

ECOLOGY AND GENETICS OF PHENOTYPIC PLASTICITY: □ A COMPARISON OF TWO STICKLEBACKS

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Abstract.—We present results of an experiment designed to address fundamental issues in the ecology and evolution of plastic trophic morphology: (1) Is observed plasticity adaptive? (2) How much interspecific morphological variation is the result of plasticity? (3) Have different selective regimes resulted in the evolution of different degrees of plasticity? (4) Is genetic variation for phenotypic plasticity present in contemporary populations? We raised fish from two recently diverged species of freshwater threespine sticklebacks on two different diets representative of the natural prey of the two species. Both species exhibited morphological plasticity in an adaptive direction: each species more closely resembled the other when raised on the latter's diet. Diet-reversal reduced the natural morphological gap between these two species, –1% to 58%, depending on the trait. One species is known to have a more variable diet in the wild than the other species, and we found that it also exhibited the greater amount of morphological plasticity. Given that the two species have recently diverged, this result is compelling evidence that diet variability is important in the evolution of plastic trophic morphology. Finally, by using a full-sib experimental design, we demonstrated that genetic variation for morphological plasticity exists in contemporary populations, thus confirming that plasticity has evolutionary potential.

Key words.—Adaptation, genotype-by-environment interaction, phenotypic plasticity, sticklebacks, trophic morphology.

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Phenotypic plasticity is environmentally induced phenotypic change that occurs within an organism's lifetime (Bradshaw 1965; Stearns 1989). A resurgence of empirical and theoretical interest in this phenomenon has brought about a reevaluation of its ecological and evolutionary significance (West Eberhard 1989). Whereas phenotypic plasticity was once thought to result primarily from developmental accidents (West Eberhard 1989), new evidence suggests that much environmentally induced phenotypic variation exhibited by organisms is selectively advantageous (Stearns 1983; Bernays 1986; Greene 1989; Spitze 1992; Thompson 1992). This has led some to view plasticity as a trait subject to evolutionary pressures, just like any other phenotypic character (Schlichting and Levin 1986; Scheiner 1993). The discovery of prevalent genotype-by-environment interaction in many natural populations (genetic variation for phenotypic plasticity) further reveals that phenotypic plasticity is a character that is evolutionarily labile.

Here we present an empirical study that addresses four fundamental questions concerning

the ecological and evolutionary significance of phenotypic plasticity. (1) Does our study organism exhibit plasticity in an adaptive direction? (2) How much of the match between the morphology of a species and its environment is a result of plasticity, and in particular, how much of the morphological difference between species inhabiting different environments is the result of plasticity? (3) Have different selective regimes resulted in the evolution of different degrees of plasticity? and (4) Is genetic variation for phenotypic plasticity present in contemporary populations? While a variety of studies have addressed (1) and (4) (Lindsey 1962; Bernays 1986; Thompson 1992), few have determined the degree to which species differences in morphology are the result of plasticity (i.e., [2]). Additionally, few studies have compared the degree of plasticity across species for which selection on plasticity is likely to differ (but see Wimberger 1991, 1992).

We studied two sympatric species of threespine sticklebacks (*Gasterosteus* spp.) from Paxton Lake, British Columbia, Canada (McPhail 1992; Schluter and McPhail 1992). These species have yet to be formally described and are referred to as the "benthic" species and the "limnetic" species, after the regions of the lake in which they usually forage (University of British Columbia

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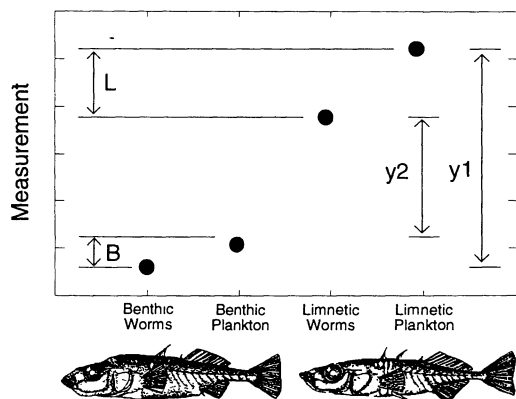


FIG. 1. The benthic species (left) and the limnetic species (right). Also the expected pattern of diet-induced morphological change. The four points represent the mean value of a trait for the four species-by-diet combinations. The diet-induced change of the limnetic species (L) is predicted to be greater than the diet-induced change of the benthic species (B) if diet variability has driven the evolution of morphological plasticity. The difference between the two species when raised on their natural diets (y_1) is expected to be greater than the difference between the two species when their diets are reversed (y_2), if morphological plasticity is adaptive.

Fish Museum Catalogue #83-351). The benthic species is specialized for littoral foraging and possesses a suite of traits suited to this. Individuals are deep-bodied, possess a small number of short gill rakers, and have a wide and terminal gape (fig. 1). The limnetic species is more slender bodied, with numerous, long gill rakers, and a narrow, upturned gape (fig. 1). It is more planktivorous, but it also exhibits a seasonal shift in habitat use. Individuals forage in the littoral zone in spring during the breeding season, and then switch to foraging in the water column in summer and fall (Schluter 1993). Morphological differences between species are largely heritable (McPhail 1992; Schluter unpubl. data) and strongly affect feeding efficiency and growth rates in different habitats (Bentzen and McPhail 1984; Lavin and McPhail 1986; Schluter 1993, unpubl. data).

The benthic and limnetic species are extremely closely related (Nei's genetic distance 0.018; McPhail 1992), and they are probably both descended from the marine threespine stickleback that colonized the lake on two separate occasions (Schluter and McPhail 1992). Comparative evidence suggests that their present morphological and habitat differences are the result of compe-

titution-induced character displacement but the exact sequence of morphological stages that occurred during their evolution is not resolved. Schluter and McPhail (1992) provide the evidence for character displacement and a discussion of the two main competing phylogenetic hypotheses. The postglacial history of this region of British Columbia indicates that these species have coexisted for no more than 13,000 yr (Mathews et al. 1970; Clague et al. 1982; Clague 1983). Thus, they are exceptionally well suited for study because many aspects of their histories are shared, their differences have evolved extremely recently, and this evolution has most likely taken place under ecological conditions still experienced by the two species.

