A TEST OF ECOLOGICALLY DEPENDENT POSTMATING ISOLATION BETWEEN SYMPATRIC STICKLEBACKS

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Abstract.—Ecological speciation occurs when reproductive isolation evolves ultimately as a result of divergent natural selection between populations inhabiting different environments or exploiting alternative resources. I tested a prediction of the ecological model concerning the fitness of hybrids between two young, sympatric species of threespine sticklebacks (Benthics and Limnetics). The two species are ecologically and morphologically divergent: the Benthic is adapted to feeding on invertebrates in the littoral zone of the lake whereas the Limnetic is adapted to feeding on zooplankton in the open water. The growth rate of two types of hybrids, the Benthic backcross and the Limnetic backcross, as well as both parent species, was evaluated in enclosures in both parental habitats in the lake. The use of backcrosses is ideal because a comparison of their growth rates in the two habitats estimates an ecologically dependent component of their fitness while controlling for any intrinsic genetic incompatibilities that may exist between the Benthic and Limnetic genomes. The backcross results revealed a striking pattern of ecological dependence: in the littoral zone, Benthic backcrosses grew at approximately twice the rate of Limnetic backcrosses, while in the open water, Limnetic backcrosses grew at approximately twice the rate of Benthic backcrosses. Such a reversal of relative fitness of the two cross-types in the two environments provides strong evidence that divergent natural selection has played a central role in the evolution of postmating isolation between Benthics and Limnetics. Although the rank order of growth rates of all cross-types in the littoral zone was Benthic > Benthic backcross > Limnetic backcross > Limnetic, neither backcross differed significantly from the parent from which it was mainly derived. Implications of this result are discussed in terms of ecological speciation and possible introgressive hybridization between the species. Results in the open water were less clear and were not fully consistent with the ecological model of speciation, mainly as a result of the low growth rate of Limnetics. However, analysis of the diet of the fish in the open water suggests that these enclosures may not have been fully successful at replicating the food regimes characteristic of this habitat.

Key words.—Backcross, ecological dependence, ecological speciation, hybrid fitness, postmating isolation, reproductive isolation, sticklebacks.

Although natural selection has long been thought to play a central role in the formation of new species, examples from nature in support of this hypothesis are scarce (Coyne 1992; Schluter 1996a; Futuyma 1998). The study of speciation has instead focused on other issues including the role of founder events in the evolution of reproductive isolation and the geographic context (i.e., sympatric vs. allopatric) of speciation. However, renewed interest in mechanisms of speciation has revived questions concerning selection's role in the evolution of reproductive isolation.

''Ecological speciation'' is a classic scenario for speciation in which selection plays a central role. Ecological speciation occurs when reproductive isolation evolves ultimately as a result of divergent natural selection between populations inhabiting distinct environments or exploiting alternative resources (Mayr 1942; Muller 1942; Dobzhansky 1951; Endler 1977; Rice and Hostert 1993; Schluter 1996a, 1998, 2000). Reproductive isolation (pre- and/or postmating) builds as populations climb separate adaptive peaks under the influence of natural selection. While laboratory experiments using *Drosophila* have demonstrated the feasibility of this model (reviewed in Rice and Hostert 1993), there are limited data concerning its importance in nature (but see MacNair and Christie 1983; Craig et al. 1997; Feder 1998, Nagel and

Schluter 1998; Filchak et al. 1999; Hatfield and Schluter 1999; Rundle et al. 2000).

Here I focus on the evolution of postmating isolation by divergent selection. Two types of postmating isolation are recognized (Rice and Hostert 1993; Coyne and Orr 1998; Rundle and Whitlock 2001). The first is ecologically dependent isolation (equivalent to Rice and Hostert's [1993] ''environment-dependent'' postzygotic isolation) and occurs when hybrids, because they are intermediate in phenotype, are less efficient at exploiting the dominant parental environment (or resource) and an intermediate environment (or resource) is lacking. In effect, these hybrids fall between niches and thus suffer reduced fitness. The second type of postmating isolation occurs when hybrids suffer reduced viability and/or fertility because of intrinsic genetic incompatibilities between the parental genomes independent of their ecological context (equivalent to Rice and Hostert's [1993] ''unconditional'' isolation). Although intrinsic genetic incompatibilities are an expected outcome of all mechanisms of speciation, ecologically dependent isolation is a unique expectation of ecological speciation (Fig. 1). I test this prediction using a recently diverged sympatric species-pair of threespine stickleback.

