

Niche Specialization Influences Adaptive Phenotypic Plasticity in the Threespine Stickleback

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ABSTRACT: Phenotypic plasticity may be favored in generalist populations if it increases niche width, even in temporally constant environments. Phenotypic plasticity can increase the frequency of extreme phenotypes in a population and thus allow it to make use of a wide resource spectrum. Here we test the prediction that generalist populations should be more plastic than specialists. In a common-garden experiment, we show that solitary, generalist populations of threespine sticklebacks inhabiting small coastal lakes of British Columbia have a higher degree of morphological plasticity than the more specialized sympatric limnetic and benthic species. The ancestral marine stickleback showed low levels of plasticity similar to those of sympatric sticklebacks, implying that the greater plasticity of the generalist population has evolved recently. Measurements of wild populations show that those with mean trait values intermediate between the benthic and limnetic values indeed have higher morphological variation. Our data indicate that plasticity can evolve rapidly after colonization of a new environment in response to changing niche use.

Keywords: phenotypic plasticity, adaptation, niche variation hypothesis, character displacement, evolution.

Introduction

Adaptive phenotypic plasticity is the ability of a single genotype to change its phenotype to better match the requirements of its environment (Newman 1992; Scheiner 1993; Gotthard and Nylin 1995; DeWitt and Scheiner 2004). The adaptive significance of phenotypic plasticity in temporally and spatially varying environments has been demonstrated repeatedly (Stearns 1989; Brönmark and Miner 1992; Scheiner 1993; Day et al. 1994; Robinson and Wilson 1996; Svanbäck and Eklöv 2006). However, plasticity may also be adaptive when a broader population niche is favored (West-Eberhard 1989; Robinson and Wil-

son 1994; Pfennig and Murphy 2000, 2002), such as when a competing species is absent. For example, Pfennig and Murphy (2000, 2002) showed that populations of the Plains spadefoot toad (*Spea bombifrons*) were more plastic when they were the only species present than were populations that were sympatric with a competitor, the New Mexico spadefoot toad (*Spea multiplicata*), although the difference in plasticity was maternally inherited rather than genetically based (Pfennig and Martin 2009).

Van Valen's (1965) "niche variation hypothesis" suggests that populations with a wider niche (i.e., generalist populations) should be phenotypically more variable than populations with narrower niches (i.e., specialist populations). The increase in phenotypic variation in a generalist population could, in principle, be achieved by two mechanisms. First, increased phenotypic variation could be caused by an increase in genetic variation (Hedrick 1986; Noy et al. 1987; Prentice et al. 1995). However, constraints on genetic variation, such as the absence of assortative mating, may limit the amount of variation that can evolve in a sexual population. Second, greater phenotypic variation can be achieved by phenotypic plasticity if plasticity itself is evolvable.

Here we test the prediction that generalist populations will have a higher degree of phenotypic plasticity, using replicate lake populations of the threespine stickleback (*Gasterosteus aculeatus*). Marine threespine sticklebacks have successfully colonized many freshwater systems throughout the Northern Hemisphere (Wootton 1976). Lake populations in southwestern British Columbia were colonized by marine sticklebacks about 12,000 years ago (Mathews et al. 1970; Clague et al. 1982; Clague 1983). Most of these lakes contain only one species of stickleback, but coexisting pairs of species have been found in a few lakes on islands in the Strait of Georgia (McPhail 1984, 1993). These two species are referred to as "benthic" and "limnetic" sticklebacks (Larson 1976; Bentzen and McPhail 1984), whereas other populations occurring as single species in lakes are known as "solitary." Benthic and limnetic species are morphologically differentiated and are

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specialized for feeding in the littoral and pelagic zones of lakes, respectively, whereas the solitary populations in our study are morphologically intermediate generalists feeding in both zones of otherwise similar lakes containing cutthroat trout as the only other fish species (Schluter and McPhail 1992). In general, while not as extreme as in the species pairs, solitary populations exhibit a wide range of phenotypes related to lake size, shape and fish species composition (e.g., Lavin and McPhail 1986; Walker 1997; Vamossi 2003; Bolnick and Lau 2008; Aguirre 2009). We first examined the relationship between phenotypic variation and specialization among wild stickleback populations. We then tested the prediction that phenotypic plasticity in the generalist solitary populations of threespine sticklebacks is greater than that in the more specialized sympatric populations. Our test used a common-garden experiment in which plasticity was measured by the phenotypic differences between siblings raised on contrasting diets. We also examined the relationship between degree of plasticity and population mean phenotype in traits to rule out scaling effects and to determine whether plasticity is indeed maximized at an intermediate phenotype. We focused on overall body shape, gill-raker length, and gape width because the mean values of these traits are known to differ among stickleback populations and to exhibit plasticity in the stickleback as well as in other fish species (Day et al. 1994; Robinson and Wilson 1994, 1996; Svanbäck and Eklöv 2006).

