A test of ecological selection against young-of-the-year hybrids of sympatric sticklebacks

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Ecological selection against hybrids, the reduction in hybrid fitness attributed solely to environmental factors, was tested by introducing young-of-the-year benthic, limnetic and F_1 hybrid sticklebacks *Gasterosteus aculeatus* to divided experimental ponds and lake enclosures. The frequency of hybrids in samples taken at the end was significantly lower than their frequency at introduction. Hybrid survival was significantly lower in pond-sides in which they were initially the most common cross type than in pond-sides in which they were initially rare, suggesting that hybrid survival may be frequency-dependent. Growth rate of F_1 hybrids was marginally lower than benthic growth rates, being significantly lower than in ponds and not different in lake enclosures. The diet of hybrids overlapped with both parent species in ponds and with benthic diets in lake enclosures. The results suggest that ecological selection is acting against young-of-the-year hybrid sticklebacks. © 2000 The Fisheries Society of the British Isles

Key words: ecological selection; fitness; *Gasterosteus aculeatus*; hybrid; postmating isolation; speciation.

INTRODUCTION

The origin and maintenance of species requires a reduction of gene flow (Dobzhansky, 1940). For sympatric populations, this involves mechanisms that reduce the amount of interbreeding between individuals of different populations (premating isolation) and, if premating isolation is imperfect, lower the fitness of offspring which result from interbreeding (postmating isolation). With inter-breeding and in the absence of selection against hybrid offspring, the two populations are expected to fuse (McPhail, 1992). Traditionally, studies of postmating isolation between sympatric species have concentrated on hybrid disadvantage in the form of laboratory-based sterility or inviability (Rice & Hostert, 1993). Numerous studies have demonstrated hybrid disadvantage in the laboratory (Ayala *et al*., 1974; Woodruff, 1979; Coyne & Orr, 1989; Orr *et al*., 1997; Lu & Bernatchez, 1998).

However, studies that measure hybrid disadvantage only in the laboratory may underestimate the amount of postmating isolation in the wild. This may be especially true of recently diverged taxa, where few genetic incompatibilities have arisen. In these cases, hybrid offspring kept in climate-controlled chambers, fed to satiation and protected from predators may not exhibit lower fitness. As Coyne & Orr (1989) point out, ' hybrids may be disadvantaged not because they

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are sterile or inviable, but because they fall between the niches of two ecologically isolated taxa. . . . ' The contribution of ecology to hybrid disadvantage has been largely overlooked, and few studies have measured the ecological component of postmating isolation (Hagen, 1967; Orr & Smith, 1998; Hatfield & Schluter, 1999).

Hatfield & Schluter (1999) extended previous work by McPhail (1984, 1992) on postmating isolation between benthic and limnetic sticklebacks *Gasterosteus aculeatus* L., by measuring the fitness of F_1 hybrids in laboratory and field settings. In the laboratory, egg fertilization and hatching success of F_1 hybrids were high and not significantly different from benthics and limnetics. Growth rates of juvenile F_1 hybrids were equal to benthic growth rates and may have been superior to limnetic growth rates. These results argue against intrinsic genetic incompatibilities in crosses between benthics and limnetics. When transplanted into the wild, however, hybrid disadvantage was detected. Adult F_1 hybrids had lower mean growth rates than benthics in the littoral zone and lower mean growth rates than limnetics in open water. Overall, the growth of F_1 hybrids was below, although not significantly, the mean of the two parental species across the two habitats. Based on these results, Hatfield & Schluter (1999) invoked ecological factors as the cause of the observed hybrid disadvantage in the field experiments.

In this paper the results are presented of several experiments that measured the fitness of hybrid sticklebacks in experimental ponds and lake enclosures. Previous experiments in the field have yielded a measure of short term (21 days) growth of individual adult F_1 (Hatfield & Schluter, 1999) and F_{10} (Schluter, 1995) hybrids. The present experiments complement and extend earlier work in several ways. First, fitness of young-of-the-year parent species and their F_1 hybrids is measured. Hybrid fitness needs to be measured at multiple life stages to gain a better understanding of the contribution of ecological selection to reproductive isolation. This is because selection pressures at one life stage may not mirror, and may even oppose, those in another life stage (Price & Grant, 1984; Monaghan & Metcalfe, 1986; Schluter *et al.*, 1991). Second, the estimate of hybrid fitness includes both survival and growth components. Finally, experiments were conducted with large numbers of individuals (2400 to 5000 fish introduced per replicate) over longer periods (45 to 90 days) than considered previously.

