Evolution, 59(2), 2005, pp. 361–373

PARALLEL EVOLUTION OF SEXUAL ISOLATION IN STICKLEBACKS

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Abstract.—Mechanisms of speciation are not well understood, despite decades of study. Recent work has focused on how natural and sexual selection cause sexual isolation. Here, we investigate the roles of divergent natural and sexual selection in the evolution of sexual isolation between sympatric species of threespine sticklebacks. We test the importance of morphological and behavioral traits in conferring sexual isolation and examine to what extent these traits have diverged in parallel between multiple, independently evolved species pairs. We use the patterns of evolution in ecological and mating traits to infer the likely nature of selection on sexual isolation. Strong parallel evolution implicates ecologically based divergent natural and/or sexual selection, whereas arbitrary directionality implicates nonecological sexual selection or drift. In multiple pairs we find that sexual isolation arises in the same way: assortative mating on body size and asymmetric isolation due to male nuptial color. Body size and color have diverged in a strongly parallel manner, similar to ecological traits. The data implicate ecologically based divergent natural and sexual selection as engines of speciation in this group.

Key words.—Body size, courtship behavior, divergent natural selection, divergent sexual selection, nuptial color, sexual isolation, speciation.

Received March 10, 2004. Accepted November 22, 2004.

How new species arise is one of the most significant unanswered questions in evolutionary biology. Current research has shifted focus from the geographic context of speciation (i.e., allopatry vs. sympatry) to the mechanisms that drive the evolution of reproductive isolation between populations (Schluter 2001). Evidence is rapidly accumulating from diverse taxa that divergent natural and sexual selection are important causes of speciation in nature (e.g., Funk 1998; Schemske and Bradshaw 1999; Rundle et al. 2000; Schluter 2000, 2001; Via et al. 2000; Boughman 2001; Podos 2001; Nosil et al. 2002). Less is known, however, about the details by which this occurs. For example, what kinds of traits cause reproductive isolation and what forms of selection act on them? Is speciation primarily the by-product of adaptive divergence, or does selection also commonly reinforce premating isolation in sympatry? When sexual selection is involved, is the divergence of male mating traits and female preferences arbitrary with respect to environment or ultimately the product of ecologically based divergent selection (e.g., sensory drive; Endler 1992, 1993; Boughman 2002)? Answers to such questions will require an understanding of the speciation process that goes beyond simply inferring a role for natural or sexual selection.

Here we focus on the evolution of sexual isolation. We address two alternative hypotheses by which natural and sexual selection may be involved in the evolution of sexual isolation that differ in the role of ecologically based selection. In the first hypothesis, termed ecological speciation, adaptation to the environment drives the evolution of sexual isolation, including the divergence among populations in both mating signals and preferences. Both natural selection and sexual selection can be involved, as long as differences in mating traits and preferences (hereafter ‘‘mating traits’’) that confer reproductive isolation arise ultimately due to ecologically based divergent selection (Schluter 2000, 2001). Ecological speciation is a general hypothesis that includes a variety of mechanisms involving contrasting selection acting on populations inhabiting separate environments or niches. For example, under this hypothesis mating traits may diverge between populations as a by-product of natural selection adapting the populations to their different environments (Mayr 1942; Dobzhansky 1951). They may also diverge due to sexual selection caused by different signaling environments (Endler 1992; Boughman 2002), or due to spatially varying selection on secondary sexual traits (Lande 1982; Day 2000) or on communication systems (Ryan and Rand 1993). Evolution of mating traits in correlation with environment is predicted not only by natural selection but by several forms of sexual selection, including sensory drive, context-dependent good genes, and spatial variation in the optimal male trait (Schluter 2000).

In the second hypothesis, termed nonecological speciation, differences in mating traits do not depend on differences in environment. Several mechanisms may again be involved. For example, mating traits may diverge between populations due to genetic drift (Gavrilets and Boake 1998), differences among populations in the amount and patterns of genetic variation (Schluter et al. 2004), or nonecological sexual selection driving traits in arbitrary directions (e.g., Parker and Partridge 1998). However, attention has focused on sexual selection because it has long been thought to be a powerful mechanism fostering speciation, and recent comparative studies implicate it (reviewed in Panhuis et al. 2001). Nonecological sexual selection causes the mating traits that confer reproductive isolation to evolve haphazardly with respect to environment. This occurs because selection on mating traits arises due to interactions between the sexes (West-Eberhard 1983) and thus there is no reason to expect parallel change in separate populations, even when they inhabit similar environments. Evolution of mating traits in arbitrary directions is predicted by various models of sexual selection such as Fisher's runaway (Lande 1981; Turner and Burrows 1995; Pomiankowski and Iwasa 1998; Higashi et al. 1999) and sexual conflict (Arnqvist et al. 2000; Gavrilets 2000; Arnqvist and Rowe 2002; Rice and Chippindale 2002). Because the directions of evolutionary change can be diverse, allopatric populations are very likely to differ in mating traits and consequently lead to sexual isolation (Turelli et al. 2001). This is the more traditional view of how sexual selection causes reproductive isolation. We refer to this form of speciation as sexual selection in arbitrary directions.

We distinguish between these hypotheses using the concept of parallel speciation. Parallel speciation is the parallel evolution of reproductive isolation in populations descended from a common ancestor that have colonized similar novel environments (Schluter and Nagel 1995). Parallel speciation occurs when reproductive isolation evolves as the by-product of ecologically based divergent selection. It predicts that the traits determining reproductive isolation should evolve in correlation with the environment. We can thus discriminate between the two hypotheses above by testing a central contrasting prediction: whether sexual isolation evolves in parallel. Finding extensive parallel evolution of sexual isolation would support the hypothesis that ecologically based divergent selection is the ultimate cause of mating trait divergence (ecological speciation). In contrast, finding nonparallel evolution of sexual isolation would support the hypothesis that nonecological processes cause mating trait divergence (e.g., sexual selection in arbitrary directions). Determining the extent of parallel evolution in sexual isolation is thus an important step toward understanding how various forms of selection contribute to speciation in nature.

Parallel speciation is not a perfect test for distinguishing ecological and nonecological speciation for two reasons. First, ecological selection could cause nonparallel divergence of mating traits that confer reproductive isolation if replicate environments vary somewhat in ecology. Thus, although finding parallel evolution of sexual isolation strongly implicates ecologically based divergent selection in the speciation process, a failure to do so does not rule it out. Doing so requires that the alternative hypothesis of adaptation to locally unique environments also be rejected. One way to distinguish between these possibilities is to compare the divergence between replicate populations in both mating and ecological traits (traits involved in resource acquisition and predator defense). Sexual selection in arbitrary directions should cause nonparallel evolution of mating traits only, whereas nonparallel adaptation to local environments will be reflected in ecological traits as well.

Here, we study parallel evolution of sexual isolation between sympatric limnetic and benthic species of threespine sticklebacks. These species pairs inhabit six lakes in southwestern British Columbia, Canada, and evolved independently following the separate colonization of each lake by the marine threespine stickleback (*Gasterosteus aculeatus*) following the retreat of the glaciers at the end of the Pleistocene 10,000–12,000 years ago (McPhail 1993, 1994). In each lake the two ecotypes are ecologically and morphologically highly differentiated and exploit alternate foraging niches: the limnetic forages primarily on zooplankton in the open water and the benthic forages on invertebrates in the littoral zone (Bentzen and McPhail 1984; McPhail 1984, 1992, 1994; Schluter and McPhail 1992).

Sexual isolation of limnetic and benthic sticklebacks has evolved, to a large extent, in parallel. Previous work has shown that, despite separate origins, limnetics from these lakes are all reproductively compatible, as are benthics, whereas limnetics and benthics are reproductively isolated no matter what combination of lakes they are from (Rundle et al. 2000). Such parallel evolution of reproductive isolation strongly implicates divergent natural selection in speciation because all limnetics have adapted to a similar environment that is distinct from that of benthics, and sexual isolation has evolved in correlation with environment. Additional evidence implicates sexual selection in the evolution of sexual isolation but shows that it also arises from differences in environment (Boughman 2001). These results support the first hypothesis above, arguing that sexual isolation has evolved ultimately due to ecologically based divergent selection. Even so, questions remain about the contribution of various forms of natural and sexual selection to stickleback speciation. In particular, we found substantial variation among pairs of populations in their reproductive compatibility that is not obviously correlated with environment (e.g., see fig. 2, Rundle et al. 2000; fig. 4, Boughman 2001). This suggests that nonecological processes, such as sexual selection in arbitrary directions, may also contribute to sexual isolation.