My study builds on previous studies of the stickleback species-pair in Paxton Lake, British Columbia, Canada (McPhail 1984, 1992, 1994; Schluter and McPhail 1992). The species are one of several pairs that evolved following the invasion of fresh water by the marine threespine stickleback

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A)

INTRINSIC GENETIC

Relative Fitness

P_1 B_1 F_1 B_2 P_2

B)   ECOLOGICALLY DEPENDENT

Env. A   Env. B

P_1 B_1 F_1 B_2 P_2 P_1 B_1 F_1 B_2 P_2
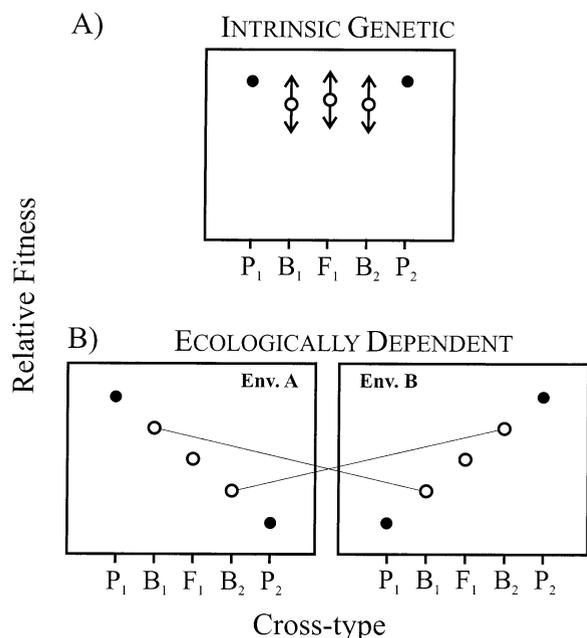
Cross-type

FIG. 1. Hypothetical examples of a reduction in the fitness of hybrids (open circles) relative to both parent species (closed circles) as a result of two alternative mechanisms. Reproductive isolation can be a function of either or both of these factors. (A) Postmating isolation results only from intrinsic genetic incompatibilities between parent populations independent of their ecological context. The relative fitness of hybrids may vary and depends on the extent of heterosis, genetic incompatibilities, and the breakup of favorable gene combinations interacting epistatically in the parents. (B) Intrinsic genetic incompatibilities are absent and reproductive isolation is solely the product of ecological mechanisms. Shown is the simplest case in which morphology is inherited in a purely additive fashion. Population P_1 is native to environment A and P_2 to B. Relative fitness increases as genotypes approach the species native to each habitat. Lines connect the same backcrosses in the two environments, demonstrating the reversal in relative fitness expected under the ecological model. Nonlinear patterns may result when dominance and epistatic effects contribute to hybrid fitness.

(*Gasterosteus aculeatus*) after the retreat of the Pleistocene glaciers 10,000–12,000 years ago (McPhail 1994). One species of every sympatric pair, referred to as the Benthic, is larger and more robust, has a wider gape and fewer, shorter gill rakers, and feeds on invertebrates in the littoral zone of

the lake. The other species, referred to as the Limnetic, is smaller and more fusiform, feeding primarily on zooplankton taken in the open water of the lake. It has a narrow gape and long and more numerous gill rakers (protuberances on the gill arches that sieve food particles or direct the movement of water through the buccal cavity [Sanderson et al. 1991]). These phenotypic differences have a polygenic basis and persist over multiple generations in a common laboratory environment (McPhail 1984, 1992; Hatfield 1997).

Premating isolation between the sympatric species is strong and previous studies indicate that it has evolved ultimately as a result of ecological mechanisms (Nagel and Schluter 1998; Rundle et al. 2000). Despite this premating isolation, F_1 hybrids are found occasionally in the wild (< 1% adults; McPhail 1992) and can be readily made in the laboratory using artificial crossing techniques. When raised in the laboratory, these hybrids are intermediate in morphology between the two parent species and are fully viable and fertile, showing no evidence of intrinsic genetic breakdown (McPhail 1984, 1992; Hatfield and Schluter 1999). However, individuals from a population established from F_1 hybrids ten generations earlier, which are also intermediate in morphology, have a foraging and growth disadvantage relative to each parent species in the habitat of that parent (Schluter 1993, 1995). Similarly, a transplant experiment in the wild revealed that F_1 hybrids between Benthics and Limnetics from Paxton Lake, despite showing no growth reduction in the laboratory, grew at 73% the rate of Benthics in the littoral zone and at 76% the rate of Limnetics in the open water (Hatfield and Schluter 1999).

The present study tests a further prediction of the ecological model, one that concerns the fitness of the backcrosses. If postmating isolation is ecologically dependent, hybrid fitness should depend on their phenotypic resemblance to the parent species. Fitness should be higher in hybrids that more closely resemble the native parent species in the habitat of that parent. The Benthic backcross and Limnetic backcross hybrids each resemble more closely the parent species from which it was mainly derived (Table 1; Hatfield 1997). Thus, the ecological model makes a clear prediction concerning their fitnesses in the wild: the Benthic backcross should outperform the Limnetic backcross in the littoral zone (the habitat preferred by the Benthic parent species), whereas the

TABLE 1. Mean ($\pm$ SE) initial body mass and seven other morphological traits for the actual fish used in the enclosure experiment. Standard length, body depth, gape width, and gill raker length were *ln*-transformed prior to analysis. Body depth, gape width, and gill raker length were size corrected by regression on *ln*(standard length).