We further compared phenotypic plasticity in the marine population ancestral to derived freshwater generalist and specialist populations. The marine stickleback is a highly successful recent invader of freshwater systems, and the evolution of plasticity has been suggested to contribute to invasion success (Losos et al. 2000; Dybdahl and Kane 2005; Spector and Putz 2006). If plasticity in foraging-related traits has been important for invasion success in lakes, then we might expect to find levels of plasticity in marine sticklebacks that equal or exceed those seen in freshwater populations. On the other hand, because marine sticklebacks live in a fairly homogenous and stable marine environment, plasticity in marine sticklebacks might be less than that in freshwater populations.

Methods

Populations and Sampling

The study was carried out on stickleback populations of small lakes in the Strait of Georgia region of British Columbia, Canada. Three categories of stickleback populations were sampled. First, we sampled sympatric benthic and limnetic species of sticklebacks from two lakes (“sympatric lakes”). Considering the sympatric sticklebacks as independent species, we thus have four populations of

specialist species. Second, we sampled sticklebacks from eight lakes containing a solitary species of stickleback (“solitary lakes”). Third, we also sampled marine (anadromous) sticklebacks from three different locations. Further information about the lakes can be found in table A1, available online. We treat limnetics and benthics from the same lake as independent populations, which assumes that there are no effects of individual lake on plasticity, except through effects on degree of specialization of populations. We tested this assumption with a two-factor ANOVA, with benthic/limnetic as one factor and lake (Paxton and Priest) as the other, and could not find any significant lake or lake \times population effects (all $P > .075$). Furthermore, using the average of the benthic and limnetic species from each sympatric lake as a replicate in our tests yielded similar results and did not change any of our conclusions.

We chose solitary lakes that had fish communities similar to those of the sympatric lakes (i.e., with cutthroat trout), but two of our eight solitary lakes (Paq Lake and Brown Lake) also contained prickly sculpins. Our results and interpretations would be similar without Paq Lake and Brown Lake, but we included these lakes to increase the power of our analyses.

We sampled males and females from all populations to generate laboratory crosses (see below). We also sampled between 25 and 40 adult individuals from each population for phenotypic measurements. We immediately anesthetized these fish with MS-222 and placed them in 10% formalin for 2 weeks before staining bony armor with alizarin red and storing the fish in 37% isopropyl alcohol.

Crossing Technique and Rearing Program

Fish from all populations were raised from artificially fertilized eggs according to the protocol of Day et al. (1994). After the eggs hatched, all fish of a brood were released into one side of a partitioned 102-L aquarium. A total of six broods were raised from each population, with two broods assigned to alternate sides of each of three randomly selected aquariums. After 3 weeks, the two broods in each aquarium were mixed together, and the number of individuals on each side of the partition was reduced to 15 by random selection. This procedure yielded three replicates (aquariums) for each population. Both sides of every aquarium received a plastic saucer (18 cm in diameter) filled with limestone to simulate a substrate. Not all fertilizations were performed at the same time because of the sporadic occurrence of gravid females. The time span between the first and last fertilizations of the whole study was approximately 1 month.

All juvenile fish were fed newly hatched brine shrimp nauplii for the first 3 weeks after hatching, after which the

two sides of the each aquarium were randomly assigned to different prey treatments. The fish on one side were fed thawed frozen bloodworms to represent a littoral habitat and diet. The bloodworms were injected into the limestone rubble at the bottom of the aquarium by means of a glass tube. The fish on the other side of the partition continued to be fed with brine shrimp nauplii, which were released into the water column to represent a pelagic habitat and diet. It has been shown that the degree of plasticity can be influenced by energy gain (Olsson et al. 2006, 2007). Therefore, all fish were fed to satiation on their assigned treatment each day, resulting in similar sizes between treatments at the end of the experiment ($P = .533$). Photoperiod was held at a constant 14L:10D cycle, and the temperature was kept between 17° and 20°C.

The experiment was terminated once the fish had been feeding on the different diet treatments for 4 months. All fish were killed by an overdose of buffered MS-222 and then fixed in a solution of 10% formalin for at least 2 weeks. Fish were stained with alizarin red before being stored in 37% isopropyl alcohol.

Morphological Measurements

We measured total length, gill-raker length, gape width, and body shape of each fish from both the natural populations and the experiment. Total length and gape width were measured with a digital caliper, whereas gill-raker length was measured with an ocular micrometer on a dissection scope and gill arches dissected and mounted on a slide. We used the average of the longest gill rakers from the left- and right-hand sides of each fish. Gill-raker length and gape width were chosen because they have been demonstrated to affect foraging efficiency in sticklebacks (Bentzen and McPhail 1984; Lavin and McPhail 1986; Schluter 1993) and because they have been shown to be plastic in both benthic and limnetic sticklebacks (Day et al. 1994). The traits were size adjusted by means of a linear regression of each trait against total length in an ANCOVA that fitted a common slope to all populations. We used the residuals and fitted values from this analysis to adjust measurements from each population to a total length of 46.7 mm, the average of the sticklebacks in this study. These size-corrected variables were used in all subsequent analyses.