MATERIALS AND METHODS

STUDY SPECIES

The experiments were carried out in 1994, 1995, and 1997 with the benthic and limnetic species of stickleback from Paxton Lake, British Columbia, Canada. The species are named for their morphological and habitat use differences: benthics are larger, deeper bodied fish that forage on invertebrates associated with vegetation and substrate, whereas limnetics are smaller, streamlined fish that forage on zooplankton in the open water zone (Larson, 1976). Benthics and limnetics exhibit strong, yet imperfect, premating isolation in the laboratory (McPhail, 1992; Hatfield & Schluter, 1996; Nagel & Schluter, 1998). Nevertheless, hybrids are detected in the wild at a low frequency. McPhail (1992) sampled Paxton Lake over 20 years and estimated that, based on morphological criteria, F_1 hybrids constitute 1% of the total adult population. Strong assortative mating is

TABLE I. Number of young-of-the-year sticklebacks in pond (volume 529 m^3) and lake enclosure (108 m^3) experiments. Ponds were divided and numbers given are the total from both pond-sides

Experiment	Replicate	Total number of sticklebacks introduced	Total number of sticklebacks recovered	Absolute survival of sticklebacks
Pond	1994-10	5000	2422	0·48
	1994-12	5000	2703	0·54
	1995-10	5000	2630	0·53
	1997-11	3000	1110	0·37
Lake enclosure	1994	2400	1308	0·55
	1995	2400	943	0·39

partially, but not wholly, responsible for this deficiency in hybrids. Stable allele frequency differences between benthics and limnetics imply that selection is also acting against hybrids. Artificial crosses in the laboratory demonstrate that F_1 hybrids are viable and fertile (Hatfield & Schluter, 1999), so it was hypothesized that ecological selection is acting against hybrids.

EXPERIMENTAL CROSSES

Wild benthic and limnetic adults in reproductive condition were used as parents of crosses. Benthic and limnetic adults in Paxton Lake are distiguished easily in the field on the basis of a number of morphological (especially length, depth and colour of body) and meristic characters (plate number, presence or absence of pelvic girdle and spines). Twenty-five benthic, 40 limnetic and 40 reciprocal F_1 hybrid (approximately equal numbers of eggs from B♀ × L♂ and L♀ × B♂) crosses were made for the lake enclosure and *c.* 50 benthic, 120 limnetic, and 100 reciprocal F_1 hybrid crosses for the experimental ponds each year. The crosses were made in May of each year by stripping eggs from gravid females into Petri dishes using gentle abdominal pressure and adding water and macerated testes from males. A single male was used per five female clutches. After 2 h had elapsed, fertilized eggs were transferred to plastic hatching cups, 175-ml containers with mesh bottoms suspended in 100-l aquaria above air stones. Often clutches were split among containers so that each aquarium received eggs from multiple crosses. The air stones provide oxygen to the eggs through the mesh. Eggs that became infected with fungus were removed daily. After hatching, fish were transferred from the containers to aquaria.

Fish were fed with infusoria cultures for the first week, then switched to diets consisting of live *Artemia* nauplii fed once or twice daily. Fish were reared for 4 weeks in aquaria before introducing them to ponds or to the lake enclosure.

POND EXPERIMENT—1994 AND 1995 REPLICATES

This experiment was carried out in two divided 23 × 23 m ponds (3 m maximum depth) on the University of British Columbia campus. On 9 and 10 June 1994 and 12 and 14 June 1995, 2500 sticklebacks were added to each side of a pond (Table I). All fish were added from a large pool created by combining fish haphazardly sampled from all conspecific stock aquaria. Densities were chosen based on previous experiments (Schluter, 1994; Pritchard, 1998) which reported growth rates similar to those measured in the wild (J. R. Pritchard & D. Schluter, pers. comm.). In addition to the main question of whether hybrids have reduced fitness due to ecological selection, this experiment examined whether the relative frequency of hybrids had an impact on their fitness. It was hypothesized that hybrid fitness would be lower when hybrids were rare, and parent species were common, due to increased competition with the parent species. Two

frequency treatments were used: 350 benthic, 1800 F_1 hybrid, and 350 limnetic individuals (=Hybrids Common treatment) were added to one randomly chosen side of each pond, and 1075 benthic, 350 F_1 hybrid, and 1075 limnetic individuals (=Hybrids Rare treatment) were added to the other side. A minimum of 200 fish of each cross type were retained and reared in the stock aquaria. The standard lengths of 30 fish of each cross type were measured to obtain estimates of starting sizes. In August 1995, the divider in pond 12 lifted off the bottom, so this replicate has been excluded.

