

SEXUAL SELECTION AGAINST HYBRIDS BETWEEN SYMPATRIC STICKLEBACK SPECIES: EVIDENCE FROM A FIELD EXPERIMENT

STEVEN M. VAMOSI¹ AND DOLPH SCHLUTER²

Department of Zoology and Centre for Biodiversity Research, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia V6T 1Z4, Canada

¹E-mail: vamosi@zoology.ubc.ca

²E-mail: schluter@zoology.ubc.ca

Abstract.—Sexual selection against viable, fertile hybrids may contribute to reproductive isolation between recently diverged species. If so, then sexual selection may be implicated in the speciation process. Laboratory measures of the mating success of hybrids may underestimate the amount of sexual selection against them if selection pressures are habitat specific. Male F₁ hybrids between sympatric benthic and limnetic sticklebacks (*Gasterosteus aculeatus* complex) do not suffer a mating disadvantage when tested in the laboratory. However, in the wild males choose different microhabitats and parental females tend to be found in the same habitats as conspecific males. This sets up the opportunity for sexual selection against male hybrids because they must compete with parental males for access to parental females. To test for sexual selection against adult F₁ hybrid males, we examined their mating success in enclosures in their preferred habitat (open, unvegetated substrate) where limnetic males and females also predominate. We found significantly reduced mating success in F₁ hybrid males compared with limnetic males. Thus, sexual selection, like other mechanisms of postzygotic isolation between young sister species, may be stronger in a wild setting than in the laboratory because of habitat-specific selection pressures. Our results are consistent with, but do not confirm, a role for sexual selection in stickleback speciation.

Key words.—*Gasterosteus*, hybrid fitness, mating discrimination, reproductive isolation, sexual selection, speciation.

Received August 17, 1998. Accepted February 2, 1999.

Speciation is the evolution of premating or postmating isolation preventing genetic exchange between populations (Dobzhansky 1940). Premating isolation is reduced interbreeding between species, whereas postmating isolation is a lower fitness of hybrid offspring (Mayr 1963). Postmating isolation is generally measured as hybrid (and backcross) inviability or sterility (Coyne and Orr 1989; Templeton 1989) and numerous examples are known (e.g., Ayala et al. 1974; Woodruff 1979; Orr et al. 1997). A largely overlooked, but potentially strong, mechanism contributing to reproductive isolation between closely related species is sexual selection against adult hybrids. If premating isolation exists between a pair of species, such that most matings are with conspecific individuals, hybrids may also be discriminated against by members of the parental species. This discrimination may be mediated through a variety of morphological (True et al. 1997), behavioral (Stratton and Uetz 1986; Noor 1997), and/or habitat-associated traits (Rolán-Alvarez et al. 1995). Few studies have tested for sexual selection against hybrids (Stratton and Uetz 1986; Krebs 1990; Price and Boake 1995; Rolán-Alvarez et al. 1995; Hatfield and Schluter 1996; Davies et al. 1997; Noor 1997), and to our knowledge only two of these have been conducted in the wild (Rolán-Alvarez et al. 1995; Davies et al. 1997).

However, reproductive isolation between recently diverged species may be underestimated if based only on laboratory measures of hybrid fitness. In their review of postmating isolation in *Drosophila*, Coyne and Orr (1989, p. 377) note that “hybrids may be disadvantaged not because they are sterile or inviable, but because they fall between the niches of two ecologically isolated taxa or cannot mate properly with either parental species.” For example, F₁ hybrids of benthic and limnetic sticklebacks (*Gasterosteus aculeatus* complex) have growth rates intermediate between those of

benthics and limnetics when grown in the laboratory on identical diets, whereas F₁ hybrids transplanted to enclosures in either of the two main habitats of the natural lake grew more slowly than the parental species specializing on that habitat (Hatfield and Schluter 1999). Therefore, selection against hybrids may be stronger in the wild than in the laboratory if there is an ecological context to postmating isolation.

Hatfield and Schluter (1996) conducted an initial test of sexual selection against hybrids of sympatric benthic and limnetics but found none. They suggested that sexual selection might arise via habitat selection by parental females in the wild. Parental males have divergent nesting sites: benthic males nest on the ground in dense cover, whereas limnetic males nest on open sediments (McPhail 1994). Hatfield (1995) found that when given a choice, laboratory-reared hybrid males also nested in the open or near sparse cover, thus showing preference for the same habitat as limnetic males. Therefore, if the habitat use of parental females matches that of conspecific males, then hybrid males will mostly encounter limnetic females that may discriminate against them compared with limnetic males. Habitat selection by females and nesting site preferences of males would then lead to sexual selection against hybrid males.