To measure body shape, we digitized the coordinates of 22 landmarks using tpsDig2 (Rohlf 2005), on digital images of the left side of each stickleback (Ingram et al. 2012; see also fig. A1, available online). Digitized landmarks were then analyzed with generalized least squares Procrustes superimposition in the program tpsRelw (Thin-Plate Spline Relative Warp; Rohlf 2005). The superimposition first centers each configuration of landmarks at the origin, which yields landmark coordinates reflecting the deviation

from the centroid. Next, the program scales each landmark configuration to unit centroid size by dividing the coordinates of each landmark by the centroid size of that configuration. Finally, it rotates all specimens to optimal alignment on the average body shape to minimize the summed-squares distances between homologous landmarks over all landmarks (Zelditch et al. 2004). These procedures resulted in 22 X- and 22 Y-coordinates for each fish, adjusted for geometric body size.

Statistics

We used a discriminant function analysis (DFA) to identify major axes of shape variation among populations relative to variation within populations. DFA was performed on the samples of wild-caught sticklebacks, with the individual populations (i.e., the lakes, except for the benthics and limnetics) as the categories or groups. Thus, importantly for this analysis, specimens were identified by unique population and not by population type (solitary, benthic, limnetic, or marine), so the DFA did not bias our tests for differences between solitary, sympatric, and marine populations. We then projected the measurements of experimental fish onto the DFA axes identified with the wild-caught fish, to ensure that we had meaningful and comparable morphological variation between the field and experimental fish. We preferred DFA to alternatives such as principal-components analysis (PCA) because DFA isolates those characters that vary among populations by finding axes that maximize variation among populations relative to variation and covariation within populations (Tabachnick and Fidell 1996). In contrast, PCA can be heavily influenced by within-population variation, including measurement error and artifacts such as upward or downward bending of specimens. However, our results were qualitatively similar when we used a PCA. The discriminant function (DF) scores from the DFA were subsequently used as independent traits in further analyses of plasticity and trait variation. For the wild fish, we determined that the different population types (solitary, benthic, limnetic, and marine) differed from each other in phenotype space by calculating the mean scores of each unique population on the first two DF axes and applying a MANOVA with these population means as replicates.

We calculated the average size-corrected phenotype and the variation (standard deviation) in phenotype for all measurements of fish sampled from the natural populations. We calculated the degree of plasticity of the experimental fish as follows. For each half of a fish tank, we calculated the average of a given trait (gill-raker length, gape width, or DF score). Then we calculated plasticity as the difference between the two halves, that is, as the mean phenotype of fish fed brine shrimp (pelagic diet) minus

the mean of fish fed bloodworms (benthic diet). Because gape width was greater in the fish fed the benthic diet than in fish fed the pelagic diet, we multiplied the calculated degree of plasticity by -1 to get a more intuitive value. When comparing the marine, solitary, and sympatric sticklebacks, we calculated the mean plasticity for each population in the study and tested for differences by using t -tests with the population mean as the replicate.

Results

The discriminant function analysis of 15 stickleback populations revealed considerable body shape variation among the four population types along the first two body shape axes (fig. 1; multivariate ANOVA with population means as replicates: Wilks $\lambda = 0.009$, $F_{3,11} = 31.58$, $P < .001$). The first axis clearly separated the ancestral marine stickleback from derived freshwater forms, which are less streamlined and have a larger head, smaller medial fins, and a more posterior position of the dorsal spines (fig. 1). Hereafter, we call this axis the “marine-freshwater” body shape axis. The second axis separated the benthic from the limnetic species, representing the extreme body shapes found in fresh water. Hereafter, we call this the “benthic-limnetic” body shape axis. Limnetic sticklebacks have narrower bodies and larger dorsal and anal fins than benthic sticklebacks (fig. 1). Solitary, generalist populations have intermediate body shapes, although they span much of the range of the benthic-limnetic axis. The benthic-limnetic axis is the primary body shape axis of interest, since it separates populations according to the diet types, benthic and pelagic, that were varied in the common-garden experiment. Because of this, we provide the analysis of the benthic-limnetic axis in the main text and leave the analyses of the marine-freshwater axis to the appendix, available online.

Wild-caught fish from generalist populations are more variable in phenotype. In agreement with the niche variation hypothesis, wild samples from the solitary, generalist populations had within-population phenotypic variation in body shape along the benthic-limnetic body shape axis 49% higher than that of sympatric, specialist populations (fig. 2A; $df = 10$, $t = 3.63$, $P = .005$). Phenotypic variation in gill-raker length was 26% greater in solitary populations than in the sympatric populations, but not significantly so (fig. 2B; $df = 10$, $t = 1.15$, $P = .28$). There was no difference among freshwater population types in the amount of variation of gape width (fig. 2C; 0.1%, $df = 10$, $t = 0.01$, $P = .99$). Within-population variation in body shape along the benthic-limnetic axis was intermediate in the marine species and not significantly different from that in the generalist species (fig. 2A; marine vs. solitary: $df = 9$, $t = 1.72$, $P = .12$) but marginally different from that in the specialist freshwater types (ma-

