



CONVERGENT EVOLUTION AS A GENERATOR OF PHENOTYPIC DIVERSITY IN THREESPINE STICKLEBACK

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Convergent evolution, in which populations produce similar phenotypes in response to similar selection pressure, is strong evidence for the role of natural selection in shaping biological diversity. In some cases, closely related populations can produce functionally similar but phenotypically divergent forms in response to selection. Functional convergence with morphological divergence has been observed in laboratory selection experiments and computer simulations, but while potentially common, is rarely recognized in nature. Here, we present data from the North Pacific threespine stickleback radiation showing that ecologically and functionally similar, but morphologically divergent phenotypes rapidly evolved when an ancestral population colonized freshwater benthic habitats in parallel. In addition, we show that in this system, functional convergence substantially increases morphospace occupation relative to ancestral phenotypes, which suggests that convergent evolution may, paradoxically, be an important and previously underappreciated source of morphological diversity.

KEY WORDS: Convergence, morphospace, multiple solutions.

The past decade has seen renewed interest in the phenomenon of convergent evolution, in which separate populations evolve similar phenotypes in response to similar selection (Harmon et al. 2005; Hoekstra et al. 2006; Østbye et al. 2006; Losos 2011). Convergence is often thought to constrain phenotypic evolution, with disparate lineages producing similar phenotypes in response to natural selection (Morris 2000). However, convergent evolution does not constrain phenotypic evolution if populations are capable of producing different phenotypes that converge in function (Alfaro et al. 2005). Evidence from artificial selection experiments and simulation studies confirm that replicate populations exposed to uniform selection will often produce different phenotypes that nonetheless converge in function (Cohan & Hoffmann 1986; Korona 1996; Yedid & Bell 2002; Alfaro et al. 2004; Garland et al. 2011).

Functional convergence is even more difficult to observe in nature, perhaps because natural systems often lack one or more of the three criteria necessary to observe the phenomenon: knowledge of the ancestral phenotype(s), replicate instances of adap-

tation, and knowledge of a pathway that connects the adaptation under selection to the component traits that produce the adaptation. The North Pacific threespine stickleback adaptive radiation meets these three criteria, making it a candidate for studying functional convergence with morphological divergence in natural populations. The criteria of knowledge of the ancestral form is met through time series studies that suggest freshwater stickleback populations are colonized by anadromous stickleback and by fossil evidence that suggests that the morphology of anadromous populations has not changed drastically for almost 15 million years (Bell et al. 2004, 2009). The criteria of repeated adaptation to the same niche is met by benthic stickleback populations, which have repeatedly specialized on diets of littoral macro-invertebrates across multiple freshwater colonization events (Bell & Foster 1994). The final criteria of a well-understood pathway is met through the use of a functional model of fish feeding performance that connects trophic specialization to its underlying morphological components. Most ray-finned fishes capture prey by expanding the buccal cavity, which generates a pressure gradient and

resulting flow of water that pulls nearby prey items into the fish's mouth (Wainwright et al. 2007). The suction index model treats the fish's buccal cavity as an expanding cylinder connected to the epaxial muscles by a first-order lever, and has been used to successfully predict suction performance in several fish species morphologically similar to threespine stickleback (Carroll et al. 2004; Wainwright et al. 2007). Suction index does not capture all facets of feeding performance but is a strong predictor of the capacity to generate suction pressure in the buccal cavity, which is one of the primary factors shaping overall ability to suction feed (Higham et al. 2006; Holzman et al. 2012).

In this study, we sought to test whether convergent evolution can drive morphological divergence, using a comparison between several anadromous populations and benthic populations. Unlike many other studies of convergence, our use of a complex functional system allows precise quantification of the level of convergence in each of the system's underlying morphological components and their emergent functional property. We expect benthics to enhance suction pressure generation relative to anadromous forms, as benthic stickleback primarily feed on attached macroinvertebrates that must be dislodged or extracted from the benthos (Schluter & McPhail 1992). If functional convergence is driving morphological divergence in benthic stickleback, we would expect benthic morphological diversity to be substantially higher than anadromous diversity. However, if a pattern of simple convergence is driving evolutionary patterns in benthic stickleback, benthic stickleback might occupy a different region in morphospace than anadromous stickleback, but both benthics and anadromous fish would each occupy a similar amount of morphospace.

