

Variation in feeding morphology between pumpkinseed populations: Phenotypic plasticity or evolution?

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ABSTRACT

Pumpkinseed sunfish exhibit considerable intraspecific variation in jaw morphology, with population-level differences in the size of key morphological structures often exceeding 200%. This inter-population variation is correlated with differences in the availability of gastropods, the pumpkinseed's primary prey. Such resource polymorphisms may be an indication of local adaptation to resource conditions. This explanation, however, assumes that the observed phenotypic variation has an underlying genetic basis. Here, we provide evidence from two types of common-garden experiments that variation in pumpkinseed pharyngeal jaw morphology is the result of phenotypic plasticity.

We collected adult fish from two populations (with the greatest observed divergence in morphology and resource use), bred them in similar environments, and then raised their young under two conditions: (1) the laboratory, where we controlled diets and fed fish either soft-bodied prey only, or a combination of soft-bodied prey and gastropods; and (2) experimental ponds in which the fish were unconstrained with respect to diet. After 1 year, we analysed the pharyngeal morphology of the fish, focusing on the size of the levator posterior, the primary muscle used to generate the force needed to crush snail shells. In the laboratory, there were no differences in morphology attributable to parental stock under either dietary treatment. In contrast, the addition of snails to the fishes' diets led to a substantial (~230%) increase in levator posterior mass. In the ponds, we observed some slight, and inconsistent, differences between populations with respect to muscle mass. In all cases, the population-level differences were very small compared to the effects of different ponds, or compared to the differences in morphology observed in the laboratory. These results show that the natural variation in pharyngeal morphology between these populations of pumpkinseeds is primarily the result of a plastic response to the environment, rather than a response to selection driven by the environmental differences.

Keywords: common-garden experiments, *Lepomis*, Michigan, phenotypic plasticity, sunfish, trophic morphology.

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INTRODUCTION

Many vertebrate species show discrete intraspecific variation in trophic morphology in association with differences in resource use (commonly called 'resource polymorphisms'; Wimberger, 1994; Smith and Skúlason, 1996). In a typical example, distinct phenotypes are associated with particular patterns of prey or habitat use, and the phenotypic differences often match the ecological segregation. These polymorphisms are commonly attributed to phenotypic variation within a population (e.g. Bentzen and McPhail, 1984; Amundson, 1988; Ehlinger and Wilson, 1988; Skúlason *et al.*, 1992); however, similar variation may also occur among populations (e.g. Svardson, 1979; Bodaly *et al.*, 1988; Witte *et al.*, 1990; Turingan *et al.*, 1995; Trussell, 1997) and may represent local adaptation to the resource environment.

Resource polymorphisms have attracted considerable attention over the years, in part because they match many of our expectations for the conditions of incipient speciation. When subgroups within a species are found to have differing resource-use patterns in association with different morphological features, it is tempting to imagine that some form of disruptive selection (or local adaptation) is at work, and that the two morphs represent the product of selection on heritable phenotypic variation. However, polymorphisms may also arise through phenotypic plasticity; for example, morphological variation might arise because of different patterns of resource use, but not be the original cause of those differences in diet. Although the morphological variation, *per se*, may not be heritable, the capacity for phenotypic plasticity may be a heritable trait that is under selection (West-Eberhard, 1989; Scheiner, 1993). The implications for the role of polymorphisms in models of speciation depend on whether the phenotypic variation is due to developmental plasticity in response to different patterns of resource use, or divergent selection on phenotypic traits that have a genetic basis (Mayr, 1963; Felsenstein, 1981; Bush, 1994; but see Waddington, 1953, for a discussion of how plasticity might ultimately lead to genetic divergence). A key step, then, in understanding the genesis and role of resource polymorphisms is to measure the relative contributions of phenotypic plasticity and genetic divergence in accounting for observed morphological differences (Day *et al.*, 1994; Robinson and Wilson, 1996).

We have previously described pronounced variation in trophic morphology between populations of the pumpkinseed sunfish, *Lepomis gibbosus* (Wainwright *et al.*, 1991a,b; Mittelbach *et al.*, 1992; Osenberg *et al.*, 1992). Here we assess the extent to which this variation represents the result of phenotypic plasticity or local evolution. In a survey of pumpkinseeds in several Wisconsin and Michigan lakes, we found a close, positive relationship among the abundance of gastropods in a lake, the amount of gastropods eaten by pumpkinseeds, and the size of the pumpkinseed's principal shell-crushing muscle (Mittelbach *et al.*, 1992). The link between jaw morphology and feeding performance is direct and readily interpretable in this predator-prey system, and the basic components involved have been observed in several other fish species (Wainwright, 1987, 1988; Hernandez and Motta, 1997). Larger jaw muscles underlie the ability of pumpkinseed to crack the shells of larger, harder snails, and allow fish to more quickly consume snails that challenge their feeding ability (Osenberg *et al.*, 1992). Morphological variation among pumpkinseed populations can be striking, with differences of almost three-fold in the size of key muscles among populations (Wainwright *et al.*, 1991a; Mittelbach *et al.*, 1992).

It is not clear, however, whether the observed variation among pumpkinseed populations is due to a plastic response of the muscle to diet (itself driven by snail abundance), or to

genetically based differences in muscle size that have arisen through local adaptation to variation in snail availability, or to a combination of these two factors. Here, we present the results of two experiments designed to measure the relative contributions of phenotypic plasticity and genetic differences in pumpkinseed feeding morphology. We focus on two Michigan populations of pumpkinseeds, those found in Three Lakes and Wintergreen Lake. Pumpkinseeds from these lakes were found in our earlier survey to exhibit the greatest phenotypic differences in trophic morphology (Wainwright *et al.*, 1991a; Mittelbach *et al.*, 1992). The lakes are located about 7 km apart within the same drainage system (Wintergreen Lake upstream from Three Lakes); however, there is little possibility of gene flow between populations because the outflow from Wintergreen Lake is closed to fish migration.

