

SEX DIFFERENCES IN MATE RECOGNITION AND CONSPECIFIC PREFERENCE IN SPECIES WITH MUTUAL MATE CHOICE

Genevieve M. Kozak,¹ Melissa Reisland,² and Janette W. Boughmann^{3,4}

¹*Department of Zoology, University of Wisconsin, Madison Wisconsin 53706*

²*Department of Anthropology, University of Wisconsin, Madison Wisconsin 53706*

³*Center of Rapid Evolution, University of Wisconsin, Madison Wisconsin 53706*

⁴*E-mail: jboughman@wisc.edu*

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Sexual isolation is often assumed to arise because choosy females recognize and reject heterospecific males as mates. Yet in taxa in which both males and females are choosy, males might also recognize and reject heterospecific females. Here, we asked about the relative contribution of the sexes to the strong sexual isolation found in limnetic–benthic species pairs of threespine sticklebacks, which show mutual mate choice. We asked whether males and females of the two species recognize conspecifics and also prefer to mate with them. We found evidence for mate recognition by both sexes but only females prefer conspecifics. The nature of male courtship depended on which species of female they were courting, indicating that males recognized conspecific females and differentiated them from heterospecifics. However, males courted both species of females with equal vigor and changed courtship in a manner that would increase the chance of mating with heterospecifics. Females both recognized conspecifics and strongly preferred them. They responded very little to heterospecific male courtship and almost never mated with them. Therefore, males are likely to undermine sexual isolation, but females uphold it. Despite mutual mate choice and mate recognition in both sexes, females are primarily responsible for sexual isolation in these taxa.

KEY WORDS: Courtship, mate recognition, mutual mate choice, sexual isolation, speciation, sticklebacks.

Sexual isolation between closely related, sympatric species is expected to be determined primarily by the choosy sex (Wirtz 1999). This is most often females who, through their preferences for trait values expressed in males of their own species, are likely to recognize and prefer conspecific males and reject heterospecific males as mates. This mate recognition limits gene flow and may be the most important component of premating reproductive isolation (Ramsey et al. 2003; Coyne and Orr 2004). In contrast, males are expected to mate more indiscriminately and may actually undermine premating reproductive isolation (Tomaru et al. 1995; Noor 1996; Jennions and Petrie 1997; Russell et al. 2006).

The different contributions of the sexes to sexual isolation may arise because they differ in their ability to discriminate the

species (differences in mate recognition), or in how strongly they prefer their own species (differences in conspecific preference). Differences in both facets likely evolve due to the distinct ways the sexes optimize reproductive success. Females typically increase reproductive success through maximizing fecundity and by increasing offspring quality from each mating, in part, by their choice of mates (Bateman 1948). Mating with the wrong species can thus reduce both the number and quality of their offspring (Noor 1997; Naisbit et al. 2001; Tech 2006); therefore, females are likely to pay high costs in choosing the wrong species and be selected to avoid doing so. In contrast, males typically increase reproductive success primarily by acquiring more mates (Bateman 1948) rather than maximizing the quality of offspring produced by

any one mating. Because it is often assumed that they invest relatively little in any one mating, they can pay relatively low costs of producing hybrid offspring (Parker and Partridge 1998) and may even benefit if this allows them to produce more offspring than they would without heterospecific matings (Nuechterlein and Buitron 1998). Males are thus less likely to reject potential mates even if they are (occasionally) females of another closely related species. Sex-specific optimization strategies arise, in part, due to differential investment by the sexes in gametes and parental care (Bateman 1948; Trivers 1972).

But what about species with mutual mate choice? In species in which males invest relatively highly in each mating, such as those with male parental care or where the costs of mating are high, male mate choice may evolve (Olsson 1993; Jones and Hunter 1993; Bonduriansky 2001; Byrne and Rice 2006). Males are expected to show preference for high-quality or highly fecund females (Johnstone et al. 1996; Bonduriansky 2001; Servedio and Lande 2006). When males invest highly and are choosy, they are also likely to experience higher costs from hybrid matings. Therefore selection should favor males who avoid such matings (Servedio 2007). This suggests that in species with mutual mate choice, sexual isolation is likely to be reciprocal, with both sexes contributing to premating isolation through their recognition of and preference for mating traits found in their own species.

Fish are a good group to explore these issues, given that many species have males that provide parental care and show mutual mate choice (Amundsen and Forsgren 2001; Wong et al. 2004; Clotfelter et al. 2006). We worked with threespine sticklebacks (*Gasterosteus* spp). Many studies have shown that female sticklebacks have strong mate preferences (Semler 1971; Milinski and Bakker 1992; Bakker and Mundwiler 1994). Moreover, several studies have shown that males also have preferences (Rowland 1982; Kraak and Bakker 1998). Thus, threespine sticklebacks show mutual mate choice. Marine stickleback males preferentially court large females with distended bellies oriented in a receptive posture (Rowland 1989b; Rowland 1994). Belly shape appears to indicate readiness to mate (Rowland et al. 2002) and large females are more fecund (Wootton 1973). Male preference therefore, seems to be driven by variation in female fecundity, as predicted by theory (Johnstone et al. 1996; Servedio and Lande 2006). Male sticklebacks are likely to experience several costs of mating, including increased energy expenditure and exposure to predators through territory defense and courtship (Moodie 1972; Whoriskey and Fitzgerald 1985; Candolin 1998). In addition, male sticklebacks are the sole providers of parental care, and spend substantial time constructing a nest, aerating and tending eggs, and defending eggs and fry. This care appears to be costly (Wootton et al. 1995; Smith and Wootton 1999), as many males do not survive the period of parental care (Chellappa et al. 1989; Whoriskey and Fitzgerald 1989). Male preference may have

evolved because males are selected to avoid wasting time courting nonreceptive or low-quality females and to invest costly parental care in large rather than small clutches.