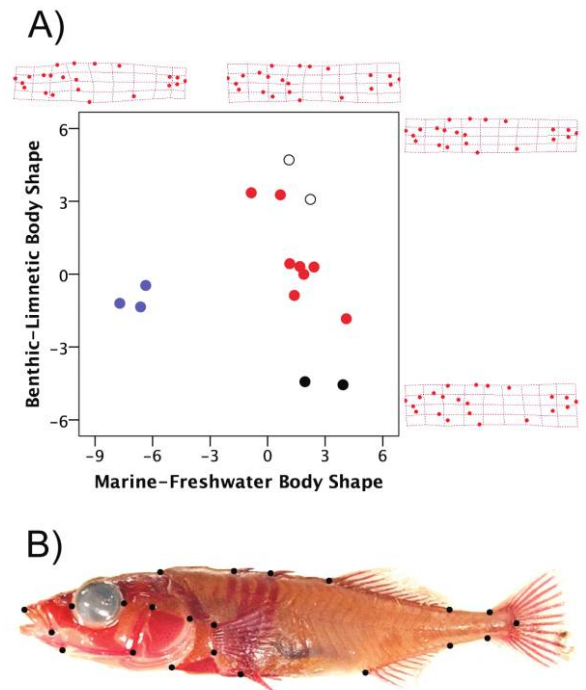
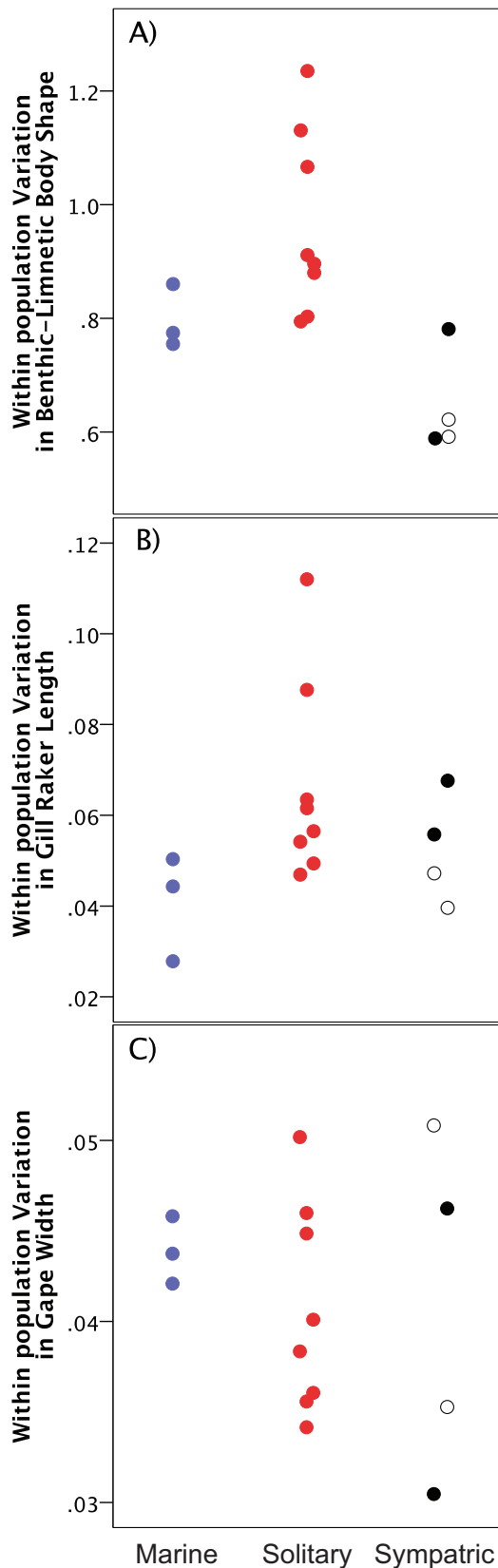


Figure 1: A, Body shape differences among the different types of stickleback populations used in this study. Data points show population means along the first two major body shape axes from a linear discriminant analysis of populations irrespective of type: marine (blue), solitary (red), benthic (black), and limnetic (open). Visualization of the morphological scores above the graph represents a marine body shape of score -9 (left) and a freshwater body shape of score $+6$ (right). Visualization of the morphological scores to the right of the graph represents a limnetic body shape of score $+6$ (top) and a benthic body shape of score -6 (bottom). B, Locations of the landmarks used in this study to help visualize the shape changes associated with the two major body shape axes. See figure A1, available online, for details about the landmarks.

rine vs. sympatric: $df = 5$, $t = 2.49$, $P = .055$). The marine species also had low variation in gill-raker length (fig. 2B; marine vs. solitary: $df = 9$, $t = 1.86$, $P = .097$; marine vs. sympatric: $df = 5$, $t = -1.30$, $P = .25$) as well as in gape width (fig. 2C; marine vs. solitary: $df = 9$, $t = 0.92$, $P = .38$; marine vs. sympatric: $df = 5$, $t = 0.56$, $P = .60$), although neither of the traits differed from those in the generalist or specialist freshwater types.