METHODS

We used two different methods, both variations of a common-garden design, to examine the extent to which variation in pumpkinseed functional morphology is genetically determined versus environmentally induced. For both studies, we collected adult pumpkinseeds from Wintergreen Lake (WG) and Three Lakes (TL) and bred them separately in ponds. By bringing adults into these ponds and raising their young outside of the maternal environments, we were able to reduce maternal effects and eliminate effects of the parental environment on early development of the offspring. Offspring from these brood ponds (young-of-year fish) were used in two experiments. In the first experiment, we raised offspring from the two lake types in the laboratory and fed them two different diets: a diet containing only soft-bodied invertebrates, and a diet containing soft-bodied invertebrates and snails. In the second experiment (run concurrently with the laboratory experiment), we raised marked offspring from the two lake types in common field environments (ponds). We refer to this as the 'pond experiment'. The laboratory experiment allowed us to control diet and therefore separate the specific effects of diet and lake stock on functional morphology and feeding performance, whereas the pond experiment allowed us to test for general effects of source lake on functional morphology and diet in environments that mimicked natural conditions and thus permitted fish to express behavioural differences, which could influence diet and therefore morphological development.

Brood ponds

Adult pumpkinseeds were collected by seining from Wintergreen Lake and Three Lakes in early May 1990. Sixty pumpkinseeds ranging in standard length (SL) from 102 to 132 mm (mean $\pm$ s.d. = 113 ± 8.1 mm) were collected from Wintergreen Lake and were stocked into three brood ponds (20 fish per pond) at the Experimental Pond Facility at the Kellogg Biological Station. Forty-four adult pumpkinseed (89–138 mm SL; mean $\pm$ s.d. = 108 ± 14.4 mm) were collected from Three Lakes and stocked into three other brood ponds (14 or 15 fish per pond). We were not able to sex fish prior to stocking as they were not yet in breeding condition; therefore, fish from a given source lake were assigned to a brood pond at random. The brood ponds were of similar size (30 m in diameter and 2 m deep) and contained vegetation and prey types that commonly occur in nearby, natural lakes (see Turner and Mittelbach, 1990; Olson *et al.*, 1995). Nevertheless, some among-pond differences in environment are unavoidable. Therefore, we used three brood ponds for

each source lake to help reduce any effects of initial environment on offspring development. Fish were observed nesting in all the brood ponds by the end of May 1990 and fish larvae were present in each of the brood ponds by the middle of June 1990.

Laboratory experiment on diet-induced morphology

We used a laboratory rearing experiment to determine the extent to which the observed variation in pumpkinseed morphology among lakes could be induced by diet or explained by genetic divergence. On 17 September 1990, we collected juvenile pumpkinseeds from the six brood ponds and brought them into the laboratory (24 fish per brood pond; 144 fish in total; $SL = 31.0 \pm 0.53$ mm, mean ± 1 s.e.). The fish were housed in 38-litre aquaria that were divided in half; each half contained one fish. For the next 2 months, fish were fed daily a diet of natural zooplankton (predominantly *Daphnia pulex*) collected from one of the experimental ponds on site. On 16 November 1991, we reduced the number of experimental fish to one per aquarium (72 fish in total; 12 per brood pond) and applied the following two diet treatments. Half the fish from each brood pond were fed a diet of soft-bodied invertebrates and the other half were fed a diet of soft-bodied invertebrates and snails (fish receiving snails were fed a reduced amount of soft-bodied prey to account for the biomass of snails consumed). Hereafter, we refer to these two diet treatments as the 'soft diet' and 'hard diet', respectively. Fish selected for each diet treatment were chosen at random and fish locations on the laboratory aquarium racks were also chosen at random.

The fish received the two diet treatments for the next 250 days. Diets varied somewhat during this period, depending on prey availability. From 20 November 1990 to 15 May 1991, all fish received a daily diet of soft-bodied prey, predominantly brine shrimp (*Artemia salina*), but occasionally larval mayflies and damselflies, amphipods (*Hyaletella*) and zooplankton (*Daphnia*). Fish on the hard diet received the same types of soft-bodied prey as the other fish, plus 1–3 snails (*Physa* and *Gyraulus* spp.) per day. After mid-May, snail availability in local lakes increased and we increased the number of snails fed to fish on the hard diet to 10–20 *Physa* per day. Live blackworms (*Tubifex*), obtained from a local aquarium dealer, were included as soft-bodied prey in both treatments from mid-May to mid-July. By mid-June, pumpkinseeds were strong enough to crush thicker-shelled snails and for the period of 11 June to 29 July 1991, fish on the hard diet received approximately 10–30 *Ammnicola*, *Potamogyrus* or *Physa* per day. The experiment was terminated on 2 August 1991 when all fish were sacrificed with an overdose of tricaine methanesulphonate (MS-222), measured and weighed, and then preserved in 10% formalin.

We conducted feeding trials just prior to the end of the experiment (29 July to 1 August 1991) to measure the abilities of pumpkinseeds raised on hard diets to feed on snails. Two snail groups were used as prey in the trials: relatively weak-shelled *Physa* collected from a laboratory culture (laboratory *Physa* crushing resistances and shell lengths were 1.98 ± 0.15 N and 5.02 ± 0.16 mm, respectively; $n = 18$), and relatively strong-shelled *Physa* collected from Gull Lake (Gull Lake *Physa* crushing resistances and shell lengths were 6.00 ± 0.42 N and 4.93 ± 0.13 mm, respectively; $n = 18$). Crushing resistances were determined using methods described in Osenberg and Mittelbach (1989). We offered snails individually to each fish and recorded the time required to crush and handle a snail, and whether or not the snail was successfully eaten. Nine fish from each source lake were used for the snail-feeding experiments. We did not test the abilities of fish raised on soft diets to

feed on snails, as we were concerned that any snail consumption might affect their final muscle masses.

Pond experiment

By 1 September, 1990, pumpkinseed offspring in the brood ponds were large enough (~25–30 mm SL) to be handled, marked and transferred to rearing ponds with minimal mortality. We collected offspring from each brood pond by seining and then marked them by clipping their pelvic fins: left fin clip for WG fish and right fin clip for TL fish. Seventy-five fish from each brood pond were stocked into each of four rearing ponds, yielding a total density of 450 pumpkinseeds per rearing pond (~0.6 fish per m²). We attempted to stock fish of similar sizes from each of the two source lakes; however, the fish available from the Wintergreen Lake brood ponds were slightly larger than those from the Three Lakes brood ponds, making some initial difference in size unavoidable. The mean (± 1 S.E.) standard length of fish stocked into the rearing ponds was 28.4 ± 0.19 mm for Wintergreen Lake and 27.3 ± 0.20 mm for Three Lakes.