In this study, we confirm that habitat use of gravid females matches that of nesting conspecific males. We test the hypothesis that F₁ hybrid male sticklebacks suffer a mating disadvantage in a natural setting by comparing their mating success in their preferred habitat with limnetic males sharing that habitat when competing for limnetic females. Our findings suggest that mating preferences are strong, indicating that sexual selection may be a potent force diminishing hybrid male fitness under the conditions prevailing in the wild. We discuss the implication of this result for our understanding of the role of sexual selection in speciation.

MATERIALS AND METHODS

Study Species

The mate choice trials were carried out on male F_1 hybrids between the "benthic" and "limnetic" species of stickleback in Paxton Lake, British Columbia, Canada (McPhail 1992). The species are morphologically, ecologically, and genetically distinct and exhibit strong premating isolation (McPhail 1992; Hatfield 1995; Nagel and Schluter 1998). Nevertheless, hybrids are produced at a low frequency. McPhail (1992) sampled Paxton Lake over 20 years and estimated that, based on morphological criteria, F_1 hybrids make up 1% of the total adult population. F_1 hybrids are intermediate between the parental species in behavior and morphology (Hatfield 1995). Laboratory experiments reveal no inherent inviability or sterility of F_1 hybrids, although they suffer a foraging and growth disadvantage in the wild (Schluter 1993, 1995; Hatfield 1995; Hatfield and Schluter 1999).

Design

We compared mating success of F_1 hybrid males with that of limnetic males in competition for limnetic females in open habitats. This plan was based on our interpretation of observations made in the wild. First, mating trials were carried out in the open habitat because nesting hybrid males prefer it to the vegetated habitat (Hatfield 1995). When introduced to enclosures in the littoral zone, which contained equal proportions of vegetated and unvegetated bottoms, benthic and limnetic males preferred divergent nest sites. Benthic males preferred vegetated areas, whereas limnetic males preferred unvegetated areas. F_1 hybrid males were not intermediate in their choice of nesting sites; they were most similar to limnetic males, tending to nest in open areas (Hatfield 1995). Second, we did not compare mating success of F_1 hybrid males to benthic males when competing for benthic females because by nesting in the open F_1 hybrid males primarily encounter limnetic females (see Results). Finally, we did not consider the mating success of F_1 hybrid males with F_1 hybrid females because, with an estimated 1% of the adult population being hybrids (McPhail 1992), the encounter rate of nesting hybrid males with gravid hybrid females will be extremely low compared with their encounter rate with limnetic females.

Fish Rearing

All males used in the mate-choice trials were laboratory-raised from experimental crosses using wild-caught individuals from Paxton Lake (Vamosi 1996). This was done to ensure that hybrid males were truly F_1 hybrids and to standardize the prior experience of males. We made crosses in May and June 1993, 1994, and 1997. Males were randomly chosen from a minimum of eight limnetic families and seven F_1 hybrid families (four $L\text{♀} \times B\text{♂}$, three $B\text{♀} \times L\text{♂}$). Fish were held at 18°C and 16:8 L:D day length for six months and then switched to a lower temperature (5–10°C) and shorter day length (8:16 L:D) to simulate winter. Fish were shifted once again to longer days (16:8 L:D) and warmer temperatures (13–18°C) one month before trials. Males were transported to Texada Island in April 1994 and May 1995 and

1998 and held in large aquaria. We trapped gravid limnetic females (standard length: 37–42 mm) from Paxton Lake immediately before using them in mating trials. Wild females were used because: (1) this increases the realism of the design; (2) it is difficult to reliably obtain a sufficient number of laboratory-reared females for use on a specific date, because gravid females are unable to retain their eggs for more than a few days; and (3) differential experience of females is unlikely to be an important factor because most limnetic females only become gravid one or two times in a breeding season (unpubl. data) and rarely survive to a second season.