Here, we explore this possibility by testing the specific prediction that the mating traits determining sexual isolation have evolved in parallel. We do this to gain insight into the relative roles of ecological and nonecological selection in the origins of these species. We focus on the sympatric species pairs because it is these combinations of populations for which reproductive isolation matters in nature; gene flow is only possible in sympatry and selection in sympatry has likely been important in the evolution of reproductive isolation (Rundle and Schluter 1998, 2004; Albert and Schluter 2004). While several traits important to sexual isolation have been identified (Nagel and Schluter 1998; Boughman 2001), the extent of their parallel evolution has not been directly tested, nor have the specific traits conferring sexual isolation in each pair been identified. We consider two morphological (body size and male nuptial color) and several behavioral traits (male and female courtship behavior), chosen because they have been implicated in previous studies (size: Nagel and Schluter 1998; color: Boughman 2001; behavior: Ridgway and McPhail 1984; McLennan and McPhail 1990). We ask about the relative contributions of these mating traits to sexual isolation and explore the form of selection causing their divergence.

We examine the extent of parallel speciation by addressing the following questions. First, are the same traits responsible for sexual isolation in all pairs? This should be true if mating traits evolve due to ecologically based divergent selection (ecological speciation), but not if sexual selection in arbitrary directions drives speciation. Second, has divergence in mating traits between sympatric limnetics and benthics occurred in parallel? If sexual isolation is evolving in parallel due to divergent selection, not only should the same traits be responsible for sexual isolation in separate lakes, but the direction of evolutionary change (i.e., increase or decrease from ancestor to descendent) and the extent of divergence between sympatric species should also be similar. In contrast, sexual selection in arbitrary directions is not expected to produce similar outcomes in replicate populations, especially not in correlation with environment.

MATERIALS AND METHODS

Study Populations

Populations used in this study originate in coastal British Columbia, Canada, in the Georgia Strait region (for locations of study populations see map in Schluter and McPhail 1992). We studied limnetics and benthics from three lakes: Enos, Paxton, and Priest Lakes. Despite the phenotypic similarity among lakes of all limnetics and of all benthics, two separate lines of genetic evidence indicate that the species pairs from these three lakes are the result of separate colonization events of the marine stickleback into freshwater (Rundle and Schluter 2004), as originally proposed by McPhail (1993). The first comes from mitochondrial DNA (mtDNA; Taylor and McPhail 1999). The limnetic-benthic pairs within each lake are characterized by unique assemblages of mtDNA haplotypes. The majority of these differ from common marine haplotypes by a single restriction site. In contrast, mtDNA haplotypes from different lakes always differ by more than one site. These patterns suggest that the separate assemblages in each lake trace their origin independently to the marine environment and not to each other. The second line of evidence comes from an analysis of allelic variation at six microsatellite loci (Taylor and McPhail 2000). If limnetics and benthics each arose only once and then colonized these lakes, genetic variation should be structured into limnetic and benthic classes. However, almost none of the variation (2.2–4.4%, not significantly different from zero) can be partitioned between these classes. In addition, although poorly resolved, the maximum-likelihood phylogeny suggests independent origins for limnetics and benthics from these lakes and is a significantly better fit to the data than one enforcing monophyly of either. For these reasons, we study the parallel evolution of reproductive isolation by focusing on limnetics and benthics from these three lakes, treating each pair as an independent evolutionary replicate. Throughout the paper, when referring to all limnetics or all benthics we use the term ‘‘ecotype,’’ and when referring to sympatric limnetics and benthics we use the terms ‘‘species’’ or ‘‘species pair.’’

Behavioral Measures

All fish used for morphological and behavioral measurements were caught in the wild using minnow traps or seine nets. We collected courtship data during 1467 mating trials conducted in April to July of eight years: 1992, 1993, 1996, 1997, 1999–2002. Not all data were collected for all populations. Males and females were collected from the wild with minnow traps and transported to Vancouver, British Columbia, where they were held in 102-L aquaria and maintained on a 16:8 L:D cycle at 18°C. All fish were fed once per day with frozen *Artemia* and chironomid larvae.

Mating traits and reproductive isolation were assessed in no-choice mating trials (described in detail in Nagel and Schluter 1998; Boughman 2001). Single males were placed in 102-L aquaria and allowed to build a nest. Once males had completed a nest and were actively courting, a single gravid female was introduced into the aquarium and interacted directly with the male until spawning occurred or for 20 min. We did not allow females to deposit eggs in a male’s nest, so as soon as a female entered a nest we gently squeezed her tail with long forceps to induce her to exit. We used females only once. We conducted multiple trials with most males, in most cases pairing each male with a single female from his own population and with a single female from one or more other populations. Males were used only once in each analysis because we did not repeat combinations. On completion of each trial, we verified that females were indeed ready to spawn by gently squeezing the abdomen and looking for ripe eggs in the oviduct. Trials in which females were deemed nonreceptive by this method were excluded. We estimated population means for behavioral traits using only trials within population. In contrast, we estimated the extent of sexual isolation using only trials between species within lakes.

We recorded behavior with an event recorder (Observer, Noldus Technologies, Wageningen, The Netherlands), and recorded the following male courtship behavior: zig-zag, bite, and lead (described in Rowland 1989). We calculated an index of courtship aggressiveness as: N(zig-zag)/N(zig-zag) + N(bite). Female response behavior included follow and examine (Rowland 1989). To adjust the number of recorded behaviors for varying trial duration, we calculated the rate of each behavior per minute. We also estimated female responsiveness to males as the proportion of follows that led to nest examination: N(examine)/N(follow). We calculated female preference for male color by regressing the rate of nest examination on male color score. The area of red color for live males was scored prior to behavioral trials by eye on a scale of 0 (no red coloration) to 5 (large area of intense red coloration). Our measure of body size was standard length, which we measured with vernier calipers accurate to 0.02 mm. We collected color, courtship, and body size data on Paxton benthics and limnetics ($N = 63$ and 78, respectively), Enos benthics and limnetics ($N = 34$ and 64), Emily limnetics ($N = 44$), and color and size data on Priest benthics and limnetics ($N = 20$ each).

Ecological Measures

To measure ecological traits (gill raker number and plate number) we anesthetized fish in MS-222, placed them in 10% formalin for at least a week, stained them with alizarin red, and then stored them in 40% isopropyl alcohol. We counted the number of gill rakers on the first gill arch. Previous studies have shown that the number of gill rakers predicts the efficiency of foraging on plankton or benthic invertebrate prey (Schluter 1993). We counted the number of armor plates on the left side of the body. Armor plates are a key adaptation against vertebrate predators (Bell et al. 1993; Reimchen

1994) and differ between ecotypes (Schluter and McPhail 1992; Vamosi 2002). We collected data on ecological traits for benthics and limnetics from Paxton ($N = 24$ and 25, respectively), Enos ($N = 30$ and 40), Priest ($N = 39$ and 23), Emily ($N = 39$ and 39), and Hadley Lakes ($N = 43$ and 10).

Data Analysis

We analyzed trait means for each population by one-way ANOVA, and we compared means with the Tukey-Kramer method. For mating trials with the male and female of opposite ecotypes, we also calculated the difference between partners in mating traits that could underlie sexual isolation. These include size difference, color difference, and courtship difference. We calculated size difference between male and female partners in standard length (male length minus female length). Thus positive values indicate that males are larger, whereas negative values indicate that females are larger. We plot these means in Figure 2, but use the absolute value of means in all analyses. We use absolute values because we are interested in how the magnitude of difference between ecotypes affects sexual isolation and have no a priori reason to consider one ecotype as the standard. For traits expressed only in males (e.g., nuptial color and courtship score), we assigned to every female the average value of the male trait in her own population. We then subtracted the value of her partner and took the absolute value of this difference. The values we calculated are thus the difference between her male partner's value and her expectation for males in her own population (best estimated as her own population mean). A significant size difference term would indicate that females reject heterospecific males who differ substantially in size, for instance, that large benthics reject small limnetics. A significant color or courtship difference term would indicate that females reject heterospecific males when they differ from the conspecific mean, for instance, that limnetic females reject dull benthic males or those that court aggressively. We measured reproductive compatibility as the proportion of trials in which spawning occurred during trials between limnetics and benthics (male and female of opposite ecotypes).

To evaluate the prediction that the same traits are responsible for sexual isolation in all pairs, we tested whether the traits that confer reproductive isolation varied among lakes. These analyses used data from trials with the male and female of opposite ecotypes. To do this required a two-step process in which we first identified mating traits that confer reproductive isolation using stepwise regression and then tested if their effects varied among lakes by testing interaction terms in a logistic regression. Testing interaction terms is a direct test of the first prediction, that sexual isolation is based on the same traits in all pairs. First, we conducted a stepwise regression to find the model that best explained variation in spawning between ecotypes, using size and male mating traits, female ecotype, and lake in the full model. We included all measured mating traits to identify the traits that actually contribute to sexual isolation, as previous work had suggested several traits might be important but had not considered them jointly (Rundle and Schluter 1998; Nagel and Schluter 1998; Boughman 2001). The reduced model included differences in size, color, and courtship (as calculated above) as continuous variables, with female ecotype and lake as categorical variables.

Next, because the response variable for sexual isolation is binomial (spawn or no-spawn) we used logistic regression with the reduced model to test the significance of terms. The direct test of the prediction for parallel sexual isolation is to test the significance of the interaction between lake and the phenotypic variables in the model. Significant interaction terms indicate that mating traits have variable effects on sexual isolation among pairs, while nonsignificant interaction terms suggest that all pairs use the same traits to discriminate against heterospecifics. We also asked if limnetics and benthics differ in the basis of sexual isolation by including interactions between female ecotype and size, color, and courtship differences. A significant interaction here indicates that the ecotypes differ in how they use a trait for reproductive isolation. The effects could be in different directions, or one ecotype may use the trait while the other does not.