We sampled fish 2 weeks after stocking by placing 4–5 minnow traps in each rearing pond between 08.30 and 11.00 h. A random subsample of the fish trapped (13–17 fish from each source lake and rearing pond) was measured for standard length. The rearing ponds were next sampled on 22 and 23 May 1991. We collected fish by seining and we recorded the mark (fin clip), sex and standard length of each fish collected. Fin clips had regrown over the winter; however, the rays of regrown fins were distinctly scarred and we were able to recognize the lake identity of >90% of the fish collected. We attempted to collect at least 10 fish of the same sex from each source lake and pond. Fish were sacrificed with an overdose of tricaine methanesulphonate (MS-222), dissected to remove their stomachs and gonads, and then preserved in 10% formalin for morphological analyses. Most fish were reproductively mature and larval pumpkinseed had appeared in all the ponds by June.

The pond experiment was terminated 18–22 July 1991 by first seining each pond to recover as many fish as possible and then draining each pond to collect all remaining fish. All fish were checked for fin clips, measured for standard length and sexed. At least 50 fish from each pond that had clearly recognizable fin clips were sacrificed with MS-222 and later dissected in the laboratory. The sex of these dissected fish was checked and their stomachs were removed and preserved in 10% formalin for later diet analyses (using the general methods described in Mittelbach, 1984). The bodies of dissected fish were preserved in 10% formalin for later analysis of pharyngeal jaw morphology.

Laboratory analyses of pharyngeal jaw morphology

In both experiments, we focused on the mass of the levator posterior muscle as a key response variable that is related to snail-crushing performance. Previous work has shown that this muscle plays a central role in applying the forces that crack mollusc shells in the pharyngeal jaw apparatus of pumpkinseeds (Lauder, 1983a,b; Wainwright *et al.*, 1991b), as in other generalized percomorph fishes (Wainwright, 1989; Galis and Drucker, 1996). Furthermore, in a survey of nine muscles and three skeletal elements, we previously found that the levator posterior muscle exhibited the greatest difference in mass between the Three Lakes and Wintergreen Lake populations (Wainwright *et al.*, 1991a). For each of the 384 fish analysed, the left levator posterior was removed and stored in 70% ethanol until it was

weighed three times to the nearest 0.1 mg after being patted twice on a dry paper towel by a worker who was blind to the source lake or diet of the fish. The average of these three measurements was used in subsequent analyses.

Statistical analyses

We conducted preliminary analyses of the laboratory and pond experiments using analysis of variance with models that included all sources of variation that were explicitly addressed in the study. For the laboratory experiment, this included effects of lake (the effect of the parental stock), brood pond (nested within lake), body size (either standard length or body mass) and all possible interactions. Given the complexity of this analysis, we reduced the full model by sequentially removing terms that did not account for a significant source of variation. However, given our interest in lake and diet effects and their interaction, as well as the *a priori* knowledge that body size strongly influences morphology and feeding performance, we retained these four factors independent of the preliminary results. The analyses of feeding performance excluded the effect of diet, and focused on the effect of lake, because we only had trials for fish raised on hard diets.

We took a similar approach with the pond experiment, where the full model included effects of lake, rearing pond, body size (standard length or mass) and all possible interactions. Again, given our primary interest in lake, pond and lake $\times$ pond effects, and the presumed importance of body size, we never reduced the final model beyond this subset of effects. The analysis of covariance model analysed is a mixed-effects model, with lake as a fixed effect and pond as a random effect. In all analyses, body size variables (including muscle mass and gonad mass) and feeding performance variables were log (base 10) transformed prior to analysis to homogenize variances and linearize allometric relationships. Other details are provided in the Results section.

RESULTS

Laboratory experiment

Diet had a strong effect on pumpkinseed morphology. Despite the fact that fish fed only soft-bodied prey (soft diet) were slightly larger at the end of the experiment, they had a smaller levator posterior muscle than fish fed a mixed diet of hard and soft-bodied prey (hard diet) (Fig. 1). There were no significant effects of source lake (i.e. Wintergreen or Three Lakes) on levator posterior muscle mass (Table 1). Average muscle masses (adjusted for standard length and back-transformed; Table 1) were 0.0064 g in Wintergreen Lake fish and 0.0061 g in Three Lakes fish (standard errors were $\pm 3.4\%$), a difference of 4.6%. In contrast, adjusted mean muscle masses were 0.01136 g for fish fed hard diets and 0.0034 g for fish fed soft diets ($\pm 4.0\%$), a difference of 234%.

Wintergreen and Three Lakes fish raised on hard diets showed no significant differences in their abilities to feed on snails at the end of the experiment. Comparisons of average crushing times and handling times for pumpkinseeds offered laboratory *Physa* or Gull Lake *Physa* showed that crushing times and handling times increased as the average crushing resistance of the snails increased (Fig. 2). However, there was no effect of source lake on any of the parameters measured (Table 2).