In addition to showing mutual mate choice, there are a number of reproductively isolated, sympatric pairs of stickleback species found in the northern hemisphere (McPhail 1994; McKinnon and Rundle 2002). This makes for an ideal system to test how mutual mate choice influences sexual isolation. We studied species pairs of limnetic and benthic threespine sticklebacks (*Gasterosteus* species complex) from three lakes in British Columbia: Enos, Paxton, and Priest. Each lake contains an independently evolved pair of species descended from the anadromous threespine stickleback (*Gasterosteus aculeatus*) (Taylor and McPhail 1999, 2000) who show strong pre- and postmating reproductive isolation (Ridgway and McPhail 1984; McPhail 1994; Nagel and Schluter 1998; Rundle et al. 2000; Gow et al. 2006, 2007). Genetic and physiological evidence suggests that each species pair was the result of two colonizations by anadromous sticklebacks, with one wave of colonists evolving into the benthic and the other into the limnetic species (McPhail 1993; Kassen et al. 1995; Taylor and McPhail 2000). The anadromous sticklebacks are thus, ancestral to the limnetic–benthic pairs (Orti et al. 1994; Taylor and McPhail 2000). Ancestral anadromous sticklebacks have large body size with little size dimorphism (M. L. Head, E. K. Price, and J. W. Boughman, unpubl. data). Males exhibit very large areas of red nuptial color (Candolin 1999).

Limnetic sticklebacks live in the open water feeding on plankton whereas benthics live in the vegetated margins of lakes feeding on macroinvertebrates (Bentzen and McPhail 1984; McPhail 1984). As limnetics and benthics adapted to their distinct niches they evolved numerous phenotypic and genetic differences (McPhail 1994). Differences in male and female reproductive traits, in body size, and in size dimorphism are extensive (Boughman 2006). Limnetic males have evolved large areas of intense red color whereas benthic males have evolved smaller areas of reduced color; both are reduced compared to the ancestral state. Color preferences also differ. Limnetic females have strong preferences for red males whereas benthics have weak or no preference (Boughman 2001). Limnetics have evolved small body size and benthics retain the large body size of anadromous ancestors (Schluter and McPhail 1992). The species also show reversed sexual size dimorphism. Male limnetics are larger whereas female benthics are larger (McPhail 1984, 1992). Limnetic and benthic males also court differently. They use many of the same elements, but limnetic males have a more display-oriented courtship whereas benthic males court more aggressively (Ridgway and McPhail 1984).

Differences in these mating traits are important to sexual isolation. Limnetic–benthic pairs are isolated by a combination of differences in body size, odor, and male color and female

color preference (Boughman et al. 2005). Limnetics recognize conspecifics using differences in body size and color, and benthics use body size and odor (Boughman 2006; Rafferty and Boughman 2006). Surprisingly, courtship behavior does not appear to contribute to sexual isolation (Boughman et al. 2005) despite differences in male courtship behavior (Ridgway and McPhail 1984; Rowland 1994), substantial evidence that males are choosy (Rowland 1982, 1989a; Rowland et al. 2002), and the very interactive nature of stickleback courtship (Tinbergen 1953). We found this puzzling. Could it be because females are solely responsible for sexual isolation in these species pairs? Although this prior work shows strong sexual isolation between species and identifies the traits responsible, it does not explore the contributions of the sexes to sexual isolation. We set out to address this question.

First, we test the relative contributions of the sexes to sexual isolation. Sexual isolation requires the ability to distinguish between one's own or another species; this is recognition of conspecifics, and would be indicated by behaving differently toward each species. But sexual isolation also requires a preference for conspecifics. This would be indicated by courting conspecifics more vigorously and in a manner that increases the chances of mating. We ask whether male sticklebacks contribute to sexual isolation, as expected in systems with mutual mate choice. To do this, we asked whether males court heterospecific females at all, and whether they show preferences for their own females by courting them more strongly. We also looked for differences in the nature of courtship directed at females of their own or the other species. Next, we explored the contributions of females to sexual isolation. Do females respond to heterospecific males at all, and do they show preferences for their own males? Does the nature of their response to males of their own or the other species differ?

Second, we explore more fully the earlier finding that courtship behavior does not contribute to sexual isolation in these species pairs. We ask whether each sex changes their behavior in a manner that decreases the probability of spawning with heterospecifics. Is sexual isolation predicted better by female or male behavior?

Methods

MATING TRIALS

We collected benthic and limnetic threespine sticklebacks from three lakes (Paxton, Priest, and Enos) using minnow traps and transported them to the University of British Columbia and the University of Wisconsin–Madison for mating trials. We housed fish separately by population and sex in 101-L aquaria. Fish were held at 18°C and fed bloodworms (*Chironomus* sp.) and brine shrimp (*Artemia* sp.) daily. We conducted mating trials with reproductively receptive individuals in the spring and summer of 1999, 2000, 2001, and 2006.

We conducted no-choice mating trials to assess male courtship and female response between conspecifics and heterospecifics (for detailed methods see Nagel and Schluter 1998). No-choice trials allow males and females to interact all the way to spawning so that we can measure the extent of premating isolation. They also allow us to exclude male–male or female–female competition. Moreover, no-choice trials mirror the mating interactions in nature where males typically encounter females one at a time. We chose males in reproductive condition from holding tanks and placed each male in a 101-L aquarium containing a sand-filled container with plants and filamentous algae as nesting material. Males in nesting tanks were exposed to a gravid female once every two days to stimulate nest building and courtship. Once a male had constructed a nest, each male courted both a limnetic and a benthic female in a random order. We ran trials for 20 min or until the female entered the nest to spawn. We removed females immediately after they entered the nest so that we could reuse males. Each female was used only once. We recorded male and female courtship behavior using Observer behavioral data recording software (Noldus Technologies, Wageningen, The Netherlands). We ran trials for 159 males (30 Enos benthic, 12 Enos limnetic, 50 Paxton benthic, 53 Paxton limnetic, and 14 Priest limnetic) for a total of 318 trials, and 354 females (106 Enos benthic, 65 Enos limnetic, 69 Paxton benthic, 68 Paxton limnetic, and 24 Priest limnetic).