Population differences in the amount of variation in samples of wild-caught fish may result from several processes. Next, we focus on the contribution of phenotypic plasticity, which we tested in our common-garden experiment.

Plasticity in the common-garden experiment was adaptive. For most traits, phenotypic plasticity was in the adaptive direction. Reaction norms for gill-raker length, gape width,



and the benthic-limnetic body shape axis exhibited a mean phenotype shifted toward the limnetic species when fish were raised on the pelagic diet and shifted toward the benthic species when fish were raised on the benthic diet (fig. 3; see also fig. A2, available online, for reaction norms for each family used in this study).

Generalist populations exhibit greater plasticity. We found a higher degree of plasticity in the generalist populations than in the specialist populations in several traits in the common garden (fig. 4). Mean plasticity of the solitary, generalist populations was greater than the mean of the four limnetic and benthic specialist populations combined along the benthic-limnetic body shape axis (203% greater in solitaries; $df = 10$, $t = 4.43$, $P = .0001$), gill-raker length (77% greater in solitaries; $df = 10$, $t = 3.92$, $P = .003$), and gape width (70% greater in solitaries; $df = 10$, $t = 2.02$, $P = .071$).

Populations with intermediate phenotypes are more plastic. The benthic and limnetic specialists lie at opposite extremes of the range of phenotypes in freshwater sticklebacks (fig. 1). Indeed, we found a significant quadratic relationship between plasticity and population mean value along the benthic-limnetic body shape axis ($R^2 = 0.580$, $P = .020$; fig. 5) as well as for gill-raker length ($R^2 = 0.508$, $P = .041$; fig. 5). This is consistent with the prediction that freshwater populations with intermediate trait values along a benthic-limnetic spectrum should have the highest plasticity. These results indicate that plasticity does not simply increase with the mean trait value, as might be expected from nonadaptive scaling relationships. We did not find any relationship between plasticity and mean trait value for gape width (fig. 5).

Marine sticklebacks have a low degree of plasticity. The marine ancestor has successfully invaded fresh water hundreds of times. Does adaptive plasticity contribute to this success? We found that the marine sticklebacks are not exceptionally plastic in the traits we measured in the face of benthic and pelagic diet regimes. Marines had lower plasticity than the solitary, generalist populations (fig. 4) along the benthic-limnetic body shape axis (286% greater in solitaries; $df = 9$, $t = 4.33$, $P = .002$), in gill-raker length (140% greater in solitaries; $df = 9$, $t = 4.51$, $P = .001$), and in gape width (102% greater in solitaries; $df = 9$, $t = 2.78$, $P = .021$). Marines and specialists had similar levels of plasticity (benthic-limnetic body shape axis: $df = 5$, $t = 0.46$, $P = .66$; gill-raker length: $df = 5$, $t = 2.24$, $P = .075$; gape width: $df = 5$, $t = 0.52$, $P = .63$).

Figure 2: Within-population phenotypic variation estimated from wild-caught individuals sampled from natural populations: benthic-limnetic body shape axis (A), gill-raker length (B), and gape width (C) for marine (blue), solitary (red), and sympatric (black for benthic and open for limnetic) populations.