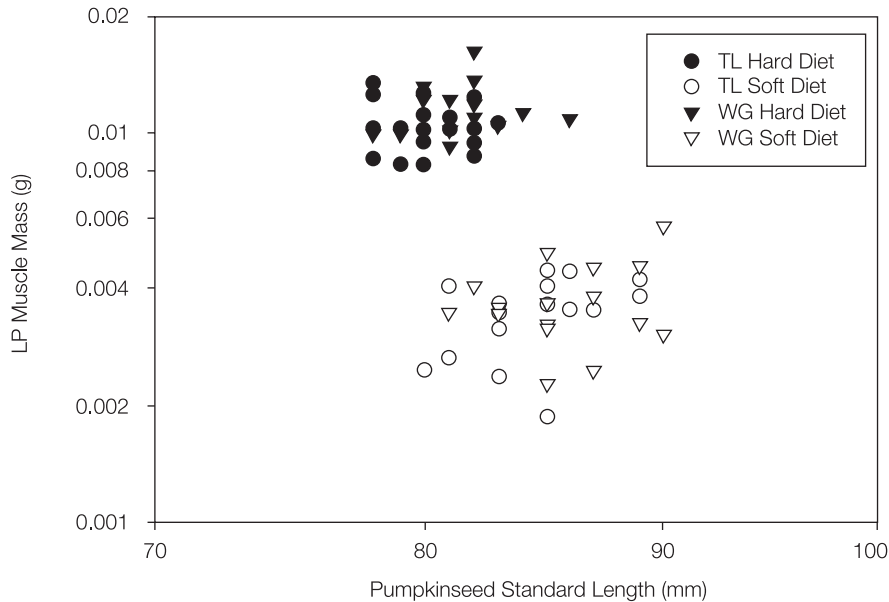


Fig. 1. Levator posterior (LP) muscle mass in pumpkinseeds raised on either a hard (snails and soft-bodied invertebrates) or a soft (soft-bodied invertebrates only) diet in the laboratory. Circles denote fish from Three Lakes (TL) parents and inverted triangles denote fish from Wintergreen Lake (WG) parents. See Table 1 for the results of the statistical analyses.

Pond experiment

Levator posterior muscle mass

The results of the pond experiment largely paralleled the laboratory results; there were only minor effects of source lake on pumpkinseed functional morphology when raised in common environments. However, there were differences among ponds, suggesting that variation in prey availability or other factors between ponds may have produced a more complicated response than that observed in the laboratory, where diets were more controlled.

We compared the size (mass) of the main snail-crushing muscle (levator posterior) in pumpkinseeds collected at two points in the experiment – May 1991 and July 1991 (final sample). Because levator posterior (LP) muscle mass scales positively with fish size (Wainwright *et al.*, 1991a), we used log SL or log fish mass as covariates in the analyses (the two measures of fish size could potentially yield different results if there were effects of rearing pond or source lake on fish condition). In all four analyses, pond had a statistically significant effect, as did the body-size covariate (Table 3). The effect of source lake was never statistically significant (Table 3). For example, if we compare mean LP muscle masses at the end of the experiment (adjusted for the covariate SL), we find that the maximum difference between lakes (within a pond) is ~19% (TL > WG), whereas lakes vary (maximally) by 30% (TL) and 44% (WG) among ponds. There was, however, a significant lake $\times$ pond interaction in three of the four analyses (Table 3; see Fig. 3 for an example), which arose because the direction of the ‘lake’ differences in LP muscle mass varied among ponds. In ponds 15

Table 1. Analysis of covariance of log LP muscle mass in pumpkinseeds from two source lakes (WG and TL) raised in the laboratory on two diets (hard and soft), using log SL as the body-size covariate (similar results were obtained using log body wet mass as the covariate)^a

Source	d.f.	MS	F	P
Lake	1,65	0.0061	0.87	0.36
Diet	1,65	2.492	361.11	<0.001
Lake × diet	1,65	0.0001	0.0001	0.91
Log SL	1,65	0.0234	3.34	0.072
Error	65	0.0070		

Least squares means (± 1 S.E.):

TL = -2.2160 ± 0.0145 Hard diet = -1.9445 ± 0.0170
 WG = -2.1965 ± 0.0145 Soft diet = -2.4680 ± 0.0170

^a Data are presented in Fig. 1.

and 9, Three Lakes and Wintergreen Lake fish had similar adjusted mean LP muscle masses (0.0058 *vs* 0.0057 in pond 15 and 0.0075 *vs* 0.0072 in pond 9). In contrast, in pond 7, Three Lakes LP muscle mass was greater than Wintergreen Lake LP muscle mass (0.0060 *vs* 0.0050), but in pond 18 the opposite was true (0.0060 *vs* 0.0068). In no case was the difference in LP muscle mass between lakes as large as the difference in LP muscle mass generated by the hard and soft diets in the laboratory experiment (lake differences in LP muscle mass within a pond were consistently <20%, whereas the difference in LP muscle mass between fish fed hard and soft diets in the laboratory was >200%).

Diets

There were statistically significant differences among ponds in terms of the extent to which pumpkinseeds fed on molluscs at the end of the experiment (Table 4), although the effects were not consistent nor easily interpreted. Overall, pumpkinseeds from Three Lakes tended to consume more molluscs than did pumpkinseeds from Wintergreen Lake (Table 4). However, this result depended on pond and there was no overall main effect of lake on either measure of molluscivory. Although pumpkinseed diets differed among ponds, these differences did not explain the among-pond variation in LP muscle mass (see Fig. 3). For example, the adjusted mean LP muscle mass for Three Lakes or Wintergreen Lake pumpkinseeds in the four ponds was not significantly correlated with the extent of molluscivory, measured either as percent molluscs or mass of molluscs eaten ($r^2 < 0.07$, $P > 0.50$, $n = 8$).

Given that we took only one diet sample at the end of the experiment, that snail densities were probably already quite low at this point, and that there was little variation in either diet or LP muscle mass, it is not surprising that diet explained little of the subtle variation in LP muscle mass observed among ponds. We have shown previously, however, a strong, positive correlation between pumpkinseed diets (percent molluscs eaten) and LP muscle mass across a gradient of natural lakes that differed greatly in the availability of molluscs and the extent of molluscs in the diet (Mittelbach *et al.*, 1992). If we compare the pond and laboratory

