Natural Selection and Parallel Speciation in Sympatric Sticklebacks

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Natural selection plays a fundamental role in most theories of speciation, but empirical evidence from the wild has been lacking. Here the post-Pleistocene radiation of threespine sticklebacks was used to infer natural selection in the origin of species. Populations of sticklebacks that evolved under different ecological conditions show strong reproductive isolation, whereas populations that evolved independently under similar ecological conditions lack isolation. Speciation has proceeded in this adaptive radiation in a repeatable fashion, ultimately as a consequence of adaptation to alternative environments.

In classic theories of speciation, reproductive isolation originates in part as the incidental by-product of adaptation to distinct environments [1, 2]. Although laboratory experiments support this view [3], the role of natural selection and the environment in the origin of reproductive isolation remains contentious because evidence from nature is lacking [4–6]. Tests of the role of natural selection in speciation have focused instead on the reinforcement of premating isolation [7, 8]. Yet reinforcement requires preexisting reproductive isolation in the form of reduced hybrid fitness and generally is considered a final step in the speciation process [1, 9]. Here we present evidence that natural selection plays a fundamental role in the early stages of speciation.

Parallel evolution of similar traits in populations that inhabit similar environments strongly implicates natural selection, as genetic drift is unlikely to produce concerted change, correlated with the environment, in multiple, independent lineages [10]. Parallel speciation is a special form of parallel evolution in which traits that determine reproductive isolation evolve repeatedly in independent, closely related populations as a by-product of adaptation to different environments [6, 11]. The outcomes are reproductive compatibility between populations that inhabit similar environments and reproductive isolation between populations that inhabit different environments. Because reproductive isolation is more strongly correlated with the environment than with geographic proximity or genetic distance, parallel speciation provides strong evidence for natural selection in the speciation process. Despite the significance of such

evidence for our understanding of mechanisms of speciation in nature, there are no conclusive tests of parallel speciation [11]. We tested parallel speciation with populations of sympatric threespine sticklebacks (*Gasterosteus* spp.).

Sympatric species of threespine sticklebacks inhabit small, low-elevation lakes in coastal British Columbia, Canada [12]. These populations are recently derived from the marine threespine stickleback (*Gasterosteus aculeatus*) that colonized freshwater after the retreat of the glaciers at the end of the Pleistocene. One species of each sympatric pair is a large-bodied Benthic that feeds on invertebrates in the littoral zone; the other species is a smaller, more slender Limnetic that feeds primarily on plankton in open water [12–14]. The Benthic and Limnetic from a given lake constitute biological species: they are reproductively isolated by strong assortative mating [15, 16], ecologically based postmating isolation [17], and probably sexual selection against hybrid males [18]. Phenotypic differences between sympatric species have a genetic basis and persist over multiple generations in a common laboratory environment [12, 19]. Both comparative [13] and direct [20] experimental evidence indicate that divergent selection caused by competition for resources has contributed to the evolution of these phenotypic differences.

The genetic evidence indicates that the Benthic-Limnetic pairs from three lakes (Priest, Paxton, and Enos Lakes) are derived independently of one another. Unique assemblages of mitochondrial DNA (mtDNA) haplotypes characterize pairs from the different lakes, and a hierarchical clustering analysis of mtDNA divergence estimates fails to detect any case in which populations of the same phenotype from different lakes cluster together [21]. Independence of these species pairs is confirmed by an analysis of six nuclear microsatellite loci [22]. Thus, neither the Benthics nor the Limnetics from different lakes are monophyletic; hence we refer to the two phenotypes as ecomorphs.

Independence of pairs allowed two tests of the predictions of parallel speciation. First, populations of the same ecomorph from different lakes (for instance, Benthics from Priest, Paxton, and Enos Lakes) should not be reproductively isolated from one another despite the known reproductive isolation between different ecomorphs within lakes [15]. Second, reproductive isolation should exist between ecomorphs from different lakes (for instance, between Benthics from Paxton Lake and Limnetics from Priest Lake). We tested reproductive isolation by conducting 753 mating trials in the laboratory with wild-caught Benthics and Limnetics from these three lakes [23]; 261 of these trials involved individuals of different ecomorphs (Limnetics with Benthics), and 492 involved individuals of the same ecomorph (Limnetics with Limnetics, Benthics with Benthics). Mean probabilities of spawning for each ecomorph combination are shown in Fig. 1.

Spawning probabilities between pairs of populations depend strongly on ecomorph identity (Fig. 2) [24]. Because pairwise comparisons between populations are not statistically independent, our analyses used conservative paired t tests that treated each of the six populations of females as a replicate and corrected for phylogeny [25]. In each test, the pair of measurements for each female population was based on averages of the corrected spawning probabilities over all the relevant male populations. Reproductive isolation between ecomorphs within a lake was strong (paired t test, $t_5 = 3.82$, $P = 0.012$), confirming past results [15, 16].