Trait	Benthic		Benthic backcross		Limnetic backcross		Limnetic	
Mass (g)	1.08	(0.03)	1.12	(0.02)	0.99	(0.03)	0.95	(0.02)
Standard length	3.85	(0.01)	3.86	(0.01)	3.83	(0.01)	3.80	(0.01)
Body depth	0.032	(0.007)	0.028	(0.008)	−0.013	(0.011)	−0.050	(0.012)
Gape width	0.12	(0.01)	0.05	(0.02)	−0.08	(0.02)	−0.10	(0.02)
Gill rake number[1]	12.75	(0.14)	13.70	(0.23)	16.23	(0.21)	16.86	(0.20)
Gill raker length[2]	−0.16	(0.02)	−0.06	(0.02)	0.06	(0.02)	0.19	(0.02)
Pelvic spine length	0.24	(0.14)	1.54	(0.36)	3.77	(0.15)	4.18	(0.14)
Plate number[3]	0.42	(0.16)	2.70	(0.25)	5.18	(0.18)	6.59	(0.11)

[1] Total number on first gill arch.
[2] Length of longest raker on first gill arch.
[3] Includes any plate, regardless of size, measured on the right side of the individual.

converse should be true in the open water zone (e.g., see the lines connecting the two backcrosses in Fig. 1B).

The use of backcrosses has an additional advantage in that it allows us to discriminate between two possible hypotheses to explain the earlier F_1 transplant results of Hatfield and Schluter (1999). The first is that the reduced fitness of F_1 hybrids in the wild is the result of ecologically dependent isolation arising from their intermediate phenotype. Alternatively, reduced F_1 hybrid fitness is the result of intrinsic genetic incompatibilities between the Benthic and Limnetic genomes, but is expressed only in the stressful natural environment and not in the benign conditions of the laboratory (where predation and disease are absent and food is abundant). Indeed, this possibility is suggested by two past experiments. In the first, an increased difference in fitness between control and mutation accumulation lines of *Drosophila* was found when fitness was assayed under more harsh conditions (Kondrashov and Houle 1994). In the second, new mutations in diploid yeast strains that had no detectable fitness consequences in a benign environment were shown to have significant deleterious effects under a more harsh environment (Szafraniec et al. 2001; see Hoffmann and Merilä [1999] for a review of the expression of genetic variation in favorable and unfavorable environments). Thus, under this second hypothesis, the expression of reduced F_1 hybrid fitness is context dependent (benign vs. harsh conditions), but fitness does not conform to the ''ecological model'' in which hybrids suffer because they fall between the two niches of the parent species. Backcrosses, but not F_1 hybrids alone, can be used to distinguish these alternatives.

Backcrosses provide a measure of ecologically dependent isolation while controlling for intrinsic genetic isolation that may be present (Rundle and Whitlock 2001). This is because backcrosses are phenotypically different yet are similarly affected by genetic incompatibilities. Thus, a comparison of the fitness of the backcrosses in the habitat of each parent species allows the contribution of these incompatibilities to be removed. This expectation derives from an analysis of a quantitative genetic model of the phenotype of various individuals under the influence of outcrossing. The model, originally developed by Lynch (1991), was expanded to include two environments and the resulting genetic effects that act in an environment-dependent manner. Details are presented in Rundle and Whitlock (2001). Analysis of this model reveals that a comparison of the fitness of both backcrosses in both environments estimates the additive effect of genes that act in an environment-dependent manner, independent of the contribution of any intrinsic genetic incompatibilities. This method is conservative as the comparison does not estimate all genetic effects that may contribute to ecologically dependent isolation (e.g., estimation of dominance effects that act in an environmentally dependent manner requires additional crosses including the F_1). The current study is the first to use this method to estimate the extent of ecologically dependent isolation while controlling for any intrinsic genetic incompatibilities.

In addition to the prediction concerning the relative fitness of the backcrosses, ecological speciation also requires that hybrid fitness be reduced related to the native parent species in each habitat (i.e., selection must be divergent). Thus, I

TABLE 2. Crossing design and number of families used in the reciprocal transplant. First letter of a cross-type indicates the species of its mother and the second indicates the species of its father. Numbers indicate the number of separate families. Wild and lab refer to whether individuals of that cross-type were wild-caught or laboratory-reared fish.