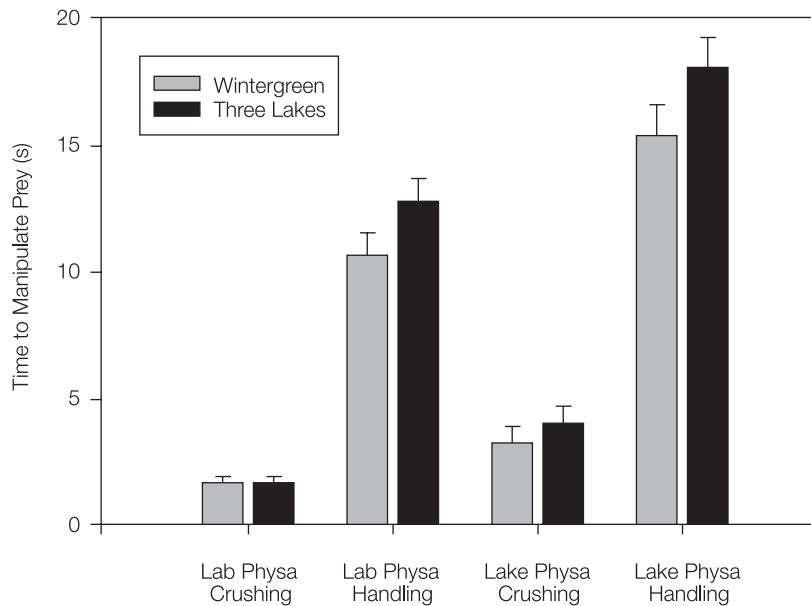


Fig. 2. Mean (± 1 s.e.) handling times and crushing times for laboratory-reared pumpkinseeds feeding on Physid snails. All pumpkinseeds tested were raised on hard diets. *Physa* were obtained from two sources: laboratory cultures (relatively weak shelled) and Gull Lake (relatively strong shelled). See Table 2 for the results of the statistical analyses.

results from the current study to this broader range of diets and LP muscle masses, we see that both the pond and laboratory results fit well within the overall relationship (Fig. 4).

DISCUSSION

Intraspecific variation in trophic morphology and resource use is extremely common in freshwater fish (for recent reviews, see Robinson and Wilson, 1994; Wimberger, 1994; Skulason and Smith, 1995; Smith and Skulason, 1996), but rarely is it known whether this variation represents a phenotypically plastic response to changing resource availability, or whether there is a genetic basis to the differentiation (Wimberger, 1994; Robinson and Wilson, 1996; Smith and Skulason, 1996). In this study, we have shown that the pronounced differences in jaw morphology and snail-crushing ability exhibited by pumpkinseed sunfish from two lakes, Wintergreen Lake and Three Lakes, are largely the result of phenotypic plasticity. In nature, adult pumpkinseeds from Three Lakes consume more snails (>80% vs < 5% of the diet: Osenberg *et al.*, 1992), have more robust pharyngeal jaw muscles and bones (up to twice as large: Wainwright *et al.*, 1991a), and are able to crush significantly harder snails and handle snails in less time (Osenberg *et al.*, 1992, unpublished data) than pumpkinseeds from Wintergreen Lake. However, when grown in common environments, both in the laboratory and in the field (ponds), offspring from these two lakes showed little difference in pharyngeal jaw morphology. Large differences in pharyngeal jaw morphology were inducible, however, by raising pumpkinseeds on diets with or without snails, and the response was the same for fish from both lake types.

Table 2. Analysis of covariance of snail-feeding performance of pumpkinseeds from two source lakes (WG and TL) raised in the laboratory on hard diets (see Fig. 2)^a

Source	d.f.	MS	F	P
Performance metric = time to crush laboratory <i>Physa</i>				
Lake	1,15	0.0075	0.67	0.43
Log SL	1,15	0.1443	12.91	<0.01
Error	15	0.4748		
Performance metric = time to crush Gull Lake <i>Physa</i>				
Lake	1,15	0.0010	0.03	0.86
Log SL	1,15	0.2674	8.17	0.01
Error	15	0.0327		
Performance metric = time to handle laboratory <i>Physa</i>				
Lake	1,15	0.0159	1.62	0.22
Log SL	1,15	0.0081	0.83	0.37
Error	15	0.0098		
Performance metric = time to handle Gull Lake <i>Physa</i>				
Lake	1,15	0.0124	0.92	0.35
Log SL	1,15	0.0091	0.67	0.42
Error	15	0.0135		

^a Performance was measured as the time required to crush or handle either laboratory-reared *Physa* or Gull Lake *Physa*. Log SL was used as a covariate in the analysis.

The results of the laboratory experiment demonstrate that there is no genetically based difference between populations in the size of the levator posterior muscle. This experiment further shows that there is no difference between populations in the response of this muscle to diet (i.e. there is no strong genotype $\times$ environment interaction). However, both populations showed considerable plasticity of muscle mass. The snail treatment resulted in muscles that were about 300% larger than among control fish, approximately the same difference in muscle mass seen in wild-caught fish from Three Lakes and Wintergreen Lake (Wainwright *et al.*, 1991a). Thus, the magnitude of the plastic response, together with the dietary differences (Wintergreen fish eat few snails, while the diet of adult Three Lakes fish is dominated by snails; Osenberg *et al.*, 1992), can account for the differences in muscle size seen in these two populations.

The importance of phenotypic plasticity rather than genetic differentiation in explaining the observed differences in pharyngeal jaw morphology between pumpkinseeds from Wintergreen Lake and Three Lakes is consistent with the relatively recent changes that have occurred in the Wintergreen ecosystem. During the winters of 1976 and 1977, Wintergreen Lake experienced selective fish kills due to heavy snowfall and severe oxygen depletion. These winterkills eliminated the pumpkinseed's major competitor (the bluegill, *L. macrochirus*) and predator (the largemouth bass, *Micropterus salmoides*) from the lake, allowing for a large increase in the pumpkinseed population (Mittelbach *et al.*, 1995). Caging experi-

Table 3. Analysis of covariance of LP muscle mass of pumpkinseeds from two source lakes (WG and TL) raised in four ponds, collected on two dates (May and July)^a

Source	d.f.	MS	<i>F</i>	<i>P</i>
May collection				
Lake	1,3	0.0330	1.71	0.25 < <i>P</i> < 0.50
Pond	3,73	0.6934	12.15	<0.001
Lake × pond	3,73	0.0193	3.38	0.023
Log SL	1,73	0.5916	103.7	<0.001
Error	73	0.0087		
May collection				
Lake	1,3	0.0149	2.50	0.10 < <i>P</i> < 0.25
Pond	3,73	0.1021	21.53	<0.001
Lake × pond	3,73	0.0060	1.26	0.30
Log body mass	1,73	0.6619	139.55	<0.001
Error	73	0.0047		
July collection				
Lake	1,3	0.0081	0.194	>0.50
Pond	3,231	0.1580	18.11	<0.001
Lake × pond	3,231	0.0418	4.79	<0.01
Log SL	1,231	1.540	176.6	<0.001
Error	231	0.0087		
July collection				
Lake	1,3	0.0210	0.461	>0.50
Pond	3,231	0.1901	23.72	<0.001
Lake × pond	3,231	0.0453	5.66	<0.001
Log body mass	1,231	1.7037	212.56	<0.001
Error	231	0.0080		