We addressed the above-mentioned four questions in the following way. First, we examined whether plasticity of trophic morphology is adaptive by reversing the natural diets of the two species and asking whether they become morphologically more similar. We conclude that phenotypic plasticity is adaptive for either or both species if the morphological distinction between them is reduced when their diets are reversed (fig. 1). Such inference is reasonable because of the effect of trophic morphology on efficiency of prey capture in the two habitats (Bentzen and McPhail 1984; Lavin and McPhail 1986; Schluter 1993). Thus, an individual whose morphology grew to resemble that of the other species when raised on a diet that is characteristic of the other species would likely enjoy an increase in foraging efficiency. An explicit test of the adaptive significance of this morphological plasticity is currently underway.

Second, by comparing morphological differences of the species observed during diet reversal with the differences exhibited when they were fed their natural diets, we determined how much of the natural morphological difference between these two species is a direct result of diet. There has been considerable debate over how much of the morphological variation among some populations is a result of plasticity, especially in systems where large-scale adaptive radiation has occurred (Witte 1984; Meyer 1987; Wimberger 1991, 1992). Cichlid radiation in the African rift lakes is probably the most frequently cited example, but other impressive examples exist (Skulason et al. 1989; Snorrason et al. 1989). Significant adaptive radiation of the *Gasterosteus* species complex has occurred through colonization of fresh water. The freshwater species have

adapted to a variety of conditions throughout the northern hemisphere (Hagen and McPhail 1970; Bell 1976; Lavin and McPhail 1985; McPhail 1993). Our study lends insight into the possible importance of morphological plasticity in these instances.

Third, we compared the degree of plasticity between the two species, one specialized for feeding in the littoral zone (the benthic), and the other which exploits both habitats seasonally (the limnetic). The limnetic species exploits the littoral habitat in the spring during reproduction (April–June). Limnetic males build and defend nests in the littoral habitat, and gravid limnetic females use the habitat when searching for prospective mates. Stomach samples indicate that littoral and planktonic prey items are nearly equally represented in the diet of limnetic fish during this life-history stage (Schluter and McPhail 1992; Schluter 1993). After reproduction, surviving limnetic fish move back into the water column, where they feed on zooplankton during the summer and fall (Schluter 1993). In contrast, fish of the benthic species forage in the littoral habitat year round.

The conventional wisdom is that plastic trophic morphology would be beneficial if organisms are faced with significant variability of resource use on the appropriate temporal scale (Gomulkiewicz and Kirkpatrick 1992). Wimberger (1991, 1992) compared two congeneric species of cichlids (*Geophagus brasiliensis* and *G. steindachneri*) and predicted that differences of diet variability would select for different degrees of morphological plasticity. His results did not bear out this prediction of an interspecific difference. Our study addresses the same issue by using a similar type of interspecific comparison. Because individuals of the limnetic species experience significantly greater variability of resource use over the course of their lifetime than individuals of the benthic species, we predicted that limnetics would be more plastic than benthics.

Finally, we employed a full-sib design in our experiment in order to estimate quantitative-genetic parameters of phenotypic plasticity. Theoretical results demonstrate that optimal levels of phenotypic plasticity can evolve, given the appropriate type of genotype-by-environment interaction (Via 1987). While some studies were able to quantify additive genetic variance for phenotypic plasticity (Via 1984a), our design only characterized family-by-diet interaction, a mea-

sure of broad-sense heritability of phenotypic plasticity. This parameter is still useful, however, because it reveals whether genetic variation for phenotypic plasticity exists. Such a crude partitioning of genetic variance is usually the norm when dealing with organisms that require extensive laboratory facilities for rearing.

MATERIALS AND METHODS

Crossing Technique and Rearing Program

Fish of both species were raised from artificially fertilized eggs. Eggs from gravid females were squeezed into a petri dish by applying gentle pressure to the abdomen in an anterior to posterior direction. Males were sacrificed in MS-222, rinsed, and their testes were removed and minced with forceps in a sterile saline solution (15% salt water by volume). This solution was poured over the egg mass and then left for 3–5 min until fertilization had taken place. The egg mass was then rinsed, and 30 fertilized eggs were selected randomly. This procedure was repeated to yield 12 full-sib broods of 30 eggs from both the benthic and limnetic species.

Each 30-egg brood was split into a pair of 15-egg half-broods, one fed live brine-shrimp (*Artemia salina*), and the other fed live blackworms (*Tubifex*) and frozen bloodworms (*Diptera* spp.). These two diet treatments were representative of the planktonic and littoral habitats, respectively. Consequently, our experiment compared the two species raised on their natural diets and on the other species' diet.

Fertilized eggs were placed in plastic cups with mesh bottoms suspended in an aquarium of continuously aerated water. After approximately 7 d, the eggs hatched and all 15 fish of each half-brood were released into one side of a partitioned 102-liter aquarium. There were a total of 24 partitioned aquariums, and each aquarium contained a half-brood of both species. Thus, the half-broods of each family were raised in different and randomly determined aquariums. Diet treatment was assigned randomly to each aquarium, with all aquariums having the same diet treatment on each side of the partition.

Not all fertilizations were performed at the same time because of the sporadic availability of adult fish. The time span between the first and last fertilizations was approximately 1 mo. The experiment was initiated in the spring of 1992 and terminated in the fall of 1992.