Mating Trials

To assess male mating success, we measured the probability of spawning in female-choice (i.e., multiple males per female) mating trials. In 1994, trials had five males of each type and multiple limnetic females in single large arenas. In 1995 and 1998, we used two males of each type in smaller enclosures and introduced only a single female to each enclosure to increase replication and avoid the nonindependence of observations of males and females. Introducing males in a 1:1 ratio to limnetic females may eliminate any rare-male advantage to hybrid males; however, we feel our design was preferable because it did not result in higher limnetic male mating success simply due to reduced probability of encounters between limnetic females and hybrid males. We conducted experiments in May–July 1994 and 1995, and May–June 1998 in enclosures in a large, shallow (maximum 1-m depth), unvegetated bay of Paxton Lake.

Two large fenced enclosures were used in 1994 (24 m \times 1.25 m each) and several smaller fenced enclosures (8 m \times 1.25 m each) were used in 1995 (four enclosures) and 1998 (10 enclosures). In 1995, more than one replicate was conducted in an enclosure; in all cases, new males and females were used. Enclosures were built using window screen mesh. All had open bottoms and fence walls buried in the sediment to prevent the escape of experimental fish. They were placed on either side of a long floating dock to permit observations during mating trials. To temporarily isolate males while they built nests, we placed small mesh cylinders (1.1-m diameter) in a single row (1.4 m between centers) down the middle of each of the fenced enclosures (10 per enclosure in 1994, four in 1995 and 1998). Each mesh cylinder was supported by three wire rings, one at either end and one in the middle. The bottom ring was buried in the sediment before introduction of males to prevent escape before nest building was complete.

At the start of a trial, males in reproductive condition were haphazardly sampled from the aquaria and introduced to the mesh cylinders, one male per cylinder. A single limnetic or hybrid male was randomly assigned to the mesh cylinder at one end of a fenced enclosure. We then alternated limnetic and hybrid males in adjacent mesh cylinders. Males were introduced in this fashion to prevent clumping of males of one type or another, thus allowing females to make direct comparisons of the two types.

Males in mesh cylinders were presented with wild gravid limnetic females in jars to stimulate them to build nests. A single female was presented in a floating glass jar for 15 min every day starting one to two days after the introduction of

males to the mesh cylinders. Males with nests "courted" these females in jars, performing many of the stereotypical courtship behaviors observed during actual encounters between nesting males and gravid females (Rowland 1994): zigzags, attempts at leading the female to the nest, and fanning and gluing while at the nest. During this time, we were able to confirm the presence and location of nests and assign a vigor rank to males, based on the number of times a male swam to his nest after zigzagging around the jar (interpreted as an attempt to lead the female to his nest) during a 10-min observation period. Vigor was scored in an ascending manner, with the most vigorous male receiving a score of one and the least vigorous male a score of four (1995, 1998) or five (1994). Males that did not nest within seven days were removed and not replaced. In 1994, three limnetic and two hybrid males remained at their nests in the first trial and two limnetic and three hybrid males remained in the second trial. In 1995, one male of each type was removed in the seventh trial; all other males remained in the other trials. All males remained in 1998. Therefore, the ratio of limnetic to hybrid males present when females were introduced was equal (i.e., 1:1) or near equal in all trials. Females used to stimulate males were not used for mating trials.

To start a trial, we lowered the sides of all mesh cylinders, allowing males to interact with one another within the fenced enclosures. The following morning we counted the number of males still present on their territories. In 1994, we released one female every 20 min until their number was about twice that of nesting males (12 females for each trial). Because of the problem of lack of independence of the number of matings within a trial, we use the trial itself as the independent observation. We released only a single female per fenced enclosure in 1995 and 1998, which allowed us to increase the number of trials.

Trials were ended two days after the release of the female(s). We caught the female(s) with a dip net and examined them to determine whether they had spawned (females that have spawned no longer have swollen abdomens). We removed the males and collected their nests using a dip net. Nests were promptly dissected to verify the presence of eggs.