COURTSHIP BEHAVIOR

Male courtship is fairly complex and includes a number of behaviors. The early phase of courtship involves initial attraction via either display or aggressive behaviors. Males display by moving in a zigzag motion toward the female (zigzag). Aggressive behaviors include chasing (chase) and nipping at the female (bite). Males then attempt to lead the female back to the nest (lead) and may prick her belly with his erect spines to deter her from following (dorsal prick). Once at the nest, the male will show the female the nest opening (show) and if she examines he will gently push her with his belly (rub). Benthic and limnetic males differ in the nature of courtship (Boughman et al. 2005). Benthics court aggressively, using primarily bites. Limnetics are more likely to display, using primarily zigzags. We categorized courtship into two groups: display oriented (zigzags, leads) and aggressive (bites, chases).

Female courtship can be divided into behaviors indicating female interest in being courted (receptivity) and those indicating female preference for particular males (choice). Females show interest in being courted through a lordosis posture when the male approaches (head up) or a relaxed form of this posture (angle). Females may also initiate courtship by approaching the male (approach). Thus, female receptive behaviors are head up, angle, and approach. Female preference behaviors include following the male who is leading her to the nest (follow) and examining when

the male has shown her the nest entrance (examine). If the female accepts the male, she enters the nest and deposits eggs (spawn). Once she leaves the nest, the male swims through the nest and releases sperm. Thus, female choice behaviors are follow, examine, and spawn.

STATISTICAL ANALYSIS

Male behavior

We calculated the rate of each male courtship behavior per minute relative to rate of total courtship. We also calculated an index of display-oriented courtship as the proportion of display behaviors (zigzag + lead) to total courtship (zigzag + lead + bite + chase + dorsal prick) and an index of aggressiveness as the proportion of aggressive behaviors (bite + chase) to total courtship. We calculated vigor as the sum of all courtship behaviors. To quantify mate recognition by males we calculated the difference between the rate of courtship to the conspecific and heterospecific females. If this value is different from zero then males differ in how they court their own and the other species of female; we tested this with *t*-tests. We analyzed this difference using ANOVA, with species, population, and their interaction as factors.

To investigate the effects of female response on male courtship, we calculated the rate of courtship by males in the first 5 min of trials. We could do this for a subset of the male trials in which the data were available (14 Enos benthics, 27 Paxton benthics, 26 Paxton limnetics, 14 Priest limnetics). We compared 5-min rates to the total rate using paired *t*-tests to test if males adjusted their courtship based on female behavior.

Previous work found that female body size influences male courtship (Nagel and Schluter 1998). To look at this effect we calculated the difference in body size between the male and the female (male length minus female length) for 11 Enos benthics, 46 Paxton benthics, 12 Enos limnetics, and 51 Paxton limnetics for which we had length data. We then used analysis of covariance (ANCOVA) with species, cross type, size difference, and their interactions to determine if body size difference explained a significant amount of variation in display or aggressive courtship. As each male in this analysis had two observations we corrected the degrees of freedom to equal the number of males (120), instead of the number of observations. None of the interaction terms with species were significant, so we removed these terms and report the reduced model. The lack of species by length difference interaction indicates that both species respond in a similar way to size differences.

Altering the nature of courtship in response to female behavior or size would indicate mate recognition by males, or the ability to distinguish between their own and the other species. Courting conspecific females more vigorously or in a manner to increase the probability of mating would indicate conspecific preference by males.

Female behavior

We calculated the rate of each female behavior per minute and used these rates in the subsequent calculations. We calculated female interest in being courted as the sum of receptive behaviors (head up + angle + approach), which we term receptivity. We did this for a subset of the trials (29 Enos benthics and 24 Priest limnetics). All other variables were calculated for the full dataset. We also calculated two indices of preference: the proportion of male leads that induced a female to follow or approach (follow + approach/lead), which we term responsiveness, and the proportion of male shows that resulted in a female examining the nest (examine/show), which we term inspection. For responsiveness, females occasionally approached even though males performed no leads. In this case, we set values to one. We analyzed the differences in receptivity and preference for conspecific and heterospecific males using ANOVA with female species, lake, cross type, and their interaction as factors. None of the terms including "lake" were significant, so we reduced the model to female species, cross type, and their interaction. Differences between mean values for cross type indicate that females differ in how they respond to their own and the other species of male.

We also used logistic regression to determine the proportion of trials in which females followed, examined, and spawned to assess female preference. Our model included female species, lake, cross type and their interaction as factors. For logistic analyses, the lake term and the three-way interaction were significant, so we kept the full model. Higher rates of behavior to conspecific males would indicate conspecific preference by females; it would also require mate recognition. All statistics were calculated using SAS (version 9.1) statistical software (SAS Institute Inc. 2007).

Results

MALE BEHAVIOR

Males courted conspecific and heterospecific females differently and tailored courtship based on the female species (Table 1; Fig. 1). Males of both species courted benthic females more aggressively and with more bites, and zigzagged and displayed more to limnetic females. To do so requires that males could distinguish between conspecific and heterospecific females and therefore, indicates male mate recognition.

Courtship behavior differed between species (Table 1), but there was little indication that males preferred conspecific females (Fig. 1E, F). Benthic males led conspecific and heterospecific females to the nest at the same rate but limnetic males led conspecific females more than heterospecifics. This was due to a single limnetic population from Paxton lake, as indicated by the significant species by lake interaction for leads (Table 1) and the test of whether lead difference equals zero for this population ($t_{52} = 2.42$, $P < 0.05$) and nonsignificance for the Priest and

Table 1. Male behavior for limnetic and benthic sticklebacks. Differences in rate of male courtship to conspecific and heterospecific females were calculated and then tested with ANOVA. Populations include both limnetics and benthics from Enos, Paxton, and Priest Lakes.