During their first month, fish in the littoral

treatment were too small to eat blackworms. Consequently, all fish were fed brine-shrimp nauplii during this period. After the first month, the two experimental diet treatments were used. Fish assigned to the littoral treatment were fed chopped frozen bloodworms for an additional 3 wk and then were fed live blackworms for the remainder of the experiment. All fish were fed to satiation on their assigned diet treatment each day. Blackworms (the littoral prey) were administered by depositing the worms into a sand-filled petri dish at the bottom of the aquariums. Brine shrimp (the planktonic prey) were released into the water column. These two methods of prey deployment mimic the natural feeding habitats of the two species. Brine shrimp were cultured in the laboratory, and blackworms were purchased weekly from a local pet store. Photoperiod was held at a constant 16 h light:8 h dark cycle, and temperature was maintained between 17°C and 20°C.

Measurements

The experiment was terminated in November 1992, when fish had been fed the different diet treatments for approximately 4 mo. By this time, both species had reached a mean size of approximately 40 mm. This is the adult body size of the limnetic species, but the benthic species typically attains sexual maturity at 50 mm or larger. All fish were sacrificed in MS-222 and fixed in a solution of 10% formalin for 1 wk. After fixation, fish were stained in a solution of Alizarin Red and 10% KOH in order to render calcified tissue more visible. The fish were then permanently stored in a solution of 37% isopropyl alcohol.

Because of mortality, not all half-aquariums contained the same number of fish when the experiment was terminated. This could potentially confound the comparison between diet treatments if mortality was nonrandom with respect to diet (Lindsey and Harrington 1972). Unfortunately, a comparison between the morphology of surviving and dying fish was not possible, because most of the mortality occurred within a few weeks of hatching. Therefore, in order to rule out natural selection as a cause of morphological differences between groups, we performed a two-way ANOVA, with diet and species as factors, and mortality level as the dependent variable. Neither factor alone, nor their interaction, was significant (diet, $0.2 < P < 0.3$; species, $0.05 <$

$P < 0.1$; interaction, $0.7 < P < 0.8$), and there were no obvious trends in the data to suggest that mortality was biased with respect to morphology. Consequently, we concluded that mortality was random with respect to the experimental treatments.

Three fish from each half-aquarium were selected randomly for measurement, in order to balance the design for analysis. This yielded a total of 6 fish per family per species (69 limnetic fish and 70 benthic fish). The number of fish of each species was slightly lower than 72 because five half-aquariums had only 2 surviving fish.

Six characters were measured on each fish, (1) standard length, (2) gape width, (3) gill-raker length, (4) gill-raker number, (5) head depth, and (6) snout length. Traits (2) through (6) were chosen a priori, since they are correlates of foraging efficiency (Bentzen and McPhail 1984; Lavin and McPhail 1986; Schluter 1993). Standard length was used as an overall size measure of each fish. These six traits are also among the most variable between sympatric benthic and limnetic species, as well as among allopatric populations of threespine sticklebacks (Hagen and Gilbertson 1972; Gross and Anderson 1984; Schluter and McPhail 1992; McPhail 1993).

All dimensions were measured using an ocular micrometer on a Wild M3C dissecting microscope, except standard length, which was measured using Vernier calipers.

Analysis

All traits except gill-raker number were correlated with body size, and consequently, a covariate was needed in order to examine diet-induced morphological changes. We used standard length as a covariate rather than a composite variable, such as a pooled first-principal component (PC1), in order to simplify interpretation. However, results were unchanged when PC1 was used instead. Additionally, we used untransformed data in all our analyses, because this resulted in homogeneous variances between species.

We examined all traits for size-by-diet interaction by using analysis of covariance. No interaction was evident in either species, implying the effect of diet was independent of an individual's size (limnetics, $0.05 < P < 0.6$ for all traits; benthics, $0.1 < P < 0.9$ for all traits). Consequently, we size-corrected all traits by least-squares regression against standard length, using

a common slope between treatments for each species. These size-corrected variables are used in all subsequent analyses.

Multiple Significance Tests.—Because five traits were examined for phenotypic plasticity, and a variety of comparisons were carried out using these traits, there was a danger that type-I error rates would escalate. We attempted to minimize the number of statistical tests by first carrying out a multivariate test of a given hypothesis using all five traits simultaneously. If the result was statistically significant ($P < 0.05$), we then attempted to decompose the multivariate result into univariate measures to determine the relative role of each trait in the significant multivariate result. There is no single established procedure for performing such a test, and Wilkinson (1975) suggests four alternatives. We used univariate tests and employed a sequential Bonferroni procedure (at $\alpha = 0.05$) to guard against type I error (Rice 1989). It should be noted, however, that it is primarily the relative magnitude of the P values for each trait that are of interest in such an analysis, rather than their absolute values, because the multivariate test establishes an a priori tablewide significance level of $\alpha = 0.05$.

Effect of Diet.—If diet-induced phenotypic plasticity is adaptive, then we expected that the benthic and limnetic species would become more similar to one another when their diets were reversed ($y_2 < y_1$ in fig. 1). Testing this is equivalent to testing whether the sum of effects L and B in figure 1 is greater than zero. An individual fish does not represent the experimental unit in our breeding design because full sibs are not statistically independent. Consequently, we calculated a mean diet-induced change for each of the 12 families of both species for each of the five traits.

We let **B** and **L** represent two matrices where b_{ij} (or l_{ij}) is the mean diet-induced difference for the i th family and the j th trait of the benthic and limnetic species, respectively. We denote the five-dimensional (co)variance matrices for **B** and **L** by S_B and S_L , and the five-dimensional vectors of mean diet-induced differences among family means by $\bar{\mathbf{b}}$ and $\bar{\mathbf{l}}$. We let the vector $\mathbf{t} = \bar{\mathbf{b}} + \bar{\mathbf{l}}$, whose (co)variance matrix is then $S_t = S_B + S_L$, and then tested whether $\mathbf{t} > \mathbf{0}$, using Hotelling's T^2 statistic, $T^2 = n(\mathbf{t} - \mathbf{0})^T S_t^{-1}(\mathbf{t} - \mathbf{0})$, where $\mathbf{0}$ is the five-dimensional zero vector and $n = 12$ is the sample size. This statistic is distributed as $[(n-1)p/(n-p)]F_{p, n-p}$, where p is the number

of dependent variables, and $F_{p, n-p}$ denotes the F distribution, with p and $n-p$ degrees of freedom (Johnson and Wichern 1982).