Materials and Methods

We obtained specimens from eight populations in Alaska and British Columbia: four anadromous (Kanaka Creek, BC; Salmon River, BC; Rabbit Slough, AK; Turnagain Arm, AK) and four benthic-adapted (Enos Lake, BC; Paxton Lake, BC; Priest Lake, BC; Mud Lake, AK). These areas were covered by glaciers until 10,000 years ago and represent a recent range expansion of threespine stickleback (Bell & Foster 1994). Three of the anadromous populations (Kanaka, Salmon, Turnagain) were museum collected, and the remaining anadromous and benthic populations were collected by previous workers (Schluter & McPhail 1992; Kimmel et al. 2005).

We used gill raker and diet data to delineate benthic populations, as benthics possess a low number of gill rakers and feed predominantly on benthic macroinvertebrates, particularly amphipods and chironomid larvae (Lavin & McPhail 1986; Schluter & McPhail 1992; Walker 1997; Purnell et al. 2006; Travis 2007). Enos, Priest, and Paxton contain both a benthic species and a lim-

netic species, whereas Mud Lake contains a benthic species and for most of the growing season, a sympatric anadromous population (Schluter and McPhail 1992; Karve et al. 2008). The Enos Lake species pair collapsed in the mid-1990s; population samples used in this study were obtained prior to its collapse (Taylor et al. 2006).

We measured a total of 198 cleared and alizarin-stained adult fish (SL > 40 mm) from eight populations, the smallest with $n = 21$ and the largest with $n = 30$. An ocular micrometer was used to measure four of the five parameters of the suction index model: length of the buccal cavity, length of the outlever, height of the epaxial muscle, and width of the epaxial muscle. The final parameter, gape width, was measured with calipers, as was the standard length of each fish.

These measurements were used to calculate the three components of the suction index model: buccal projected area, lever ratio, and the cross-sectional area of the epaxial muscles. Buccal area was calculated by multiplying gape width times buccal length, lever ratio was calculated by dividing the inlever (one-half epaxial height) by the outlever, and epaxial cross-sectional area was calculated as the area of a semi-ellipse with a major axis equal to epaxial width and a semi-minor axis equal to epaxial height. Suction index was then calculated by dividing epaxial cross-sectional area by buccal area, then multiplying the resulting quantity by the lever ratio.

To test for functional convergence and divergence among different populations we compared suction index among populations using pairwise Mann-Whitney tests with Bonferroni correction (Fig. 1A). First, we wished to see if anadromous populations exhibit similar suction index values, suggesting that they represent a similar functional starting point for freshwater populations. Second, we wished to test whether benthic populations diverge from anadromous populations in a consistent way, preferably by increasing suction index to increase suction pressure generation for benthic macroinvertebrate foraging.

To compare morphologies, we log-transformed each suction index component variable and performed a regression on log of standard length using a generalized linear model, and used ANOVA to verify that no significant ($P < 0.05$) population by standard length interactions occurred. We used the regression residuals of the standard length term as the size-corrected trait variables. A principal components analysis was conducted on the correlation matrix of the size-corrected suction index components (Fig. 1B). A broken-stick model (Jackson 1993) implemented via the R package 'vegan' was used to examine whether each PC axis contained more variation than would be expected by chance. The model retained PC axes 1 and 2 and discarded 3 through 5. We compared PC 1 and 2 values between populations via pairwise Mann-Whitney tests with Bonferroni correction. As before, we wished to see if anadromous populations are morphologically