The experiments were terminated after 3 months. On 7 and 9 September 1994 and 12 September 1995, fish were harvested by the use of minnow traps. The following day, 0·5 kg of 5% rotenone ($C_{23}H_{22}O_6$) was added to each pond-side and the remaining fish were collected with a dip net as they swam to the surface. Fish were anaesthetized immediately with MS-222 (tricaine methanesulfonate; Syndel Laboratories, Vancouver, BC) and fixed in 10% formalin. Thirty individuals of each cross type, to be used as reference fish for identification of experimental fish, were sampled haphazardly from stock aquaria on these dates. After a week, all fish from a pond-side were counted, 200 individuals were sampled randomly and, along with reference fish, were rinsed in water, stained in alizarin red, and preserved in 37% isopropyl alcohol for identification and measurement.

POND EXPERIMENT—1997 REPLICATE

This replicate was conducted using the same pond facilities as in 1994 and 1995. On 22 June, as part of a larger experiment investigating hybrid fitness in the presence of predators, 2100 individuals (=high density treatment) were added to one randomly chosen side and 900 individuals (=low density treatment) to the other side of a divided pond (Table I). Benthic, limnetic, and F_1 hybrid young-of-the-year sticklebacks were added at equal frequencies. Constraints on the number of fish needed for the entire experiment (>17 000 individuals) prevented the use of 2500 individuals in the high density side as in the 1994 and 1995 replicates. On 20 September all fish were removed by the addition of rotenone to the pond. Preservation and preparation of fish for identification and measurement was conducted as in the previous pond replicates.

LAKE ENCLOSURE EXPERIMENT

The experiment was conducted in an enclosure placed in a shallow bay (1·5 m depth) of Paxton Lake. The 8·5 × 8·5 m enclosure, constructed of ' no-see-em ' fabric mesh (<0·5 mm mesh size; Allander & Co., Michigan, U.S.A.) impermeable to juvenile fish, was erected in April 1994 and again in May 1995. The bottom of the enclosure was open, allowing fish to forage on the substrate, and the top extended out of the water. Before each trial, any sticklebacks found inside the enclosure were removed with minnow traps and with a dip net while snorkeling. This procedure was repeated until no sticklebacks were caught in traps and three consecutive sweeps of the entire enclosure yielded no sightings. In each year, fewer than five fish were removed.

The enclosure was stocked with 2400 sticklebacks (Table I) on 11 June 1994 and 15 June 1995. The number of sticklebacks introduced was chosen to match those used in pond experiments. The smaller size of the enclosures (Table I) resulted in densities of fish that were roughly twice as high as in the ponds. Despite this difference in starting densities, absolute survival and growth rates in enclosures were similar to those seen in the wild and in pond experiments (see Results). The experiment was terminated on 28 July 1994 and 3 August 1995. Juveniles were removed by snorkeling in the enclosure with a large dip net over a period of 5 days in 1994 and 7 days in 1995. Again, this was done until three consecutive sweeps of the entire enclosure yielded no sightings. Fish were anaesthetized immediately with MS-222 and fixed in 10% formalin. After a week had elapsed, all fish were counted and 200 individuals were sampled randomly rinsed in water, stained with alizarin red, and preserved in 37% isopropyl alcohol for identification and measurement.

IDENTIFICATION OF FISH

Because of the relatively small size of fish at the end of the experiment, positive discrimination of fish required quantitative measurements from each individual. Six

morphometric measurements were made on the left side of reference and experimental fish following the methods of Lavin & McPhail (1985): standard length, body depth, gape width, first dorsal spine length, pelvic spine length and pelvic girdle length. All six measurements were ln-transformed [ln(trait value+1) for traits with zero values] before analysis. Two meristic traits were added: number of pelvic spines and a pelvic girdle score. Pelvic girdles were scored as follows: no pelvic girdle=0, partial girdle=1, and full girdle=2. Pelvic characters were used because often hybrids express partial girdles or only one pelvic girdle (S. M. Vamosi et al., unpubl. data).

For each morphometric measurement other than standard length, the trait values for 60 reference fish (30 F_1 hybrids and 30 limnetics combined) were regressed against standard length. Benthics were not included in these analyses because often they have zero values for the armour characters (first dorsal spine length, pelvic spine length, and pelvic girdle length). All five variables were related significantly to standard length ($P<0.05$ in all cases). Using the regression equation obtained for each trait, residual values were obtained for the five morphometric measurements for each fish, including benthics. Residuals, rather than raw values, were used in subsequent discriminant function analyses (for both reference and experimental fish) to allow for comparison of fish of different standard lengths.