The above multivariate analysis revealed whether or not the overall morphological gap between the two species was narrowed. Subsequently, we carried out univariate t tests to determine if the sum of L and B in figure 1 was greater than zero using the sequential Bonferroni procedure described above. This revealed the nature of the multivariate difference.

Interspecific Comparison.—The above analysis does not reveal whether diet-induced changes were exhibited equally by both species or if one species was primarily responsible for the narrowing of the gap when both were diet reversed. We expected the gap would be narrowed primarily by diet-induced change in the limnetic species (i.e., $L > B$ in fig. 1), thus reflecting greater adaptive phenotypic plasticity in the species with the more variable natural diet.

To test this expectation, we performed a one-way multivariate analysis of variance (MANOVA) (Johnson and Wichern 1982) comparing the mean diet-induced change of the 12 benthic families with the mean diet-induced change of the 12 limnetic families for all five traits. A significant Wilks' lambda indicates that there is a significant overall difference between species in phenotypic plasticity. The multivariate result was then decomposed using a sequential Bonferroni procedure (Rice 1989) on univariate ANOVA results for each trait.

Family-by-Diet Interaction.—For optimal levels of phenotypic plasticity to evolve, genetic variation for plasticity must exist in the population (Via 1987). Here we estimated family-by-diet interaction. This is a measure of broad-sense genetic variation for phenotypic plasticity and thus reflects whether plasticity has a genetic component. We estimated the significance of this interaction using a two-way mixed-model MANOVA (Johnson and Wichern 1982). Separate MANOVAs were carried out for each species. Family was considered a random factor here, and diet was considered a fixed factor.

A potential complication in calculating family-by-diet interaction arises from the confounding effects of microenvironment. Each of the 15 fish from a half-brood raised on the same diet was also raised in the same half-aquarium, and thus, they are not strictly independent. All of these fish experienced the same microenvironmental

(aquarium) effects during their growth. Because we assumed that sibs are independent when calculating the family-by-diet interaction term, our findings must be regarded as tentative. However, a previous quantitative-genetic analysis of sticklebacks by Lavin and McPhail (1987) demonstrated that aquarium effects on size-corrected measurements are negligible, lending support to our assumption.

There is considerable difficulty in interpreting the relationship between the dependent variables and the factors in a two-way MANOVA when there is a significant interaction effect (Morrison 1976; Johnson and Wichern 1982). Our purpose with this test was mainly to determine whether there was significant overall genetic variation for plasticity, and consequently, we did not attempt to examine the effect of diet or family in each species using this procedure. To probe the nature of the genetic variation for plasticity, we calculated two-way univariate ANOVAs for each trait of both species.

All analyses were carried out using Systat 5.01 on an IBM-compatible microcomputer (Wilkinson et al. 1992).

RESULTS

Effect of Diet

All diet-induced changes were in a direction that is suggestive of adaptive phenotypic plasticity. Limnetic fish raised on a littoral diet developed a morphology that was displaced toward that of the benthic species, relative to control fish raised on a planktonic diet. Benthic fish raised on a planktonic diet developed a morphology that was displaced toward that of the limnetic species, relative to control fish raised on a littoral diet. This trend was exhibited by all morphological characters examined except gill-raker number, which displayed no trend in change (fig. 2).

The results of the multivariate analysis reveal that the morphological gap between the benthic and limnetic species was significantly reduced by diet reversal (Hotelling's $T^2 = 45.14$; $df = 5, 7$; $0.01 < P < 0.05$). Thus, when considering all traits simultaneously, diet affects the degree to which these species differ morphologically. This suggests that differences in diet may contribute to the morphological difference between the two species in the wild.

Table 1 presents the percentage of reduction

of the morphological gap between the two species caused by diet reversal for each of the five traits. The percentage of reductions ranged from -1% for gill-raker number, to 58% for head depth. Also presented in table 1 are the results of the univariate t tests for each trait. Gill-raker length was the only trait that was significant using the sequential Bonferroni procedure. It is informative, however, that all traits except gill-raker number exhibited a diet-induced change in the same direction. Additionally, head depth had a P value less than 0.05, which suggests that it also contributed to the multivariate significance.

Interspecific Comparison

Figure 2 and table 2 suggest that the limnetic species tends to be more plastic than the benthic species in all traits except snout length and gill-raker number (which showed virtually no plasticity). A MANOVA confirmed that the two species differed in their amount of plasticity, although statistical significance is marginal ($P = 0.05$). Univariate ANOVAs (table 2) revealed that only gill-raker length displayed a significant difference in the magnitude of plasticity between the two species. All other traits had P values at least an order of magnitude larger than gill-raker length. Thus, the degree of plasticity of the two species appears more distinct in some traits than in others, suggesting the benthic and the limnetic species differ not only in the magnitude of plasticity but in the pattern of plasticity among these morphological characters as well.

Family-by-Diet Interaction

Multivariate analysis revealed the presence of significant family-by-diet interaction in both species ($P < 0.001$), indicating differences among families in the extent, or direction, or both, of diet-induced change. This is a measure of broad-sense heritability of phenotypic plasticity. Thus, there is genetic variability for morphological plasticity present in both species' populations.

Results of the univariate analysis are presented in table 3. Family-by-diet interaction in the benthic species is exhibited primarily by gape width, although gill-raker length and snout length had relatively small P values as well. Family-by-diet interaction in the limnetic species is exhibited primarily by gape width. This suggests that morphological plasticity has evolutionary potential and could respond to natural selection in both species of sticklebacks.