We also delved into how body size differences influence mating probability. Because our analyses showed that the magnitude of difference in body size affected the probability of spawning between ecotypes, we used multiple regression to investigate whether this size effect was due to male size, female size, or their difference. We found no effect of male size or female size, so used only the difference variable in all analyses. We looked for size assortative mating between ecotypes by calculating the mean size difference in trials with and without spawning. Then, we compared this to the within-ecotype pattern. We analyzed both between- and within-ecotype size differences using *t*-tests.

We transformed data for all analyses to better meet assumptions of ANOVA using square-root transformations for behavioral and count data and log transformations for size data. However, we present back-transformed values in tables and figures for ease of interpretation.

We then turned to testing the second prediction, that divergence in mating traits has occurred in parallel for sympatric pairs. For all traits, we quantified the degree of parallel evolution between pairs using the quantity γ, which compares the magnitude of the difference in a trait between ecotypes (limnetic or benthic) averaged across the three pairs, with the magnitude of the interaction between ecotype and pair (Schluter et al. 2004). Parallel evolution is indicated by a significant main effect of ecotype and weak interaction between ecotype and pair. The quantities are provided by fitting the two-factor fixed-effects ANOVA model to the data,

$$\mathrm{Y}_{ijk} = \mu + \alpha_{\mathrm{i}} + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}, \tag{1}$$

where Y_{ijk} is the trait value of individual k, modeled as the sum of a constant (μ), the effects of its ecotype i (α_i) and pair j (β_j), the deviation ($\alpha\beta_{ij}$) resulting from an interaction between its ecotype and pair, and a random error term (ε_{ijk}). The measure of parallel evolution is quantified as the difference between the variance components for ecotype and the interaction relative to their sum,

$$\mathrm{Y} = \frac{A - AB}{A + AB}, \tag{2}$$

where

TABLE 1. Predictors of spawning between ecotypes for limnetic-benthic pairs. Data are for trials between ecotypes (benthics and limnetics). Interaction terms test for parallel slopes of absolute differences in mate recognition traits on ecotype or lake. Populations include Paxton, Enos, and Priest benthics and limnetics. Data were analyzed with logistic regression on transformed variables.

Source of variation	df	χ^2
Lake	1	0.14
Female ecotype	1	1.16
Absolute length difference	1	1.03
Absolute male color difference	1	0.50
Absolute courtship difference	1	0.67
Length difference × lake	1	0.01
Male color difference × lake	1	1.03
Courtship difference × lake	1	0.02
Length difference × female ecotype	1	5.18**
Male color difference × female ecotype	1	4.35*
Courtship difference × female ecotype	1	0.31
Error	98	

* $P < 0.05$; ** $P < 0.01$.

$$A = \frac{b}{a - 1} \sum \alpha_i^2, \tag{3}$$

represents the main effect of ecotype, and

$$AB = \frac{1}{(a - 1)(b - 1)} \sum \alpha\beta_{ij}^2 \tag{4}$$

represents the interaction between ecotype and pair. The constants a and b are the numbers of ecotypes and pairs, respectively.

The quantity γ ranges between -1 and $+1$. A value of $+1$ represents pure parallel evolution, occurring when trait means of all pairs shift between ecotypes by precisely the same amounts. A value of 0 represents no parallel evolution and occurs when shifts in trait means between ecotypes are uncorrelated among pairs. A value of -1 represents pure antiparallel evolution, occurring when shifts of trait mean between ecotypes in one pair are exactly opposed by shifts in trait means of a second pair.

The quantity γ is estimated by substituting the fitted estimates of ecotype main effects, $\hat{\alpha}_{ij}$, and interaction deviations $\widehat{\alpha\beta}_{ij}$ into the above equations. In the case of a balanced design (equal sample size for all ecotype and pair combinations), the fitted estimates may be extracted using the mean squares from the fitted ANOVA model and the formulas for the expected mean squares (e.g., Sokal and Rohlf 1995, p. 333). In the more typical case of unequal sample sizes the fitted estimates $\hat{\alpha}_{ij}$ and $\widehat{\alpha\beta}_{ij}$ must be extracted from the coefficients of the two-factor linear ANOVA model. We obtained these in S+ using the dummy.coef command.

Confidence limits for $\hat{\gamma}$ were calculated using the bootstrap (Efron 1982). On each iteration a new sample of the data was generated by resampling with replacement from the original data. This was repeated 1000 times, leading to 1000 resampled values for $\hat{\gamma}$. The standard deviation of the 1000 values is the standard error of $\hat{\gamma}$. The fraction of the 1000 values of $\hat{\gamma}$ falling at or below zero provides an approximate P-value for a one-tailed test of the null hypothesis that $\gamma = 0$.

We used S+ (Mathsoft, Seattle, WA) for all statistical analyses.

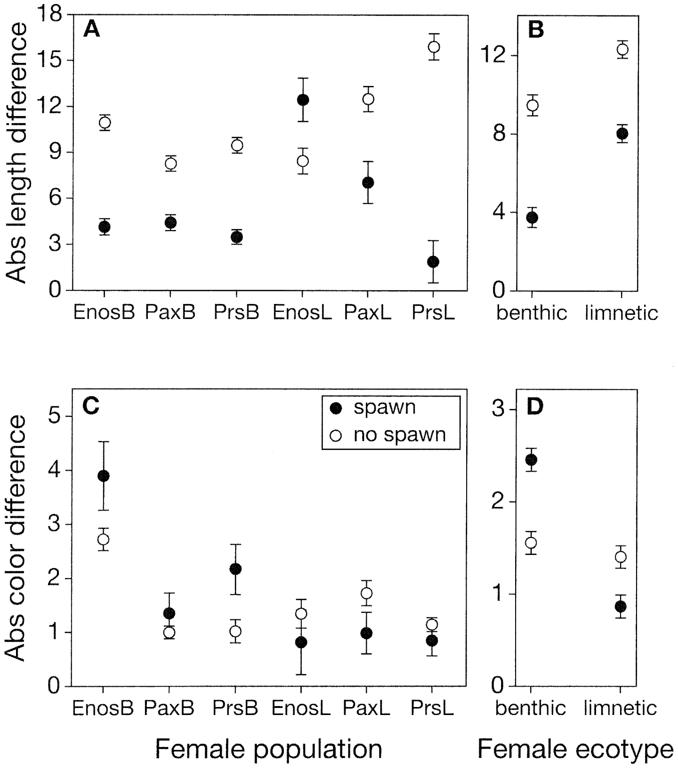

FIG. 1. The effect of differences in body size and nuptial color on spawning between benthic and limnetic sticklebacks. (A) Difference in size for all populations, and (B) for both ecotypes. (C) Difference in nuptial color for all populations, and (D) for both ecotypes. The absolute value of mean difference between male and female ± standard error is shown for trials where spawning did (closed circles) and did not (open circles) occur. Populations include Enos, Paxton, and Priest benthics (EnosB, PaxB, and PriestB, respectively) and limnetics (EnosL, PaxL, and PriestL, respectively). Values shown in (B) and (D) are means for each ecotype.

RESULTS

Parallel Evolution of Sexual Isolation

Limnetics and benthics are reproductively isolated from one another (assortative mating by ecotype: $\chi_1^2 = 26.6$, $P < 0.0001$), as found in previous studies. Our analyses indicate that body size and color play a role, but we found no evidence that differences in courtship behavior contribute to sexual isolation (Table 1). There are no main effects for phenotypic traits, nor are there interactions between the traits and lake. That we did not find such interactions suggests that all pairs use traits in the same way to avoid heterospecific matings, or that any differences among pairs are small relative to other effects. This confirms the first prediction of the ecological hypothesis, that the same traits are responsible for sexual isolation in all pairs.

We did find significant interactions between ecotype and both body size and color differences, suggesting that limnetics and benthics differ in how these traits cause sexual isolation (Table 1, Fig. 1). Females of both ecotypes reject heterospecific males who differ substantially in size, although the effect is stronger for benthic females, and Enos limnetic females do not show this pattern. Thus, size contributes to sexual isolation in both ecotypes and leads to assortative

TABLE 2. Mean values (on transformed scale) for limnetics and benthics, P-values for differences between ecotypes, and sample sizes. Courtship, color, and ecological variables are square root transformations, whereas length variables are $\log_{10}$ transformed. Sample size is given first for benthics and then limnetics.