	Male species		Lake		Male species × Lake	
	$F_{1,154}$	P	$F_{2,154}$	P	$F_{1,154}$	P
Zigzag	19.15	<0.001	1.22	0.30	3.61	0.06
Display	22.10	<0.001	2.38	0.09	0.43	0.23
Bite	32.37	<0.001	2.93	0.06	0.31	0.58
Aggression	27.04	<0.001	2.50	0.10	1.46	0.52
Lead	3.33	0.07	1.17	0.31	6.39	0.01
Vigor	0.96	0.33	0.65	0.52	1.99	0.16

Enos limnetic populations ($P > 0.45$ for both). Males courted both conspecific and heterospecific females with equal vigor, indicating a lack of preference, and that differences in the nature of courtship directed to limnetic and benthic females were not due to overall differences in courtship vigor. Limnetic males did have a nonsignificant tendency to show more vigorous courtship to heterospecifics (Fig. 1F). The lack of differences in these two preference measures for benthic males and the finding that they were in opposite directions for limnetic males suggest that males did not prefer conspecific females.

Both species of males responded to body size differences between themselves and females in a similar manner, as shown by the lack of species by length difference interaction (display $F_{1,113} = 1.18$, $P = 0.27$, aggression $F_{1,113} = 0.56$, $P = 0.46$) and the parallel slopes (Table 2; Fig. 2). However, there was a large effect of cross type. Body size differences did not influence display or aggressive courtship toward conspecific females for either species (all $P > 0.25$; Table 2; Fig. 2). In contrast, heterospecific female size differences strongly influenced courtship by both limnetic and benthic males (Table 2; Fig. 2). Small heterospecific females received more display and less aggression. Benthic males courted limnetic females who were much smaller than them with more display. Limnetic males courted benthic females who were similar in size with more display, but because the species differ in body size, these were the smaller benthic females. Thus, small benthic and small limnetic females were courted similarly, even though small benthic females are the same size as large limnetic males. Large heterospecific females were courted aggressively by both species of males. These results indicate that males responded not to absolute size or absolute size differences, but to female size when they recognized the female was heterospecific.

Males did not adjust their courtship based on female response over the course of the trial. The courtship rates in the first 5 min were not significantly different than those in the total trial for any

behavior (paired t -tests: benthics: $df = 40$, all $P > 0.05$; limnetics: $df = 39$, all $P > 0.10$).

FEMALE BEHAVIOR

Females responded differently to conspecific and heterospecific male courtship, and showed both mate recognition and conspecific preference (Table 3; Fig. 3). Females of both species were significantly more receptive toward conspecific males. They also preferred conspecific males by all measures. Females approached, followed, and examined at a higher rate when paired with a conspecific than with a heterospecific male. They also were more responsive toward conspecifics and inspected their own males' nests more. The species responded similarly for most measures, but there was a significant interaction between cross type and species for examine rate. Limnetic females examined conspecific male nests at a higher rate but benthic females did not, and examined very infrequently. The population of benthic females from Paxton Lake was unusual, so we compared their responses to conspecific and heterospecific males using t -tests. They did not show a preference for conspecific males by any measure (Fig. 3; all $t_{95} < 1.5$; all $P > 0.15$).

In addition to greater receptivity and preference for conspecifics as measured by the rates of these behaviors, logistic regression analyses showed that females were more likely to follow conspecific males (Table 4; Fig. 4). They were also more likely to examine conspecific nests and spawn with conspecific males. Limnetic females were more likely to spawn than benthic females. Here again, Paxton benthic females stood out. They were no more likely to follow, examine, or spawn with benthic males than limnetic males (follow $t_{95} = 0.58$, $P > 0.5$; examine $t_{95} = 0.13$, $P > 0.7$; spawn $t_{95} = 0.18$, $P > 0.9$).

Discussion

SEX DIFFERENCES IN MATE RECOGNITION AND THE COSTS OF ERRORS IN STICKLEBACKS

Both sexes appear to discriminate between their own and the other species. Mate recognition is shown for males because they court limnetic females differently than benthic females. Limnetic females are courted in the same way by both species of males. This is also true for benthic females, suggesting that the females induce particular courtship behavior from males. Males do not alter behavior over the course of courtship, suggesting that they recognize and respond to some aspect of female morphology rather than female behavior. A likely candidate is size, based on male responses to female size differences, although body shape or other traits probably also play a role, given that the response to female size depends on species identity. Despite good mate recognition, there is little indication of conspecific preference on the part of males. Leading females to the nest is essential for spawning to occur,