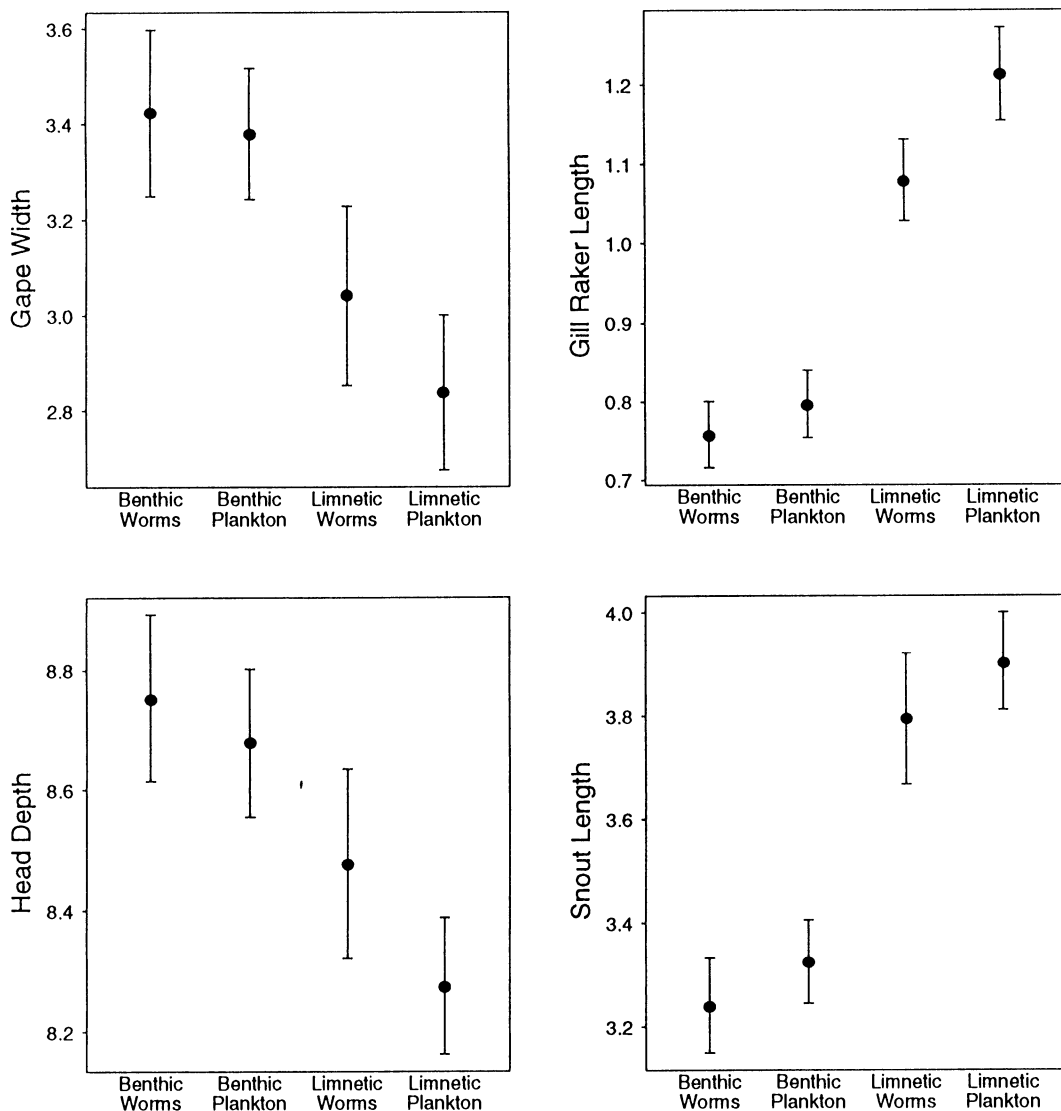


FIG. 2. Plots of the four species-by-diet combinations for all traits except gill-raker number (which exhibited no plasticity). Plots are the mean of the 12 family means in each combination (i.e., $N = 12$ for each of the means for each trait) and the 95% confidence intervals. Units are in millimeters.

DISCUSSION

The ecology of morphological plasticity is not well understood and is just beginning to receive empirical attention (Meyer 1990; Witte et al. 1990; Wimberger 1991, 1992). The ecological conditions that select for morphological plasticity are still unclear. Yet this knowledge is necessary to understand the interplay between selective regimes, plasticity, and morphological

evolution. The wider effects of morphological plasticity on niche partitioning, speciation, and adaptive radiation are also poorly understood. The extent to which plasticity accounts for morphological variation within and between populations would lend some insight into this problem.

Our study addressed four important issues of morphological plasticity using two sympatric species of sticklebacks. First, we demonstrated

TABLE 1. Absolute magnitude of the "morphological gap" between species raised on their "natural" diets and reversed diets. Values are calculated using the mean of the 12 family means from each species for each trait. Units are in millimeters except for gill-raker number. Asterisks indicate the results of univariate *t* tests for a reduction of the morphological gap between species.

Trait	Natural diet	Reversed diet	% Reduction
Gape width	0.585	0.339	42
Gill-raker length	0.456	0.283	38***
Gill-raker number	5.291	5.319	-1
Head depth	0.477	0.201	58*
Snout length	0.665	0.469	29

* $0.01 < P < 0.05$; *** $P < 0.001$.

that when the diets of the two species are reversed, their morphologies become more similar overall. This suggests that either or both species exhibit adaptive morphological plasticity. The individual traits that contributed most to this overall morphological change were gill-raker length and perhaps head depth. Both of these traits are probably important with respect to feeding efficiency (Bentzen and McPhail 1984; Lavin and McPhail 1986; Schluter 1993).