Traits	N	Benthic	Limnetic	P
Male courtship				
Color	104, 163	1.13 ± 0.063	1.65 ± 0.050	0.0001
Court	33, 133	1.43 ± 0.195	1.96 ± 0.097	0.01
Zig-zag/min	69, 130	1.39 ± 0.119	2.21 ± 0.087	0.0001
Bite/min	69, 130	1.86 ± 0.082	1.14 ± 0.059	0.0001
Lead	69, 130	0.35 ± 0.058	0.66 ± 0.042	0.0001
Female courtship				
Follow/min	69, 130	0.71 ± 0.066	0.83 ± 0.048	0.14
Examine/min	69, 130	0.22 ± 0.056	0.50 ± 0.040	0.0001
Responsiveness	52, 109	0.22 ± 0.121	0.60 ± 0.084	0.01
Red preference[1]	4, 3	−0.06 ± 0.057	0.09 ± 0.050	0.39
Body size				
Female length	112, 159	1.77 ± 0.004	1.65 ± 0.004	0.0001
Male length	112, 159	1.75 ± 0.003	1.69 ± 0.003	0.0001
Length difference	112, 159	0.51 ± 0.059	0.64 ± 0.031	0.05
Ecological				
No. gill rakers	132, 127	4.31 ± 0.013	4.86 ± 0.013	0.0001
No. armor plates	132, 127	1.69 ± 0.049	2.43 ± 0.050	0.0001

[1] N is the number of populations rather than the number of individuals for this variable.

mating. Oddly, size differences contribute to sexual isolation between ecotypes ($t_{10} = -2.6$, $P = 0.026$) even though there is no evidence for size assortative mating within ecotype ($t_{12} = -0.5$, $P = 0.65$).

In contrast, color has asymmetric effects on sexual isolation (Fig. 1). Benthic males have less color on average than limnetic males. Limnetic females reject the dullest benthic males, who differ most from conspecific males. Therefore, large color differences increase sexual isolation. The combination of reduced nuptial color in benthic males and strong color preference in limnetic females contributes to sexual isolation between limnetic females and benthic males. Color differences have the opposite effect on sexual isolation for benthics. Benthic females reject the dullest limnetic males, who are most similar to conspecific males. Therefore, it is small rather than large color differences that increase sexual isolation between benthic females and limnetic males. In both ecotypes, females are more likely to mate with bright heterospecific males than with dull ones. The similar tendency to reject dull males contributes to sexual isolation in one direction but not in the other.

TABLE 3. Test of parallel divergence in phenotypic traits for limnetic-benthic pairs. Lakes include Paxton and Enos for all traits, and Priest for size and morphological traits as well. γ ranges from −1 (anti-parallel evolution) to +1 (pure parallel evolution). See text for details.

Traits	γ	SE	P
Male courtship			
Color	0.64	0.11	0.0001
Court	0.60	0.25	0.03
Zig-zag/min	0.09	0.46	0.44
Bite/min	0.47	0.25	0.04
Lead	0.51	0.46	0.17
Female courtship			
Follow/min	−0.94	0.49	0.87
Examine/min	0.88	0.20	0.0001
Responsiveness	0.24	0.24	0.11
Red preference	0.98	0.35	0.39
Body size			
Female length	0.92	0.06	0.0001
Male length	0.96	0.05	0.0001
Length difference	0.89	0.14	0.0001
Ecological			
No. gill rakers	0.99	0.00	0.0001
No. armor plates	0.62	0.05	0.0001

Parallel Divergence of Phenotypic Traits

Mating, behavioral, and ecological traits all show substantial parallel evolutionary divergence between ecotypes. This is evidenced both by the number of traits that have diverged and the extensive parallel nature of this divergence. Limnetics and benthics differ for 12 of 14 traits, including all male mating, body size and ecological traits, and two female mating traits (Table 2).

The extensive divergence between sympatric limnetics and benthics is fully in parallel for size, color, and ecological traits (Table 3; Figs. 2, 3) and partly in parallel for behavioral traits (Figs. 4, 5). Although divergence in behavioral traits is more variable among lakes, our results provide no evidence that variation in these traits significantly influence sexual isolation between ecotypes. Thus, the available evidence suggests that divergence in behavioral traits does not contribute to sexual isolation. The two traits that do contribute to sexual isolation between ecotypes—size and color—show strong parallel divergence in sympatry.

DISCUSSION

Our primary aim was to understand the nature of selection acting on the traits that confer sexual isolation in threespine stickleback species pairs. In particular, we were interested in

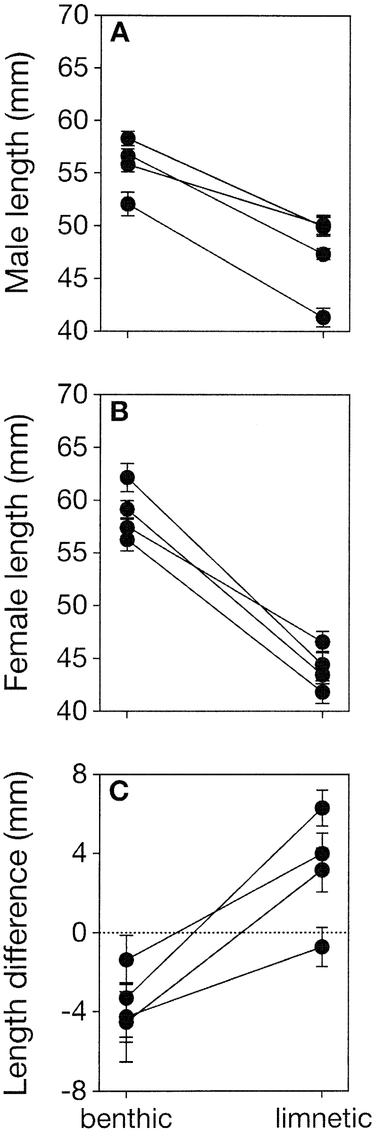

FIG. 2. Mean body size (± standard errors) for benthic and limnetic sticklebacks from Paxton, Priest, Emily, and Enos Lakes. (A) male length, (B) female length, and (C) length difference between male and female. Positive differences indicate larger males and negative differences indicate larger females. Lines connect limnetics and benthics from the same lake.

the relative roles of ecological and nonecological selection in the evolution of sexual isolation. Two lines of evidence support our first hypothesis: that the ultimate cause of sexual isolation is adaptation to environment. First, all three pairs have independently evolved to recognize mates based on differences in body size, and all limnetics use nuptial color. Second, both of these mating traits have diverged between benthics and limnetics in parallel, as have all size and ecological traits. The eight behavioral traits that we examined show more haphazard evolutionary divergence and do not appear to contribute to sexual isolation in sympatry. We further explore these lines of evidence and their implications below.

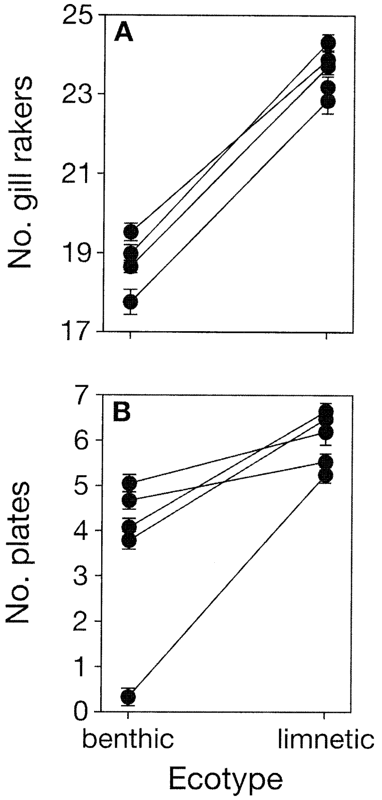

FIG. 3. Ecological trait means (± standard errors) for benthic and limnetic sticklebacks from Paxton, Priest, Emily, Enos, and Hadley Lakes: (A) number of gill rakers, and (B) number of armor plates. Lines connect limnetics and benthics from the same lake.

Parallel Sexual Isolation

Despite measuring a number of behavioral traits that form key components of stickleback courtship, sexual isolation in all three species pairs depended on the same two morphological traits: assortative mating based on differences in body size and asymmetric isolation based on male nuptial color. This confirms that sexual isolation has evolved in parallel. All benthics use the same traits as do all limnetics; however, limnetics and benthics base sexual isolation to a different extent on these two traits. Benthics base sexual isolation primarily on differences in body size, whereas limnetics base sexual isolation on a combination of differences in body size and nuptial color.