Habitat Use by Females

We examined the habitat use of gravid benthic and limnetic females during the breeding season by trapping in the littoral zone of Paxton Lake. We trapped at two vegetated and two open sites, all located in water less than 2 m in depth. Vegetated station 1 (1994–1996, 1998) was located beside a large, partially submerged log (0.5 m × 5 m) at the water surface, and cover consisted of large beds of aquatic macrophytes such as *Chara* and *Potamogeton*. Vegetated station 2 (1994) was located between a floating dock and a large bed of cattails (*Typha*), with *Chara* beds covering the substrate. Open station 1 (1994–1996) was in a sparsely vegetated bay, with open substrate and low numbers of yellow water lilies (*Nuphar*) present near (> 2 m) one end of the station. Open station 2 (1998) was located along an open, rocky face bordering a deep bay. We trapped once every seven to 14 days. A trapping session consisted of setting 12 standard minnow traps spaced 2 m apart along the bottom. Traps were set

between 10:00 h and 16:00 h and retrieved 24 h later. Trapped fish were placed in a bucket of water and classified as benthic or limnetic based on meristic and morphometric characters given by McPhail (1992). Gravid females were identified by a distended abdomen.

Statistical Analysis

To determine the habitat use of gravid females, we compared the number of benthic and limnetic females caught during trapping sessions at each station with paired *t*-tests. Data from the same stations in different years were combined after verifying with a one-way ANOVA that catches did not differ between years.

We determined whether limnetic females mate with limnetic males more often than with hybrid males with a one-tailed binomial test. For the two trials in 1994, the successful male was considered the one that had mated with the most females. By dividing the number of eggs in the nest of a male into the total number of eggs retrieved from all the nests, we estimate that the successful male mated with nine of the 12 females in trial 1 and eight of the 12 females in trial 2. Furthermore, in the first trial we observed that the male chosen as the successful male on the basis of the number of eggs in his nest was also the first male to successfully mate with a female (Vamosi 1996). In 1995 and 1998, the successful male was identified as the only one with eggs in his nest. In all cases, females spawned with only a single male (unpubl. data), as in the wild.

To determine the basis of any differential mating success of limnetic and hybrid males, we examined the influence of pretrial vigor and size of males on their mating success. We compared the pretrial vigor of successful males with the mean vigor of remaining males using a Wilcoxon signed rank test. In addition, we compared the mean pretrial vigor of limnetic males with that of the hybrid males in a trial with a Wilcoxon signed rank test to determine whether the vigor of limnetic males was higher than that of hybrid males. The same comparisons were carried out with standard length, our measure of body size, using paired *t*-tests.

RESULTS

Wild gravid benthic and limnetic females exhibit distinct habitat use within the littoral zone (Fig. 1). At both vegetated stations, there were significantly more benthic females trapped than limnetic females (paired *t*-test; vegetated 1: $t_{16} = 5.349$, $P < 0.001$; vegetated 2: $t_7 = 3.710$, $P = 0.008$). This pattern was reversed at the open stations, where significantly more limnetic females than benthic females were trapped (paired *t*-test; open 1: $t_{14} = 4.751$, $P < 0.001$; open 2: $t_2 = 4.359$, $P = 0.049$). These observations indicate that the habitat use of gravid females matches that of nesting conspecific males.

The hypothesis that limnetic females prefer conspecific males to hybrid males was supported. Limnetic females preferred limnetic males in 18 of the 22 trials (binomial test; $P = 0.0043$). This preference is significant even if the two trials from 1994 are not considered (16 of 20 trials, $P = 0.0118$).

Vigor of a male, measured as the relative courtship intensity toward females in jars prior to the start of a trial, was a

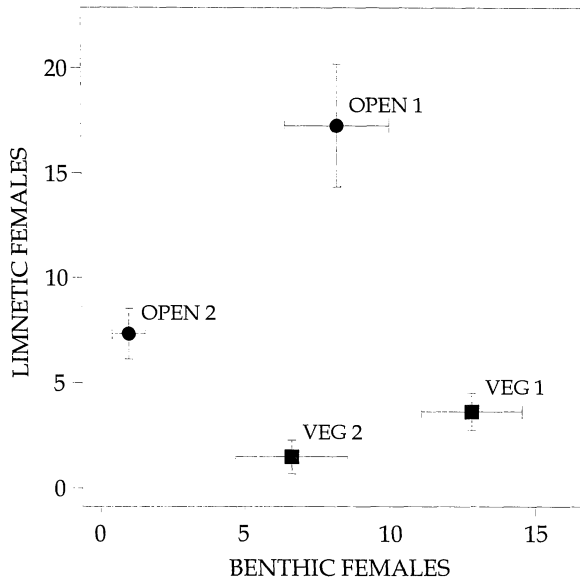


FIG. 1. Mean numbers (\pm SEM) of gravid benthic and limnetic females captured at four trapping stations (■ vegetated stations; ●, open stations) in the littoral zone of Paxton Lake. Number of trapping sessions: vegetated 1, 17; vegetated 2, 8; open 1, 15; open 2, 3. Limnetic females selected the open habitat, whereas benthic females selected the vegetated habitat.

