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Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*)

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Summary

Adaptive variation can exist at a variety of scales in biological systems, including among species, among local populations of a single species and among individuals within a single population. Trophic or resource polymorphisms in fishes are a good example of the lowest level of this hierarchy. In lakes without bluegill sunfish (*Lepomis macrochirus*), pumpkinseed sunfish (*Lepomis gibbosus*) can be trophically polymorphic, including a planktivorous limnetic form found in the pelagic habitat, in addition to the usual benthic form found in the littoral zone. In this paper we examine the degree to which morphological differences between the two forms are caused by genetic differences versus phenotypic plasticity. Adults from pelagic and littoral sites in Paradox Lake, NY, were bred separately and their progeny were raised in cages both in the open water and shallow water habitats of an artificial pond. The experimental design permitted two tests of genetic differences between the breeding stocks (in open and shallow water cages, respectively) and two tests of phenotypic plasticity (in the limnetic and benthic offspring, respectively). Limnetic progeny were more fusiform than benthic progeny raised in the same habitat. In addition, progeny of both stocks displayed limnetic-type characteristics when raised in the open water and benthic-type characteristics in the shallow water. Thus, genetic differences and phenotypic plasticity both contributed to the trophic polymorphism. Phenotypic plasticity and genetic differentiation accounted for 53 and 14%, respectively, of the variation in morphology. This study addresses the nature of subtle phenotypic differences among individuals from a single population that is embedded within a complex community, a condition that is likely to be the norm for most natural populations, as opposed to very large differences that have evolved in relatively few populations that reside in species-poor environments.

Keywords: common environment; reciprocal transplant; genetic differentiation; phenotypic plasticity; trophic polymorphism; evolution; specialization; fish

Introduction

Morphological and behavioural variation exists at a variety of scales, including among species, among local populations of the same species and among individuals within local populations. At each scale, variation can be interpreted as adaptive or non-adaptive. For instance, when David Lack first visited the Galapagos Islands in the 1940s, the prevailing view was that most differences between closely related species arose from processes such as genetic drift and did not have an adaptive explanation. Lack (1947) showed that adaptation to multiple niches could explain many differences among the Galapagos finch species. His and other studies resulted in a paradigm shift in which adaptation became the primary explanation of species-level differences, rather than an explanation of last resort.

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A similar shift occurred in the interpretation of population-level differences during the 1960s and 1970s. Bradshaw *et al.* (1965), Ehrlich and Raven (1969), Endler (1973, 1980, 1982), Boag and Grant (1981) and others showed that gene flow is sufficiently weak and opposing selection pressures are sufficiently strong for differences among local populations to be explained in the same way as species-level differences; that is, as the product of natural selection. This was not obvious to the architects of the modern synthesis, who tended to assume that selection pressures were too weak and gene flow too strong for local adaptation to occur (e.g. Mayr, 1963).

The interpretation of variation at the smallest scale remains uncertain. If two individuals from a single population differ in their morphology and behaviour, do we interpret the differences as adaptive or non-adaptive? If the differences are adaptive, do they reflect genetic differences or the ability of single genotypes to change their phenotype in response to local conditions? Obviously, there is no single answer to this question and we cannot expect *all* differences to be adaptive at any scale. Nevertheless, in the past there has been a tendency to interpret individual differences within single populations as non-adaptive noise around an adaptive mean. Studies during the 1980s and 1990s, however, are increasingly showing that the differences themselves are adaptive. Thus, the same shift that occurred at the scale of species and local populations seems to be occurring at the scale of individuals within populations. Because it is an ongoing shift, however, it is taking place unequally in the various subdisciplines of evolutionary ecology. For example, it is now common to expect alternative mating strategies within a single population, in particular among males (e.g. Dominey, 1980; Gross and Charnov, 1980; Gross, 1985; Ryan and Causey, 1989; Shuster, 1989; van den Berghe *et al.*, 1989; Lodi and Malacarne, 1991). It is less common to expect alternative feeding strategies.

These differences among subdisciplines can be partially explained by the theoretical models on which they are based. Studies of mating behaviour are based on evolutionary game theory, in which the fitness of a given strategy depends on what other strategies are present in the population, often leading to stable phenotypic polymorphisms (Maynard Smith, 1982). Studies of foraging behaviour, on the other hand, are based on optimization theory, which assumes that individuals interact with a static environment, leading to a single best strategy for any given environmental situation (Stephens and Krebs, 1986). It is therefore not surprising that virtually all empirical tests of optimal foraging theory compare the average behaviour of the study population to the predictions of a given model and do not attempt to interpret individual differences that are present within the study population (see, for example, all of the studies listed in Table 9.1 of Stephens and Krebs, 1986).

Recently, we reviewed a large literature on fish which shows that variation in foraging behaviour within single populations is often adaptive (Robinson and Wilson, 1994; see also Skulason and Smith, 1995). In other words, individuals are often morphologically and behaviourally specialized to forage on different resources or in different habitats, much as species are adapted to exploit different niches. Indeed, there is a strong relationship between inter- and intraspecific variation in fishes, since the most impressive examples of adaptive individual differences within populations occur in species-poor lakes. The relationship between form and function is well known among different fish taxa (Lauder, 1989). For example, a streamlined body is energetically efficient for foraging for prey that are patchily distributed in large volumes of open water. In contrast, shorter and deeper-bodied plans with paired lateral fins off centre are better for navigating through structurally complex habitats, such as the littoral zones of lakes and ocean reefs (Webb, 1975, 1984a, b; Weihs, 1989; Videler, 1993). Functional diversification also includes differences in mouth shape and location, eye size (Lindsey, 1981), gill raker morphology (Magnuson and Heitz, 1971) and specialized feeding structures such as pharyngeal jaws (Liem, 1974), to name a few. Trophic diversification at the intraspecific level often parallels these patterns in many lake fishes.

Single populations can be composed of both open water types (often termed 'limnetic' or 'pelagic' forms) and shallow water types (termed 'benthic' or 'littoral' forms; reviewed in Robinson and Wilson, 1994).

At the intrapopulation level, adaptive variation in foraging behaviour is expected for the same reason as adaptive variation in mating behaviour, because the fitness of a given phenotype depends on what other phenotypes are present in the population. In the case of foraging, however, the interaction among phenotypes occurs indirectly, through their effects on resource abundance (Pimm, 1978; Rosenzweig, 1978; Wilson and Turelli, 1986; Wilson, 1989; Hori, 1993; Schluter, 1994), rather than directly as in the case of mating. These indirect effects are not included in most optimal foraging models, resulting in a misleading expectation of a single optimal strategy.