^a Log SL or log body mass were used as covariates in the analysis. Data presented in Fig. 3.

ments in Wintergreen Lake showed that the high density of pumpkinseeds was capable of maintaining snail densities at very low levels and that, in the absence of pumpkinseeds, snail abundances increased dramatically (Osenberg *et al.*, 1992). Thus, it appears that the extreme rarity of snails in Wintergreen Lake is a recent phenomenon, brought about by a large increase in the pumpkinseed population, and that while snails make up a small fraction of the diets of individual pumpkinseeds, the population as a whole is able to maintain snails at a very low level. If this is correct, then the differences in pharyngeal jaw anatomy observed between the lakes most probably reflect recent divergences and we would expect them to result largely from developmental plasticity rather than rapid genetic divergence. We note, however, that other work with vertebrates has documented rapid responses to natural selection mediated by changes, for example, in resource availability (Boag and Grant, 1981; Price *et al.*, 1984) or predator density (Reznick *et al.*, 1990).

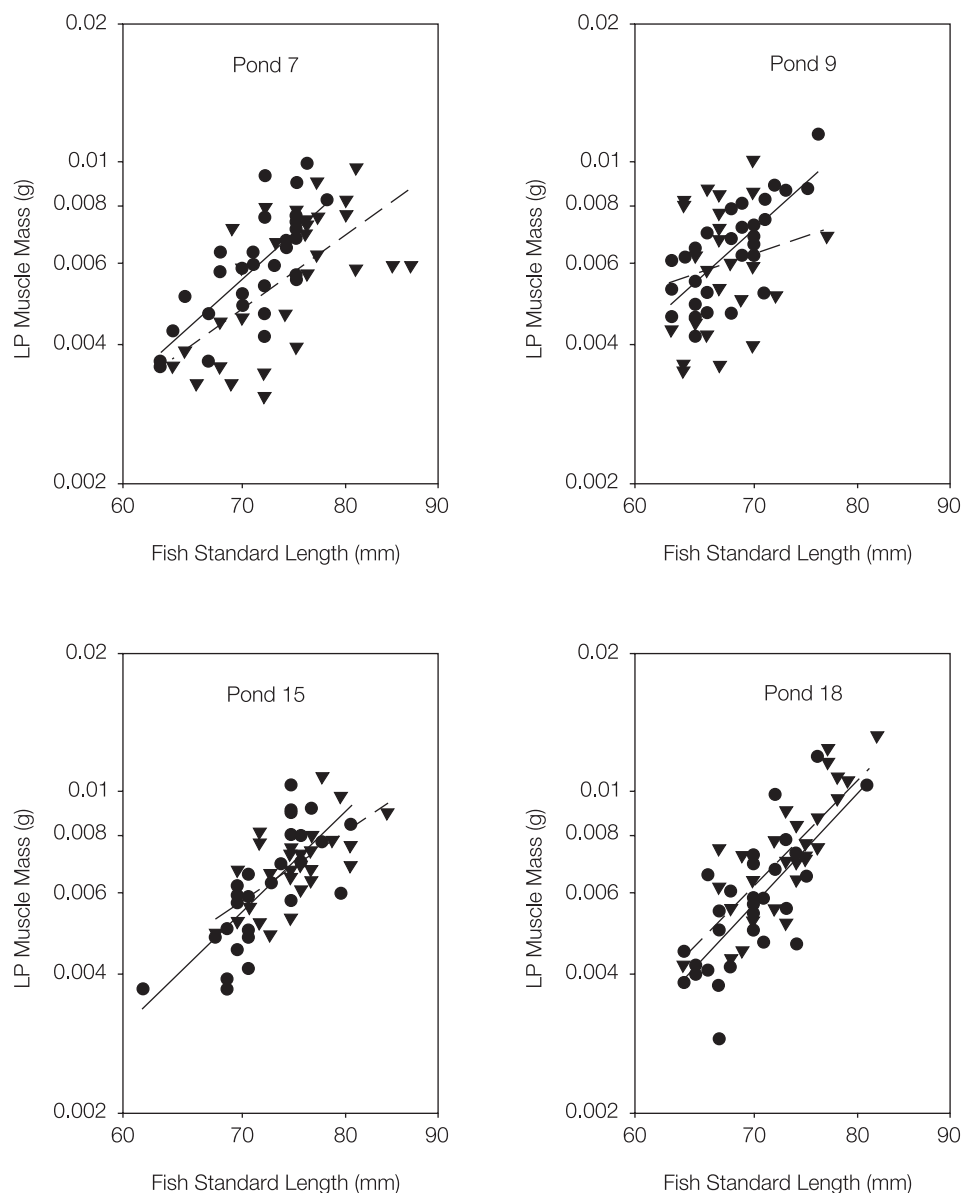


Fig. 3. Levator posterior muscle mass as a function of pumpkinseed standard length in the four common-garden ponds. Lines are least-squares regressions for pumpkinseeds from either Three Lakes parents (●) or Wintergreen Lake parents (▼). See Table 3 for the results of the statistical analyses.

