



## Selection for phenotypic plasticity in *Rana sylvatica* tadpoles

JOSH VAN BUSKIRK<sup>1</sup> AND RICK A. RELYEA

Department of Biology, University of Michigan, Ann Arbor, MI 48109, U.S.A.

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The hypothesis that phenotypic plasticity is an adaptation to environmental variation rests on the two assumptions that plasticity improves the performance of individuals that possess it, and that it evolved in response to selection imposed in heterogeneous environments. The first assumption has been upheld by studies showing the beneficial nature of plasticity. The second assumption is difficult to test since it requires knowing about selection acting in the past. However, it can be tested in its general form by asking whether natural selection currently acts to maintain phenotypic plasticity. We adopted this approach in a study of plastic morphological traits in larvae of the wood frog, *Rana sylvatica*. First we reared tadpoles in artificial ponds for 18 days, in either the presence or absence of *Anax* dragonfly larvae (confined within cages to prevent them from killing the tadpoles). These conditioning treatments produced dramatic differences in size and shape: tadpoles from ponds with predators were smaller and had relatively short bodies and deep tail fins. We estimated selection by *Anax* on the two kinds of tadpoles by testing for non-random mortality in overnight predation trials. Dragonflies imposed strong selection by preferentially killing individuals with relatively shallow and short tail fins, and narrow tail muscles. The same traits that exhibited the strongest plasticity were under the strongest selection, except that tail muscle width exhibited no plasticity but experienced strong increasing selection. A laboratory competition experiment, testing for selection in the absence of predators, showed that tadpoles with deep tail fins grew relatively slowly. In the cattle tanks, where there were also no free predators, the predator-induced phenotype survived more poorly and developed slowly, but this cost was apparently not associated with particular morphological traits. These results indicate that selection is currently promoting morphological plasticity in *R. sylvatica*, and support the hypothesis that plasticity represents an adaptation to variable predator environments.

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<sup>1</sup>Correspondence to J. Van Buskirk. Present address: Institute of Zoology, University of Zürich, CH-8057 Zürich, Switzerland. Email: jvb@zool.unizh.ch

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## INTRODUCTION

The performance of an individual, and ultimately its fitness, results from complex relationships between the traits it possesses and the environment it occupies. Individuals with high relative fitness presumably have achieved an appropriate match between their phenotype and the environment. Most organisms achieve this match to some extent by selecting habitats at various scales, but they also match their phenotype to the environment by means of plasticity, in which individuals alter their phenotypes during development in response to environmental cues.

It has long been recognized that plasticity might represent an adaptation allowing individuals to match their phenotype to the local environment, given that reliable cues are available to signal the condition of the environment (Wright, 1931; Dobzhansky, 1951; Bradshaw, 1965; Levins, 1968; Stearns, 1989; West-Eberhard, 1989). This hypothesis constitutes an important and general conclusion of theoretical treatments of plasticity as well (Via & Lande, 1985; Lively, 1986a; Gomulkiewicz & Kirkpatrick, 1992; Moran, 1992; van Tienderen, 1997). Confirmation of the adaptive plasticity hypothesis (Schmitt, McCormac & Smith, 1995) calls for evidence concerning both the present function of plasticity and the evolutionary forces that produced it in the past and presently maintain it (Williams, 1966; Lewontin, 1978; Brandon & Rausher, 1996). Our goal is to assess one part of this hypothesis, the assumption that natural selection currently acts on plastic traits, and maintains plasticity by favouring different phenotypes in different environments.