Once adaptive phenotypic variation has been documented within a single population, it becomes important to know how it is produced. In particular, are the foraging specialists genetically different or do they reflect the ability of single genotypes to produce a range of morphologies and behaviours based on local conditions? The answer to this question has important implications for a number of important issues in evolutionary ecology, including speciation (Meyer, 1987; West Eberhard, 1989; Stearns, 1989; Skulason and Smith, 1995), the evolution of ecological specialization (Via and Lande, 1985; Futuyma and Moreno, 1988; Joshi and Thompson, 1995) and the implications of phenotypic plasticity for evolutionary change (Bull, 1987; Maynard Smith, 1987; Schlichting, 1989; Cheplick, 1991).

In this paper we attempt to measure the relative importance of genetic differences versus phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). The pumpkinseed trophic polymorphism is especially interesting because it is *not* particularly dramatic. As mentioned above, the most impressive trophic polymorphisms occur in species-poor lakes and can rival differences between species in magnitude (e.g. Behnke, 1972; Lindsey, 1981; Todd *et al.*, 1981; Sandlund *et al.*, 1987; Skulason *et al.*, 1992; Snorrason *et al.*, 1994b). By contrast, the pumpkinseeds in our population co-exist with at least 15 other fish species. Intraspecific morphological variation can only be detected with multivariate statistical methods and is not obvious to the human eye. Nevertheless, morphological variation is both highly replicable (Robinson *et al.*, 1993; Robinson, 1994) and has important consequences for fitness, measured in terms of growth rate and stored fat (Robinson *et al.*, 1996). Thus, our study addresses the nature of very small differences among individuals that probably occur in most populations, as opposed to the very large differences that have evolved in just a few populations.

Methods

We used a reciprocal transplant method, where the progeny of limnetic and benthic types were reared in two common environments: the open water and shallow littoral habitats. This allowed us to test for four particular features of this trophic polymorphism.

(1) Genetic differences in morphology between offspring types (morphological comparison between the two offspring types in each environment). This is the classic test for genetic effects between the different types of offspring.

(2) Morphological plasticity of each offspring type (comparison within each offspring type between environments). This is the classic test for phenotypic plasticity that yields norms of reaction for each type of offspring across environments.

(3) Comparison of phenotypic plasticity between progeny types, measured as the interaction between offspring and environment effects.

(4) The relative importance of each mechanism to the morphological differentiation.

Breeding and stocking of cages in common environments

The research was conducted at Cornell University's experimental pond facility. The square ponds are 30.5 m on a side at the surface, have a maximum depth of 2.4 m and contain 1000 m^3 of water. Three ponds without other fish species were used. One each for breeding the limnetic and benthic adults and the third for rearing their offspring in cages placed in common environments. All ponds were qualitatively similar with respect to macrophyte composition, water clarity and open water zones free of vegetation.

Parental stocks were collected from Paradox Lake (Essex Co., NY) in the autumn of 1991 and overwintered in aquaria at Binghamton University. This overwintering period would have mitigated many short-term environmental differences between the two samples. The benthic sample was collected from a large zone of vegetation at the eastern (inlet) end of the lake, while the limnetic sample was collected 1.3 km away in the open water habitat surrounding Grass Island. Although we did not analyse morphological differences between these parental stocks, other samples collected on four separate occasions between 1989 and 1993 from these sites were always morphologically differentiated (unpublished results). Fish were angled with small barbless hooks baited with worms. The 23 benthic and 26 limnetic adults that made up the parental groups were almost evenly composed of mature males and females. Each breeder group was released into a separate pond on 3 June 1992. Male pumpkinseeds nested by 7 June and within 1 week at least eight occupied nests were found in each breeding pond (more than half of which contained fertilized eggs). Schools of free-swimming fry were observed in both ponds by 25 June.

Offspring were collected from their natal ponds and stocked in cylindrical cages placed in the open water and the shallow vegetated zone of the third pond. Because progeny were too small to be individually marked and mixed together in single cages, the cages were stocked separately with the two offspring types and placed in close proximity to each other in a common environment. Cages placed in the shallow zone of the pond were open ended so that they could be anchored around growing vegetation. Cages suspended in the open water habitat were entirely enclosed. Cages were constructed of flexible black nylon screening (mesh size = 1.1 mm^2) sewn around an open-ended cylinder of galvanized steel fencing (1.04 m high, 1.02 m in diameter and 0.85 m^3 in volume). Four replicate cages for each offspring type were placed in the shallow and the open water habitats for a total of 16 cages. Shallow water cages were sealed to the bottom by attaching a chain to the bottom edge of the mesh which was pushed into the soft substrate, by staking the wire frame to the bottom and by pouring gravel around the outside of each cage. The seal was then checked by a diver. Open water cages were wired shut at the top and suspended 50 cm below the surface from a raft in the centre of the pond.

One hundred pumpkinseed fry were stocked into each cage between 24 and 30 July for a total of 1600 fry (e.g. 800 limnetic and benthic progeny). The juveniles were not older than 51 days and the majority were less than 38 days of age because large fry were not used. Ideally, larvae or even fertilized eggs may have been stocked in order to minimize any natal pond effects. However, this was not possible because sunfish eggs and fry are extremely fragile. Fry at 1 month of age were the youngest and smallest that could be collected in any numbers without severe handling effects (even at this size, significant mortality occurred within cages as discussed below). On each stocking day, fry were collected from their natal ponds using a seine (mesh size = 0.8 mm^2) and immediately placed into a wading pool. Individuals were then sampled from the pool with dip nets, placed into buckets and released into randomly chosen cages for that offspring type. Cages were stocked in the following schedule (two environments: open and shallow; two progeny types in each environment: limnetics (L) and benthics (B)). Open water cages were stocked twice: on 24 July (40 B and 40 L into their respective cages) and 29 July (60 B and 60 L). Shallow water cages were stocked three

times: on 24 July (40 B and 40 L), 28 July (15 B and 15 L) and 29 and 30 July (respectively, 45 L and 45 B). Adding individuals to each cage on at least two separate occasions decreased the chance of a consistent sampling bias. Although we did not measure each contributed fish because of adverse handling effects, on each day the largest and smallest fish were measured. The sizes overlapped among all cages between 9 and 19 mm standard length. A diver checked the cages every 10 days and brushed their outsides to minimize fouling. After 40 days, 15–20 individuals were trapped from each cage, narcotized in MS-222 and placed in 10% neutral buffered formalin. After a further 35 days (11 November) all remaining fish were collected. Following 2.5 months of preservation in formalin, the samples were rinsed in water and stored in 70% ethyl alcohol.

Morphometrics and statistical analyses

The experimental results presented in this paper are based on the 534 juveniles collected at the end of the rearing period at approximately 113 days of age (38 days old at the start plus 75 treatment days), because these fish were larger and easier to digitize than the samples collected a month earlier. Thirteen landmark points were digitized on the right side of each fish and 19 distances were measured between pairs of points with an image analysis system (Meacham and Duncan, 1989). Two additional measurements were calculated: the pectoral fin aspect ratio (the ratio of its width at insertion to its maximum length) and the multivariate centroid body size (the sum of squared distance measurements whose end-points lie on the periphery of a shape; Ehlinger, 1991; Bookstein *et al.*, 1985). With the exception of the aspect ratio and pectoral fin insertion width, these are the same measurements used to analyse morphological differentiation in adult phenotypes sampled from Paradox Lake and reported in Robinson *et al.* (1993). All measurements were transformed to their natural logarithms to assure linearity in the analyses below (the aspect ratio was multiplied by a factor of 10 before transformation).