Female	Male			
	BB (wild)	BL (lab)	LB (lab)	LL (wild)
BB (wild)	8	2	2	
BL (lab)	2			2
LB (lab)	2			2
LL (wild)		2	2	8

included both parent species in both habitats as a reference. Including the parent species also allows a test of a more general prediction of ecological speciation. In the absence of any intrinsic genetic isolation, ecological speciation predicts the rank order of the fitnesses of all cross-types. Basically, the closer in phenotype a cross-type is to the parent species native to that habitat, the higher its fitness. Thus, the rank order of fitness in the littoral zone of the lake is predicted to be: Benthic > Benthic backcross > Limnetic backcross > Limnetic. This order should be reversed in the open water.

MATERIALS AND METHODS

Experimental Crosses

All crosses were made in the laboratory in the summer of 1999. Eight families each of both parent species (Benthic and Limnetic) and both backcrosses (F_1 crossed with a Benthic or with a Limnetic) were made and raised separately using the fully balanced design shown in Table 2. All Benthic and Limnetic individuals used to make these crosses were wild-caught fish recently trapped from Paxton Lake on Texada Island, British Columbia. F_1 hybrids used in the crosses were laboratory-reared fish made the previous summer (1998) using wild-caught individuals from Paxton lake.

To make crosses, gentle pressure was applied to the abdomen of a gravid females causing her eggs to be released into a petri dish filled with water. Fertilization was achieved by macerating the testes dissected from a single male in the same petri dish. Each clutch was then hatched separately in an aerated, mesh-bottomed plastic cup suspended in one half of a divided 100 L aquarium. Hatchlings were fed daily a diet of live *Artemia* nauplii. As they grew, their diets were supplemented with frozen bloodworms (chironomid sp.) and frozen adult brine shrimp (*Artemia* sp.). Approximately two to three months after hatching, 30 individuals from each clutch were transferred into separate, undivided 100 L aquaria to equalize densities among families.

Juveniles were overwintered in the laboratory at a variable temperature (laboratory open to outside ambient temperature, 8–16°C) and a constant light regime (10L:14D). Fish were fed daily a mixed diet of live *Artemia* nauplii, frozen bloodworms and adult brine shrimp. These conditions were maintained until the fish were used in the transplant experiment to reduce the likelihood of individuals entering reproductive condition. At approximately nine months of age, family sizes were reduced to 20 individuals each, with individuals selected

nonrandomly within each family to minimize body size differences among families and among cross-types. In addition, the first dorsal spine of every fish was clipped to permit identification at the end of the transplant experiment. From the remaining 20 fish in each family, 12 were haphazardly selected for use in the transplant experiment.

Reciprocal Transplant

The design of the transplant experiment was similar to earlier experiments (Schluter 1995; Hatfield and Schluter 1999). The fitness surrogate was growth rate, measured over a three-week period, of individual fish held separately in enclosures in either parental habitat (littoral zone or open water) in the lake. In May 2000, a total of 96 enclosures were placed in Paxton Lake: 48 in the littoral zone and 48 in the open water of the lake. The study was conducted at this time because open water enclosures have the fewest benthic organisms colonizing them and previous studies have shown that the diet of fish inside the enclosures is similar to wild caught fish outside the enclosures at this time (Schluter 1995; Hatfield and Schluter 1999).

Littoral zone enclosures were 1 m $\times$ 1 m square, made of 6–10 mm knotless nylon mesh, with open bottoms and tops and a metal frame attached around the bottom margin. They were secured by sinking the metal frame into the sediment of the lake bottom with the top edge of each enclosure held out of the water on wooden stakes placed in each of the four corners. The enclosures were located haphazardly around the margin of the lake at a depth of approximately 1 m and were emptied of wild fish prior to the experiment. Open water enclosures were cylinders, made of the same nylon mesh, 1 m in diameter and 5 m deep, with a closed bottom and an open top encircled by a metal hoop. Eight enclosures were suspended from each of six rafts anchored in the deepest part of the lake.

Prior to introduction, each fish was individually weighed to within 0.01 g using an Ohaus CT 200 portable balance (Ohaus Corporation, Florham, NJ). On May 1, 2000, a single individual was placed in each enclosure. Twelve individuals (from eight families) of each of the four cross-types were used in each habitat. All fish were approximately 11 months old when introduced to the enclosures and were large enough to prevent their escape but small enough to allow for significant further growth. Past studies have shown that calanoid copepods, a large component of the diet of wild Limnetics, tend to abandon open water enclosures (Schluter 1995). For this reason, the contents of a single plankton tow, taken in the open water of the lake at a depth of 0–2 m over an approximately 100 m distance, was proportioned among the open water enclosures daily.

After three weeks, all fish were removed from their enclosures, weighed, anaesthetized, and then placed in 10% Formalin. Growth rate (mg day^{-1}) of the fish from each enclosure was used as the independent observation. Fish were recovered from 45 of the littoral zone enclosures (two Limnetics and one Limnetic backcross were not retrieved) and from all 48 of the open water enclosures. Although none of the fish were in reproductive condition at the start of the experiment, a few were by the end. The weight of both males

and females may change when they enter reproductive condition, so to avoid confounding effects, two gravid backcrosses (one Benthic and one Limnetic) were deleted from the littoral zone analysis, leaving 43 replicates. Conclusions are similar when they are included.