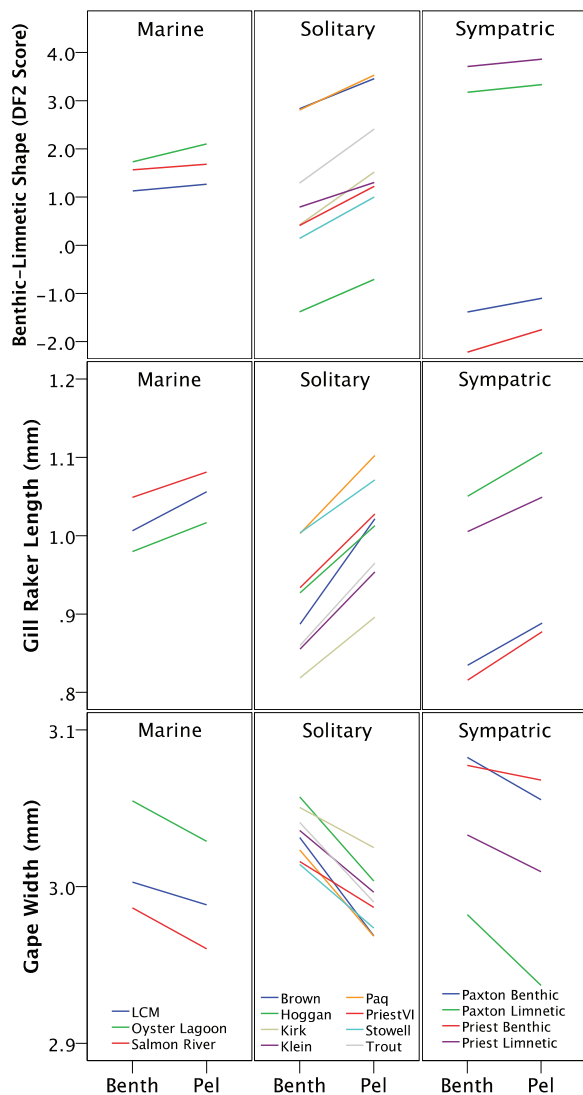


Figure 3: Reaction norms for marine, solitary, and sympatric populations raised on benthic and pelagic diets. Each line connects the average phenotype of replicate families from a single population raised in a common environment. See also fig. A2, available online, for reaction norms for each family used in this study.

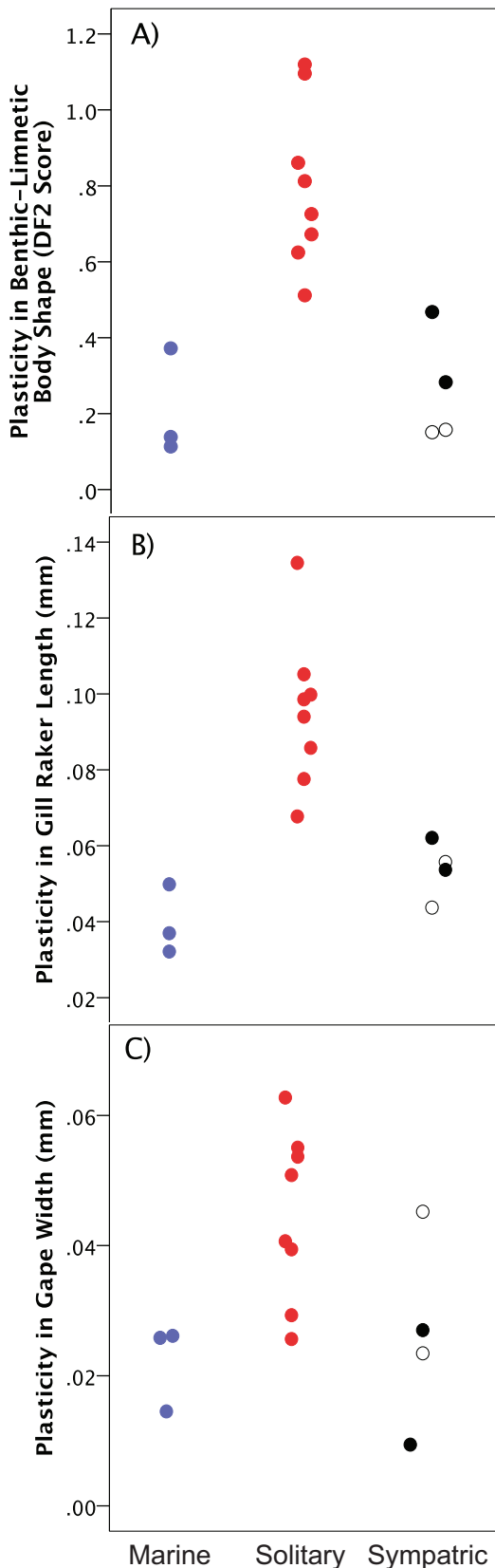
Discussion

Our results provide evidence that adaptive phenotypic plasticity is tightly connected to the ecological niche width of a population. In our study, the solitary, generalist populations had a higher degree of phenotypic plasticity along the benthic-pelagic gradient than the more specialized sympatric populations. This is in agreement with the hypothesis that phenotypic plasticity can be favored if it broadens the niche, allowing populations to exploit a broad resource spectrum (West-Eberhard 1989; Robinson

and Wilson 1994). Our generalist populations also had higher plasticity than the marine ancestral species, which colonized fresh water only about 12,000 years ago, indicating that plasticity can evolve rapidly.

The niche variation hypothesis (Van Valen 1965) and theories on character displacement and character release (Brown and Wilson 1956; Schluter and McPhail 1993; Robinson and Wilson 1994) suggest that in the presence of a wide resource spectrum, allopatric populations should become generalists and broaden their niche. Earlier studies have focused on the increase in niche width caused by genetic changes (Hedrick 1986; Noy et al. 1987; Prentice et al. 1995). However, phenotypic plasticity has also been suggested as a means to broaden the niche (West-Eberhard 1989; Robinson and Wilson 1994). For example, Pfennig and Murphy (2000, 2002) showed that sympatric and allopatric populations of Plains spadefoot toads differed in the degree of plasticity, although the difference in plasticity were due to maternal effects (Pfennig and Martin 2009). Our results provide the first demonstration that morphological plasticity can evolve to higher degrees in replicate allopatric populations, compared with sympatric populations. Plasticity has likely evolved in allopatry in response to a broad resource spectrum in the absence of interspecific competition. Previous work has shown that the sympatric sticklebacks have undergone ecological character displacement in mean phenotype in response to interspecific resource competition and that solitary populations in otherwise similar lakes are phenotypically intermediate and exploit the resources of both specialist species (Schluter and McPhail 1992; Schluter 1994). However, variation in a trait in natural populations (fig. 2) does not perfectly match the degree of plasticity found in our experiment (fig. 4). This is probably because plasticity in relation to benthic and pelagic foraging is not the only factor determining variation in a trait in nature. Other factors that can affect trait variation in nature are, for example, genetic variation (Hedrick 1986; Noy et al. 1987; Prentice et al. 1995) and plasticity in response to other, unmeasured aspects of the environment, such as habitat structure or presence of predators (e.g., Brönmark and Miner 1992; Olsson and Eklöv 2005; Eklöv and Svanbäck 2006).