Three statistical analyses were performed on the morphological data, two that tested the significance of morphological differentiation among the four treatment groups (two progeny types reared in two environments) and one that identified the nature of the morphological differences.

(1) We used a multivariate discriminant function analysis (DFA; Pedhazur, 1982) to detect significant variation in morphology among the four treatment groups. This method calculated the optimal discrimination among treatment groups in three-dimensional morphological space (each dimension described by a canonical factor or linear combination of the weighted measurements). Because we wanted to discriminate groups using body shape, we needed to minimize any effect of body size. To do this, we first regressed each morphological measurement alone against centroid size and calculated residual values. These size-free residuals were then analysed in the DFA.

(2) We also performed a more conservative multivariate test to confirm the results of the DFA above. This compared the morphological variation among groups against the random variation among replicate cages. The three sets of discriminant function scores above (one for each factor) were analysed together using a multivariate analysis of variance (type II MANOVA factors: offspring type and rearing environment; Sokal and Rohlf, 1981). The main effects and their interaction were tested against the mean-squared replicate variance, which reduced the overall degrees of freedom to 16, making this analysis more conservative than the DFA. In addition, each factor's scores were analysed using a type II ANOVA to estimate the components of variance due to environment and offspring effects and their interaction.

(3) While both multivariate methods tested the significance of morphological differences among treatment groups, they did not quantify how fish from alternate treatments differed with regard to single body measurements. In order to do this, we compared the four groups trait-by-trait, using a

two-factor analysis of covariance (ANCOVA factors: offspring type and rearing environment) with the centroid size as covariate. For each morphological measurement, group means were calculated adjusted for centroid size. Because the body measurements are intercorrelated, the individual test statistics from these ANCOVAs are inflated (type 1 error) and are not reported. We provide these results only to demonstrate the relative change among groups for each measurement, and not to assess statistical significance among groups (which is provided by the two multivariate analyses above). All analyses were performed using Systat 5.2 software (Wilkinson, 1989).

Results

Survivorship within cages averaged 50%, which is typical for experiments that require the collection and handling of juvenile fish of this size (C.W. Osenberg, personal communication). Environment and offspring effects had no influence on survivorship (type II ANOVA of final density: environment $F = 0.419$, $p = 0.530$; offspring type $F = 0.006$, $p = 0.939$; interaction $F = 0.081$, $p = 0.781$; $n = 16$, $R^2 = 0.04$), nor on final body size (type II ANOVA of centroid size: environment $F = 4.03$, $p = 0.068$; offspring type $F = 0.735$, $p = 0.408$; interaction $F = 3.17$, $p = 0.100$), although significant differences in body size occurred among replicates (replicate effects: $F = 4.80$, df = 12, 519, $p < 0.001$). Despite this, body sizes broadly overlapped among all cages as indicated by the relatively low explanatory power of the analysis ($R^2 = 0.168$).

Morphological differentiation

The discriminant function analysis clearly indicated that the four treatment groups were distinct in multivariate morphological space (Wilks' $\lambda = 0.322$, $F = 11.75$, $p < 0.0001$), with 343 of the 535 fish (64%) classified to their correct treatment group (compare with the random expectation of 134 or 25% correctly classified). Figure 1 displays a scatter plot of the first two factor scores from this analysis with 50% ellipsoids about the centre of each group for reference. Note that in this experiment, the first factor represented variation within offspring types across the two environments and, thus, seemed to reflect morphological plasticity. Morphological variation on the second factor represented differences between offspring types within environments and, hence, reflected genetic differences between the two progeny types in each environment. Variation among groups on the third factor (not shown in Fig. 1) included both effects. The canonical loadings of each body measure on the three factors are given in Table 1 along with the canonical correlations for each factor. The canonical correlations indicate the relative importance of each factor in discriminating among the treatment groups (respectively, 65, 26 and 9% for the first to third factors; Pedhazur, 1982, p. 757). Because the third factor added little additional discriminating power, we limit subsequent analyses to the first two factors except where noted.

Significant morphological variation among the four treatment groups was confirmed with the more conservative two-factor MANOVA of the three sets of factor scores (environment Wilks' $\lambda = 0.02$, $F = 164$, $p < 0.0001$; offspring type Wilks' $\lambda = 0.22$, $F = 11.84$, $p = 0.001$; interaction Wilks' $\lambda = 0.425$, $F = 4.51$, $p = 0.03$; replicates Wilks' $\lambda = 0.781$, $F = 3.71$, $p < 0.0001$). We now examine these results in greater detail in order to contrast what effects offspring type and rearing environment had on the observed morphological differentiation.

Genetic differentiation

We confirmed that only offspring effects had a significant influence on morphological variation on the second factor by independently analysing these scores with a two-way ANOVA (factors: offspring $p = 0.0001$; environment $p =$ ns; see caption of Fig. 2 for details). The variance components on this factor due to offspring type, environment and their interaction are 0.52, 0 and