Discriminant function analysis (Pimentel, 1979) was used to identify experimental individuals of the three cross types. This method was applied first to the 90 reference fish to generate the discriminant functions and establish their accuracy. Two discriminant functions were calculated, the first to separate benthics from hybrids and limnetics combined, and the second to separate hybrids from limnetics. This two-step approach improved discrimination of hybrids from limnetics. The success rate of the first classification was 100%, separating all 30 benthics from hybrids and limnetics combined. The second discriminant function identified 58 of 60 (96·7%) fish correctly. One limnetic was identified incorrectly as a hybrid, and one hybrid was identified as a limnetic. In total, the two discriminant functions identified 88 of the 90 fish correctly, a 97·8% success rate.

STOMACH CONTENTS

To determine the feeding ecology of juveniles in the experiments, stomach contents of parent species and hybrids ($n=10$ for each cross type) were examined from lake and 1994 and 1995 replicates of the pond experiment. No diet data are available for 1997 pond replicates. Prey items were identified to order and occasionally to family or genus using the keys in Pennack (1978). Most prey were assigned to one of two groups based on the habitat in which they are most likely to occur (modified from Schluter & McPhail, 1992). Benthos includes those items generally associated with sediment or vegetation (e.g. chironomid larvae, ostracods, gammarids, dipteran larvae, and harpactacoid copepods). Items classified as plankton inhabit the water column [e.g. brachionid rotifers, calanoid copepods, transparent cyclopoid copepods, and bosminid and daphnid cladocerans (*Bosmina*, *Daphnia* and *Diaphanosoma*)]. Chydorids were not assigned to either category because, although they are associated typically with vegetation in ponds and shallow areas of lakes (Edmondson, 1959; Pennack, 1978), they are known to migrate between the water column and vegetation in the experimental ponds (W. E. Neill, pers. comm.).

STATISTICAL ANALYSES

It was examined whether the frequency of hybrids at the end of experiments was lower than their frequency at introduction, comparable to cohort analyses of hybrid survival in the wild (Dowling & Moore, 1985). It was tested whether hybrid relative survival was lower than expected by applying Fisher's method of combining probabilities (Sokal & Rohlf, 1995) obtained from goodness-of-fit tests (Sokal & Rohlf, 1995) of the six replicates. The combined probability test is commonly applied to multiple tests of the same hypothesis and is calculated as: $-2\Sigma\ln P$, where the test statistic ($-2\Sigma\ln P$) is distributed as χ^2 with $2k$ degrees of freedom, P is the probability obtained in each separate test and k is the number of separate tests ($k=6$). To simplify comparisons

between different replicates, the relative survival of a cross type is reported as: relative survival $=1+(F_e - F_s)$, where F_e is the frequency of the cross type in samples at the end of the experiment and F_s is the starting frequency of the cross type. The effect of frequency treatment on hybrid survival was tested using a paired *t*-test on arcsin transformed relative survival of hybrids. A goodness-of-fit test was not used for this analysis because the expected frequency of hybrids was different among pond-sides.

After individual fish were identified to cross type, their growth rates were calculated as: growth rate (mm day^{-1}) $=(L_{S_e} - L_{S_s})D^{-1}$, where L_{S_e} is the standard length of an individual at the end of experiment, L_{S_s} is the mean standard length of its cross type at the start of the experiment and D is the duration of the experiment in days. Growth rates were ln-transformed prior to analysis. To determine whether there was hybrid deficiency in growth in the experiments, their growth rates were compared with those of benthics. This was done because lab experiments revealed that benthic and hybrid growth rates were equal in the absence of ecological selection whereas limnetics appear to have lower intrinsic growth rates than hybrids (Hatfield & Schluter, 1999). Mean hybrid and benthic growth rates were compared with a one-tailed paired *t*-test. It was also examined whether the genes that determine growth rate in the parent species combine additively in hybrid crosses. Only pond replicates, which provided both benthic and planktonic resources (see Results) and should allow for high growth rates of all three cross types, were used for this comparison. A regression of growth rate on cross type was calculated, assigning benthics a value of $+1$ and limnetics a value of -1. Using this regression, the predicted growth rate of hybrids, assigned a cross score of 0, could be compared with observed growth rates using a *t*-test.