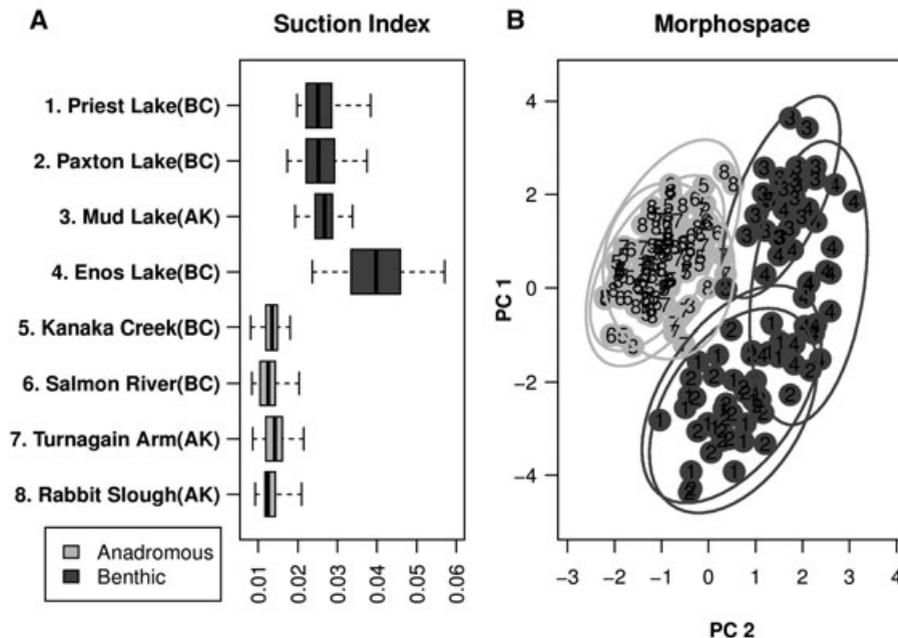


Figure 1. (A) Suction Index values of four benthic and four anadromous populations from Alaska(AK) and British Columbia(BC). Each box represents the difference between the 75% quartile and the 25% quartile, the bar represents the median, and the whiskers represent the extremes for each population. (B) Size-free morphospace of the constituent traits of Suction Index, expressed as the two principal component axes retained by a broken-stick model (Jackson 1993). 95 percent confidence ellipses surround each population. Numbers from (A) correspond to population in (B).

similar to each other, representing the likely ancestral form of the freshwater benthic populations. We also wished to see whether benthic populations exhibit a pattern of simple convergent evolution by converging to a similar morphology, or whether benthic populations instead exhibit a pattern of convergent evolution with multiple solutions by diversifying into different morphologies relative to each other.

To explicitly quantify the morphological diversity of benthic and anadromous morphologies relative to one another, we examined patterns of morphospace occupation by calculating the mean Euclidean pairwise distance, a common measure of morphological diversity, on a principal components analysis of the correlation matrix of the size-corrected suction index components. We used the program morphospace disparity analysis (MDA) in MATLAB to compare the bootstrap distribution of a reference group's mean Euclidean pairwise distance on the five principle component axes to the Euclidean pairwise distance of a focal group (Navarro 2003). *P*-values were generated by bootstrap resampling the data 100,000 times, counting the number of times the focal group exceeded the observed value of the analyzed group, then dividing by the number of resampling operations. We used anadromous populations as our reference group and compared them to our focal group of benthic populations. If functional convergence is producing substantial amounts of morphological diversity, we would expect to see a substantially elevated mean Euclidean pairwise

distance within benthics relative to anadromous fish, with benthic populations evolving multiple high-suction phenotypes from the ancestral anadromous phenotype. If, however, functional convergence is not appreciably increasing morphological diversity, the mean Euclidean pairwise benthic within benthics will resemble that within anadromous populations.

Results and Discussion

We compared suction index values across four anadromous and four benthic populations (Fig. 1A). Anadromous populations exhibited similar suction index values, and were not statistically distinguishable from each other ($P = 1.0$ for each comparison). Benthic populations exhibited a higher suction index than anadromous populations (P from $2.0e-9$ to $9.0e-14$), but did not differ from each other ($P = 1.0$ for each comparison), with the exception of Enos Lake benthics, which exhibited higher values of suction index than the other benthic populations (P from $2.0e-6$ to $7.2e-7$). This result is consistent with our functional predictions: benthic fish feed on attached and buried prey, which requires the generation of higher suction pressure for a successful foraging attempt, whereas anadromous stickleback are thought to be generalists, with populations observed feeding on both zooplankton and benthic invertebrates (Schluter 2000, McGee pers. obs.).