Second, we considered how much of the interspecific difference in morphology of these species can be attributed to plasticity. The percentage of the morphological gap between the two species that was closed by diet reversal ranged from -1% for gill-raker number, to 58% for head depth. Thus a considerable amount of the morphological difference between the two species in the wild might be attributable to diet-induced morphological change.

TABLE 2. Percentage of difference in each morphological character under diet reversal. Each value was calculated by dividing the absolute value of mean diet-induced change by the mean value of that character when the species was raised on its "natural" diet. Asterisks indicate a significant difference between species as tested using univariate ANOVAs.

Character	Benthic difference	Limnetic difference
	%	%
Gape width	1.8	7.1
Gill-raker length**	5.1	11.0
Gill-raker number	1.4	1.0
Head depth	0.8	2.5
Snout length	2.6	2.8

** $P < 0.01$.

TABLE 3. *F* ratios from univariate ANOVAs for family-times-diet interaction ($F \times D$ df = 11, Error df = 46 for all values).

Trait	Species	
	Benthic	Limnetic
Gape width	7.34***	13.76***
Gill-raker length	2.29*	0.68
Gill-raker number	1.58	1.99
Head depth	1.55	1.44
Snout length	2.18*	1.52

* $0.01 < P < 0.05$; *** $P < 0.001$.

Third, we compared the degree of plasticity between the two species, on the expectation that greater natural diet variability in the limnetic species would drive the evolution of greater plasticity in that species. The two species did exhibit a significant difference in overall level of phenotypic plasticity, and this difference was primarily attributable to a greater plasticity of gill-raker length in the limnetic than in the benthic species. Gill-raker length has been strongly implicated as a determinant of foraging efficiency in sticklebacks (Bentzen and McPhail 1984; Lavin and McPhail 1986; Schluter 1993), and therefore, these results lend support to the hypothesis that diet variability can select for morphological plasticity. Head depth and gape width also tended to be more plastic in the limnetic than in the benthic species, but these differences were not statistically significant (fig. 2).

Lastly, we addressed whether contemporary populations of benthics and limnetics maintain genetic variation for morphological plasticity. Theoretical work suggests that optimal levels of phenotypic plasticity can evolve, given appropriate genotype-by-environment interaction (Via and Lande 1985; Via 1987). The family-by-diet interaction we demonstrate reveals a broad-sense heritability of plasticity (Via 1984a, 1984b). This heritability includes variation resulting from dominance and epistatic interactions, as well as additive genetic variance (Falconer 1989). Although this does not completely satisfy the conditions required for the evolution of phenotypic plasticity, it does reveal that plasticity is genetically determined to some extent.

When making comparisons between species, it is often difficult to distinguish the effect of phylogeny from the effect of recent natural selection (Lauder 1982). Because the benthic and limnetic species have both recently evolved from

a common marine ancestor (McPhail 1993), and because both are relatively young species, phylogenetic effects are not responsible for their differences in plasticity.

Additionally, the wealth of natural-history information on these two species of sticklebacks and the well documented glacial history of this region make us confident that the ecological conditions under which these two species have evolved are relatively well understood. Consequently, the adaptive nature of all diet-induced morphological changes, the greater degree of plasticity in the limnetic species, and the presence of genetic variation for plasticity, together provide compelling evidence to support the hypothesis that morphological plasticity has evolved as a result of diet variability. It has often been suggested that diet variability might drive the evolution of trophic morphological plasticity.

At this point, however, two other hypotheses for the observed results deserve mention. One involves the possibility of differences between the two species in their ability to ingest prey. Because benthic fish are generally larger than limnetic fish in most dimensions, it may be easier for a benthic fish to ingest a planktonic prey than for a limnetic fish to ingest a littoral prey. Assuming that diet-induced morphological change results from mechanical stress (as discussed below), limnetic fish would probably exhibit greater morphological plasticity than benthic fish. However, if this were the case, an overall effect of body size on the degree of plasticity would also be expected. No interaction between size and the effect of diet was evident in the data, and consequently, we do not believe this to be the explanation for our results.

The second alternative basis for the interspecific difference in plasticity may lie in differences in the way benthic fish and limnetic fish are constructed. Since their divergence from a common ancestor, the limnetic species has evolved a less robust morphology than the benthic species. For example, gill rakers of the limnetic species are not only longer and more numerous than those of the benthic species, but they are more slender as well. Thus, if slender-built morphology is more susceptible to stress-induced change, this would explain how the difference in plasticity between the two species is determined. Conceivably, such differences in robustness have evolved merely as an incidental by-product of evolutionary divergence in body form, in which case interspecific

differences in plasticity are simply a (nonadaptive) correlated response to selection on the mean value of each trait. However, if robustness of morphology can evolve independent of the mean values of morphological traits, then such structural differences may be the proximate mechanism by which adaptive evolution of plasticity is realized. An experiment designed to distinguish between these alternatives is underway.

Diet-induced morphological plasticity could result from either nutritional differences between diets, or from differences in the mechanics of prey ingestion. Nutritional effects are confounded with mechanical effects in our experiment. Nutrition can be an important determinant of fish morphology (Halver 1984; Wimberger 1993). However, we feel that differences in the mechanics of prey ingestion are most important in our study. If nutritional differences were important, diet-induced morphological changes would likely have exhibited a more random pattern (Wimberger 1993). The pattern of diet-induced change demonstrated is consistent with the difference in morphology observed between many littoral-foraging and plankton-foraging species (Lavin and McPhail 1985, 1986; Schluter and McPhail 1993). Littoral and planktonic ecomorphs likely result from the mechanical requirements of foraging in these habitats, rather than from nutritional effects.

We suspect that mechanical stress is the cause of observed morphological plasticity in the traits we examined, because these traits are composed of either cartilage or bone. It has long been realized that structures made of bone remodel and change shape depending on the stresses imposed upon them (Lanyon 1984; Lanyon and Rubin 1985). The lack of diet-induced change in gill-raker number supports this hypothesis, because whereas mechanical stress can change the shape of particular structures, it cannot easily alter their number.