good predictor of subsequent mating success in a trial. The ranked vigor score of the successful male was significantly higher than the mean vigor score of the remaining males in a trial (Wilcoxon signed rank test; $T_s = 3.393$, $P < 0.001$). Limnetic males tended to display more vigorously toward limnetic females than did hybrid males (Wilcoxon signed rank test; $T_s = 3.074$, $P = 0.002$; Table 1).

The size of a male, measured as standard body length, also had a significant impact on mating success. The size of the successful male was significantly smaller than the mean size of the other males in a trial (paired t -test; $t_{21} = 3.358$, $P = 0.001$). This pattern is most easily attributable to the relatively smaller size of limnetic males (Table 1; paired t -test; $t_{21} = 5.985$, $P < 0.001$). Therefore size differences between limnetic and F_1 hybrid males may have contributed to mating success.

DISCUSSION

The sympatric species of sticklebacks in Paxton Lake are genetically isolated despite the presence at low frequency of hybrids in the adult population (McPhail 1992). Therefore, strong selection must be acting against hybrids in the wild. In the present study, we addressed whether there is sexual selection against hybrids by verifying that the habitat use of parental females matches that of conspecific males and by showing that the mating success of male F_1 hybrids is reduced when in competition with limnetic males for limnetic females in the habitat where limnetic females are typically found. The reduced mating success of male adult F_1 hybrids in the field may be a significant component of postmating isolation between benthic and limnetic species.

We found evidence for differential habitat use between limnetic and benthic females matching the habitat preference

TABLE 1. Mean standard body lengths (mm) and ranked vigor (\pm SEM) of limnetic and hybrid males ($n = 44$ for each) in mating trials in 1994, 1995, and 1998 ($n = 22$ trials). Vigor was scored in an ascending fashion, with the most vigorous male given a score of one and least vigorous male given a score of four (1995, 1998) or five (1994).

	Limnetic males	Hybrid males	
Standard length	45.90 \pm 0.58	48.99 \pm 0.70	$P < 0.001$
Ranked vigor	2.02 \pm 0.19	2.95 \pm 0.23	$P = 0.002$

of nesting conspecific males. Limnetic females selected open sites, with over four times as many limnetic females being trapped there than at the vegetated sites. Although benthic females were also trapped at open sites, they are not likely to spawn with males there. Benthic females have been observed foraging or nest raiding in open areas of the littoral zone, but never spawning with a male. Overall, this habitat selection by parental females, especially limnetics, biases the encounter rates between hybrid males and creates the conditions necessary for sexual selection against F_1 hybrids (Hatfield and Schluter 1996). This is another case of ecological factors, absent in the laboratory setting, crucially affecting reproductive isolation. Because sexual selection was detected in the field but not in the laboratory (Hatfield and Schluter 1996), we feel that studies examining postmating isolation between recently diverged species should take into consideration ecological factors contributing to hybrid disadvantage. Perhaps ecological factors are more important in preventing the fusion of young species than is commonly appreciated, with the traditional criteria for reproductive isolation (i.e., hybrid inviability and sterility; Coyne and Orr 1989; Templeton 1989) becoming important only after sufficient divergence has occurred.

Our field results are much stronger than those recorded in the laboratory tests of Hatfield and Schluter (1996), even when we confine our attention to tests involving limnetic and F_1 hybrid males and limnetic females. In their experiment, limnetic females eventually spawned with hybrid males in 10 of 17 (59%) trials compared with 13 of 20 (65%) with limnetic males. Our data from 1995 and 1998 show that limnetic males had a much higher success rate (16 of 20 trials) at the expense of hybrid male success (four of 20 trials). One reason for the stronger result here is that our design enabled females to choose males, whereas Hatfield and Schluter (1996) gave no choice. A more realistic field setting (e.g., light, substrate, nest materials) may also have contributed.