During the past 2 decades, empirical evidence has accumulated that suggests (1) that phenotypically plastic genotypes can have fitness advantages relative to nonplastic genotypes (Schlichting 1986; Sultan 1987) and (2) that additive genetic variation exists for phenotypic plasticity (Schlichting 1986; Sultan 1987; Scheiner 1993). Appreciating that phenotypic plasticity can be selected for raises questions about the ecological conditions that promote or prevent its evolutionary emergence. Phenotypic plasticity has been shown in many studies to be an adaptive response to environmental variability (Levins 1963; West-Eberhard

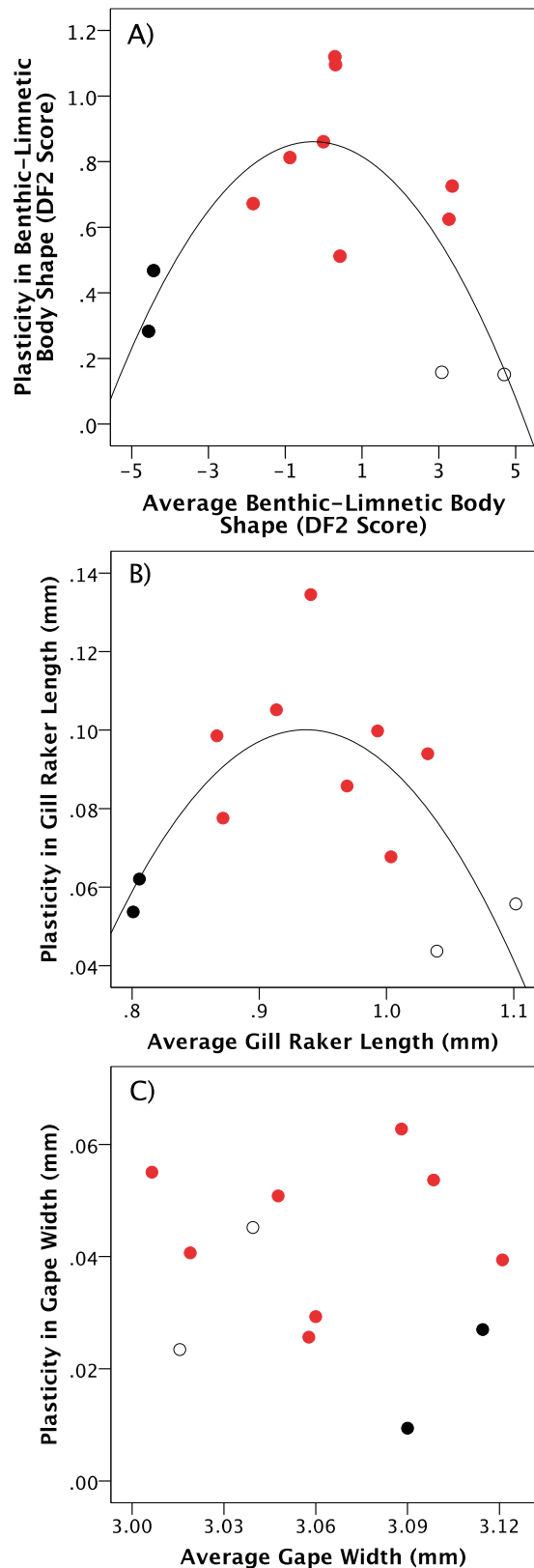


1989; Scheiner 1993; DeWitt and Scheiner 2004; Merilä et al. 2004; Lind and Johansson 2007). For example, in a recent well-documented study of adaptive phenotypic plasticity, Lind and Johansson (2007) found that the degree of plasticity in developmental time was positively related to spatial variation in pool-drying regimes for populations of a common frog (*Rana temporaria*). Sultan et al. (2009) also found a greater degree of plasticity in a generalist species than in a specialist species. Our study used replicated populations to show that phenotypic plasticity is indeed correlated with a generalist diet within a species complex undergoing adaptive radiation.