Behavioral plasticity also most likely plays an important role in adaptation to resource variability. Changes in foraging behavior have large effects on foraging efficiency (Dill 1983; Ehlinger 1989), and behavior is probably amenable to more rapid change than morphology. For example, very different modes of foraging are used to exploit plankton and benthos, and individual fish switch rapidly between them when moving between habitats (Schluter 1993). An experiment that tests the adaptive significance of behavioral

plasticity versus morphological plasticity is also currently underway.

Evolutionary Implications

Whether phenotypic plasticity retards or enhances evolution is still a matter of some debate (Stearns 1989; West Eberhard 1989). The distinction may be particularly important when considering the *Gasterosteus* species complex. Invasion of fresh water by marine *G. aculeatus* is pervasive throughout the holarctic region (Wootton 1976). Colonization of fresh water occurred as the Pleistocene glaciation ended and dramatic adaptive radiation ensued (Hagen and McPhail 1970; Bell 1976; McPhail 1993). Even slight differences among bodies of fresh water in the same drainage basin have led to fine-scale adaptation in resident sticklebacks (Lavin and McPhail 1985). Trophic morphology maps remarkably well onto lake ecology in all populations examined (Lavin and McPhail 1985; Schluter and McPhail 1992). Heritability of trophic morphology also provides compelling evidence that this diversity is the result of evolutionary change.

What has not been known is the extent to which this radiation owes its diversity to phenotypic plasticity. A comparison of several populations of sticklebacks in British Columbia (Schluter and McPhail 1992) has shown that gill-raker length, gape width, and gill-raker number are among the most variable traits among stickleback populations. Given that gill-raker length has exhibited considerable phenotypic plasticity, it is possible that some of this interpopulation variability is environmentally induced. However, there is no general relationship between trophic plasticity within populations and diet variability between populations. For example, gill-raker number was not affected by diet, though it differs markedly between populations (Schluter and McPhail 1992).

The effects of phenotypic plasticity on adaptive radiation and speciation in *Gasterosteus* fishes are not clear. It is evident that not all sticklebacks are equally phenotypically plastic, but the extent to which plasticity plays a role in the evolution of species pairs, such as the benthic and the limnetic, is unknown. Given that trophic character displacement is an important component of evolution and speciation in sticklebacks (Schluter and McPhail 1992), it is possible that plasticity of trophic traits plays a very important role as well.

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LITERATURE CITED

- Bell, M. A. 1976. Evolution of phenotypic diversity in *Gasterosteus aculeatus* superspecies on the Pacific Coast of North America. *Systematic Zoology* 25:211–227.
- 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. *Canadian Journal of Zoology* 62:2280–2286.
- Bernays, E. 1986. Diet-induced head allometry among foliage-chewing insects and its importance for graminivores. *Science* 231:495–497.
- Bradshaw, A. D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics* 13:115–155.
- Clague, J. J. 1983. Glacio-isostatic effects of the Cordilleran Ice Sheet, British Columbia, Canada. Pp. 321–343 in D. E. Smith and A. G. Dawson, eds. *Shorelines and isostasy*. Academic Press, London.
- Clague, J. J., J. R. Harper, R. J. Hebda, and D. E. Howes. 1982. Late Quaternary sea levels and crustal movements, coastal British Columbia. *Canadian Journal of Earth Sciences* 19:597–618.
- Dill, L. 1983. Adaptive flexibility in the foraging behaviour of fishes. *Canadian Journal of Fisheries and Aquatic Science* 40:398–408.
- Ehlinger, T. J. 1989. Learning and individual variation in bluegill foraging: habitat-specific techniques. *Animal Behaviour* 38:643–658.
- Falconer, D. S. 1989. *Introduction to quantitative genetics*, 3d ed. Longman, Essex, U.K.
- Gomulkiewicz, R., and M. Kirkpatrick. 1992. Quantitative genetics and the evolution of reaction norms. *Evolution* 46:390–411.
- Greene, E. 1989. A diet-induced developmental polymorphism in a caterpillar. *Science* 243:643–646.
- Gross, H. P., and J. M. Anderson. 1984. Geographic variation in the gillrakers and diet of European threespine sticklebacks, *Gasterosteus aculeatus*. *Copeia* 1984:87–97.
- Hagen, D. W., and L. G. Gilbertson. 1972. Geographic variation and environmental selection in *Gasterosteus aculeatus* L. in the Pacific Northwest, America. *Evolution* 26:32–51.
- Hagen, D. W., and J. D. McPhail. 1970. The species