Females of both ecotypes spawn most readily with bright heterospecific males, leading to asymmetric sexual isolation based on color. Previous work has shown that there is evolutionary change in the strength but not direction of female color preferences: female preference for colorful males is strong in limnetics but weak in benthics (Boughman 2001). The opposite effect of color differences on sexual isolation for limnetics and benthics is consistent with expectations from open-ended preference functions, where sexual isolation is predicted to be asymmetric (Lande 1981; Turelli et al. 2001). The ecotype with strong female preferences and exaggerated male traits (limnetics) is expected to have stronger reproductive isolation because females will reject heterospecific males with low trait values. However, females of the

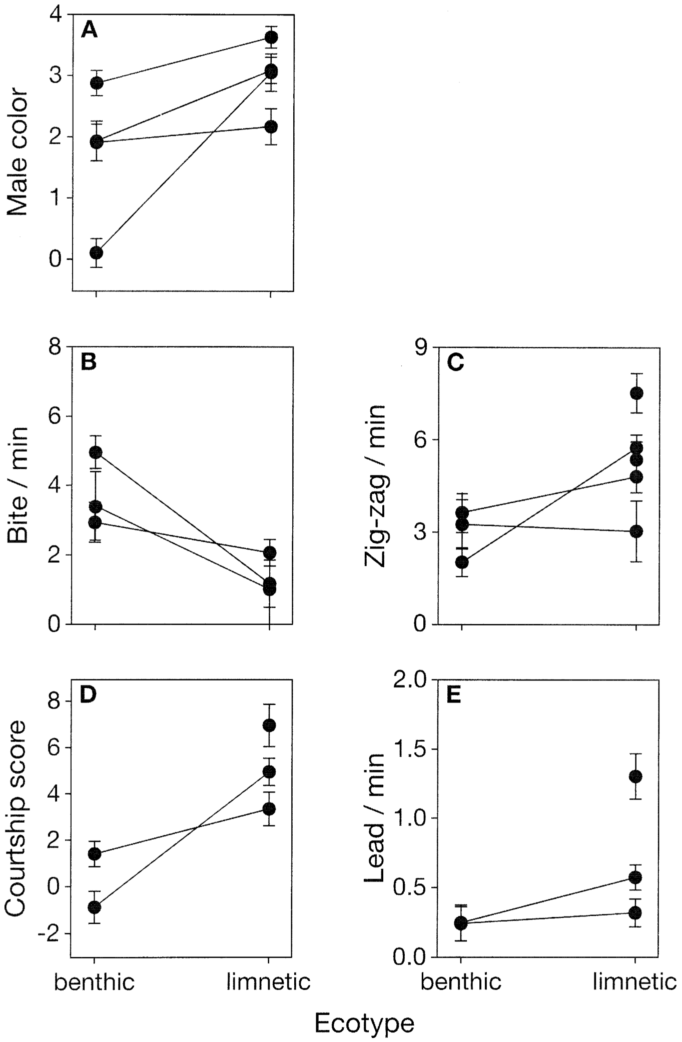

FIG. 4. Male mating trait means (± standard errors) for benthic and limnetic sticklebacks from Paxton, Priest, Emily, and Enos Lakes. Traits include: (A) male nuptial color score, (B) number of bites per minute, (C) number of zig-zags per minute, (D) courtship score, and (E) number of times male led female toward the nest per minute. Lines connect limnetics and benthics from the same lake.

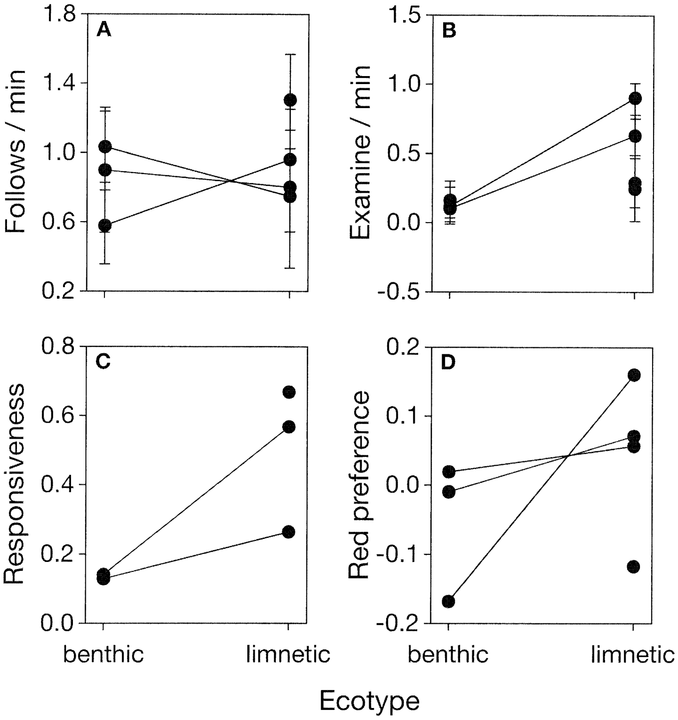

FIG. 5. Female mating trait means (± standard errors) for benthic and limnetic sticklebacks from Paxton, Priest, Emily, and Enos Lakes. Traits include: (A) number of times female followed male toward the nest per min, (B) number of times female examined the nest per min, (C) female responsiveness, and (D) preference for red nuptial color. Lines connect limnetics and benthics from the same lake.

ecotype whose males have low trait values (benthics) are expected to accept heterospecific mates with high trait values, allowing gene flow. This asymmetry means that color differences alone could not restrict gene flow. Sexual isolation requires a combination of differences in body size and color.

Asymmetric sexual isolation based on differences in male nuptial color is likely the extension of within-population mating patterns, and ecologically based sexual selection is almost certainly involved. In contrast, at present it is unclear if size based sexual isolation is an extension of within-population mating behavior, because we found no evidence for size preferences or size assortative mating within ecotype. Thus, sexual selection on body size within species does not appear to give rise to sexual isolation between species. Additional experiments need to be done to test directly for such preferences, especially given that the range of sizes within a species is not as great as that between species so it might be more difficult to detect. However, if no such within population size preferences or assortative mating are found, this raises the possibility that size assortative mating between ecotypes evolved as a consequence of secondary contact. This could occur through a number of mechanisms, including reinforcement or direct selection on mate preferences (Servedio 2000, 2001, 2004). Evidence for both processes has been found for sticklebacks (Rundle and Schluter 1998; Albert and Schluter 2004).

Several studies have found differences in courtship behavior between stickleback populations, as we did here (Ridgway and McPhail 1984; Foster and Baker 1995; Hay and McPhail 2000; Ishikawa and Mori 2000). For example, Ridgway and McPhail (1984) found strong reproductive isolation between Enos Lake limnetics and benthics and described courtship differences, but they did not test that it was courtship that caused the reproductive isolation. In their study males courted conspecific and heterospecific females differently. Others have shown that males adjust courtship in part based on female body size, especially when females are much larger (Nagel and Schluter 1998; Rundle and Schluter 1998). Ridgway and McPhail (1984) did not consider size or color in their trials. If courtship behavior depends on size traits, then including both variables in a statistical model estimates their independent effect on spawning. Our analyses use this approach and find that differences in courtship behavior do not have an effect on spawning that is independent of the size effect. We did not manipulate male traits, but rather selected males haphazardly with respect to size and color, endeavoring to use males from all parts of the size and color distributions for each population. Thus, we controlled statistically but not experimentally for correlated effects. Future work could use experimental manipulations of color, size, and courtship behavior to investigate their independent effects on sexual isolation. Future work could also explore the possibility that some traits we did not measure, such as body shape or chemical cues, may contribute to sexual isolation.

Traits Conferring Sexual Isolation

Variation in the two morphological traits we measured function in sexual isolation, whereas no behavioral traits do so. Is this likely to be a common pattern? Are mating traits that confer reproductive isolation more likely to be morphological than behavioral? Certainly differences in body size and color have been found to confer reproductive isolation in several taxa. For example, differences in color pattern isolate mimetic *Heliconius* butterflies (Jiggins et al. 2001; Naisbit et al. 2001), *Colias* butterflies (Ellers and Boggs 2003), *Ficedula* flycatchers (Saetre et al. 1997; Dale et al. 1999), and many haplochromine cichlid species (e.g., Seehausen et al. 1997; Couldridge and Alexander 2002; Allender et al. 2003). In contrast to our findings, in most of these systems color contributes to sexual isolation in a symmetric way. Differences in body size also isolate many taxa, including sympatric cichlids (Schliewen et al. 2001), skinks (Richmond and Reeder 2002), Darwin's finches (Ratcliffe and Grant 1983), lake whitefish (Lu and Bernatchez 1999), and lake and anadromous forms of sockeye salmon (Foote and Larkin 1988). Body size contributes asymmetrically in sockeye because males of the smaller lake form steal spawnings of the larger male anadromous sockeye (Wood and Foote 1996).

What about behavioral traits? Much of the evidence that behavioral traits confer sexual isolation comes from studies of acoustic signals, including anurans (Ryan and Wilczynski 1991; Pfennig 2000; Hobel and Gerhardt 2003), crickets (Otte 1992; Gray and Cade 2000; Shaw and Lugo 2001), *Drosophila* (Gleason and Ritchie 1998), and *Crysoperla* lacewings (Wells and Henry 1998). However, stickleback courtship is movement based. Despite commonly found differences among populations in courtship behavior in many taxa, we have little data to suggest that movement behavior underlies sexual isolation. Spiders do provide some examples (Stratton and Uetz 1986; Miller et al. 1998; Hebets and Uetz 2000). The paltry number of examples for movement could reflect a bias in what is studied. Perhaps movement traits are more difficult to quantify and study than acoustic or morphological traits, so fewer studies do so. But if this pattern is real, why should some kinds of traits more commonly confer sexual isolation? Part of the answer might lie in the nature of selection acting on those traits.