While both the laboratory and pond ‘common-garden’ experiments showed that variation in diet and developmental plasticity are the predominant factors explaining variation in pumpkinseed pharyngeal jaw morphology, there was evidence from the pond experiment of an effect of source lake on the fishes’ response to the pond environment. In particular, both levator posterior muscle mass and diet showed significant lake $\times$ pond interactions. The diet

Table 4. Comparison of pumpkinseed molluscivory among ponds^a

Pond	Three Lakes fish		Wintergreen Lake fish	
	Mean dry mass of molluscs eaten (mg)	Mean % molluscs in diet	Mean dry mass of molluscs eaten (mg)	Mean % molluscs in diet
7	0.70	13.1 ± 3.4	0.50	13.8 ± 4.6
9	0.21	10.8 ± 3.7	0.24	11.4 ± 5.8
15	0.13	16.5 ± 5.2	0.15	3.5 ± 1.6
18	1.19	33.3 ± 6.4	0.40	14.3 ± 4.9
Average	0.56 ± 0.24	18.4 ± 5.1	0.32 ± 0.08	10.8 ± 2.5

^a Pumpkinseed diets within a pond (mean ± 1 S.E.) are based on 15 fish per source lake, collected at the end of the experiment (July). Average diets are the mean (+ 1 S.E.) of the four ponds ($n = 4$). Two-way analysis of variance revealed significant effects of pond on the percent of molluscs in the diet ($F_{3,111} = 4.82$, $P < 0.01$) and on the mass of molluscs eaten ($F_{3,111} = 11.19$, $P < 0.01$). There was also a significant pond × lake interaction for mass of molluscs eaten ($F_{3,111} = 4.31$, $P < 0.01$), and a marginally significant pond × lake interaction for the percent of molluscs in the diet ($F_{3,111} = 2.42$, $P = 0.07$). There was no effect of lake on either the mass of molluscs eaten ($F_{1,111} = 1.474$, $P > 0.50$) or the percent of molluscs in the diet ($F_{1,111} = 2.772$, $P > 0.20$).

results suggest that there may be behavioural differences between fish from the two lakes that led to different diets within the same pond, and there is the suggestion that Three Lakes fish tend to feed more extensively on molluscs than do Wintergreen Lake fish. However, between-lake dietary differences were not expressed in all ponds. Moreover, differences in diet were not related to the differences in levator posterior muscle mass among ponds. Therefore, while the common-garden experiment shows subtle, but significant, interactions between source lake and rearing environment, we do not have the ability in this experiment to determine the mechanisms by which these lake effects are manifest in either diet or jaw morphology. It is clear, however, that differences due to source lake were only expressed in complex, natural environments. In the controlled environment of the laboratory, where fish were not allowed any behavioural choice of diet or habitat, there was no effect of source lake on jaw morphology.

The variation in pumpkinseed morphology observed between Wintergreen Lake and Three Lakes represents variation among pumpkinseed populations (see also Fig. 4). We have, in addition, observed variation in morphology within populations, which also appears related to diet. For example, in lakes with abundant snails, pumpkinseed shift from a diet dominated by soft-bodied invertebrates to a diet dominated by gastropods between approximately 50 and 80 mm standard length (Mittelbach, 1984; Osenberg *et al.*, 1994). During this transition, however, individuals seem to differ in the timing of the transition. For example, in one collection of fish from Three Lakes, in which we have both diets and measurements of the mass of the levator posterior, there were 10 pumpkinseeds between 59 and 80 mm standard length, of which 4 had diets dominated by snails (>80% of prey biomass) and 6 had diets dominated by soft-bodied prey (<20% snail biomass). We classified these fish as either ‘snail specialists’ or ‘insect specialists’, and then examined the relationship between LP muscle mass and fish size. The snail specialists had an LP muscle mass almost twice as large as the insect specialists, when compared at a common size (Fig. 5).

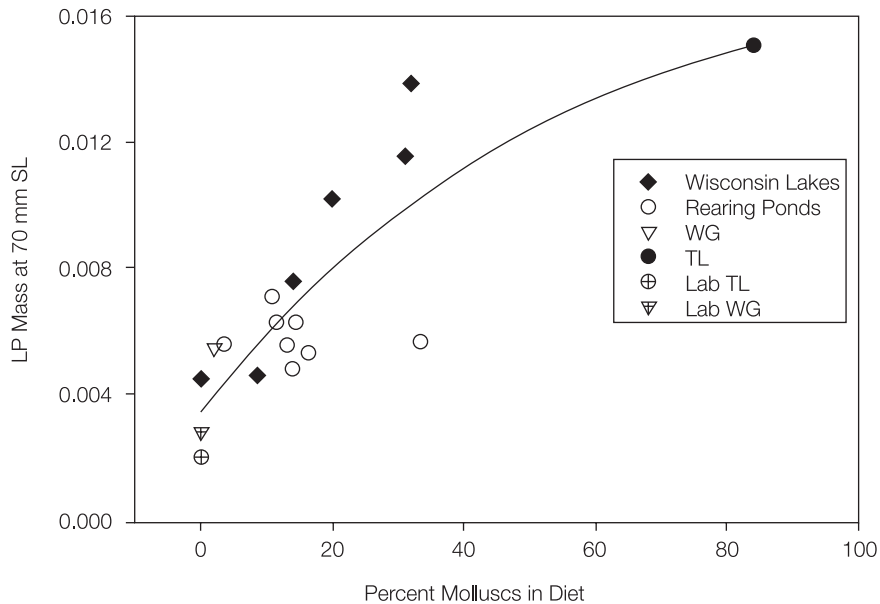


Fig. 4. Mean levator posterior (LP) muscle mass in pumpkinseeds collected from Michigan and Wisconsin lakes, raised in the Kellogg Biological Station experimental ponds, or raised in the laboratory. For each environment, log LP muscle mass was regressed against log pumpkinseed standard length, and the predicted LP mass at a standardized fish length (70 mm) was used to compare among environments. The data were fit by non-linear regression to the equation: $y = a + b(1 - c^x)$, where $a = 0.00345$, $b = 0.01488$, $c = 0.9819$, $r^2 = 0.71$.

Robinson *et al.* (1993, 1996) and Robinson and Wilson (1996) have also demonstrated a trophic or resource-based polymorphism within a population of pumpkinseeds from a single lake. Robinson *et al.* (1993) collected pumpkinseeds from the shallow littoral zone and from rocky outcrops in the open water of Paradox Lake, NY, and found that individuals from these two areas differed in diet. Pumpkinseeds from rocky outcrops feed on zooplankton and snails, while those in the littoral zone feed on snails and other benthic invertebrates. Individuals from the two areas also differed in body shape, gill-raker width and pectoral fin length. In a common-garden type experiment, Robinson and Wilson (1996) found that phenotypic plasticity and genetic differentiation accounted for 53 and 14%, respectively, of the variation in body shape. Therefore, like our study, phenotypic plasticity was the major factor accounting for morphological variation among pumpkinseeds, although genetic differentiation also appeared to contribute significantly to the Paradox Lake polymorphism.