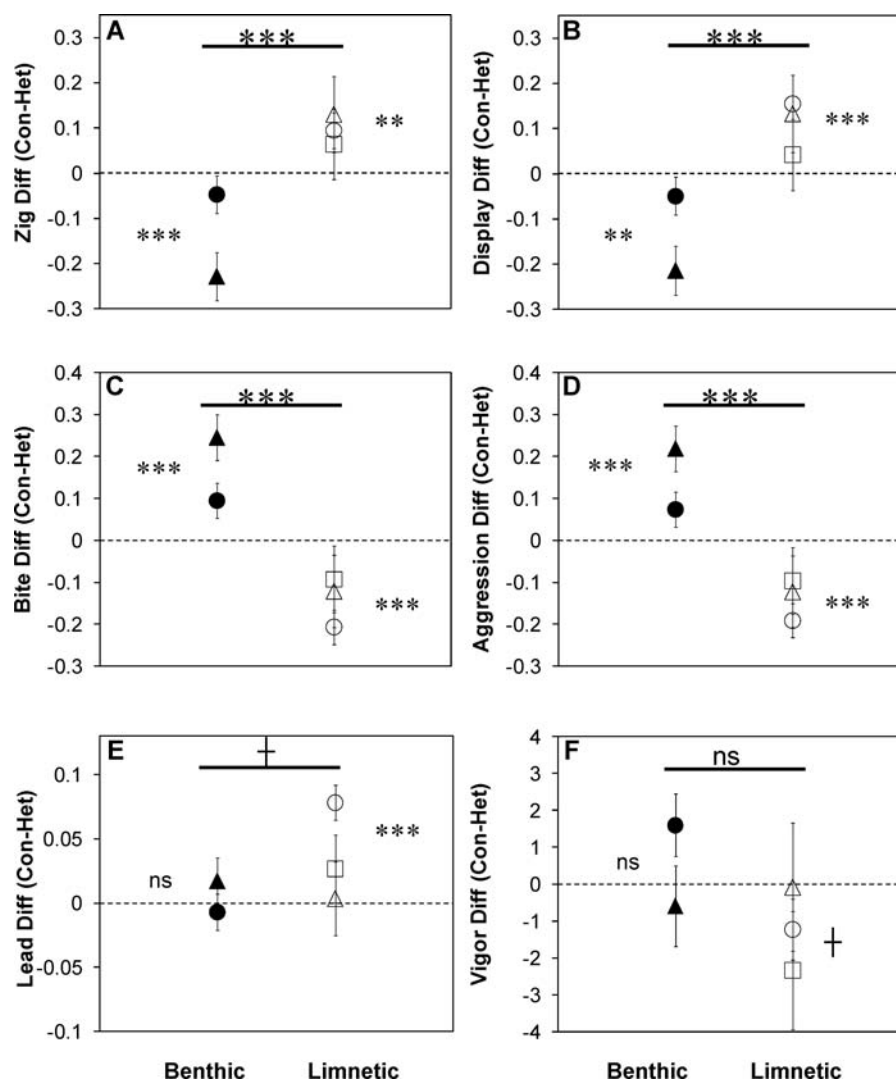


Figure 1. Male courtship to conspecific and heterospecific females. Least-squares means \pm standard errors of the difference in courtship (conspecific minus heterospecific) shown for each species and population from ANOVA. Positive values signify a higher rate of behavior to conspecifics, negative values a higher rate to heterospecifics. Filled symbols are benthics, open symbols are limnetics. Triangles represent fish from Enos Lake, circles represent fish from Paxton Lake, and squares represent fish from Priest Lake. Significance for tests that courtship by each male species differs to conspecific and heterospecific females is tested with t -tests and shown next to population means. Significance for tests of differences between the species is shown across the top of figures. Differences are shown for (A) zigzag rate, (B) display courtship, (C) bite rate, (D) aggressive courtship, (E) lead rate, and (F) courtship vigor. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, + $P < 0.10$.

yet there is no difference in the rate at which benthic and most populations of limnetic males lead conspecific and heterospecific females. Moreover, males do not court conspecific females more vigorously, suggesting that males are not ignoring heterospecific females in favor of conspecifics. Males courted both species of females, showing little, if any preference. Although our tests were designed so that each male was presented with one female at a time, this mirrors the typical situation in nature in which females search for males in nesting areas. The data here cannot speak to whether males would show preferences if given a simultaneous choice, but other data from our laboratory using choice tests sug-

gest that benthics may bite conspecifics slightly more but that limnetics show no change from no-choice behavior (G. M. Kozak unpubl. data). In any case, our results clearly show that males do not reject the opportunity to court heterospecific females. Instead, males court females with the behavior that is most likely to result in spawning whether or not females are conspecifics. Instead of decreasing the probability of heterospecific matings, changes to male courtship should increase it. These results indicate that although males show mate recognition they do not strongly prefer conspecific females. Rather than contributing to sexual isolation, males may actually undermine it.

Table 2. Male response to female body size for limnetic and benthic sticklebacks. Length diff is calculated as male length minus female length. Cross type refers to conspecific or heterospecific trials. Data are analyzed with ANCOVA in the first part of the table, and slopes are reported separately for each species to both conspecific and heterospecific females in the second part of the table.

	Male species		Cross type		Length diff		Length diff \times Cross type	
	$F_{1,120}$	P	$F_{1,120}$	P	$F_{1,120}$	P	$F_{1,115}$	P
Display	32.71	<0.0001	1.34	0.25	11.52	<0.001	4.71	0.032
Aggression	41.34	<0.0001	2.41	0.12	11.19	<0.001	5.54	0.020
	Benthic to benthic		Benthic to limnetic		Limnetic to limnetic		Limnetic to benthic	
	$\beta \pm SE$	P	$\beta \pm SE$	P	$\beta \pm SE$	P	$\beta \pm SE$	P
Display	-0.0002 ± 0.0052	0.97	0.010 ± 0.0056	0.073	0.0051 ± 0.0056	0.36	0.016 ± 0.0040	<0.001
Aggression	0.0002 ± 0.0052	0.98	-0.0096 ± 0.0055	0.083	-0.0028 ± 0.0055	0.33	-0.014 ± 0.0040	<0.001

Mate recognition is shown for females because they respond to limnetic males differently from benthic males. They also strongly prefer conspecific males, as shown by the significant reduction in response to heterospecifics for all variables. Females from both species nearly always reject heterospecifics even when these males court them the same way their own males would. This suggests that females use male morphology rather

than male behavior in mate recognition and prefer conspecific morphological traits. Morphology appears to be the primary basis of mate recognition in both sexes.

Although our data suggest Paxton benthic females show reduced mate recognition and conspecific preference, other studies have demonstrated that these females do prefer conspecifics (Rundle and Schluter 1998; Nagel and Schluter 1998;