To be convincing, a test of adaptation requires evidence in support of two central assumptions, neither of which implies that the other is true (Brandon & Rausher, 1996). The first assumption is that the existing phenotype is optimal, at least locally. In the context of plasticity, this assumption implies that the average reaction norm within a population confers higher fitness than any alternative possible reaction norm. That is, the population should be at or near an evolutionary stable strategy that could not be invaded by genotypes displaying a different configuration or degree of plasticity. At this stage, predictions of the general form of the reaction norm may be constructed, based on knowledge of the functional consequences of phenotypic variation and the constraints operating within the system (Stearns, 1989; Werner & Anholt, 1993; Smith & Van Buskirk, 1995; Dudley & Schmitt, 1996). Tests of these predictions typically compare the performance of the phenotype induced by an

environment with the performance of other phenotypes induced by other environments. Many of these studies report that the phenotype that confers the highest performance within a particular environment is that which was induced by prior exposure to the environment (Harvell, 1986; Lively, 1986b; Spitze, 1992; McCollum & Van Buskirk, 1996). This approach has not upheld the adaptive plasticity hypothesis in all cases, however (O'Brien *et al.*, 1980; Baldwin, Sims & Kean, 1990; Spitze, 1992; Schmitt, 1993; Kingsolver, 1995), reflecting extensive variability in performance or suggesting that constraints might often prevent organisms from evolving adaptive plasticity.

The second assumption of the adaptive plasticity hypothesis is that the reaction norm is a result of natural selection acting in the past. This is a primarily historical assumption, but it can be tested in at least two ways. First, phylogenetic tests of character evolution (Baum & Larson, 1991; Leroi, Rose & Lauder, 1994) can demonstrate an association between plasticity and environmental heterogeneity. The predicted outcome of such analyses is that taxa that routinely experience variable environments should display greater plasticity than taxa from constant environments, because only organisms exposed to multiple environments will undergo selection for plasticity. This prediction appears reasonable, but has not been uniformly upheld by empirical results (e.g. Semlitsch, Harris & Wilbur, 1990; Galloway, 1995).

The assumption that a reaction norm resulted from natural selection can also be tested indirectly by measuring whether selection is currently maintaining plasticity. Such evidence would not demonstrate that selection in the past, across the existing set of environments, produced the reaction norm in the specific case, but it would help argue the general case that divergent selection produces phenotypic plasticity (Brandon & Rausher, 1996). This is the approach we take in this study. We ask whether selection is currently acting to maintain plasticity, using a species that has an apparently adaptive morphological response to predators. If selection is currently maintaining plasticity, then it should be possible to show that there are differences among environments in the relationship between fitness and phenotype. A few earlier studies of plastic traits have demonstrated that selection currently favours different phenotypes in contrasting environments, and that the direction of selection is consistent with the adaptive plasticity hypothesis (Dudley & Schmitt, 1996; Van Buskirk, McCollum & Werner, 1997). If these results turn out to be general, then we will have strengthened the case that some types of plasticity evolve in response to divergent selection regimes in heterogeneous environments.

Amphibian larvae display extensive plasticity in life history, morphology, diet, and behaviour in response to variation in environmental conditions such as food, competitors, and predators (Skelly & Werner, 1990; Newman, 1992; McCollum & Van Buskirk, 1996). In many cases phenotypes are affected in such a way that plasticity appears to be beneficial, but specific assumptions of the adaptive plasticity hypothesis have not often been tested. For example, in ponds containing predatory insects, tadpoles of several species become relatively inactive and develop tail muscles and fins that are deep, broad, and sometimes brightly-coloured in comparison with those of tadpoles from predator-free habitats (Smith & Van Buskirk, 1995; McCollum & Van Buskirk, 1996). Each of these components of the phenotype that respond to the presence or absence of predators might contribute to an overall adaptive response to predators, because one can hypothesize functional connections between traits (e.g. behaviour, shape, colour) and individual performance with or without predators. However, it remains to be shown that the plastic traits are consistently beneficial

when expressed in the appropriate environment (e.g. Anholt & Werner, 1995; McCollum & Van Buskirk, 1996), are targets of divergent selection across environments, or vary among species as predicted by the adaptive plasticity hypothesis.