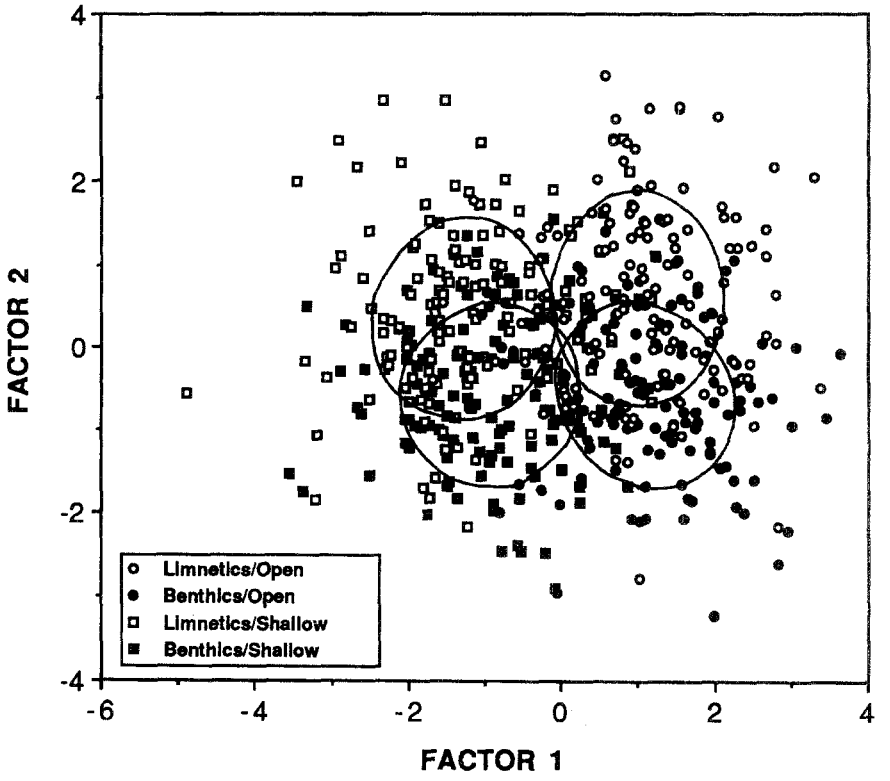

Figure 1. Distribution of the factor scores from the discriminant function analysis (DFA) of the four offspring–environment groups with 50% ellipsoids about the centroid of each group plotted on the first two factors. Sixty-four percent of the fish were classified to the correct treatment group ($p < 0.0001$). Note that the first factor predominantly represented morphological variation within offspring types across environments or variation due to phenotypic plasticity. Morphological variation on the second factor represented differences between offspring types within environments indicating genetic differences between the two progeny types. A third factor from the DFA is not shown. Size effects were removed by analysing residual values calculated by regressing each body measurement against centroid size. All shape and size data were first transformed to their natural logarithms.

0.002 respectively (Sokal and Rohlf, 1981, p. 216). The morphological differentiation of the two offspring types was qualitatively the same regardless of rearing environment (offspring effect in Table 2). Thirteen of the 20 body measures responded to offspring effects in the same direction in both rearing environments. Focusing on these environmentally constant morphological differences between offspring types, limnetic offspring generally displayed longer heads, longer pectoral fins with a smaller aspect ratio positioned lower on the body, a shorter anal fin base length, generally shallower bodies and longer standard lengths in comparison to benthic offspring.

Phenotypic plasticity

Morphological variation on the first factor alone was significantly influenced by rearing environment and to a much lesser extent by offspring effects (two-way ANOVA: environment $p < 0.0001$; offspring $p = 0.022$; see caption to Fig. 3 for details). The variance components on the first factor due to environment, offspring type and their interaction are, respectively, 0.81, 0.012

and 0.0003 (e.g. the environmental response was almost 68 times stronger than offspring effects on this factor). The morphological responses of both types of offspring to the rearing environment were also similar (environment effect in Table 2). Seventeen of the 20 body measures responded to the rearing environment in the same direction. Graphical examples of the similarity in the two progeny's mean norms of reaction across environments are given in Fig. 4 for two size-adjusted measurements: the pre-pectoral length and pectoral fin aspect ratio. The open water environment induced somewhat longer heads (except for the predorsal length), longer and thinner pectoral fins with a smaller aspect ratio positioned lower on the body and longer caudal peduncles.

Table 1. Canonical loadings (the correlation) of each body measurement with each of the three factors or axes in the discriminant function analysis

Body measurement	Factor 1 (environment)	Factor 2 (offspring)	Factor 3 (mixed)
Body lengths			
Head			
Pre-pelvic length	0.184	0.107	0.109
Pre-dorsal length	−0.202	0.364	0.26
Pre-pectoral length	0.153	0.352	0.008
Mid-body			
Dorsal fin base length	0.086	0.092	0.406
Anal fin base length	−0.208	−0.029	0.041
Anterior anal fin to anterior pelvic fin	−0.054	−0.079	−0.205
Tail			
Dorsal caudal peduncle length	0.13	−0.066	−0.333
Ventral caudal peduncle length	0.144	−0.14	−0.133
Standard length	−0.075	0.35	−0.124
Body depths			
Mid-body			
Anterior dorsal fin to anterior pelvic fin	0.42	−0.072	0.041
Anterior dorsal fin to anterior anal fin	0.145	−0.116	0.217
Posterior dorsal fin to anterior anal fin	−0.115	−0.168	0.232
Tail			
Anterior caudal peduncle depth	0.157	−0.167	0.129
Posterior caudal peduncle depth	−0.117	−0.279	0.25
Posterior anal fin to dorsal tail fin	0.075	−0.241	0.03
Fin size and position			
Pectoral altitude (from dorsal)	0.251	0.094	−0.039
Pectoral fin maximum length	0.037	0.316	0.08
Pectoral fin width at insertion	−0.163	−0.031	0.047
Pectoral fin aspect ratio	−0.16	−0.186	0.015
Pelvic fin maximum length	0.162	−0.233	0.024
Canonical correlations	0.743	0.468	0.282

The four offspring–environment treatments were significantly differentiated from each other in morphological space ($p < 0.0001$) as described in the text. Note that a measure of body depth is the most important element of the first factor (phenotypic plasticity) and that body length measures are important on the second factor (genetic differentiation). Size effects were removed by analysing residual values calculated by regressing each body measurement against centroid size. The canonical correlations for each factor are given at the bottom of the table. Factors 1 and 2, respectively, represent morphological variation due to phenotypic plasticity or genetic differences between offspring types as shown in Fig. 1.

Morphological differences between the two environments also arose on a variety of body depth and length measures, but these did not uniformly respond to the environment. For example, body depth was measured at the front and back of two distinct body regions: the mid-body and the caudal peduncle. The open water induced the greatest body depth at the front of both of these regions, while the shallow water resulted in deeper bodies at the rear of these two regions. Our measurements of body length were across three regions: head length, mid-body length and caudal peduncle length. While the open water induced longer heads and tails (and relatively short mid-bodies), the shallow water environment induced longer mid-bodies (and shorter heads and tails) to