What other factors may have produced the difference in mating success between limnetic and F_1 hybrid males? Some of the discrimination may be the result of lower courting intensity by hybrid males, as has been observed in certain hybrid crosses in *Drosophila* (e.g., Noor 1997) and *Anartia* butterflies (Davies et al. 1997). Females may also be discriminating against hybrid males based on male body size (or some correlated body trait), as evidenced by the tendency for the winning male to be smaller than the mean size of the other males. Nagel and Schluter (1998) have demonstrated that much of the reproductive isolation between benthics and limnetics is a result of body size differences, and this may lead to discrimination by limnetic females against hybrid

males. Our explanation assumes that females alone are exhibiting "choice," although it is also possible that hybrid males are rejecting limnetic females. However, at no stage of the trial did hybrids cease courtship of females, which suggests that females are mainly responsible for their lowered mating success. Another possibility is that hybrid males may have lower mating success due to direct intermale competition, although the number of aggressive encounters initiated by males of the two types does not appear to be different (unpubl. data).

Two questions need to be considered in the future before we can be certain of the role of mating disadvantage of hybrids in maintaining reproductive isolation between the species pairs. First, the mating success of hybrid females in the wild is unknown. If hybrid females can mate with males of the parental species, this would reduce the overall hybrid mating disadvantage. Second, the mating success of hybrid males with benthic females in the wild is unknown. Benthic females prefer vegetated areas of the littoral zone and observations suggest that benthic females are foraging rather than actively searching for mates in the open. Because of the relative rarity of hybrid females and the low probability that hybrid males can successfully court benthic females in open areas, we do not feel that either of these factors are likely to substantially augment the mating success of hybrids.

Studies of sexual selection on hybrids are principally interesting for what they may reveal about the role of this mechanism in speciation. Our results are consistent with two models of sexual selection in speciation. The first model is reinforcement, which facilitates speciation by selection for increased assortative mating (Butlin 1995). Traditionally, studies have sought to demonstrate reduced mating between two incipient species; however, assortative mating should also produce low mating success of adult hybrids, which was observed in this study. This low mating success could constitute the strong selection against hybrids that is required for reinforcement to work (Coyne and Orr 1989; Liou and Price 1994; Butlin 1995; Rundle and Schluter 1998). The second model posits a direct role for divergent sexual selection: changes in the traits that affect mating success in a population either via genetic drift or natural selection on preferences should lead to rapid differentiation. Both models predict reduced mating success of intermediate phenotypes, including hybrids. However, our results are also consistent with a third possibility, namely that reduced hybrid mating success is a by-product of genomic incompatibilities between species that arose for other reasons (e.g., Noor 1997). Further experimental work on the mating success of viable, fertile hybrids is likely to contribute exciting insights into the role of sexual selection in speciation.

ACKNOWLEDGMENTS

This paper is dedicated to the memory of Queets. Our work was made possible by the pioneering work of M. Linden and T. Hatfield. H. Rundle kindly provided some males in 1998. We are grateful to S. Anderson, K. Feldman, S. Hicks, and S. Leary for assistance in the field. H. Barclay, H. Diggon, O. Jespersen, D. Sayers, and Mr. and Mrs. B. Williams kindly provided logistic support on Texada Island. E. Brodie III, J.

McPhail, M. Noor, P. Verrell, an anonymous reviewer, and members of the SOW discussion group provided insightful comments on earlier versions of the manuscript. The Natural Sciences and Engineering Research Council (Canada) funded the research with a scholarship (PGS A) to SMV and grants to DS.