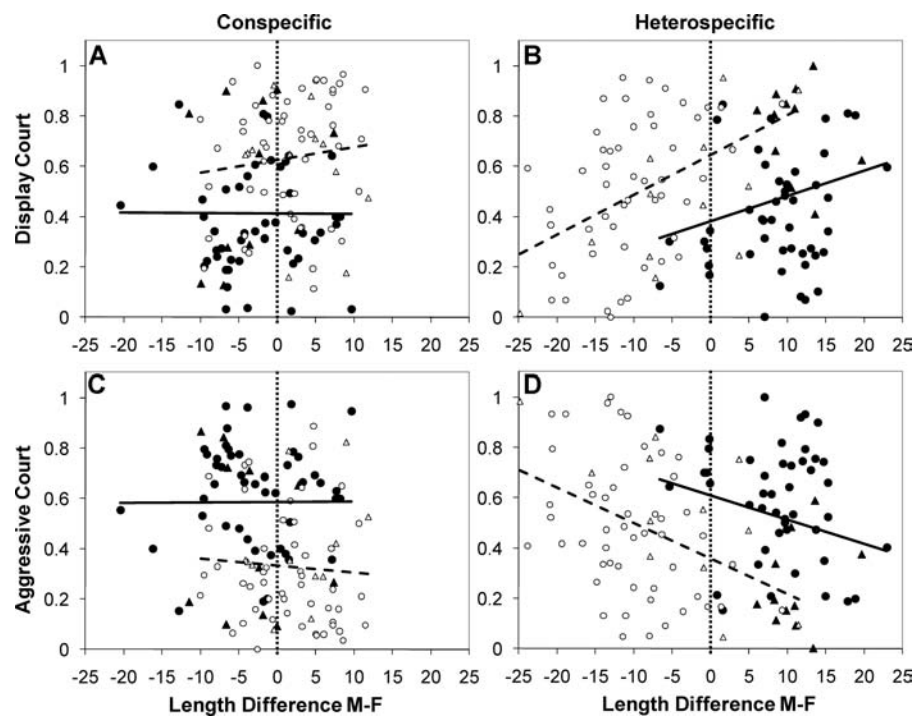


Figure 2. Relationship between display-oriented and aggressive male courtship to size differences between the sexes. Amount of display-oriented (top) and aggressive courtship (bottom) is shown in relation to the difference in length between males and females (male minus female length). Positive values indicate that males are larger, negative values indicate that females are larger, and the reference line indicates equal size. Courtship to conspecific females (left) and heterospecific females (right) is shown. Lines represent the linear response to size difference. Slope values with significance are reported in Table 2. Filled symbols and solid lines are for benthic males, open symbols and dashed lines are for limnetic males. Triangles are for Enos Lake, circles are for Paxton Lake. (A) Display courtship to conspecific females and (B) heterospecific females; (C) aggressive courtship to conspecific females and (D) heterospecific females.

Table 3. Female behavior for limnetic and benthic sticklebacks. Differences in rate of female response to conspecific and heterospecific males tested with ANOVA. Cross type refers to conspecific or heterospecific trials.

	Species		Cross type		Species × Cross type	
	$F_{1,350}$	P	$F_{1,350}$	P	$F_{1,350}$	P
Receptivity ¹	0.15	0.70	11.27	0.0015	0.002	0.97
Follow rate	0.24	0.62	15.07	0.0001	1.52	0.21
Examine rate	18.75	0.0001	11.23	0.0009	5.45	0.02
Responsiveness (follow/lead)	0.88	0.35	9.75	0.0019	0.20	0.65
Inspection (examine/show)	12.86	0.0004	10.32	0.0014	0.01	0.93

¹Denominator degrees of freedom for receptivity are 49.

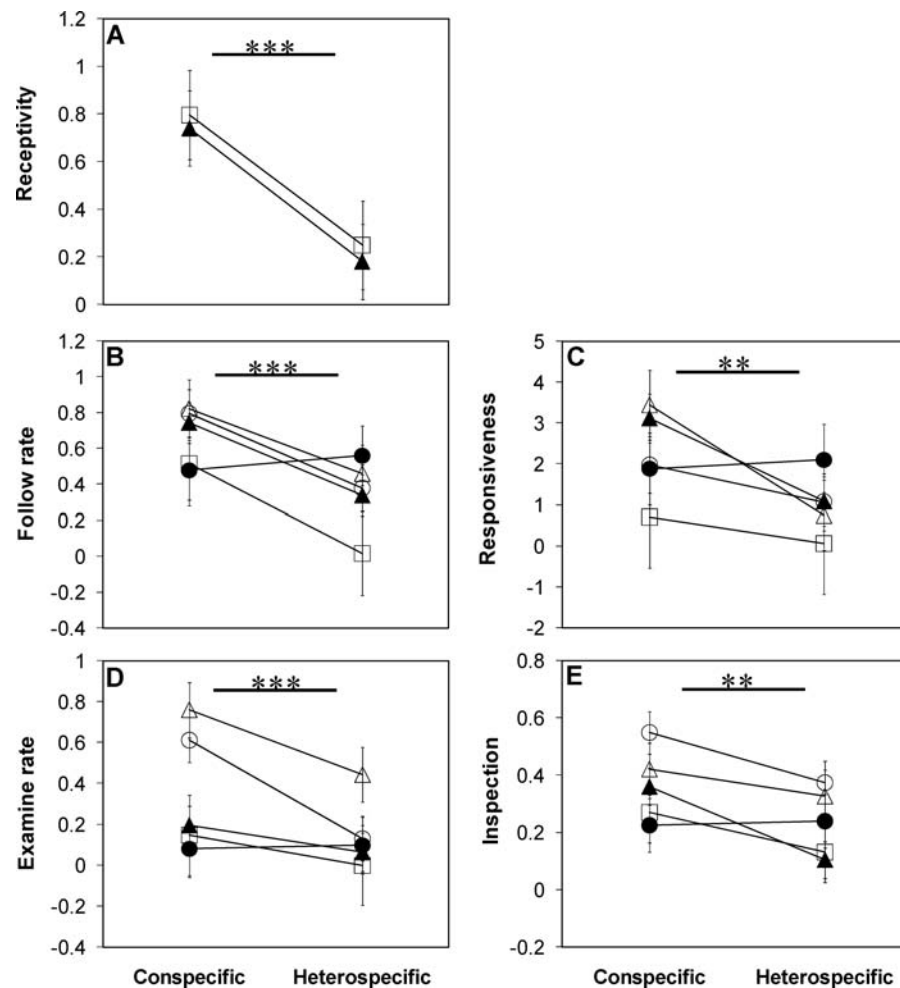


Figure 3. Rate of female responses to conspecific and heterospecific males. Least-square means ± standard errors for each species and population from ANOVA. Open symbols represent limnetics and closed symbols benthics. Triangles represent fish from Enos Lake, circles represent fish from Paxton Lake, and squares represent fish from Priest Lake. Lines connect mean responses for the same population. (A) receptivity (head up + angle + approach), (B) follow rate (follow + approach/minute), (C) responsiveness (follow + approach/lead), (D) examine rate (examine/minute), and (E) inspection (examine/show). *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Table 4. Female response for limnetic and benthic sticklebacks from Paxton, Priest, and Enos Lakes. Probability of female response to conspecific and heterospecific males tested with logistic regression. Likelihood-ratio tests were used to evaluate the significance of effects. Cross type refers to conspecific or heterospecific trials. Two-way interactions were tested in the full model but are not shown here because none were significant for any variable.