Diet overlap between cross types was calculated using the index: $a_{xy}=1-0.5(\Sigma_{i=1}^{n}|p_{xi} - p_{yi}|)$, where: p_{xi} is the proportion of food category i in the diet of cross type x, p_{yi} is the proportion of food category i in the diet of cross type y, and n is the number of food categories (Schoener, 1970). This index gives values from 0 (no overlap) to 1 (complete overlap). An a value >0.60 is thought to be biologically significant (Zaret & Rand, 1971; Wallace, 1981) and this convention was followed in this study. Diet composition data were bootstrapped to provide multiple estimates of diet overlap between the three cross types for each replicate. It was determined whether mean diet overlap between two cross types was significant (i.e. >0.60) using a *t*-test on bootstrapped values. To correct for multiple comparisons per replicate, $a'=1-(1-a)^{1/k}$ was calculated, where a' is error rate for each individual test, a is the experimentwise error rate and k is the number of tests (Sokal & Rohlf, 1995). For these comparisons, $a'=0.02$ ($a=0.05$; $k=3$) were used.

RESULTS

Absolute survival of young-of-the-year sticklebacks was relatively high and similar among pond (mean $\pm$ S.E. $48.0 \pm 3.9\%$) and lake ($46.9 \pm 7.7\%$) experiments (Table I). Growth of young-of-the-year sticklebacks was marginally higher in pond (0.30 ± 0.004 mm day^{-1}) than in lake enclosure (0.24 ± 0.004 mm day^{-1}) experiments. A larger representation of limnetics, the cross type with the lowest growth rates, in lake enclosure samples (43.5% of experimental fish) compared with pond samples (29.5%) contributed to this difference. Growth rates were comparable to those measured in the wild (J. R. Pritchard & D. Schluter, pers. comm.; 0.19 ± 0.003 mm day^{-1}). These observations suggest that the two environments replicated conditions in the wild adequately and, thus, their results are considered together.

Relative survival of young-of-the-year hybrid sticklebacks was lower than expected (Table II; $-2\Sigma\ln P=24.41$, d.f. $=12$, $P<0.025$). The frequency of hybrids in samples taken at the end of experiments was $3.7 \pm 1.6\%$ lower than

TABLE II. Relative survival of young-of-the-year benthics, F_1 hybrids, and limnetics in samples taken from ponds and lake enclosures

Experiment	Replicate	n	Benthic relative survival	F_1 hybrid relative survival	Limnetic relative survival
Pond	1994-10	400	1·08	0·96	0·96
	1994-12	400	1·07	1·00	0·93
	1995-10	386	0·97	0·99	1·04
	1997-11	402	1·03	0·91	1·07
Lake enclosure	1994	200	0·97	0·93	1·11
	1995	200	0·92	0·99	1·10

TABLE III. Frequency-dependent relative survival of young-of-the-year hybrids in ponds

Pond	Treatment	
	Hybrids rare	Hybrids common
1994-10	0·98	0·96
1994-12	1·04	0·96
1995-10	1·09	0·89

their frequency at introduction. A significant effect of treatment on the relative survival of hybrids was detected in divided ponds (Table III: $t_2 = 3·19$, $P < 0·05$). The relative survival of hybrids when initially common was $10·0 \pm 5·3\%$ lower than when hybrids were initially rare.

Mean growth rate of young-of-the-year hybrid sticklebacks was marginally lower than mean growth rate of benthics ($t_5 = 1·92$, $P = 0·057$). Hybrids grew at $95·2 \pm 2·9\%$ the rate of benthics. Hybrid growth rates were significantly lower in ponds (Fig. 1; $t_3 = 2·91$, $P < 0·05$) and not significantly different from benthic growth rates in lake enclosures (Fig. 1; $t_1 = 0·93$, $P > 0·05$). Growth of hybrids, unlike relative survival, did not depend on initial frequency ($t_2 = 0·81$, $P > 0·05$). In ponds, hybrid growth rates were not significantly different from predicted growth rates (Fig. 2; $t_3 = 0·25$, $P > 0·05$) calculated using a regression of cross type on benthic and limnetic growth rates.