The Nature of Selection on Mating Traits

Traits under ecological selection may be especially likely to contribute to sexual isolation for two reasons. First, speciation should be facilitated when reproductive isolation is based on ecological traits that differentiate species (e.g., Dieckmann and Doebeli 1999; Servedio 2000, 2004) such as host choice in phytophagous insects that also mate on their host (Funk 1998; Dres and Mallet 2002; Nosil et al. 2002, 2003). Second, speciation should be facilitated when ecological traits and mating traits diverge in concert (Schluter 2000; Turelli et al. 2001). This is especially likely when mating traits are under ecologically dependent selection and habitats differ. Body size fits the first scenario and nuptial color the second. Courtship behavior fits neither.

Body size affects exploitation of the different niches that limnetics and benthics inhabit (Bentzen and McPhail 1984; Schluter 1993). Therefore, natural selection arising from resource use and competition for those resources contributes to divergence in body size (Schluter and McPhail 1992; Schluter 1994). We find here that this adaptive divergence in size is an essential component of sexual isolation. Assortative mating on body size has also been found in anadromous-stream pairs of sticklebacks (McKinnon et al. 2004), who also show strong parallel evolution of body size. Thus, body size appears to be a trait under strong ecological selection that causes sexual isolation between species in both limnetic-benthic pairs and anadromous-stream pairs.

Differences in nuptial color and color preference are due, in part, to ecologically mediated sexual selection arising from differences in light environment (Boughman 2001), because the environment determines effective signaling colors, color perception, and preference (Endler 1992; Fleishman et al. 1997; Fuller 2002; Leal and Fleishman 2002). Given that limnetics and benthics mate in different light environments, both color and color preference have diverged between them. Thus, sexual isolation based on differences in color appears to be a case of ecological and mating traits evolving in correlation with the same aspect of environment.

Ecologically Based Selection and Genetic Constraints

An alternative to the hypotheses we test here is that parallel evolution of sexual isolation results because closely related populations share biases in both their standing genetic variation and their production of new, heritable variation (Haldane 1932; reviewed in Schluter et al. 2004). Such genetic biases may influence evolutionary change as populations colonize new habitats. For example, some traits may have more genetic variance than others, and evolution is expected to proceed most rapidly for traits with the greatest genetic variance. However, these biases should result in parallel or divergent evolution among replicate populations only to the extent that selection acts in a parallel or divergent manner on this variance. Although shared genetic biases may cause nonecological processes to produce similar trait changes in independent populations, these changes will not be correlated with environment. Parallel evolution in correlation with environment is only expected when selection is ecologically based.

Substantial data demonstrate that competition between ecotypes and divergent natural selection causes divergence in ecological traits and reproductive isolation (Schluter and McPhail 1992, 1993; Schluter 1993, 1994, 1995, 2003; Hatfield and Schluter 1999; Rundle et al. 2000; Pritchard and Schluter 2001). We find here that limnetics and benthics have undergone parallel divergence in ecological traits and body size, which suggests not only that each ecotype adapts to a particular niche, but that the magnitude of difference between them is important. This would occur if competition drives the ecotypes apart, and a constant magnitude of difference decreases the intensity of competition to the point where evolutionary change halts (Pacala and Roughgarden 1985; Pritchard and Schluter 2001; Gray and Robinson 2002; Pfennig and Murphy 2003). Our data bolster the already strong case for ecological selection.

Sexual Selection and Speciation: Arbitrary or Ecological?

We found differences among lakes in absolute trait values for size and color, which could be due to differences in local ecology or to sexual selection acting independently of environment. We can rule out sexual selection in arbitrary directions by comparing ecological and mating traits. Sexual selection is expected to affect only mating traits, but instead we find that ecological traits and mating traits show similar patterns of divergence. Thus, adaptation to locally unique environments is more likely.

However, our results suggest that sexual selection acts on behavioral traits at least partly independently of environment. Thus, sexual selection in arbitrary directions may be operating in our populations. Yet, despite extensive evolutionary change in courtship behavior, behavioral traits do not appear to confer sexual isolation. Therefore, this arbitrary divergence arising from interactions between the sexes does not appear to play a major role in speciation for stickleback pairs. This is a surprise. Several models of sexual selection and speciation suggest that it is just this haphazard evolutionary change that enables reproductive isolation to evolve. In fact, this is the traditional view of how sexual selection is likely to lead to sexual isolation. Further work will be required to test this definitively, but the present results provide no evidence that this mode of speciation by sexual selection operates for stickleback pairs.

At present, it is unclear if this is an unusual or common outcome. Data from some systems supports the arbitrary mode of sexual selection and speciation. Examples include: egg-sperm recognition in broadcast spawners (e.g., Palumbi 1999; Swanson and Vacquier 2002), male-female reproductive tract coupling (e.g., Eberhard 1992; Presgraves et al. 1999), and conspecific sperm precedence (e.g., Price 1997; Brown and Eady 2001; Dixon et al. 2003). However, these examples are primarily for fertilization traits. There appear to be relatively few examples of arbitrary divergence in premating, preinsemination traits with concomitant evidence that such divergence plays a critical role in reproductive isolation. This is, however, an area of very active research and more data become available all the time.

Evidence is accumulating that environmentally dependent sexual selection contributes to divergence in mating traits and sexual isolation. A number of examples invoke sensory drive, where mating signals adapt to local environments to enhance their transmission (Ryan and Wilczynski 1991; Marchetti 1993; Endler and Thery 1996; Uy and Borgia 2000; Boughman 2002; Leal and Fleishman 2002; McNaught and Owens 2002), or perception adapts to local environments and results in altered preferences (Boughman 2001; Smith et al. 2004). Changes in light environment have even led to the collapse of premating isolation in some Lake Victoria cichlids (Seehausen et al. 1997). Premating isolation is based heavily on conspicuous color differences between species in both Lake Victoria (Seehausen et al. 1998; Couldridge and Alexander 2002) and Malawi cichlids (Allender et al. 2003; Knight and Turner 2004). It is increasingly recognized that many forms of sexual selection may depend, at least in part, on the environment. This recognition lies at the heart of the view we test here that ecologically dependent selection causes divergence in mating traits. Ecological differences among populations are likely to be ubiquitous, so investigating this mode of speciation is likely to prove fruitful.

Many other mating traits that confer sexual isolation appear to be by-products of adaptation, as is the case for body size in sticklebacks. These include Darwin’s finches, where beak shape is an adaptation for exploiting seeds and also affects vocal tract resonance properties, causing differences among species in song that contribute to sexual isolation (Podos 2001). There are likely to be many examples for host races of phytophagous insects (Funk et al. 2002). Even cuticular hydrocarbon differences between populations of *Drosophila* have adaptive function, as shown elegantly by Greenberg et al. (2003) and others (Markow and Toolson 1990; Blows 2002). Such differences cause sexual isolation between several *Drosophila* species (e.g., Coyne 1996; Higgie et al. 2000; Etges and Ahrens 2001). In all these cases, sexual selection does not act on sexual isolation alone, although it could contribute to trait divergence in concert with natural selection.

In conclusion, we found evidence that ecologically based sexual and natural selection have caused sexual isolation in species pairs of threespine sticklebacks. The basis for sexual isolation is similar across multiple species pairs, and the traits that confer reproductive isolation have evolved in parallel. Both findings invoke a strong role for ecologically based divergent selection and provide additional data in support of the hypothesis that the ultimate cause of speciation in this system is adaptation to environment. Whether this holds true for other systems remains to be determined.

ACKNOWLEDGMENTS

Thanks to S. Morgan, B. Harvey, K. Rosalska, A. Agrawal, and M. McDonald for help with data collection. Comments by U. Candolin, J. Mallet, K. Shaw, and three anonymous reviewers improved the paper. JWB was supported by the Natural Science Foundation and the National Science and Engineering Research Council of Canada; HDR by Killam (University of British Columbia), Eastburn (Hamilton Community Foundation), and Australian Research Council Fellowships; and DS by the Natural Science and Engineering Research Council of Canada.

LITERATURE CITED

Albert, A. Y. K., and D. Schluter. 2004. Reproductive character displacement of male stickleback mate preference: reinforcement or direct selection? Evolution 58:1099–1107.

Allender, C. J., O. Seehausen, M. E. Knight, G. F. Turner, and N. Maclean. 2003. Divergent selection during speciation of Lake

Malawi cichlid fishes inferred from parallel radiations in nuptial coloration. Proc. Natl. Acad. Sci. USA 100:14074–14079.

Arnqvist, G., and L. Rowe. 2002. Antagonistic coevolution between the sexes in a group of insects. Nature 415:787–789.

Arnqvist, G., M. Edvardsson, U. Friberg, and T. Nilsson. 2000. Sexual conflict promotes speciation in insects. Proc. Natl. Acad. Sci. USA 97:10460–10464.

Bell, M. A., G. Orti, J. A. Walker, and J. P. Koenings. 1993. Evolution of pelvic reduction in threespine stickleback fish: a test of competing hypotheses. Evolution 47:906–914.

Bentzen, P., and J. D. McPhail. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): specialization for alternative trophic niches in the Enos Lake species pair. Can. J. Zool. 62:2280–2286.