- problem within *Gasterosteus aculeatus* on the Pacific Coast of North America. *Journal of Fisheries Research Board of Canada* 27:147–155.
- Halver, J. E. 1972. The vitamins. Pp. 30–97 in J. E. Halver, ed. *Fish nutrition*. Academic Press, San Diego, Calif.
- Johnson, R. A., and D. W. Wichern. 1982. *Applied multivariate statistical analysis*. Prentice-Hall, Englewood Cliffs, N.J.
- Lanyon, L. E. 1984. Functional strain as a determinant for bone remodeling. *Calcified Tissue International* 36:S56–S61.
- Lanyon, L. E., and C. T. Rubin. 1985. Functional adaptations in skeletal structures. Pp. 1–25 in M. Hildebrand, D. M. Bramble, K. F. Liem, and D. B. Wake, eds. *Functional vertebrate morphology*. Belknap Press, Cambridge, Mass.
- Lauder, G. V. 1982. Historical biology and the problem of design. *Journal of Theoretical Biology* 97: 57–67.
- Lavin, P. A., and J. D. McPhail. 1985. The evolution of freshwater diversity in the threespine stickleback (*Gasterosteus aculeatus*): site-specific differentiation of trophic morphology. *Canadian Journal of Zoology* 63:2632–2638.
- . 1986. Adaptive divergence of trophic phenotype among freshwater populations of threespine stickleback (*Gasterosteus aculeatus*). *Canadian Journal of Fisheries and Aquatic Science* 43:2455–2463.
- . 1987. Morphological divergence and the organization of trophic characters among lacustrine populations of threespine stickleback (*Gasterosteus aculeatus*). *Canadian Journal of Fisheries and Aquatic Science* 44:1820–1829.
- Lindsey, C. C. 1962. Observations on meristic variation in ninespine sticklebacks, *Pungitius pungitius*, reared at different temperatures. *Canadian Journal of Zoology* 40:1237–1247.
- Lindsey, C. C., and R. W. Harrington. 1972. Extreme vertebral variation induced by temperature in a homozygous clone of self-fertilizing cyprinodontid fish *Rivulus marmoratus*. *Canadian Journal of Zoology* 50:733–744.
- Mathews, W. H., J. G. Fryles, and H. W. Nasmith. 1970. Postglacial crustal movements in southwestern British Columbia and adjacent Washington state. *Canadian Journal of Earth Sciences* 7:690–702.
- McPhail, J. D. 1992. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): evidence for a species pair in Paxton Lake, Texada Island, British Columbia. *Canadian Journal of Zoology* 70:361–369.
- . 1993. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of southwestern British Columbia. Pp. 399–437 in M. A. Bell and S. A. Foster, eds. *Evolutionary biology of the threespine stickleback*. Oxford University Press, Oxford.
- Meyer, A. 1987. Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution* 41(6):1357–1369.
- . 1990. Ecological and evolutionary consequences of the trophic polymorphism in *Cichlasoma citrinellum* (Pisces: Cichlidae). *Biological Journal of the Linnean Society* 39:279–299.
- Morrison, D. F. 1976. *Multivariate statistical methods*. McGraw-Hill, New York.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225.
- Scheiner, S. M. 1993. Plasticity as a selectable trait: reply to Via. *American Naturalist* 142:371–373.
- Schlichting, C. D., and D. A. Levin. 1986. Phenotypic plasticity: an evolving plant character. *Biological Journal of the Linnean Society* 29:37–47.
- Schluter, D. 1993. Adaptive radiation in sticklebacks: size, shape and habitat use efficiency. *Ecology* 74:699–709.
- Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. *American Naturalist* 140:85–108.
- . 1993. Character displacement and replicate adaptive radiation. *Trends in Ecology and Evolution* 8:193–227.
- Skulason, S., Noakes, D. L. G., and S. S. Snorrason. 1989. Ontogeny of trophic morphology in four sympatric morphs of arctic char *Salvelinus alpinus* in Thingvallavatn, Iceland. *Biological Journal of the Linnean Society* 38:281–301.
- Snorrason, S. S., S. Skulason, O. T. Sandlund, H. J. Malmquist, B. Jonsson, and P. M. Jonasson. 1989. Shape polymorphism in arctic char, *Salvelinus alpinus*, in Thingvallavatn, Iceland. *Physiology and Ecology Japan* 1:393–404.
- Spitze, K. 1992. Predator-mediated plasticity of prey life history and morphology: *Chaoborus americanus* predation on *Daphnia pulex*. *American Naturalist* 139:229–247.
- Stearns, S. C. 1983. The evolution of life-history traits in mosquitofish since their introduction to Hawaii in 1905: rates of evolution, heritabilities, and developmental plasticity. *American Zoologist* 23:65–76.
- . 1989. The evolutionary significance of phenotypic plasticity. *Bioscience* 39:436–445.
- Thompson, D. B. 1992. Consumption rates and evolution of diet-induced plasticity in the head morphology of *Melanoplus femurrubrum* (Orthoptera: Acrididae). *Oecologia* 89:204–213.
- Via, S. 1984a. The quantitative genetics of polyphagy in an insect herbivore. I. Genotype–environment interaction in larval performance on different host plant species. *Evolution* 38:881–895.
- . 1984b. The quantitative genetics of polyphagy in an insect herbivore. II. Genetic correlations in larval performance within and among host plants. *Evolution* 38:896–905.
- . 1987. Genetic constraints on the evolution of phenotypic plasticity. Pp. 46–71 in V. Loeschcke, ed. *Genetic constraints on adaptive evolution*. Springer, Berlin.
- Via, S., and R. Lande. 1985. Genotype–environment interaction and the evolution of phenotypic plasticity. *Evolution* 39:505–522.
- West Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* 20:249–278.
- Wilkinson, L. 1975. Response variable hypotheses in the multivariate analysis of variance. *Psychological Bulletin* 82:408–412.

- Wilkinson, L., M. Hill, J. P. Welna, and G. K. Birkenbeuel. 1992. Systat for windows. Systat Inc., Evanston, Ill.
- Wimberger, P. H. 1991. Plasticity of jaw and skull morphology in the Neotropical cichlids *Geophagus brasiliensis* and *G. steindachneri*. *Evolution* 45:1545–1563.
- . 1992. Plasticity of fish body shape. The effects of diet, development, family and age in two species of *Geophagus* (Pisces: Cichlidae). *Biological Journal of the Linnean Society* 45:197–218.
- . 1993. The effects of vitamin C deficiency on body shape and skull osteology in *Geophagus brasiliensis*: implications for interpretations of morphological plasticity. *Copeia* 1993:343–351.
- Witte, F. 1984. Consistency and functional significance of morphological differences between wild-caught and domestic *Haplochromis squamipinnis* (Pisces: Cichlidae). *Netherlands Journal of Zoology* 34:596–612.
- Witte, F., C. D. N. Barel, and R. J. C. Hoogerhoud. 1990. Phenotypic plasticity of anatomical structures and its ecomorphological significance. *Netherlands Journal of Zoology* 40:278–298.
- Wootton, R. J. 1976. *The functional biology of sticklebacks*. Academic Press, London.

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