Young-of-the-year sticklebacks foraged on benthos and plankton in ponds [Fig. 3(a)] and predominantly on benthos in lake enclosures [Fig. 3(b)]. Hybrids consumed intermediate amounts of benthos in ponds ($33·9 \pm 3·6\%$) compared with benthics ($48·8 + 3·2\%$) and limnetics ($17·9 \pm 3·0\%$). Limnetics consumed more plankton ($37·0 \pm 4·5\%$) than benthics ($28·2 \pm 2·4\%$) or hybrids ($22·7 \pm 2·6\%$). Both hybrids and limnetics consumed large numbers of chydorids (hybrid: $41·5 \pm 4·2\%$; limnetic: $44·4 \pm 4·6\%$) compared with benthics
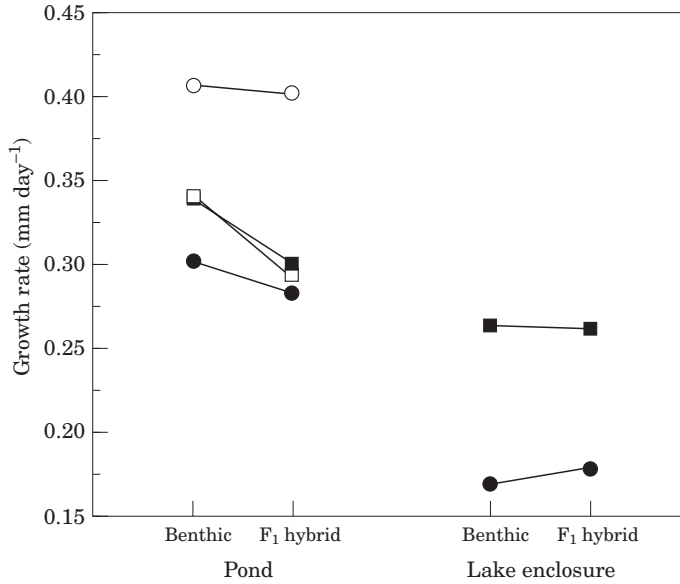
FIG. 1. Mean growth rates of benthic and F_1 hybrid young-of-the-year sticklebacks in pond and lake enclosure experiments. Standard error bars are not shown because all are less than, or equal to, the height of symbols. Ponds: ■, 1994–10; □, 1994–12; ●, 1995–10; ○, 1997–11. Lake enclosure: ■, 1994; ●, 1995.
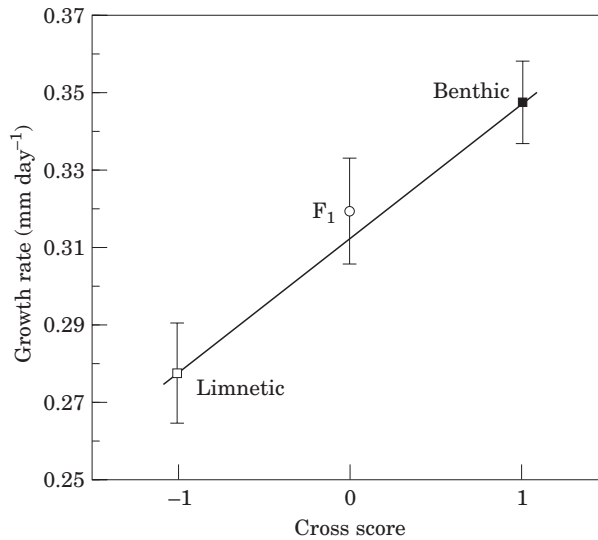

FIG. 2. Mean growth rates of the three cross types ($\pm$ S.E.) based on pond means ($n=4$ for each cross type). The line is the regression of parent species means on cross score and shows the predicted growth rate of F_1 hybrids if genes for growth rates from the two parent species are expressed additively in hybrid crosses. Linear regression: $y=0.312+0.035x$; $r^2=0.46$, $P=0.066$.