Blows, M. W. 2002. Interaction between natural and sexual selection during the evolution of mate recognition. Proc. R. Soc. Lond. B 269:1113–1118.

Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411:944–948.

———. 2002. How sensory drive can promote speciation. Trends Ecol. Evol. 17:571–577.

Brown, D. V., and P. E. Eady. 2001. Functional incompatibility between the fertilization systems of two allopatric populations of *Callosobruchus maculatus* (Coleoptera: Bruchidae). Evolution 55:2257–2262.

Couldridge, V. C. K., and G. J. Alexander. 2002. Color patterns and species recognition in four closely related species of Lake Malawi cichlid. Behav. Ecol. 13:59–64.

Coyne, J. A. 1996. Genetics of sexual isolation in male hybrids of *Drosophila simulans* and *D. mauritiana*. Genet. Res. 68:211–220.

Dale, S., T. Slagsvold, H. M. Lampe, and G. P. Saetre. 1999. Population divergence in sexual ornaments: the white forehead patch of Norwegian pied flycatchers is small and unsexy. Evolution 53:1235–1246.

Day, T. 2000. Sexual selection and the evolution of costly female preferences: spatial effects. Evolution 54:715–730.

Dieckmann, U., and M. Doebeli. 1999. On the origin of species by sympatric speciation. Nature 400:354–357.

Dixon, S. M., J. A. Coyne, and M. A. F. Noor. 2003. The evolution of conspecific sperm precedence in *Drosophila*. Mol. Ecol. 12: 1179–1184.

Dobzhansky, T. 1951. Genetics and the origin of species. Columbia Univ. Press, New York.

Dres, M., and J. Mallet. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. Philos. Trans. R. Soc. B 357:471–492.

Eberhard, W. G. 1992. Species isolation, genital mechanics, and the evolution of species-specific genitalia in three species of *Macrodactylus* beetles (Coleoptera, Scarabeidae, Melolonthinae). Evolution 46:1774–1783.

Efron, B. 1982. The jackknife, the bootstrap, and other resampling plans. Society for Industrial and Applied Mathematics, Philadelphia, PA.

Ellers, J., and C. L. Boggs. 2003. The evolution of wing color: male mate choice opposes adaptive wing color divergence in *Colias* butterflies. Evolution 57:1100–1106.

Endler, J. A. 1992. Signals, signal conditions, and the direction of evolution. Am. Nat. 139:125–153.

———. 1993. Some general comments on the evolution and design of animal communication systems. Philos. Trans. R. Soc. B 340: 215–225.

Endler, J. A., and M. Thery. 1996. Interacting effects of lek placement, display behavior, ambient light, and color patterns in three Neotropical forest-dwelling birds. Am. Nat. 148:421–452.

Etges, W. J., and M. A. Ahrens. 2001. Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. V. Deep geographic variation in epicuticular hydrocarbons among isolated populations. Am. Nat. 158:585–598.

Fleishman, L. J., M. Bowman, D. Saunders, W. E. Miller, M. J. Rury, and E. R. Loew. 1997. The visual ecology of Puerto Rican anoline lizards: habitat light and spectral sensitivity. J. Comp. Physiol. A 181:446–460.

Foster, S. A., and J. A. Baker. 1995. Evolutionary interplay between ecology, morphology and reproductive-behavior in threespine stickleback, *Gasterosteus aculeatus*. Environ. Biol. Fish. 44: 213–223.

Foote, C. J., and P. A. Larkin. 1988. The role of male choice in the assortative mating of anadromous and non-anadromous sockeye salmon (*Oncorhynchus nerka*). Behaviour 106:43–62.

Fuller, R. C. 2002. Lighting environment predicts the relative abundance of male colour morphs in bluefin killifish (*Lucania goodei*) populations. Proc. R. Soc. Lond. B 269:1457–1465.

Funk, D. J. 1998. Isolating a role for natural selection in speciation: host adaptation and sexual isolation in *Neochlamisus bebbianae* leaf beetles. Evolution 52:1744–1759.

Funk, D. J., K. E. Filchak, and J. L. Feder. 2002. Herbivorous insects: model systems for the comparative study of speciation ecology. Genetica 116:251–267.

Gavrilets, S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. Nature 403:886–889.

Gavrilets, S., and C. R. B. Boake. 1998. On the evolution of premating isolation after a founder event. Am. Nat. 152:706–716.

Gleason, J. M., and M. G. Ritchie. 1998. Evolution of courtship song and reproductive isolation in the *Drosophila willistoni* species complex: Do sexual signals diverge the most quickly? Evolution 52:1493–1500.

Gray, D. A., and W. H. Cade. 2000. Sexual selection and speciation in field crickets. Proc. Natl. Acad. Sci. USA 97:14449–14454.

Gray, S. M., and B. W. Robinson. 2002. Experimental evidence that competition between stickleback species favours adaptive character divergence. Ecol. Lett. 5:264–272.

Greenberg, A. J., J. R. Moran, J. A. Coyne, and C. I. Wu. 2003. Ecological adaptation during incipient speciation revealed by precise gene replacement. Science 302:1754–1757.

Haldane, J. B. S. 1932. The causes of evolution. Longman, London.

Hatfield, T., and D. Schluter. 1999. Ecological speciation in sticklebacks: environment-dependent hybrid fitness. Evolution 53: 866–873.

Hay, D. E., and J. D. McPhail. 2000. Courtship behaviour of male threespine sticklebacks (*Gasterosteus aculeatus*) from old and new hybrid zones. Behaviour 137:1047–1063.

Hebets, E. A., and G. W. Uetz. 2000. Leg ornamentation and the efficacy of courtship display in four species of wolf spider (Araneae: Lycosidae). Behav. Ecol. Sociobiol. 47:280–286.

Higashi, M., G. Takimoto, and N. Yamamura. 1999. Sympatric speciation by sexual selection. Nature 402:523–526.

Higgie, M., S. F. Chenoweth, and M. W. Blows. 2000. Natural selection and the reinforcement of mate recognition. Science 20: 519–521.

Hobel, G., and H. C. Gerhardt. 2003. Reproductive character displacement in the acoustic communication system of green tree frogs (*Hyla cinerea*). Evolution 57:894–904.

Ishikawa, M., and S. Mori. 2000. Mating success and male courtship behaviors in three populations of the threespine stickleback. Behaviour 137:1065–1080.

Jiggins, C. D., R. E. Naisbit, R. L. Coe, and J. Mallet. 2001. Reproductive isolation caused by colour pattern mimicry. Nature 411:302–305.

Knight, M. E., and G. F. Turner. 2004. Laboratory mating trials indicate incipient speciation by sexual selection among populations of the cichlid fish *Pseudotropheus zebra* from Lake Malawi. Proc. R. Soc. Lond. B 271:675–680.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. Proc. Natl. Acad. Sci. USA 78:3721–3725.

———. 1982. Rapid origin of sexual isolation and character divergence in a cline. Evolution 36:213–223.

Leal, M., and L. J. Fleishman. 2002. Evidence for habitat partioning based on adaptation to environmental light in a pair of sympatric lizard species. Proc. R. Soc. Lond. B 269:351–359.

Lu, G. Q., and L. Bernatchez. 1999. Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. Evolution 53:1491–1505.

Marchetti, K. 1993. Dark habitats and bright birds illustrate the role of the environment in species divergence. Nature 362:149–152.

Markow, T. A., and E. C. Toolson. 1990. Temperature effects on epicuticular hydrocarbons and sexual isolation in *Drosophila mojavensis*. Pp. 315–331 *in* J. S. F. Barker, W. T. Starmer, and R. J. MacIntyre, eds. Ecological and evolutionary genetics of *Drosophila*. Plenum, New York.

Mayr, E. 1942. Systematics and the origin of species. Columbia Univ. Press, New York.

McKinnon, J., S. Mori, B. K. Blackman, L. David, D. M. Kingsley, L. Jamieson, J. Chou, and D. Schluter. 2004. Evidence for ecology's role in speciation. Nature 429:294–298.

McLennan, D. A., and J. D. McPhail. 1990. Experimental investigations of the evolutionary significance of sexually dimorphic nuptial coloration in *Gasterosteus aculeatus* (L.): The relationship between male color and female behavior. Can. J. Zool. 68: 482–492.

McNaught, M. K., and I. P. F. Owens. 2002. Interspecific variation in plumage colour among birds: species recognition or light environment? J. Evol. Biol. 15:505–514.

McPhail, J. D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. Can. J. Zool. 62: 1402–1408.

———. 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.

———. 1993. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): origin of the species pairs. Can. J. Zool. 71: 515–523.

———. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of 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.

Miller, G. L., G. E. Stratton, P. R. Miller, and E. Hebets. 1998. Geographical variation in male courtship behaviour and sexual isolation in wolf spiders of the genus *Schizocosa*. Anim. Behav. 56:937–951.

Nagel, L. M., and D. Schluter. 1998. Body size, natural selection, and speciation in sticklebacks. Evolution 52:209–218.