LITERATURE CITED

- AYALA, F. J., M. L. TRACEY, D. HEDGECOCK, AND R. C. RICHMOND. 1974. Genetic differentiation during the speciation process in *Drosophila*. *Evolution* 28:576–592.
- BUTLIN, R. K. 1995. Reinforcement: an idea evolving. *Trends Ecol. Evol.* 10:432–434.
- COYNE, J. A., AND H. A. ORR. 1989. Patterns of speciation in *Drosophila*. *Evolution* 43:362–381.
- DAVIES, N., A. AIELLO, J. MALLET, A. POMIANKOWSKI, AND R. E. SILBERGLIED. 1997. Speciation in two neotropical butterflies: extending Haldane's rule. *Proc. R. Soc. Lond. B Biol. Sci.* 264: 845–851.
- DOBZHANSKY, T. 1940. Speciation as a stage in evolutionary divergence. *Am. Nat.* 74:312–321.
- HATFIELD, T. 1995. Speciation in sympatric sticklebacks: hybridization, reproductive isolation and the maintenance of diversity. Ph.D. diss., University of British Columbia, Vancouver, BC.
- HATFIELD, T., AND D. SCHLUTER. 1996. A test for sexual selection on hybrids of two sympatric sticklebacks. *Evolution* 50:2429–2434.
- . 1999. Ecological speciation in sticklebacks: environment-dependent hybrid fitness. *Evolution* 53: *In press*.
- KREBS, R. A. 1990. Courtship behavior and control of reproductive isolation in *Drosophila mojavensis*: genetic analysis of population hybrids. *Behav. Genet.* 20:535–543.
- LIU, L. W., AND T. D. PRICE. 1994. Speciation by reinforcement of premating isolation. *Evolution* 48:1451–1459.
- MAYR, E. 1963. *Animal species and evolution*. Harvard Univ. Press, Cambridge, MA.
- MCPHAIL, J. D. 1992. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): evidence for a species-pair in Paxton Lake, Texada Island, British Columbia. *Can. J. Zool.* 70:361–369.
- . 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) in south-western British Columbia. Pp. 399–437 in M. A. Bell and S. A. Foster, eds. *The evolutionary biology of the threespine stickleback*. Oxford Univ. Press, Oxford, U.K.
- NAGEL, L., AND D. SCHLUTER. 1998. Body size, natural selection, and speciation in sticklebacks. *Evolution* 52:209–218.
- NOOR, M. A. F. 1997. Genetics of sexual isolation and courtship dysfunction in male hybrids of *Drosophila pseudoobscura* and *Drosophila persimilis*. *Evolution* 51:809–815.
- ORR, H. A., L. D. MADDEN, J. A. COYNE, R. GOODWIN, AND R. S. HAWLEY. 1997. The developmental genetics of hybrid inviability: a mitotic defect in *Drosophila* hybrids. *Genetics* 145: 1031–1040.
- PRICE, D. K., AND C. R. B. BOAKE. 1995. Behavioral reproductive isolation in *Drosophila silvestris*, *D. heteroneura*, and their F₁ hybrids (Diptera: Drosophilidae). *J. Insect Behav.* 8:595–616.
- ROLÁN-ALVAREZ, E., K. JOHANNESSON, AND A. EKENDAHL. 1995. Frequency- and density-dependent sexual selection in natural populations of Galician *Littorina saxatilis* Oliv. *Hydrobiologia* 309:167–172.
- ROWLAND, W. J. 1994. Proximate determinants of stickleback behaviour: an evolutionary perspective. Pp. 297–344 in M. A. Bell and S. A. Foster, eds. *The evolutionary biology of the threespine stickleback*. Oxford Univ. Press, Oxford, U.K.
- RUNDLE, H. D., AND D. SCHLUTER. 1998. Reinforcement of stickleback mate preferences: sympatry breeds contempt. *Evolution* 52:200–208.
- SCHLUTER, D. 1993. Adaptive radiation in sticklebacks: size, shape, and habitat use efficiency. *Ecology* 74:699–709.

- . 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. *Ecology* 76:82–90.
- SOKAL, R. R., AND F. J. ROHLF. 1995. *Biometry: the principles and practice of statistics in biological research*. 3d ed. Freeman, New York.
- STRATTON, G. E., AND G. W. UETZ. 1986. The inheritance of courtship behavior and its role as a reproductive isolating mechanism in two species of *Schizocosa* wolf spiders (Araneae; Lycosidae). *Evolution* 40:129–141.
- TEMPLETON, A. R. 1989. The meaning of species and speciation: a genetic perspective. Pp. 3–27 in D. Otte and J. A. Endler, eds. *Speciation and its consequences*. Sinauer, Sunderland, MA.
- TRUE, J. R., J. LIU, L. F. STAM, Z.-B. ZENG, AND C. C. LAURIE. 1997. Quantitative genetic analysis of divergence in male secondary sexual traits between *Drosophila simulans* and *Drosophila mauritiana*. *Evolution* 51:816–832.
- VAMOSI, S. M. 1996. Postmating isolation mechanisms between sympatric populations of three-spined sticklebacks. M.Sc. thesis. University of British Columbia, Vancouver, BC.
- WOODRUFF, D. S. 1979. Postmating reproductive isolation in *Pseudophryne* and the evolutionary significance of hybrid zones. *Science* 203:561–563.

Corresponding Editor: E. Brodie III