Why should plasticity be favored in a generalist population? Divergent natural selection between two contrasting habitats, such as the littoral and pelagic habitats of lakes, has been shown to favor different phenotypes (genetic or plastic) in the two habitats (Robinson et al. 1996; Svanbäck and Eklöv 2003; Bolnick and Lau 2008; Svanbäck and Persson 2009). Furthermore, disruptive selection has been detected in intermediate solitary populations (Bolnick and Lau 2008). In theory, such selection may be strong enough to lead to sympatric speciation if assortative mating evolves (Dieckmann and Doebeli 1999; Kondrashov and Kondrashov 1999), although this has apparently not happened in sticklebacks (Schluter and McPhail 1992, 1993; Bolnick and Lau 2008). Possibly, genetic or other constraints prevent assortative mating from evolving to high levels in sticklebacks (Bolnick and Lau 2008). In such cases, other evolutionary responses to disruptive or divergent selection, such as sexual dimorphism of feeding-related traits and enhanced phenotypic plasticity (Rueffler et al. 2006; Bolnick and Lau 2008; Svanbäck et al. 2009), may be favored to take advantage of a wide resource spectrum in the absence of specialist phenotypes.

There are now several lines of evidence that the plastic responses in sticklebacks are adaptive. For example, this study and others (Day et al. 1994; Wund et al. 2008) have shown that sticklebacks develop different phenotypes in different environments: sticklebacks raised in a benthic environment develop a deeper body, shorter gill rakers, and a larger mouth, compared to sticklebacks raised in an open-water environment. The adaptive significance of such plasticity is highlighted by the observation that deeper-bodied individuals with larger gapes and shorter gill rakers have higher foraging success in benthic environments, whereas streamlined individuals with smaller gapes and longer gill

Figure 4: Plasticity in benthic-limnetic body shape (A), gill-raker length (B), and gape width (C) for marine (blue), solitary (red), and sympatric (black for benthic and open for limnetic) populations. Plasticity is measured as the average difference between siblings raised on contrasting diets in a laboratory common garden (see “Methods” for more information).



rakers have higher foraging success in open water (e.g., Schluter 1993; Robinson 2000). Yet finding differences in phenotypic plasticity among populations that are related to niche variation, as we did in this study, also strongly suggests that plasticity is adaptive (Newman 1992; Gotthard and Nylin 1995). The results imply that genetic variation exists for plasticity and that there is an adaptive match between plasticity and niche variation.

When colonizing new and previously unoccupied areas, colonist populations often find themselves in selective environments radically different from that previously experienced. Phenotypic plasticity has long been thought to be key to the persistence of colonists of new areas (Losos et al. 2000; Dybdahl and Kane 2005; Spector and Putz 2006). The marine threespine stickleback has repeatedly colonized many freshwater systems, and significant plasticity in response to changing salinity (McCairns and Bernatchez 2010) and reproductive behavior (Shaw et al. 2007), as well as diet (this study; Wund et al. 2008), may have contributed to their success. Nevertheless, we found that marine sticklebacks exhibit relatively low levels of phenotypic plasticity in key traits, compared to their generalist descendants in fresh water. This implies that a high degree of plasticity is not always necessary in a good colonizer. This conclusion is in line with recent studies suggesting that rapid, genetically based adaptations to novel environments are also important in the evolutionary ecology of colonization (Huey et al. 2000; Maron et al. 2004; Barrett et al. 2010).

In results similar to our own, Wund et al. (2008) found that the direction of dietary reaction norms in mean phenotype in ancestral marine sticklebacks paralleled differences among solitary freshwater populations varying along a benthic-pelagic resource gradient. In contrast to our results, Wund et al. (2008) found no significant differences in plasticity between marine and solitary freshwater sticklebacks, although on average, their single-species populations tended to be about 80% more plastic than their marine population. However, the solitary freshwater populations investigated by Wund et al. (2008) were more benthic-like or limnetic-like than the strictly intermediate forms used in our study, though not as differentiated phenotypically as the sympatric benthic and limnetic species. Hence, the quantitative differences between our studies

Figure 5: Relationship between plasticity in a trait and the population mean phenotype for that trait in the benthic-limnetic body shape axis (A), gill-raker length (B), and gape width (C). Plasticity was measured in the common garden, whereas mean phenotype was based on measurements of wild-caught individuals. Solitary stickleback populations are represented by red circles, benthics by black circles, and limnetics by open circles. Lines represent significant quadratic relationships.

might lie in the use of more specialized solitary populations by Wund et al. (2008). The quantitative difference between our studies in the magnitude of population differences in plasticity may also lie in the fact that Wund et al. reared their fish only to a size of 2.5 cm, whereas our fish were reared to a size of 4.2 cm and so had more opportunity to develop plasticity.

In conclusion, our results indicate that there is a repeated tendency for more generalized populations to evolve a higher degree of phenotypic plasticity over the past 10,000 years. This plasticity is probably an adaptation to broaden the niche of the population. Although the exact nature of the genetic architecture is not known, it is clear that adaptive phenotypic plasticity might be common (DeWitt and Scheiner 2004), and documenting comparative evidence between niche variation and plasticity is a first step toward understanding its basis and implications.

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