Stomach contents were examined from all fish in both habitats. Variation in diet was measured as percent organism number with whole prey items identified and grouped into broad categories (e.g., insect larvae, ostracods, chydorids, pelagic copepods) indicative of the habitat in which they are primarily found (e.g., littoral vs. open water).

RESULTS

Individual growth rates varied widely, within and between cross-types, in both the littoral zone and open water enclosures (Fig. 2A). In the littoral zone, Benthics grew fastest on average (17.2 mg day^{-1}), followed very closely by Benthic backcrosses (16.7 mg day^{-1}), with Limnetic backcrosses growing more slowly (7.2 mg day^{-1}), but still outperforming Limnetics (3.7 mg day^{-1}; Fig. 2B). In the open water, Benthics and Benthic backcrosses grew slowly and at similar rates (5.2 and 4.2 mg day^{-1}, respectively). Limnetic backcrosses performed better (10.1 mg day^{-1}), whereas Limnetics performed relatively poorly, growing at the same rate as Benthics (5.2 mg day^{-1}; Fig. 2B).

In accord with the prediction of ecological speciation, the fitnesses of the two backcrosses were ecologically dependent and reversed in the two habitats. In the littoral zone Benthic backcrosses grew at more than twice the rate of Limnetic backcrosses, whereas in the open water Limnetic backcrosses outperformed Benthic backcrosses by a similar margin (Fig. 2B). This difference was confirmed by a significant interaction between habitat (littoral vs. open water) and backcross type (Benthic vs. Limnetic) in a two-way ANOVA on growth rates ($F_{1,41} = 17.61$, $P < 0.0001$). The main effect of habitat was also significant, indicating that overall the growth rate of backcrosses was higher in the littoral zone than in the open water ($F_{1,41} = 6.72$, $P = 0.013$). The other main effect of backcross was not significant, indicating that the difference in growth rates of the backcrosses was similar in the two habitats ($F_{1,41} = 0.91$, $P = 0.346$). These conclusions are not altered if the data are *ln*-transformed (with the addition of a constant to make every growth rate positive), with the exception that the habitat term becomes marginally nonsignificant ($P = 0.057$).

Regarding the second prediction of ecological speciation concerning the rank order of fitnesses of the cross-types in each habitat, results were mixed. In the littoral zone, cross-type (i.e., Benthic, Benthic backcross, Limnetic backcross, Limnetic) had a significant effect on growth rate ($F_{3,39} = 9.18$, $P < 0.0001$) and, although the rank order of the cross-types was as expected, not all differences were significant. A Tukey posthoc comparison of means (Zar 1996) indicated that the growth rates of Benthics and Benthic backcrosses were significantly higher than that of Limnetic backcrosses and Limnetics, but no other comparisons were significant (Fig. 2B). Thus, Benthic backcross fitness was not significantly lower than that of Benthics, the type native to the littoral zone. In addition, growth rates of Benthic backcross
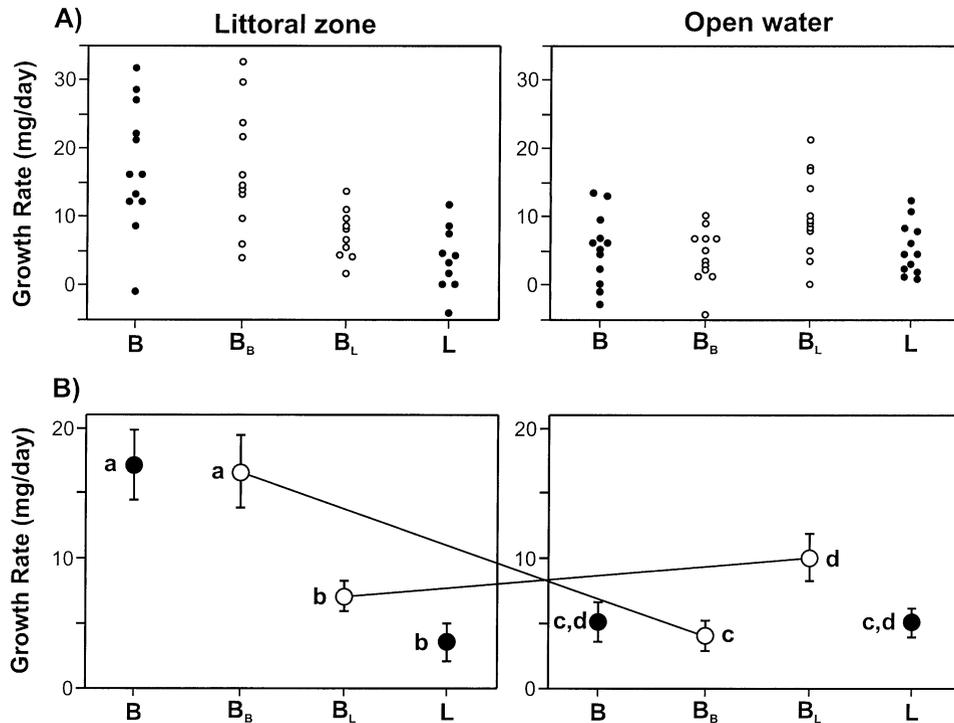
FIG. 2. (A) Growth rates of hybrid (open circles) and parent species (closed circles) individuals when held for three weeks in enclosures in the littoral zone and open water of Paxton Lake. Individuals belong to one of four cross-types: Benthic, B; Benthic backcross, B_B; Limnetic backcross, B_L; and Limnetic, L. (B) Mean growth rate ($\pm$ SE) of each cross-type. Lines connect the same backcrosses in the two habitats. Letters (a,b and c,d) indicate significantly different means using Tukey's post hoc comparison of means (Zar 1996). Comparisons are performed among the four cross-types separately for each habitat.