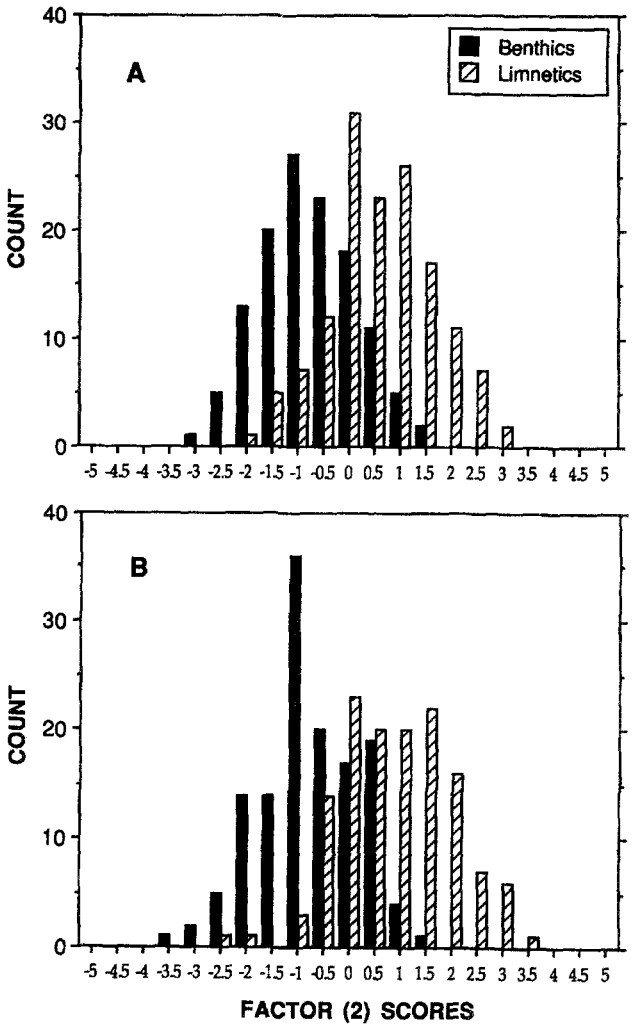

Figure 2. Histograms of the second factor's scores from the discriminant function analysis showing genetically based differences in morphology between offspring types within (A) the shallow and (B) the open water environment. Filled and hatched bars, respectively, represent the benthic and limnetic progeny types in each environment. Only offspring effects influenced morphological variation on the second factor (type II ANOVA of the second factor's scores alone: offspring $F = 35.47, p = 0.0001$; environment $F = 0.126$, $p = 0.729$; interaction $F = 0.363, p = 0.558$; replicates $F = 4.20, p < 0.0001$; $R^2 = 0.288$; main effects tested against mean-squared replicate variance).

such an extent that the overall standard length was unexpectedly longer in the shallow water cages.

There were only minor differences between benthics and limnetics in their plastic response to the rearing environment. While the MANOVA analysis of all three sets of factor scores combined suggested a significant interaction between offspring type and environment (see above), this was not present in the separate analyses of the scores from either the first or second factors (see the captions to Figs 2 and 3). A significant interaction effect was detected in the third factor's scores

Table 2. Mean differences between the four offspring–environment treatment groups nested under the main effects of the two-factor ANCOVA with centroid size as covariate

	Main effects			
	Offspring (limnetics–benthics)		Environment (open–shallow)	
Body measurement	Open	Shallow	Limnetics	Benthics
Body lengths (mm)				
Head				
Pre-pelvic length	0.03	− 0.03	0.14	0.08
Pre-dorsal length	0.13	0.05	− 0.06	− 0.14
Pre-pectoral length	0.11	0.08	0.14	0.11
Mid-body				
Dorsal fin base length	0.08	− 0.06	0.12	− 0.02
Anal fin base length	− 0.01	− 0.01	− 0.12	− 0.12
Anterior anal fin to anterior pelvic fin	− 0.04	0.02	− 0.05	0.01
Tail				
Dorsal caudal peduncle length	− 0.09	0.03	0.01	0.13
Ventral caudal peduncle length	− 0.09	− 0.03	0.05	0.11
Standard length	0.06	0.06	− 0.03	− 0.03
Body depths (mm)				
Mid-body				
Anterior dorsal fin to anterior pelvic fin	− 0.03	− 0.07	0.25	0.21
Anterior dorsal fin to anterior anal fin	0	− 0.07	0.11	0.04
Posterior dorsal fin to anterior anal fin	− 0.01	− 0.05	− 0.03	− 0.07
Tail				
Anterior caudal peduncle depth	− 0.03	− 0.04	0.06	0.05
Posterior caudal peduncle depth	− 0.03	− 0.04	− 0.03	− 0.04
Posterior anal fin to dorsal tail fin	− 0.08	− 0.05	0.02	0.05
Fin size and position (mm)				
Pectoral altitude (from dorsal)	0.03	0.02	0.16	0.15
Pectoral fin maximum length	0.13	0.08	0.06	0.01
Pectoral fin width at insertion	− 0.01	0.01	− 0.08	− 0.06
Pectoral fin aspect ratio	− 0.005	− 0.004	− 0.01	− 0.01
Pelvic fin maximum length	*	*	*	*

Pair-wise contrasts are calculated within each main effect as specified at the top of each column. All body measurements and sizes were first transformed to their natural logarithms for analysis. Slopes were not different between contrasts in the ANCOVA unless indicated by an asterisk.

alone which may account for the significant interaction detected in the MANOVA analysis of all three sets of factor scores combined (type II ANOVA of the scores from the third factor alone: environment $p = 0.545$; offspring $p = 0.796$; interaction $F = 9.23$, $p = 0.010$). Because the third factor explained only 9% of the total variation in morphology and because neither offspring was consistently more responsive to the rearing environment (see Table 2), we conclude that any

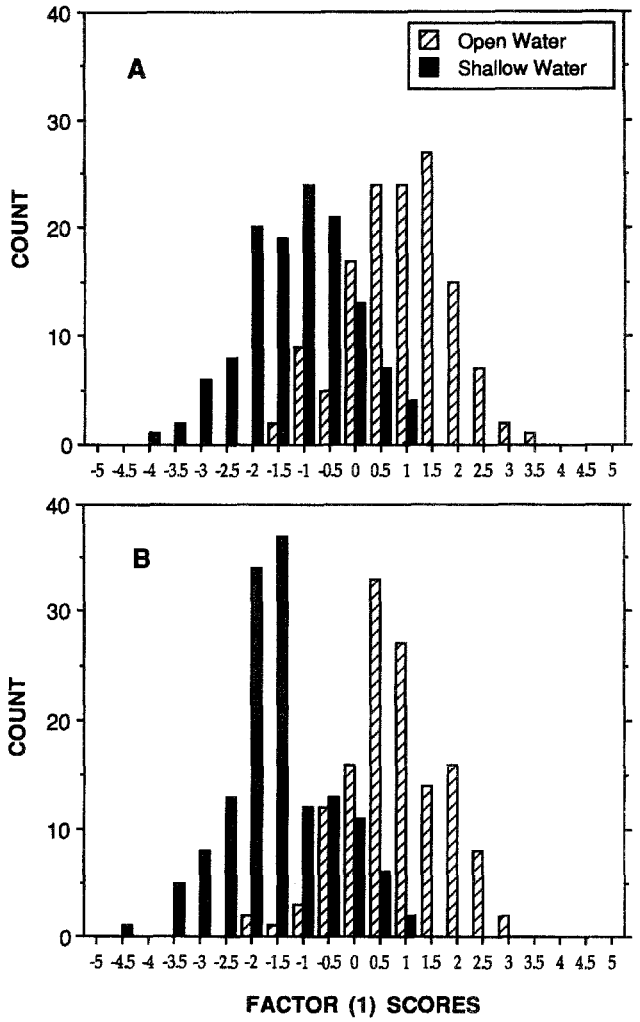

Figure 3. Histograms of the first factor's scores from the discriminant function analysis showing the morphological plasticity of (A) the benthic and (B) the limnetic progeny in response to rearing environment. Filled and hatched bars, respectively, represent the shallow and open water environments for each progeny type. While morphological variation on the first factor was predominantly influenced by environmental effects, a small but significant offspring effect was also detected (type II ANOVA of the first factor's scores: environment $F = 426$, $p < 0.0001$; offspring $F = 6.98$, $p = 0.022$; interaction $F = 0.861$, $p = 0.372$; replicates $F = 1.46$, $p = 0.134$; $R^2 = 0.567$; main effects tested against mean-squared replicate variance).

differences in the plastic responses of the two types of offspring were minor compared to the effects due to offspring and environment.