There are some important differences between the studies of Robinson *et al.* (1993, 1996) and our own work on pumpkinseed trophic variation. For one, the morphological differences observed by Robinson *et al.* (1993, 1996) are relatively small in magnitude (individual traits differed by <5% on average) and the distinction between morphs can only be detected by a multivariate analysis of many characters. In contrast, the observed differences in pharyngeal jaw muscle or bone masses between pumpkinseeds from Wintergreen Lake and Three Lakes are large (up to a 230% difference) and are detectable on individual traits.

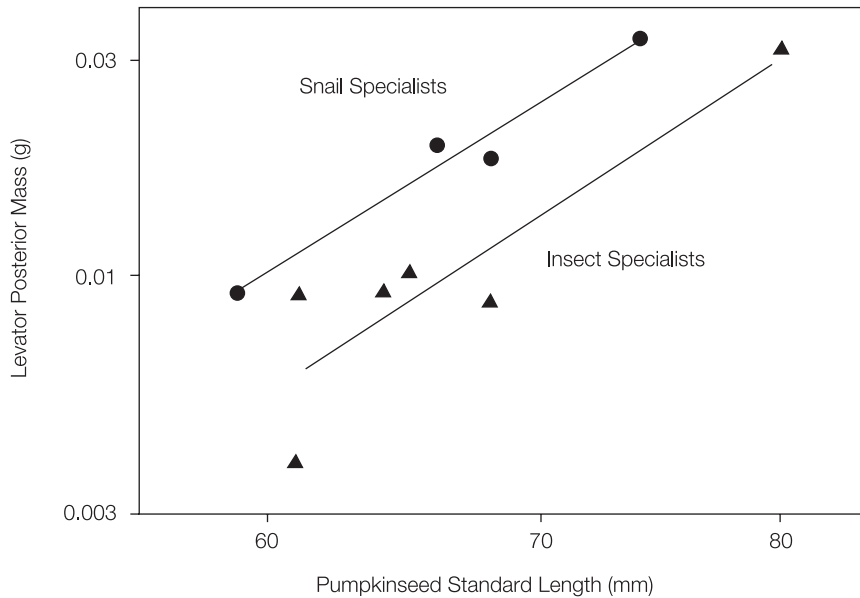


Fig. 5. Levator posterior muscle mass in fish collected from Three Lakes, which had diets dominated by either snails or soft-bodied prey (notably insect larvae). All fish smaller than 59 mm SL had diets dominated by soft-bodied prey, whereas fish > 80 mm SL had diets dominated by snails (e.g. Mittelbach, 1984; Osenberg *et al.*, 1994). The 59–80 mm SL size range is the range at which the ontogenetic shift to snails occurs. Analysis of covariance revealed statistically significant effects of diet type ($F_{1,9} = 11.23$, $P = 0.0122$) and fish size ($F_{1,9} = 36.68$, $P = 0.0005$) on LP muscle mass. Snail specialists had LP muscle masses ~80% larger than insect specialists.

Second, the link between morphology and ecological function is clear and well-documented for the muscles and bones used by pumpkinseeds to crush snails (i.e. Lauder, 1983a; Wainwright, 1989; Wainwright *et al.*, 1991a; Wainwright and Lauder, 1992), whereas it is less clear how the differences in body shape observed by Robinson *et al.* (1993) for pumpkinseeds from the littoral-zone and the open-water habitats in Paradox Lake actually determine their abilities to acquire resources in each habitat. However, despite the lack of a strong mechanistic link between morphology and ecological function in the study of Robinson *et al.* (1993), they did find evidence for a trade-off between general body form and feeding performance. Pumpkinseeds with an intermediate body form tended to be in poorer condition and have slower growth compared to extreme open-water forms found in the open-water habitat and extreme littoral forms found in the littoral habitat (Robinson and Wilson, 1996).

Are there similar adaptive advantages to enlarged pharyngeal jaw muscles and bone due to feeding on snails, and are there any associated trade-offs on other resources? It appears clear that there is a significant advantage associated with hypertrophied pharyngeal muscles in snail-rich environments. For example, in many crushing fishes, there is a direct correlation between the mass of the pharyngeal jaw muscles and the strength of snail shell that can be crushed (Wainwright, 1987, 1988), a pattern also observed in pumpkinseeds (Osenberg *et al.*, 1994). Because there is a fraction of the snail assemblage of lakes that includes species and sizes beyond the crushing abilities of a fish (Osenberg and Mittelbach, 1989; Osenberg

et al., 1994), increasing jaw muscle mass would allow pumpkinseeds to exploit a greater portion of the snail resource. Furthermore, because pumpkinseeds make the switch to feeding on snails gradually during their ontogeny (e.g. approximately between 50 and 80 mm SL in many Michigan lakes; Osenberg *et al.*, 1994), increasing muscle mass would allow fish to switch to feeding on snails at a smaller size (earlier age). Therefore, there is a strong, positive feedback between the consumption of snails, an increase in pharyngeal jaw strength and the ability of an individual to exploit a larger fraction of the snail resource. Induced morphological differences thus reinforce foraging specialization by increasing foraging efficiency on a prey type (Wimberger, 1994), as suggested by the variation in diet and morphology observed within Three Lakes (Fig. 5).

Thus, although our results provide no evidence of a genetic basis for variation in functional morphology, the observed phenotypic plasticity represents an important mechanism that can mould a fish's morphology to the resource base of a lake. Ultimately, this would be most adaptive if reduced crushing morphology resulted in the more efficient use of soft-bodied prey. To date, we have no evidence for such a trade-off in pumpkinseed, although work on its sister species, the redear sunfish, has shown that such a trade-off exists (Huckins, 1997; see also Ehlinger, 1990; Schluter, 1995; Robinson *et al.*, 1996, for other examples of trade-offs). Now that we know we can manipulate the pharyngeal jaw structure of pumpkinseed through variation in diet, we are in a position to induce variation experimentally and use this to examine more carefully trade-offs between feeding on hard- and soft-bodied prey. Such work can provide a powerful approach to understanding the functional basis of trophic polymorphisms and the evolution of phenotypic plasticity.

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