivory they experience (Floate et al. 1993). Reciprocal transplants along an elevational gradient of two subspecies of sagebrush, *Artemisia tridentata*, and their hybrids indicated that each of the three populations has highest fitness in its own environment, with the hybrids most fit at intermediate elevation (Wang et al. 1997). Hybrids in the last two studies were not F_1 or F_2 hybrids, but rather were individuals from populations of hybrid origin.

All these studies emphasize the need to measure fitness of hybrids in their natural environment. The selection pressures responsible for low hybrid fitness in the wild may be responsible for the origin of the species themselves.

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