individuals were highly variable, with some individuals outperforming the average Benthic (the type native to the littoral zone; Fig. 2A).

In the open water, growth rate was also dependent on cross-type ($F_{3,44} = 3.69$, $P = 0.018$) but results were less clear and the rank order of cross-types was not fully as expected under the ecological model of speciation (Fig. 2B). As expected, Benthics and Benthic backcrosses grew slowly; these were the only cross-types in which some individuals lost weight. Also as expected, Limnetic backcrosses performed better. However, contrary to expectation Limnetics, the type native to this habitat, performed relatively poorly and grew at the same rate as Benthics. A Tukey posthoc comparison of means (Zar 1996) indicated that the only significant difference in growth rate was between Benthic backcrosses and Limnetic backcrosses.

Analysis of the diet of the fish indicated that the littoral enclosures were successful at replicating the food regimes of this habitat. The gut content of fish in these enclosures consisted primarily of benthic invertebrates such as insect larvae (41%, 36%, 38%, 33%; mean percent in diet for Benthics, Benthic backcrosses, Limnetic backcrosses, and Limnetics, respectively) and ostracods and chydorids (56%, 52%, 38%, 57%). For neither of these prey types do the proportions in the diet differ significantly among cross-types when tested using a one-way ANOVA (proportions were arcsine transformed prior to testing; insect larvae: $F_{3,37} = 0.11$, $P = 0.95$; ostracods and chydorids: $F_{3,37} = 0.78$, $P = 0.51$).

In the open water enclosures, diets were similar to that of wild Limnetics in the lake, consisting mainly of zooplankton such as pelagic copepods (51%, 37%, 77%, 62%). Differences in these proportions among cross-types approached significance however ($F_{3,37} = 2.44$, $P = 0.08$), suggesting that different cross-types may have been exploiting different food resources. These differences in diet among cross-types mirror their differences in growth rate, with Benthic backcrosses consuming the lowest fraction of zooplankton and also growing slowest, while Limnetic backcrosses consumed the highest proportion of zooplankton and also grew fastest (Fig. 2B). Ostracods were also found in the diets of the open water fish (29%, 40%, 15%, 26%), although the proportion in the diet does not differ significantly among cross-types ($F_{3,37} = 1.56$, $P = 0.21$). Ostracods are a prey item generally associated with the littoral zone of the lake and not normally found in significant numbers in the diet of wild Limnetics (Schluter 1993). Their presence also suggests that the environment within these enclosures was not fully representative of the open water environment of the lake.

DISCUSSION

Under the hypothesis of ecological speciation, reproductive isolation arises ultimately as a consequence of divergent natural selection between environments or niches (Schluter 1996a, 2000). Here I have focused on the evolution of ecologically dependent postmating isolation, a unique prediction

of the ecological model, between a young, sympatric species-pair of threespine stickleback. I have extended earlier work by measuring growth rate, a component of fitness, of Benthic and Limnetic backcrosses, as well as both parent species, in enclosures in the wild. The two types of backcross differ considerably in morphology; the Benthic backcross is intermediate in morphology between the Benthic and F_1 hybrid while the Limnetic backcross is intermediate between the Limnetic and F_1 hybrid (Table 1). My results demonstrate a large, environmentally dependent genetic effect in the growth rate of the backcrosses, indicating a striking pattern of ecological dependence. In the littoral zone Benthic backcrosses grew at more than twice the rate of Limnetic backcrosses, while in the open water Limnetic backcrosses grew at more than twice the rate of Benthic backcrosses (Fig. 2B). Such a reversal of relative fitnesses of these two hybrids in the two habitats can only be the product of divergent natural selection and is strong evidence that ecological mechanisms have contributed to the evolution of postmating isolation between these species (Rundle and Whitlock 2001). In addition, neither backcross showed a substantial reduction in growth relative to the parent species from which it was mainly derived, supporting past work which suggested the absence of any strong intrinsic genetic mechanisms of postmating isolation between Benthics and Limnetics (Hatfield and Schluter 1999). High intrinsic viability and fertility of hybrids has been observed in other fishes (e.g., Wood and Foote 1990) and is consistent with hybrid inferiority having an ecological basis (Schluter 1996b). However, few studies of hybrid fitness have attempted to determine the separate contribution of these two forms of postmating isolation (but see Lu and Bernatchez 1998). Doing so is crucial if we wish to make inferences about mechanisms of speciation in nature (Rundle and Whitlock 2001).