In accord with the first prediction of parallel speciation, reproductive isolation was absent among lakes within an ecomorph ($t_5 = 0.56$, $P = 0.599$) (Fig. 2, comparison A). A female was just as likely to mate with a male of the same ecomorph from a different lake as with a male of the same ecomorph from her own lake [26]. In agreement with the second prediction, reproductive isolation was present between ecomorphs from different lakes ($t_5 = 2.61$, $P = 0.048$) (Fig. 2, comparison B). A female from a given population spawned more frequently with males of her own ecomorph from a different lake than
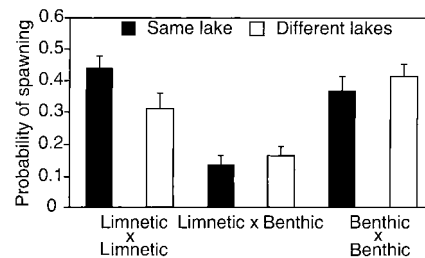

Fig. 1. Uncorrected probability of spawning in no-choice mating trials for various combinations of populations. Error bars are ±1 SE and represent the amount of variation in spawning rate among the various combinations.

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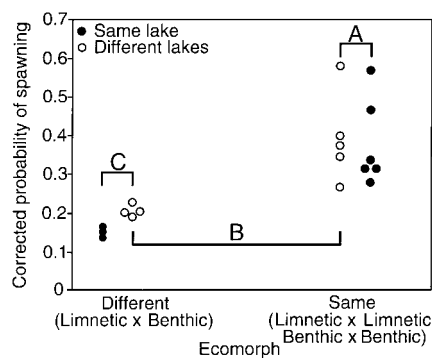
with males of the other ecomorph from a different lake. The probability of spawning was slightly higher among ecomorphs from different lakes than among ecomorphs from the same lake and approached statistical significance ($t_5 = 2.36$, $P = 0.065$) (Fig. 2, comparison C).

Correcting for phylogeny had a negligible effect on these statistical results (27), confirming that parallel speciation and not shared history is responsible for the observed mating patterns. For phylogeny to have a significant influence, populations of the same ecomorph must be more closely related to each other than to populations of different ecomorphs. Phylogenetic trees based on mtDNA and microsatellite DNA reject this hypothesis (28).

The parallel evolution of reproductive isolation in these sticklebacks in nature provides some of the strongest evidence yet for a role of divergent natural selection in speciation. Two studies similar to ours suggest that reproductive isolation also may have evolved in parallel: populations of stream-resident sticklebacks from Japan and North America, and populations of herbivorous leaf beetles adapted to similar host plants (29). This suggests that parallel speciation may be widespread. Our results complement and strengthen another form of evidence in which key traits under divergent selection form the proximate basis of reproduc-

Fig. 2. Population mean probabilities of spawning as a function of shared ecomorph. Each point is the corrected fraction of all trials in which spawning resulted when individuals from a given pair of populations were tested. Comparisons A and B highlight the tests of the two predictions of parallel speciation. First, within an ecomorph the probability of spawning is compared for combinations of populations from the same or different lakes (comparison A). Second, the probability of spawning between populations of the same ecomorph from different lakes is compared with that between ecomorphs from different lakes (comparison B). Comparison C tests for a difference in the strength of reproductive isolation between populations of different ecomorphs from the same and different lakes. Because our statistical analysis used conservative paired t tests that treated each population of females as a replicate (24, 25), the comparisons shown here represent the nature of the tests but do not depict the exact analyses performed.

tive isolation (15, 30). The absence of premating isolation between independently derived stickleback populations of the same ecomorph suggests that such key traits can evolve repeatedly in similar environments, yielding parallel speciation. We have not identified the trait or traits that underlie parallel mate preferences in sticklebacks but body size is a strong candidate (15, 31).

Reproductive isolation between these sympatric species is not just a by-product of phenotypic divergence, but it also may have involved reinforcement in sympatry (8). This suggests a scenario in which premating isolation between ecomorphs arose initially as a simple by-product of divergent natural selection on key traits and was later reinforced in sympatry. Whether reinforcement occurred in parallel among lakes is not known. In addition, the reduced probability of spawning between Limnetics from different lakes (Fig. 1) (26) and the slight reduction in reproductive isolation between ecomorphs from different lakes (Fig. 2, comparison C) suggest that a small degree of independent evolution has occurred within lakes. It is not known whether this independent evolution is a product of reinforcement or a by-product of unique adaptations to each lake. Regardless, under a common selection regime speciation was repeatable. The contribution of both divergent natural selection and reinforcement to speciation may explain the high rates of phenotypic divergence that characterize adaptive radiations (5).