Relative importance of genetic versus phenotypically plastic differentiation

While we have demonstrated that both offspring (e.g. genotype) and rearing environment (e.g. phenotypic plasticity) affected morphological differentiation in these pumpkinseeds, we have yet to assess the relative importance of each mechanism. Three lines of evidence indicated that morphological differentiation was influenced more by the phenotypic response to the environment than by the genetic differences between benthics and limnetics. First, the 50% ellipses of each group in Fig. 1 overlapped less on the first factor (rearing environment) compared with the second factor (offspring type). Second, the MANOVA results indicated that the rearing environment had a greater effect on the morphology than offspring type (compare F statistics between the effects in the MANOVA analysis above). Third, the canonical correlations calculated for each factor of the DFA indicated that the first factor (representing predominantly environmental effects) accounted for 65%, while the second factor (representing offspring effects) only accounted for 26% of the

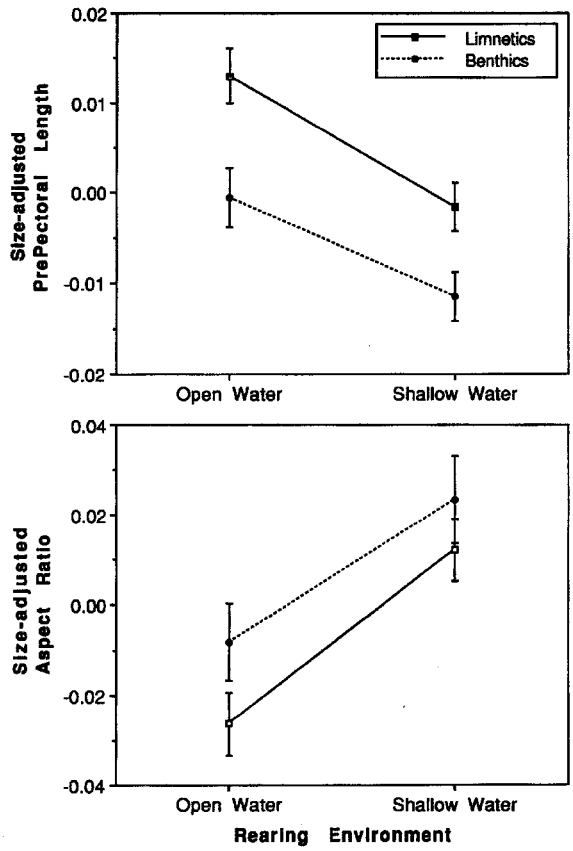

Figure 4. Morphological responses by the limnetics (open square) and benthics (solid circles) to rearing environment for two size-adjusted measurements. The overall mean and standard error of residuals within groups is plotted in each environment. Each value is size adjusted by calculating the residual after regression against centroid size and averaging over all individuals. Shape and size data were first transformed to their natural logarithms.

discriminatory power of the DFA. These values must be adjusted, however, to reflect the variance components due to the environment, offspring and their interaction within each factor (e.g. overall environmental effect: 0.813(65) + 0(26) + 0.006(9) = 53%). The adjusted morphological variance components due to plasticity, offspring effects and their interaction are, respectively, 53, 14 and 2%, making the morphological response to environment almost four times more powerful than the genetic differences between offspring types.

At the level of individual measurements, are some traits more phenotypically plastic or more genetically fixed than others? The most important trait that contributed to the first factor is a body depth measure: the distance from the anterior dorsal fin to the anterior pelvic fin insertion points (see the canonical loadings for each body measure in Table 1). On the second factor, measures of body and fin length were important including the standard length, pre-dorsal and pre-pectoral lengths and pectoral fin length. Similar differences in the responses of single measurements to offspring and environmental effects were reflected in the ANCOVA results in Table 2. Overall, body depth characteristics responded more to the rearing environment while length responded better to genetic differences between offspring types (respectively, factors 1 and 2 in Fig. 1).

Discussion

The body shape of juvenile pumpkinseed sunfish varied among the four offspring–environment treatments in this experiment. While the morphologies of the four groups were consistent with our ecological expectations, the differences in morphology were relatively small. The morphological differentiation had significant contributions from both offspring effects (e.g. genotype) and the ability of each progeny type to respond to the rearing environments (e.g. morphological plasticity). Our analyses also indicated that phenotypic plasticity was almost four times as important as the genetic variation in explaining the observed morphological diversification. Finally, there were only very minor differences in the plastic responses of the benthic and limnetic progeny in this experiment.

Our study has four strengths. (1) We examined the nature of subtle as opposed to extreme phenotypic differentiation within a single population. (2) We found evidence of genetic differences between benthic and limnetic phenotypes. (3) Functional trade-offs between these phenotypes have been demonstrated in a concurrent study. (4) We estimated the relative importance of genetic differentiation and phenotypic plasticity. We discuss each of these topics below in relation to the functional diversification of single populations, then focus on the evolution of ecological specialization and finish by concluding that the adaptive diversification of single populations is not only possible but likely in many taxa.

The value of studying subtle phenotypic diversification within single populations

This study addressed the nature of very small phenotypic differences among individuals within a single population. The phenotypic diversification of sunfish in Paradox Lake is of particular interest because this population does not consist of two discrete morphs but rather of a unimodal morphological distribution in which most phenotypes are intermediate (Robinson *et al.*, 1993). While the morphological differences among phenotypes in the field (or among juveniles in this experiment) were statistically significant and have been demonstrated on four separate samples of adults collected between 1989 and 1993 (unpublished results), they were small in magnitude (e.g. individual body measurements differed on average by 3%). We used a multivariate analysis of 20 different body measurements from each individual in order to discriminate between benthic and limnetic phenotypes. In Paradox Lake, benthic and limnetic pumpkinseeds occur in very close proximity to one another (for example, the parental stocks were collected from sites 1.3 km apart).

Finally, the trophic diversification of these sunfish does not occur in a species-poor lake as in many other fishes that are trophically polymorphic, but is embedded within a community of at least 15 other fish species (at least three of which are zooplanktivorous; Robinson *et al.*, 1993). Relatively minor phenotypic diversification by a population embedded in a complex community, as studied here, is likely to be the norm for most natural populations, as opposed to the very large differences that have evolved in just a few populations residing in species-poor environments (e.g. Behnke, 1972; Skulason *et al.*, 1992; Snorrason *et al.*, 1994a; Skulason and Smith, 1995). Our research indicates that natural selection can favour subtle phenotypic differences at the intraspecific level in vertebrates over very small spatial scales.