The hypothesis of ecological speciation also makes predictions concerning the rank order of fitnesses of all four cross-types in both habitats and requires that hybrid fitness be reduced relative to the native species in each environment. Given the intermediate morphologies of the backcrosses (Table 1), the rank order in the littoral zone was predicted to be: Benthic > Benthic backcross > Limnetic backcross > Limnetic. This order should be reversed in the open water. Although the rank order of cross-types in the littoral zone was as predicted, differences in growth rate between pairs of cross-types were not all significant. Specifically, neither backcross differed significantly from the parent species from which it was mainly derived, whereas the two backcrosses differed significantly from one another (Fig. 2B). Such a result is not unexpected given our knowledge of the quantitative genetics of the morphological differences between these species. With the exception of armor traits (that also include a significant dominance component), the morphological differences between Benthics and Limnetics are primarily additive (Hatfield 1997). Thus, if growth rate is a function of morphology and morphology is inherited in a roughly additive fashion, each backcross is expected to be closer in growth rate to the parent species from which it was mainly derived, and the difference between the two backcrosses should be greater.

Nevertheless, the fitness of Benthic backcrosses in the littoral zone was variable and high, with some individuals exceeding the growth rates observed in some Benthic individuals (Fig 2A). This mirrors results from other systems in which the fitness of some hybrid genotypes equals or exceeds that of nonhybrids (Arnold 1997; Arnold and Emms 1998). The high fitness of some hybrids suggests that hybridization may have significant evolutionary consequences, such as the introgression of genetic material between the parent species (Dowling and DeMarais 1993; Arnold 1997). Introgressive hybridization is common in fish (Arnold 1997; Wilson and Bernatchez 1998) and has been suggested to explain the discrepancy between the nuclear and mtDNA phylogenies of Benthics and Limnetics (Taylor and McPhail 1999, 2000). It is hypothesized that gene flow between the Benthic-Limnetic pair within a lake following secondary contact may be responsible for their near-indistinguishable mtDNA (Taylor and McPhail 2000); the high fitness of some hybrid genotypes, such as observed in the current study, may facilitate such a process.

The high relative fitness of the backcrosses in both habitats also appears inconsistent with the ecological model of speciation, suggesting weak postmating isolation overall between Benthics and Limnetics. The production of backcrosses, however, is dependent on F_1 hybrid fitness, which is reduced as a result of ecological mechanisms. A previous reciprocal transplant experiment showed that F_1 hybrids grew at approximately 75% the rate of the native parent species in each habitat (Hatfield and Schluter 1999). Individual fitness, however, was variable in this study as well and in both habitats some hybrids grew as fast or faster than some individuals belonging to the native parent species. Although suggestive of relatively weak postmating isolation between Benthics and Limnetics, as noted by Hatfield and Schluter (1999), we are a long way from estimates of lifetime fitness. To date we have measured only one component of hybrid fitness, growth rate, over only a small portion (three weeks) of their total life span (maximum one to three years). Other forms of selection may also act against hybrids, further reducing their fitness. For example, it has been shown that F_1 hybrid males suffer reduced mating success when in competition with Limnetic males, the predominate type of male in their preferred mating habitat (Vamosi and Schluter 1999). Other components of fitness, including predator avoidance, disease resistance, and fecundity, measured over more inclusive portions of their life span, remain to be addressed. Thus, final conclusions as to the relative fitness of various hybrids await more inclusive fitness measures.

Returning to the prediction of ecological speciation concerning the rank order of the cross-types, results in the open water were less clear, mainly because of the low growth rate of Limnetics (the type expected to perform best in this habitat). Limnetics grew at the same average rate as Benthics and did not differ significantly in growth rate from the Benthic backcrosses (Fig. 2B). In contrast to these results, two past studies that used the same open water enclosures, in the same lake, at the same time of the year, revealed that Limnetics grew faster than Benthics (Schluter 1995) and faster than F_1 hybrids (Hatfield and Schluter 1999). If biologically accurate, the current result stands in contrast to these past studies and suggests that the relative fitness of various cross-

types may vary temporally. Although such temporal variation in hybrid fitness has been observed in other systems (e.g., Grant and Grant 1992, 1993; Britch et al. 2001) and its presence does not eliminate a role for divergent natural selection in speciation (indeed, its presence may actually be indicative of ecological mechanisms playing a central role in reproductive isolation; see Schluter 2000), there is an alternative scenario that must be considered.