	Species		Lake		Cross type		Species \times Lake \times Cross type	
	$\chi^2_{1,3}$	<i>P</i>	$\chi^2_{1,3}$	<i>P</i>	$\chi^2_{1,3}$	<i>P</i>	$\chi^2_{1,3}$	<i>P</i>
Follow	0.07	0.79	15.02	0.0005	11.26	0.0008	2.93	0.087
Examine	9.86	0.0017	12.13	0.0023	13.53	0.0002	4.36	0.037
Spawn	2.83	0.093	3.68	0.16	5.01	0.025	0.25	0.62

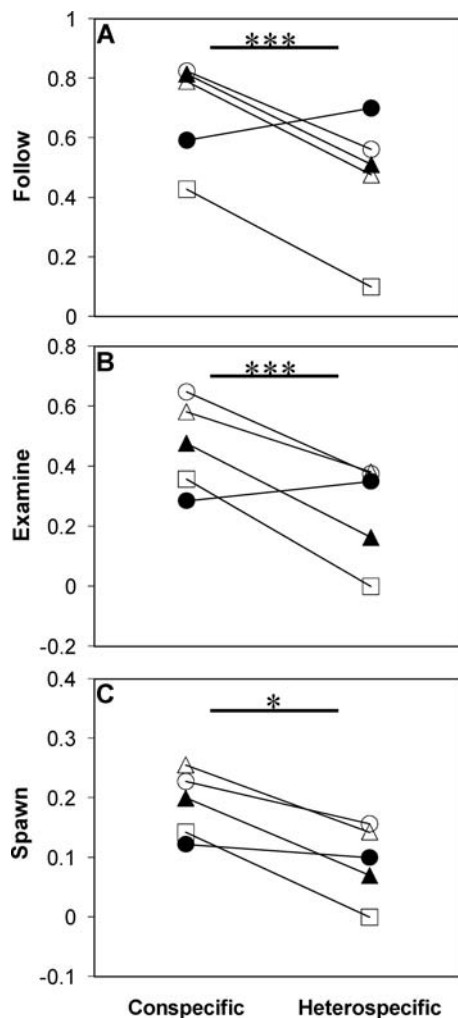


Figure 4. Probability of female response to male courtship. Probabilities are estimated by logistic regression. Open symbols represent limnetics and closed symbols benthics. Triangles represent fish from Enos Lake, circles represent fish from Paxton Lake, and squares represent fish from Priest Lake. Proportion of females that (A) followed, (B) examined, and (C) spawned in trials. ****P* < 0.001, **P* < 0.05.

Rundle et al. 2000). Moreover, our data indicate that sexual isolation in Paxton Lake would be maintained by the conspecific preferences of female limnetics in this lake.

Even though both sexes recognize conspecific mates, only females reject heterospecifics consistently. Their stronger preference for conspecifics shows that females are primarily responsible for sexual isolation in this system. This is in contrast to our hypothesis that both sexes would contribute to sexual isolation because sticklebacks show mutual mate choice. Instead, it is in line with expectations from systems with female only choice. This is somewhat surprising. Reinforcement could help to explain the difference in the strength of male and female conspecific mate preference. Reinforcement has contributed to the evolution of conspecific mate recognition in stickleback species pairs, including both female (Rundle and Schluter 1998) and male recognition (Albert and Schluter 2004). Although Servedio (2007) showed that reinforcement can favor the evolution of male choice and male mate recognition, her model predicted that male choice for viability traits in females should have weaker effects on premating isolation than female choice for male traits. Our results are broadly consistent with these predictions in that males do recognize and differentially court conspecifics, but the effects of female choice on premating isolation outweigh male effects.

Another study on the limnetic–benthic species pairs showed differences in male courtship directed toward females of their own or another species, suggesting that males can distinguish females of their own species (Albert and Schluter 2004). In that study, limnetic males displayed less to benthic females but bit them more, which corroborates our findings and indicates that recognition does occur. Benthic and benthic-like females are known to cannibalize eggs (Ridgway and McPhail 1988; Foster 1995) so both limnetic and benthic males may court aggressively to assess a female's potential for cannibalism versus spawning (Shaw et al. 2007). Albert and Schluter (2004) suggested that this threat of cannibalism would exert direct selection on mate recognition for limnetic males. It might also influence the nature of courtship for the benthic species. However, our results suggest that these

differences in male courtship may not indicate conspecific preference and may contribute little to total sexual isolation.

The reliance on morphology for mate recognition probably explains why courtship behavior contributes little to sexual isolation in the limnetic–benthic pairs (Boughman et al. 2005). Even though males adjust courtship depending on which species of female they are courting, this does not appear to override female preference. Females still reject heterospecific males. Males can alter courtship behavior rapidly so behavior is less closely associated with species identity, and females may use morphology rather than behavior because it is a more reliable cue.

Female sticklebacks may have stronger conspecific preference than males because the costs of hybrid matings are higher for them; this is likely to be particularly true for direct costs (Servedio 2001). Females are sensitive to the costs and benefits of mate choice and become more choosy when the costs of choice decrease (Milinski and Bakker 1992). Other studies have shown that female sticklebacks have stronger mate preferences than males, which will result in stronger sexual selection on males (Fitzgerald 1983; Whoriskey and FitzGerald 1994). Because preferences evolve in response to the costs and benefits of choice this suggests that the benefits of choice are likely to be larger for females (Kraak et al. 1999b). By extension, the costs of recognition errors may also be larger. In sticklebacks, female parental investment is restricted to egg provisioning and thus occurs before spawning (Wootton et al. 1995). This means that a hybrid mating undermines a female's full investment in a clutch because the survival and mating prospects of those hybrid offspring are low (Schluter 1995; Hatfield and Schluter 1996; Vamosi and Schluter 2002; Gow et al. 2007). This imposes a large indirect cost on females for mate recognition errors and when coupled with direct costs may result in large total costs.