Genetic differentiation

The results of our reciprocal transplant experiment indicated that genetic-based differences in morphology existed between the progeny of benthic and limnetic pumpkinseed phenotypes in Paradox Lake. An alternative explanation is that natal pond effects resulted in the morphological differences between progeny. The ideal design would have included replicated parental stocks that contributed progeny to the experiment in order to include variation due to breeding ponds. This was not feasible because we had a limited number of parental pumpkinseeds available from the previous autumn, and a limited amount of time for breeding, for rearing offspring to a size large enough to handle and for growing out progeny in the experimental cages. This problem is solved in a second common garden analysis that focused solely on measuring genetic differentiation and will be published elsewhere. While this is a potential problem for this study, it is less likely because the research ponds used in this study were qualitatively similar based on an inspection by a diver, were all side-by-side and were artificial in construction.

Inferring genetic differentiation through morphological comparisons among phenotypes reared in common environments is a powerful technique for populations characterized by only subtle diversification (see also Skulason *et al.*, 1993). Biochemical genetic and mtDNA techniques are likely to be unsatisfactory under these conditions because (1) geneflow among phenotypes may be high, (2) the likelihood of finding loci or genes that are polymorphic is small and (3) any loci or genes that are polymorphic may be neutral and have no known phenotypic expression. Indeed, the literature on phenotypic polymorphisms in fishes is replete with studies that demonstrate little if any genetic differentiation at the intraspecific level using these genetic techniques (e.g. Sage and Selander, 1975; Ryman *et al.*, 1979; Ferguson and Mason, 1981; Kornfield *et al.*, 1982; Turner *et al.*, 1983; Grudzien and Turner, 1984; Hindar *et al.*, 1986; Magnusson and Ferguson, 1987; Danzmann *et al.*, 1991). Our common garden results suggest that genetic differences underlie potentially important phenotypic traits in these sunfish, such as body shape. As expected, limnetic offspring were generally longer and shallower of body with longer pectoral fins in comparison to benthics regardless of the rearing environment (Table 2). Differences in body shape are regularly interpreted to be functional at the species level in fishes (Webb, 1975, 1984a, b; Weihs, 1989; Videler, 1993). Because intraspecific and interspecific patterns of morphological differentiation are so similar to each other in fishes (Lindsey, 1981; Robinson and Wilson, 1994), it is reasonable to expect that functional trade-offs exist between benthics and limnetics within a single population.

Functional trade-offs

We have presented evidence of functional trade-offs among the sunfish phenotypes of Paradox Lake elsewhere (Robinson *et al.*, 1996). Fish with intermediate phenotypes are on average inferior to limnetics and to a much lesser extent benthic types within their respective habitats. Extreme phenotypes grew faster and had higher reserves of stored fat in the early spring in comparison to

intermediate phenotypes particularly in the pelagic habitat. Growth rate and condition factor are important components of fitness in fishes because they can influence fecundity, survivorship, competitive ability and patterns of prey selection (see the references in Robinson *et al.*, 1996). We now know that differences in relative fitness exist among sunfish phenotypes which can be favoured by natural selection, although we do not know if the functional trade-offs reside in the morphological differences outlined above or in other correlated traits. The results suggest a two-peak fitness landscape that favours phenotypes specialized for either the littoral or pelagic habitats and not a single phenotype that can optimally exploit both lake habitats (for other examples of trophic specialization in fishes, see Bentzen and McPhail (1984), Malmquist (1992), Snorrason *et al.* (1994a) and Schluter (1995)). Disruptive selection between littoral and pelagic habitats and/or reproductive segregation (Robinson *et al.*, 1993) could have facilitated the genetic diversification of this pumpkinseed population.

Phenotypic plasticity versus genetic differentiation

Our data also demonstrated that the phenotypes of both benthic and limnetic progeny were plastic in response to the rearing environment. For example, in the open water environment both progeny displayed longer heads, longer pectoral fins with a smaller aspect ratio placed lower on the body and somewhat shallower posterior body depths than in the shallow water environment (Table 2). Body depth has also been found to be plastic in response to predator cues in European carp (Brönmark and Miner, 1992). After trophic polymorphisms are documented at the phenotypic level, it becomes important to determine the genetic and developmental mechanisms that cause their expression. The common environment approach used here is an effective method for inferring mechanisms of phenotypic diversification because it tests for (1) genetic differences at the phenotypic level where relative fitness and functional trade-offs can be assayed within common environments, (2) phenotypic plasticity across different rearing environments and (3) the relative importance of phenotypic plasticity and genetic differentiation and their interaction.

Evolutionary ecologists are only beginning to understand the conditions that favour either genetic differentiation or phenotypic plasticity within natural populations (Via and Lande, 1985; Bull, 1987; Schlichting, 1989; West Eberhard, 1989). One problem with most studies of phenotypic polymorphisms in fishes is that usually only one mechanism has been demonstrated (e.g. the existence of genetic differences or the existence of phenotypic plasticity), which makes it impossible to measure the relative importance or the interaction between these two mechanisms. (For studies of phenotypic plasticity see Lindsey (1981), Meyer (1987, 1989, 1990), Wainwright *et al.* (1991), Wimberger (1991, 1992), Brönmark and Miner (1992), Mittelbach *et al.* (1992) and Robinson and Wilson (1995) and for studies of genetic differentiation see Lindsey (1981), McPhail (1984, 1992), Lavin and McPhail (1987), Schluter and McPhail (1992) and Skulason *et al.* (1993); but see Todd *et al.* (1981).)

While the relative importance of genetic differentiation and phenotypic plasticity has been studied in other taxa, this has generally been performed among allopatric populations largely isolated from each other. For example, experiments on phenotypic polymorphisms in vertebrate taxa have been performed at a spatial scale large enough for geographic mechanisms to interrupt gene flow among different phenotypes significantly (for example, in birds (James, 1983; Jehl *et al.*, 1990), in fish (Felley and Smith, 1978; Felley and Avise, 1980; Trexler *et al.*, 1990) and in mammals (Patton and Brylski, 1987)). While plant studies have focused on diversification over a much smaller spatial scale, this can also be interpreted as a population-level difference because dispersal in plants is so limited (Bradshaw *et al.*, 1965; Ehrlich and Raven, 1969; Levin, 1988; Galen *et al.*, 1991). These examples of adaptive diversification generally do not represent cases of intraspecific diversification within single populations. Our research demonstrates that both genetic

and phenotypically plastic components underlie the diversification of a single trophically polymorphic population of fish.