An alternative scenario for the poor relative performance of Limnetics is that it was an artifact of the experimental design. A number of such possibilities exist, including natural or artificial selection acting in the laboratory, the use of poor fitness surrogates, and ''enclosure effects'' in the lake. Natural and/or artificial selection that acted when the fish were being raised in the laboratory could have changed the mean phenotype of certain cross-types, thus altering their relative fitnesses when transplanted to enclosures in the lake. Although I did not conduct quantitative measure of mortality when raising the fish, few individuals died of ''natural'' causes during this time, suggesting that natural selection was weak. I did attempt, however, to minimize size differences between cross-types by nonrandomly selecting individuals when reducing family sizes (see Methods), a procedure that entailed discarding the smallest Limnetics (Limnetics are generally smaller than Benthics; Schluter 1993, 1995). If body size is adaptive for foraging in the open water as a past study suggests (Schluter 1993), such artificial selection on body size may have disproportionately affected Limnetic fitness, reducing their growth advantage in the open water. Nevertheless, it appears unlikely that this hypothesis could explain the discrepancy between current and past results for two reasons. First, mean body size of the various cross-types in the current experiment was within in the range of that used in past studies (e.g., Schluter 1995; Hatfield and Schluter 1999). Second, in this type of transplant study the correlation between initial body size and subsequent growth within a cross-type has been found to be weak (Schluter 1995; H. D. Rundle, unpubl. data).

An alternative possibility is that growth rate may be a poor fitness surrogate for individuals exploiting the open water environment of the lake. As noted above, Limnetics are smaller on average than Benthics and this size difference is thought to be adaptive for foraging on zooplankton in the open water (Schluter 1993). It is interesting to note that in both past studies the difference in growth rate between cross-types in the open water was smaller than in the littoral zone (Schluter 1995; Hatfield and Schluter 1999). However, limnetics did outperform F_1 hybrids and Benthics in both of these studies so this hypothesis alone cannot explain the discrepancy between current and past results. Nevertheless, as noted earlier, it would be beneficial to obtain measurements of traits that are more closely correlated with lifetime fitness.

Finally, the poor relative growth rate of Limnetics could be the result of ''enclosure effects'' in the open water. If benthic species of invertebrates colonize the sides of the open water enclosures, they may create a small littoral zone-like niche. The presence of ''alien'' resources may favor phenotypes intermediate between Benthics and Limnetics, or may result in higher growth rates of more benthic-like phenotypes than would normally be possible in the open water

environment. The extent of colonization may vary year-to-year depending on environmental conditions during the experiment, such as the amount of incident sunlight and water temperature. This possibility is supported by two observations on the diet of the fish in the open water enclosures. First, there was near-significant heterogeneity among cross-types in the proportion of their diet consisting of zooplankton, suggesting that the different cross-types may have been exploiting alternative food resources. Second, the presence of ostracods, a prey item generally found in the diet of Benthics, but not Limnetics, in the wild (Schluter 1993), suggests that the environment in these enclosures may not have been fully representative of the open water of the lake. In comparison, ostracods formed only a small component of the diet of fish in open water enclosures in past experiments (2–3% Schluter 1995; < 1% Hatfield 1995). For these reasons I conclude that the low growth rate of Limnetics in the open water may not accurately represent the performance of these individuals in this habitat.

In conclusion, the reversal of the relative fitnesses of the two types of backcrosses in the two habitats can only be caused by ecologically dependent postmating isolation, the presence of which is a unique prediction of the ecological model of speciation (Rundle and Whitlock 2001). This result adds to a growing body of evidence that postmating isolation between sympatric Limnetic and Benthic sticklebacks in Paxton Lake is ecologically dependent (Schluter 1993, 1995; Hatfield and Schluter 1999), providing strong evidence that postmating isolation has arisen between these sympatric species as a result of divergent natural selection.

Past studies have also demonstrated a role of divergent selection in the evolution of premating isolation between Limnetics and Benthics. In laboratory mating trials, hybridization was found to be dependent on body size, a trait which evidence suggests is an adaptation to their alternative environments (Nagel and Schluter 1998). Premating isolation may also have been reinforced directly by selection in sympatry (Rundle and Schluter 1998). In addition, premating isolation has evolved in parallel, in correlation with the environment, among independently evolved populations of Limnetics and Benthics in at least three lakes, thus implicating natural selection (Rundle et al. 2000). The conclusion, when all of these studies are taken together, is that reproductive isolation, both pre- and postmating, has evolved via ecological mechanisms and that Limnetic and Benthic sticklebacks are prime examples of ecological speciation in nature.

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