($22.2 \pm 2.8\%$). Diet overlap was high between hybrids and benthics in one pond and high between hybrids and limnetics in two ponds (Table IV). The mean proportion of benthos in stomach contents from lake enclosure samples was high
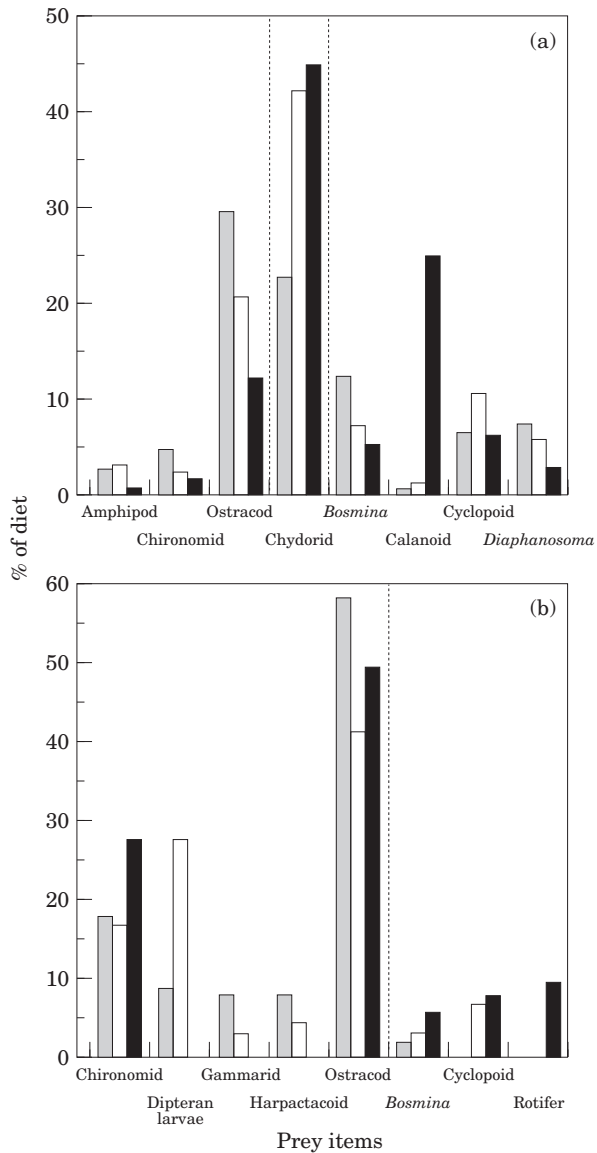
FIG. 3. Diets of benthic (▨), F$_1$ hybrid (□), and limnetic (■) young-of-the-year sticklebacks in pond (a) (1994 and 1994 only) and lake enclosure (b) experiments. Only prey items which constituted ≥2% of the diet for at least one cross type are shown. Benthos is presented to the left of the dotted line, plankton to the right and chydorids in between for ponds.

for benthics (97·8 ± 1·2%) and hybrids (91·3 ± 2·5%). In addition to consuming benthos, limnetics foraged on plankton (21·4 ± 5·8%), especially rotifers and cyclopoid copepods. Diet overlap was high between hybrids and benthics in both lake enclosure replicates (Table IV). Mean diet overlap, across both experiments, was highest between hybrids and benthics and lowest between benthics and limnetics.

TABLE IV. Diet overlap (mean $\pm$ S.E.) between young-of-the-year sticklebacks in pond and lake enclosure experiments

Experiment	Replicate	$a_{Ben,Lim}$	$a_{Hyb,Ben}$	$a_{Hyb,Lim}$
Pond	1994-10	0.40 ± 0.04	0.46 ± 0.02	$\mathbf{0.75 \pm 0.04}$
	1994-12	$\mathbf{0.69 \pm 0.02}$	$\mathbf{0.67 \pm 0.03}$	$\mathbf{0.67 \pm 0.03}$
	1995-10	0.35 ± 0.05	0.62 ± 0.02	0.41 ± 0.04
Lake enclosure	1994	0.36 ± 0.03	$\mathbf{0.71 \pm 0.04}$	0.39 ± 0.03
	1995	0.66 ± 0.03	$\mathbf{0.69 \pm 0.06}$	0.54 ± 0.05

Schoener's (1970) diet overlap index is presented for the overlap between the parent species and each species with the hybrids. Overlap values significantly >0.60 are in boldface.

DISCUSSION

The morphologically distinct species pair of sticklebacks in Paxton Lake persist in the face of interspecific hybridization (McPhail, 1992). Because F_1 hybrids are viable and fertile in the laboratory, ecological causes of hybrid disadvantage have been sought. Previous studies have examined the short-term growth of non-breeding adult F_1 and F_{10} hybrid sticklebacks in small enclosures (Schluter, 1995; Hatfield & Schluter, 1999). This study extended previous work by measuring the fitness of young-of-the-year F_1 hybrids introduced with benthics and limnetics into lake enclosures and experimental ponds.