While plastic responses to the local environment explained four times the variation in morphology compared to offspring effects in this experiment, this does not lead us to the conclusion that genetic variation is unimportant in this system. The pattern of phenotypic diversification that we observed has two interesting features. First, genetic differentiation has evolved *despite* a functionally interpretable plastic response to the rearing environment. Second, plasticity and genetic differentiation appear to interact to fine-tune the phenotype to the local habitat. For example, fusiform limnetics can gain a further morphological advantage in the open water by reducing body depth characters and increasing head and fin lengths (becoming even more streamlined) or can increase body depth traits and reduce head and fin lengths to ameliorate the effects of their phenotype in a littoral habitat (shifting right or left, respectively, on the first factor in Fig. 1). Benthic types, despite having a less fusiform phenotype that appears adapted to the littoral habitat, also have this capacity to become less or more streamlined (in littoral and pelagic habitats, respectively). This suggests that genetic variation and phenotypic plasticity may interact to match the phenotype to the local environment in these sunfish.

Magurran (1990) makes a similar argument in reference to the antipredator behaviour of minnows. She found that inheritance and experience interact adaptively in the development of minnow behaviour, making it difficult to ascribe such patterns solely to genetic or environmental factors. While we cannot conclude that the combination of genetic differences and phenotypic plasticity is adaptive in our study (it may equally well represent an evolutionary disequilibrium in which one mechanism is replacing the other; Bull, 1987), we have demonstrated that both mechanisms can operate additively to produce the morphological differentiation observed in this pumpkinseed system.

The pumpkinseed sunfish of Paradox Lake should be viewed as marking one end of a spectrum of trophic polymorphisms in fishes that ranges from barely detectable to rivalling differences between species. It will be interesting to compare these results with an analysis of more extreme trophic polymorphisms to see how the interaction between genetic differentiation and phenotypic plasticity changes with increasing phenotypic diversification. We have completed a survey of 29 isolated populations of pumpkinseed sunfish in the Adirondack region and have found that they differ in the degree of trophic polymorphism at the phenotypic level (B.W. Robinson, D.S. Wilson and A.S. Margosian, undated). For example, pumpkinseed sunfish from Paradox Lake display only a moderate amount of differentiation, while phenotypic differentiation in Round Lake is bimodally distributed with significantly fewer intermediate phenotypes. Between-lake differences in trophic polymorphisms within a single fish species will allow the evolution of genetic differentiation and the intensity of functional trade-offs to be studied in considerable detail.

Evolution of specialization

Ecologists attempt to explain the diversity and abundance of organisms in biological systems. A fundamental concept that has proved useful in this endeavour is that organisms evolve to become specialized to a particular niche. While our understanding of how specializations evolve has been well advanced both theoretically and empirically (e.g. Moore, 1952; MacArthur and Levins, 1964, 1967; Grant, 1972, 1975; Lawlor and Maynard Smith, 1976; Rosenzweig, 1981, 1991; Wilson and Yoshimura, 1994), important questions remain unresolved. For example, Futuyma and Moreno (1988) recently criticized empirical studies of specialization on three grounds. First, they frequently lack evidence of underlying genetic variation, which makes an evolutionary history of specialization difficult to infer. Futuyma and Moreno (1988) suggested that behavioural mechanisms are more important than genetic variation in populations composed of phenotypes that

occupy multiple niches (with the exception of sexual dimorphisms). Second, studies of functional trade-offs between species are inappropriate because these trade-offs often evolved after ecological specialization. Therefore, they are of little use in explaining how specializations first arise. Intraspecific, not interspecific, comparisons are the appropriate level to study phenotypic trade-offs. Third, evidence of functional trade-offs within single species is rare. While Futuyma and Moreno (1988) have performed a useful service by suggesting stringent criteria for empirical studies of specialization, their review also leaves the disturbing impression that there is little evidence to support some of our most basic ideas of how specializations evolve.

In this paper, we have provided evidence that the progeny of limnetic and benthic pumpkinseeds *are* genetically differentiated with respect to morphology, because differences are maintained regardless of the rearing environment. Therefore, this study meets Futuyma and Moreno's (1988) first criterion for demonstrating genetic differentiation at the intraspecific level. Robinson *et al.* (1996) have also demonstrated that intermediate phenotypes are on average inferior to limnetics and less so to benthics in their respective habitats using growth rate and stored fat content as two components of fitness. This suggests that functional trade-offs exist between benthic and limnetic phenotypes (Futuyma and Moreno's (1988) second criterion). In addition, trophic polymorphisms in freshwater fishes are widely replicated both among different taxa and within single species sometimes across hundreds of isolated lakes (Robinson and Wilson, 1994). The diversification can exist as discrete morphs or as a continuum of morphological variation. Intraspecific and interspecific trophic variation in fish are so similar to each other, that it is probably not the case that the interspecific differences evolved after ecological specialization. These unique features make trophic polymorphisms in fishes a model system in which we can study intraspecific trade-offs (Futuyma and Moreno's (1988) third criterion). Our research on the pumpkinseed polymorphism, therefore, supports some of our basic intuitions about specialization, such as the jack-of-all-trades as a master of none. Rather than rejecting interspecific differences as evidence for functional trade-offs as argued by Futuyma and Moreno (1988), we should study the relationship between interspecific and intraspecific differentiation at the level of phenotypic trade-offs in a single biological system. Freshwater fish communities are ideal for this kind of study.

Domains of adaptive variation

Adaptive variation in biological systems is hierarchical, existing among species, among isolated populations of a single species and among individuals within a single population. This is particularly apparent in fishes, where patterns of trophic diversification at the inter- and intraspecific levels parallel one another. Trophic or resource polymorphisms have been documented in a variety of vertebrate taxa including fish (Robinson and Wilson, 1994), birds (Smith and Temple, 1982; Smith, 1987, 1990a,b), lizards (Schluter and McPhail, 1993), salamanders (Collins and Cheek, 1983) and whales (Baird *et al.*, 1992; see the review in Skulason and Smith (1995) for further examples). Trophic polymorphisms, along with mating polymorphisms (Dominey, 1980; Gross and Charnov, 1980) and defensive phenotypes (Jones *et al.*, 1977; Owen and Whiteley, 1989; Brodie, 1990) are important examples of adaptive variation at the lowest level of the biological hierarchy. A growing appreciation for adaptive variation at the intrapopulation level may favour a shift along the lines seen with the recognition of adaptation at higher taxonomic levels, such as among populations and among species. Evolutionary ecologists will have to answer a variety of important questions. Whether intrapopulation variation is non-random? What is the importance of frequency-dependent selection? Whether indirect competitive effects are as strong or common as direct effects? How natural selection counters gene flow among phenotypes? Whether habitat selection is a necessary component for the evolution of adaptive intrapopulation variation? These questions highlight weaknesses in our understanding of some of the most basic issues in ecology

and evolution. The intrapopulation domain of biological variation provides a largely unexplored and fertile realm for our investigations into these and related questions.

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