Naisbit, R. E., C. D. Jiggins, and J. Mallet. 2001. Disruptive sexual selection against hybrids contributes to speciation between *Heliconius cydno* and *Heliconius melpomene*. Proc. R. Soc. Lond. B 268:1849–1854.

Nosil, P., B. J. Crespi, and C. P. Sandoval. 2002. Host-plant adaptation drives the parallel evolution of reproductive isolation. Nature 417:440–443.

———. 2003. Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. Proc. R. Soc. Lond. B 270:1911–1918.

Otte, D. 1992. Evolution of cricket songs. J Orthoptera Res 1:25–49.

Pacala, S., and J. Roughgarden. 1985. Population experiments with the *Anolis* lizards of St. Maarten and St. Eustatius. Ecology 66: 129–141.

Palumbi, S. R. 1999. All males are not created equal: fertility differences depend on gamete recognition polymorphisms in sea urchins. Proc. Natl. Acad. Sci. USA 96:12632–12637.

Panhuis, T. M., R. K. Butlin, M. Zuk, and T. Tregenza. 2001. Sexual selection and speciation. Trends Ecol. Evol. 16:364–371.

Parker, G. A., and L. Partridge. 1998. Sexual conflict and speciation. Philos. Trans. R. Soc. Lond. B 353:261–274.

Pfennig, D. W., and P. J. Murphy. 2003. A test of alternative hypotheses for character divergence between coexisting species. Ecology 84:1288–1297.

Pfennig, K. S. 2000. Female spadefoot toads compromise on mate quality to ensure conspecific matings. Behav. Ecol. 11:220–227.

Podos, J. 2001. Correlated evolution of morphology and vocal signal structure in Darwin's finches. Nature 409:185–188.

Pomiankowski, A., and Y. Iwasa. 1998. Runaway ornament diversity caused by Fisherian sexual selection. Proc. Natl. Acad. Sci. USA 95:5106–5111.

Presgraves, D. C., R. H. Baker, and G. S. Wilkinson. 1999. Coevolution of sperm and female reproductive tract morphology in stalk-eyed flies. Proc. R. Soc. Lond. B 266:1041–1047.

Price, C. S. C. 1997. Conspecific sperm precedence in *Drosophila*. Nature 388:663–666.

Pritchard, J. R., and D. Schluter. 2001. Declining interspecific competition during character displacement: summoning the ghost of competition past. Evol. Ecol. Res. 3:209–220.

Ratcliffe, L. M., and P. R. Grant. 1983. Species recognition in Darwins finches (*Geospiza*, Gould). 1. Discrimination by morphological cues. Anim. Behav. 31:1139–1153.

Reimchen, T. E. 1994. Predators and morphological evolution in threespine stickleback. Pp. 240–276 *in* M. A. Bell and S. A. Foster, eds. The evolutionary biology of the threespine stickleback. Oxford Univ. Press, Oxford, U.K.

Rice, W. R., and A. K. Chippindale. 2002. The evolution of hybrid infertility: perpetual coevolution between gender-specific and sexually antagonistic genes. Genetica 116:179–188.

Richmond, J. Q., and T. W. Reeder. 2002. Evidence for parallel ecological speciation in scincid lizards of the *Eumeces skiltonianus* species group (Squamata: Scincidae). Evolution 56: 1498–1513.

Ridgway, M. S., and J. D. McPhail. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): mate choice and reproductive isolation in the Enos Lake species pair. Can. J. Zool. 62:1813–1818.

Rowland, W. J. 1989. The ethological basis of mate choice in male threespine sticklebacks, *Gasterosteus aculeatus*. Anim. Behav. 38:112–120.

Rundle, H. D., and D. Schluter. 1998. Reinforcement of stickleback mate preferences: sympatry breeds contempt. Evolution 52: 200–208.

———. 2004. Natural selection and speciation in sticklebacks. Pp. 1–21 *in* U. Dieckmann, H. Metz, M. Doebeli, and D. Tautz, eds. Adaptive speciation. Cambridge Univ. Press, Cambridge, U.K.

Rundle, H. D., L. M. Nagel, J. W. Boughman, and D. Schluter. 2000. Natural selection and parallel speciation in sympatric sticklebacks. Science 287:306–308.

Ryan, M. J., and A. S. Rand. 1993. Species recognition and sexual selection as a unitary problem in animal communication. Evolution 47:647–657.

Ryan, M. J., and W. Wilczynski. 1991. Evolution of intraspecific variation in the advertisement call of a cricket frog (*Acris crepitans*, Hylidae). Biol. J. Linn. Soc. 44:249–271.

Saetre, G.-P., S. G. Weller, W. L. Wagern, P. S. Soltis, and D. E. Soltis. 1997. A sexually selected character displacement in flycatchers reinforces premating isolation. Nature 387:589–592.

Schemske, D. W., and H. D. Bradshaw. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). Proc. Natl. Acad. Sci. USA 96:11910–11915.

Schliewen, U., K. Rassmann, M. Markmann, J. A. Markert, T. D. Kocher, and D. Tautz. 2001. Genetic and ecological divergence of a monophyletic cichlid species pair under fully sympatric conditions in Lake Ejagham, Cameroon. Mol. Ecol. 10: 1471–1488.

Schluter, D. 1993. Adaptive radiation in sticklebacks: size, shape, and habitat use efficiency. Ecology 74:699–709.

———. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. Science 266:798–801.

———. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. Ecology 76:82–90.

———. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774.

———. 2000. The ecology of adaptive radiation. Oxford Univ. Press, Oxford, U.K.

———. 2001. Ecology and the origin of species. Trends Ecol. Evol. 16:372–380.

———. 2003. Frequency dependent natural selection during character displacement in sticklebacks. Evolution 57:1142–1150.

Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. Am. Nat. 140:85–108.

———. 1993. Character displacement and replicate adaptive radiation. Trends Ecol. Evol. 8:197–200.

Schluter, D., and L. M. Nagel. 1995. Natural selection and parallel speciation. Am. Nat. 146:292–301.

Schluter, D., E. A. Clifford, M. Nemethy, and J. S. McKinnon.

2004. Parallel evolution and inheritance of quantitative traits. Am. Nat. 163:809–822.

Seehausen, O., J. J. M. van Alphen, and F. Witte. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. Science 277:1808–1811.

Seehausen, O., F. Witte, J. J. M. van Alphen, and N. Bouton. 1998. Direct mate choice maintains diversity among sympatric cichlids in Lake Victoria. J. Fish Biol. 53:37–55.

Servedio, M. R. 2000. Reinforcement and the genetics of nonrandom mating. Evolution 54:21–29.

———. 2001. Beyond reinforcement: the evolution of premating isolation by direct selection on preferences and postmating, prezygotic incompatibilities. Evolution 55:1909–1920.

———. 2004. The evolution of premating isolation: local adaptation and natural and sexual selection against hybrids. Evolution 58:913–924.

Shaw, K. L., and E. Lugo. 2001. Mating asymmetry and the direction of evolution in the Hawaiian cricket genus *Laupala*. Mol. Ecol. 10:751–759.

Smith, C., I. Barber, R. J. Wootton, and L. Chittka. 2004. A receiver bias in the origin of three-spined stickleback mate choice. Proc. R. Soc. Lond. B 271:949–955.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. 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.

Swanson, W. J., and V. D. Vacquier. 2002. Reproductive protein evolution. Annu. Rev. Ecol. Syst. 33:161–179.

Taylor, E. B., and J. D. McPhail. 1999. Evolutionary history of an adaptive radiation in species pairs of threespine sticklebacks (*Gasterosteus*): insights from mitochondrial DNA. Biol. J. Linn. Soc. 66:271–291.

Taylor, E. B., and J. D. McPhail. 2000. Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus*. Proc. R. Soc. Lond. B 267:2375–2384.

Turelli, M., N. H. Barton, and J. A. Coyne. 2001. Theory and speciation. Trends Ecol. Evol. 16:330–343.

Turner, G. F., and M. T. Burrows. 1995. A model of sympatric speciation by sexual selection. Proc. R. Soc. Lond. B 260: 287–292.

Uy, J. A. C., and G. Borgia. 2000. Sexual selection drives rapid divergence in bowerbird display traits. Evolution 54:273–278.

Vamosi, S. M. 2002. Predation sharpens the adaptive peaks: survival trade-offs in sympatric sticklebacks. Ann. Zool. Fenn. 39: 237–248.

Via, S., A. C. Bouck, and S. Skillman. 2000. Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. Evolution 54:1626–1637.

Wells, M. L. M., and C. S. Henry. 1998. Songs, reproductive isolation, and speciation in cryptic species of insects: a case study using green lacewings. Pp. 217–233 *in* D. J. Howard and S. H. Berlocher, eds. Endless forms: species and speciation. Oxford Univ. Press, Oxford, U.K.

West-Eberhard, M. J. 1983. Sexual selection, social competition, and speciation. Q. Rev. Biol. 58:155–183.

Wood, C. C., and C. J. Foote. 1996. Evidence for sympatric genetic divergence of anadromous and nonanadromous morphs of sockeye salmon (*Oncorhynchus nerka*). Evolution 50:1265–1279.

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