A significant reduction was detected in the proportion of hybrids surviving in these experiments. The relative survival of hybrids measured in the experiments can be used to make a rough estimate of the strength of selection against hybrids over a year, if the assumption is made that the strength of selection is equal over the life span of hybrids. Using the mean of the intensity of selection against hybrids and the 95% CL (lower: -13.2%, upper: $+5.7\%$), it is estimated that the mean change in the relative frequency of hybrids would be -11.1% (range -39.6%–$+17.0\%$) per year. Dowling & Moore (1985) determined that 9.2% of hybrids between two cyprinid species, the common shiner *Luxilus* (*Notropis*) *cornutus* (Mitchill), and the striped shiner *L. chrysocephalus* (Rafinesque), were selected against per year and concluded that this was evidence for strong selection against hybrids. Attempts to validate the present estimate in the wild will be hampered by the low numbers of hybrids in the field and the lack of genetic markers that are unique to hybrids.

Relative hybrid survival may be dependent on their initial frequency in a cohort. In all three ponds, the recovery of hybrids was higher in the pond-side where hybrids were initially rare (14% of cohort) than from the pond-side where hybrids were initially common (72% of cohort). Such negative frequency-dependent selection may contribute to their low, but relatively constant, frequency in adult samples (McPhail, 1992). The mechanism(s) which produced this result is not known, which is contrary to the initial hypothesis based on a two-niche model and the assumption that an increased frequency of the parent species, presumed to be superior competitors in each niche, would lead to reduced fitness of hybrids. Perhaps predators or parasites were also important in

determining relative survival of hybrids. The mechanisms which result in reduced hybrid survival need further study in the wild.

The mean growth rate of hybrids was lower, albeit marginally, than mean benthic growth rates. These results contrast with laboratory experiments which found that the intrinsic growth rate of F_1 hybrids was not significantly different from benthic growth rates (Hatfield & Schluter, 1999), suggesting a contribution of ecological selection to this disadvantage. The genes that determine growth rate in the parent species appear to be expressed in an additive fashion in hybrid crosses grown under field conditions. Ecological selection may also be implicated in this measure, as hybrid growth rates are much closer to predicted in these experiments than in lab experiments, where there appeared to be hybrid superiority in growth rates (Hatfield & Schluter, 1999). It is suggested that the mechanisms contributing to reduced hybrid growth rates are a lack of a unique niche, revealed by their high degree of diet overlap with the ecologically divergent parent species, and a feeding disadvantage when feeding on the prey items preferred by the parent species (Schluter, 1993).

The coexistence of benthic and limnetic sticklebacks appears to be facilitated by a combination of mechanisms. There is evidence for strong premating isolation, with mate choice dependent largely on size differences (Nagel & Schluter, 1998) and microhabitat differences in spawning sites (McPhail, 1994; Hatfield & Schluter, 1996; Vamosi & Schluter, 1999). Postmating isolation may act throughout the life span of hybrids. Survival of young-of-the-year F_1 hybrids was lower than expected and their growth rates were inferior to benthics. This reduction in fitness appears to be more pronounced when predators are considered (S. M. Vamosi & D. Schluter, unpubl. data). Hybrids are less efficient foragers than benthics and limnetics (Schluter, 1993) and, hence, growth rates of adult hybrids are lower than the mean of benthics and limnetics (Schluter, 1995; Hatfield & Schluter, 1999). F_1 hybrid males that survive to sexual maturity have low mating success with limnetic females (Vamosi & Schluter, 1999). Finally, backcrosses may have reduced hatching success and growth rates (Hatfield & Schluter, 1999).

Multiple genetic and ecological mechanisms may prevent introgression between other sympatric or parapatric species that interbreed occasionally (Hagen, 1967; Taylor & Foote, 1991; Aspinwall & McPhail, 1995; Wood & Foote, 1996; Bernatchez et al., 1999). Bernatchez et al. (1999), for example, summarize two observations on reproductive isolation between dwarf and normal lake whitefish *Coregonus clupeaformis* (Mitchill) ecotypes: (1) gene flow is reduced with increased morphological differentiation associated with trophic ecology (Bernatchez et al., 1996), and (2) mortality rates are 2·4 to 4·7 times higher in reciprocal hybrid crosses compared with pure crosses (Lu & Bernatchez, 1998). The former observation suggests a role for ecology, whereas the latter study, which was conducted in the laboratory, argues for the presence of genetic incompatibilities between the two ecotypes.

The role of ecology in promoting divergence and speciation has long been emphasized (e.g. Dobzhansky, 1941; Simpson, 1953; Schluter, 1998), yet the empirical tests have been lacking. It is concluded that ecological factors contribute to postmating isolation between benthics and limnetics by causing reduced survival and growth rates of young-of-the-year F_1 hybrids. Further

work on these and other sympatric species promises to yield continued insights into the early stages of speciation.

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