We used a principal component analysis of the five size-corrected components of suction index—gape, buccal cavity length, outlever length, epaxial height, and epaxial width—to examine whether anadromous populations are similar morphologically and whether benthic stickleback have diverged morphologically from anadromous populations (Fig. 1B). Anadromous stickleback populations were statistically indistinguishable ($P = 1.0$ for each comparison) from each other on PC1 and PC2. This result supports results from previous workers indicating that anadromous fish are panmictic and show fewer signatures of local adaptation than freshwater stickleback (Bell & Foster 1994; Hohenlohe et al. 2010). If P -values for anadromous populations are not corrected for multiple comparisons, they range from $P = 0.095$ (PC2, Rabbit vs. Turnagain) to $P = 0.83$ (PC2, Konoko vs. Salmon). In contrast, each population of benthic stickleback is strongly divergent from all anadromous stickleback on PC1 and/or PC2 (P from 0.00032 to $9.8e-13$), Mann–Whitney test with Bonferroni correction). Among benthics, Priest and Paxton fish do not differ from each other on PC1 and PC2 ($P = 1.0$) but substantial variation exists between benthics for all other population comparisons (P from 0.0076 to $5.3e-11$).

Further comparison of suction index component traits reveals at least three “solutions” that give benthics increased values of suction index relative to anadromous populations. Mud Lake benthic stickleback diverged from anadromous stickleback by decreasing buccal cavity length, outlever length, and gape width. In contrast, Priest and Paxton benthic stickleback diverged from anadromous stickleback by increasing all five suction index components relative to the anadromous form, especially gape, epaxial height, and epaxial width. Enos Lake stickleback decreased buccal length and outlever length similar to Mud Lake fish, but also increased epaxial height and width similar to Priest and Paxton Lake fish.

Each morphological change in the Enos and Mud Lake benthic populations operates to increase suction index. In particular, the high suction index values of Enos Lake fish occur because the four component changes each increase suction index. However, changes in the Paxton and Priest Lake benthic populations operate antagonistically. Increases in gape, buccal cavity length, and outlever length decrease maximum suction pressure by increasing the area over which buccal expansion force is distributed and decreasing the mechanical advantage of expansion forces, but these decreases in suction pressure are counterbalanced by extreme increases in the cross-sectional area of the epaxial muscles, which increase the force that is transmitted to the expanding buccal cavity during suction feeding.

After comparing population morphologies, we examined patterns of morphospace occupation by calculating the bootstrapped mean Euclidian pairwise distance on a principal components analysis (Fig. 1B) of the five size-corrected components of suction

index—gape, buccal cavity length, outlever length, epaxial height, and epaxial width. Anadromous populations show less morphological diversity relative to benthic populations (1.63 vs. 3.04, $P < 1.0e-7$). If the analysis is restricted to only the two principal component axes that explain more variation than would be expected by chance, benthic populations have more than double the mean Euclidean pairwise distance of anadromous populations (1.26 vs. 2.74, $P < 1.0e-7$). These patterns are largely driven by benthics occupying the extremes of morphospace on PC1, with anadromous populations concentrated in a smaller region near the center. If each benthic population is centered to the origin and benthic disparity is compared to anadromous disparity on PC1 and PC2, both exhibit similar mean Euclidean pairwise distances (1.26 vs. 1.35, $P = 0.09$), suggesting that increased interpopulation variation drives benthic disparity.

Our work suggests that convergence, traditionally thought to constrain phenotypic evolution, is capable of generating substantial morphological diversity. Because we use an explicit functional model to quantify convergence, we are able to observe that radically different phenotypes are nonetheless producing similar functional outputs. This pattern may also help to explain why convergent or parallel evolution does not always produce identical phenotypes, even among close relatives (Stein 2000; Young et al. 2009). Functional convergence may also contribute to a sort of cryptic diversity, as morphologically distinct phenotypes may appear ecologically divergent in the absence of functional information, when in reality these phenotypes are derived from selection for similar function. Convergence can be a source of constraint, but functional convergence can result in phenotypic divergence and may be an underappreciated generator of morphological diversity.

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