In contrast, male parental investment is primarily in the form of paternal care and thus occurs after mating (Wootton et al. 1995). Males can adjust their investment based on the number of eggs and quality of offspring, and therefore, could withhold care for low-quality hybrid eggs (M. L. Head, E. K. Price, and J. W. Boughman, unpubl. data). In this way, males may be able to reduce costs of hybrid matings. In many species parental care is adjusted according to the quality of the breeding attempt (Pressley 1981; Dale et al. 1996; Listoen et al. 2000) in accordance with the differential allocation hypothesis (Burley 1988; Sheldon 2000). Doing so imposes direct costs on females who mate heterospecifically (Servedio 2001).

In addition, hybrid matings may not reduce further mating opportunities for males. Male sticklebacks often remate (Kraak et al. 1999a), and can actually benefit from having eggs in their nest, possibly even if they are hybrid eggs. Eggs increase attractiveness to subsequent females (Kraak and Groothuis 1994; Kraak et al. 1999a) and so, might increase a male's mating success. More-

over, males often consume eggs and the nutrition they gain can increase their survival and parental success (Smith 1992). Hybrid eggs might be especially prone to consumption because of their low quality; slow developing eggs are selectively cannibalized in other species (Klug and Lindstrom 2008). In addition, if care effort is not linearly related to egg number and males remate with conspecifics, then the cost of additional care for hybrids might be low relative to the number of additional offspring reared, and males may benefit from these additional offspring even if some are low-quality hybrids. The high fecundity of large females may partly offset the indirect cost of producing low-quality hybrid offspring, again reducing total cost for males. These considerations suggest that when males provide parental care but do not forgo further mating opportunities, they may not show strong preference for conspecific females. Such conditions are likely to be true for many fish species (Rios-Cardenas 2005). Further work is needed to test for differential costs of hybridization for males and females in sticklebacks and in other taxa.

MUTUAL MATE CHOICE AND MATE RECOGNITION IN OTHER SPECIES

The same relative costs and benefits that select for males to show mate choice may also select for males to show mate recognition and conspecific preference (Servedio 2007). Therefore, species in which males have strong mating preferences are those for which males are expected to contribute to sexual isolation. Such taxa are likely to have high parental investment by males, a female-biased operational sex ratio (OSR), high costs to mating or sexual signaling, or variation in female fecundity (Owens and Thompson 1994). Theory implicates variation in female fecundity as a primary cause for the evolution of male mate choice (Johnstone et al. 1996; Servedio and Lande 2006), and data from several systems suggest it may also be involved in the evolution of male mate recognition. For example, male preferences contribute substantially to sexual isolation in several species of garter snakes (Shine et al. 2002). Costs of mating may select for this fecundity-based mate choice (Shine 2003) and conspecific preference (Shine et al. 2004) because male garter snakes produce costly mating plugs and thus, can copulate only a limited number of times in a mating season (Shine et al. 2001). However, multiple factors appear to operate in several systems, and we lack direct tests of the influence these factors have on the evolution of both male mate choice and mate recognition, so much more work is needed. There is almost no theoretical work on the evolution of male mate recognition, so more theoretical work is also needed.

Male mate recognition is also found in other systems in which males experience high costs of mating and/or sexual signaling. In sister species of leaf beetles males appear to contribute more to sexual isolation than females (Peterson et al. 2001, 2005a). Males engage in prolonged periods of mate guarding (Dickinson

1995), which reduces future mating opportunities in these very promiscuous species (Schwartz and Peterson 2006). Hybrids have very low fitness; therefore, males that avoid heterospecific females should be favored (Peterson et al. 2005a,b). One of the classic examples of reproductive character displacement was described for the damselflies *Calopteryx maculata* and *C. aequabilis* in North America (Waage 1979). Reproductive character displacement has occurred in parallel in two European species (*C. virgo* and *C. splendens*), and recent data show that it arises from male conspecific preference. The European species show strong but asymmetric sexual isolation (Svensson et al. 2004, 2006, 2007). This asymmetry arises because male *C. virgo* damselflies discriminate more strongly against *C. splendens* females than any of the other combinations (Svensson et al. 2007). Risk of predation is thought to be especially high on male *C. virgo* because their heavily melanized wings make them conspicuous to predators (Svensson and Friberg 2007). Such reproductive costs could select for strong conspecific preference by *C. virgo* males, and could also help explain character displacement of wing color in the North American species. *Drosophila persimilis* and *D. pseudoobscura* provide a counter example because males do not show mate recognition despite mating and hybridization costs (Noor 1996; Ortiz-Barrientos and Noor 2005; Noor and Ortiz-Barrientos 2006), and contributions to sexual isolation by both sexes in other drosophilids (Wood and Ringo 1980).

At present, we cannot fully explain or predict variation among taxa in male mate recognition and conspecific preference. Further work is needed before generalizations can be made about the role of males in sexual isolation and the extent to which it correlates with the strength of male mate choice. It will be especially interesting to investigate how differences between the sexes in costs and benefits of reproduction and hybridization affect their relative roles in sexual isolation. Some surprises might await us. Research in several taxa indicates that one or both sexes can reduce the costs or actually benefit from hybridization under some circumstances (Veen et al. 2001; Pfennig 2007; Wiley et al. 2007), and the results of our study suggest that whether each sex contributes to sexual isolation is determined by complex interactions of these costs and benefits. Interactions between the sexes add another layer of complexity and will ultimately determine the direction and course of evolution.

Despite mutual mate choice and high parental investment by male sticklebacks, sexual isolation in the limnetic–benthic pairs is determined primarily by females. Our results suggest that if males contribute to reproductive isolation, they will most likely do so after mating and during the period of parental care, and thus contribute to postmating isolation. Therefore, to understand sexual isolation in stickleback species pairs will require a focus on the contributions of females. Research efforts in these areas are likely to be fruitful because greater insight into mate recogni-

tion and conspecific preference in taxa with mutual mate choice will improve our understanding of both sexual selection and the evolution of reproductive isolation.

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