References and Notes

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23. A trial involved placing a single, gravid female into a 100-liter aquarium (or one-half of a divided 100-liter aquarium) in which a single male had built a nest. The pair was allowed to interact directly. Whether or not they spawned was recorded after 30 min. A detailed description of this protocol (8, 15) and information about the populations used (13–15) are provided elsewhere. Combinations of populations tested (PaL = Paxton Limnetic, PaB = Paxton Benthic, PrL = Priest Limnetic, PrB = Priest Benthic, EnL = Enos Limnetic, EnB = Enos Benthic) and the number of replicate trials (in parentheses) are as follows: (i) same ecomorph and same lake, PaL × PaL (54), PrL × PrL (45), EnL × EnL (43), PaB × PaB (66), PrB ×PrB (20), EnB × EnB (21); (ii) same ecomorph and different lake, PaL × PrL (28), PaL × EnL (75), PaB × PrB (86), PaB × EnB (45), PrB × EnB (9); (iii) different ecomorph and same lake, PaL × PaB (64), PrL × PrB (40), EnL × EnB (10); (iv) different ecomorph and different lake, PaL × PrB (44), PaL × EnB (26), PrL × PaB (44), EnL × PaB (33).
24. Before we calculated population averages, we corrected spawning probabilities for main effects of year and male population by logistic regression; then the probabilities were arcsin square-root transformed. No other main effects were present. Year controls for differences in the propensity to spawn between years, and male population controls for varying propensity to spawn of males from different populations.
25. We performed phylogenetic correction on population-level data by the general least-squares method [N. Draper and H. Smith, *Applied Regression Analysis* (Wiley, Chichester, UK, ed. 2, 1981); E. P. Martins and T. F. Hansen, *Am. Nat.* **149**, 646 (1997)]. We performed the equivalent of a one-sample paired t test by testing the significance of the intercept in a weighted regression fitted to a zero slope. We performed weighted regression with a matrix of weights whose elements were correlations specifying the degree of phylogenetic similarity (proportion of total branch length shared from root to tip) of pairs of populations. Larger correlations resulted in lower weights. Similar methods have been applied in previous studies [M. Lynch, *Evolution* **45**, 1065 (1991); M. Lynch and P. E. Jarrell, *Genetics* **135**, 1197 (1993); D. Schluter, *Evolution* **50**, 1766 (1997)]. We calculated correlations from the phylogenetic tree constructed from microsatellite distance data (22) by using the UPGMA method in PHYLIP [J. Felsenstein, Phylogeny Inference Package, version 3.57c (Univ. of Washington, Seattle, WA, 1995)]. Similar results were obtained with the Kitsch algorithm [W. M. Fitch and E. Margoliash, *Science* **155**, 279 (1967)].
26. The reduced probability of spawning between Limnetics from different lakes shown in Fig. 1 is present in two of three Limnetic populations. Although suggestive, the difference is not significant when comparison A is repeated with Limnetics only (paired t test, $t_2 = 1.154$, $P = 0.368$).
27. Statistical results differed little when phylogenetic correction was not done. Reproductive isolation between Benthics and Limnetics within a lake remained strong ($t_5 = 5.26$, $P < 0.003$). A female from a given population remained as likely to spawn with males of the same ecomorph from a different lake as with males of the same ecomorph from her own lake ($t_5 = 0.075$, $P = 0.943$) (Fig. 2, comparison A). The probability of spawning for populations of females with males of the other ecomorph from different lakes remained significantly lower than with males of the same ecomorph from different lakes ($t_5 = 3.97$, $P = 0.011$) (Fig. 2, comparison B). Finally, however, the small and marginally sig-

nificant increase in the probability of spawning observed for populations of females with males of the other ecomorph from different lakes versus males of the other ecomorph from the same lake was significant in the absence of the phylogenetic correction (paired t test, $t_5 = 3.37$, $P = 0.020$) (Fig. 2, comparison C).

28. No significant difference in microsatellite or mtDNA divergence is detected between pairs of populations (conspecific pairs excluded) from the same versus a different environment [microsatellite, analysis of variance (ANOVA), $F_{1,10} = 0.22$, $P = 0.65$; mtDNA, ANOVA, $F_{1,10} = 0.76$, $P = 0.40$].

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30. Two other cases exist in which the traits that form the proximate basis of reproductive isolation have known adaptive significance. These are beak and body size in Darwin's finches in the Galápagos Islands [P. T. Boag and P. R. Grant, *Science* **214**, 82 (1981); L. M. Ratcliffe and P. R. Grant, *Anim. Behav.* **31**, 1139 (1983); T. D. Price, P. R. Grant, H. L. Gibbs, P. T. Boag, *Nature* **309**, 787 (1984)] and copper tolerance in *Mimulus* [M. R. MacNair and P. Christie, *Heredity* **50**, 295 (1983); P. Christie and M. R. MacNair, *J. Hered.* **75**, 510 (1984)].

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