We measured morphological plasticity and selection in tadpoles of the wood frog, *Rana sylvatica* (Anura: Ranidae), because this species occurs in ponds with highly variable predator faunas and the tadpoles show strong morphological and behavioural responses to predators. *Rana sylvatica* tadpoles exposed to dragonflies consistently have deeper tail fins and shorter bodies than tadpoles reared in the absence of predators. In this study, we ask whether selection on tadpole morphology, imposed by predatory dragonflies and competing tadpoles of a different species, is consistent with the hypothesis that plasticity in shape is maintained by divergent selection in the presence and absence of predators. Specifically, we predict that traits showing the most extensive plasticity also currently undergo the strongest selection, and that the directions of plasticity and selection are congruent.

#### METHODS

We collected *Rana sylvatica* eggs in early April 1996 from eight populations on and near the University of Michigan's E.S. George Reserve in southeastern Michigan, USA. Three clutches of full-sibs were taken from each population, and the clutches were maintained separately in well water within 300-L outdoor wading pools until the experiment began, 19–21 days after the tadpoles hatched. The populations came from fish-free temporary or semi-permanent ponds, including four open marshes and four closed-canopy woodland ponds. The choice of populations and clutches was dictated by the design of a separate experiment (R.A. Relyea, unpublished).

The study consisted of three phases. First we reared the tadpoles in the presence or absence of caged dragonfly larvae for several weeks in order to estimate morphological plasticity. Then we took tadpoles from the two environments and subjected them to free dragonflies in overnight predation trials to determine whether predators killed tadpoles non-randomly with respect to body size and shape. Finally, we estimated selection in the absence of free predators by comparing tadpole phenotypes with tadpole performance in cattle tanks over the entire larval period, and by relating growth rate to morphology in a laboratory competition experiment.

#### *Plasticity experiment*

The plasticity experiment was conducted in outdoor artificial ponds (plastic stock tanks). Tadpoles do well in the semi-natural conditions of these ponds, which are large enough to support a sufficient number of tadpoles for predation trials. We filled the tanks with well water on 24–26 April 1996 to a depth of 41 cm (1100 L volume, 2.6 m<sup>2</sup> surface area), and stocked them with 0.3 kg oak leaf litter, aliquots of phytoplankton and zooplankton collected from nearby ponds, and 25 g commercial rabbit chow to provide an initial nutrient source for the food web. Tanks were kept covered with lids constructed of fibreglass window screen to prevent colonization by unwanted predators and amphibians. We introduced 110 tadpoles to each tank on 6 May 1996 (42 individuals/m<sup>2</sup>).

The experiment included 24 replicates of two treatments, *Anax* present or no predators present, with each replicate comprised of 110 full sibs from one of the eight source populations. The treatments were assigned at random to 48 tanks arranged in an hexagonal array in an open field at the George Reserve. Four late-instar *Anax longipes* were placed within each of the *Anax* tanks, confined inside floating cages constructed of a 12 cm length of plastic drainpipe (11 cm diameter) with the ends covered by fibreglass window screening. Tanks in the no-predator treatment contained four empty cages. Each *Anax* was fed 4–7 *R. sylvatica* tadpoles (300 mg total), three times per week, throughout the experiment.

Groups of tadpoles were removed from the tanks after 18 days for predation trials and estimates of plasticity (see below), after 23 days for a competition experiment (see below), after 35 days for a sample of growth rate (20 tadpoles weighed to the nearest mg), and for unrelated experiments on one other occasion. In all cases, the same number of individuals was removed from every tank.

Once metamorphosis was underway we collected all metamorphosing froglets from the tanks 3–5 times per week, and recorded the date upon which each one resorbed its tail (stage 46; Gosner, 1960). For each tank we determined the average date of metamorphosis, and calculated survival to metamorphosis as the number of froglets divided by 30 (the number of tadpoles not removed for ancillary experiments during the course of the study).

There were no significant differences among populations in the impact of selection, and we will hereafter disregard the population of origin and treat each clutch as an independent replicate. Population-level differences in phenotype and plasticity will be reported elsewhere (R.A. Relyea, *unpublished*).

We measured the morphology of tadpoles using a dissecting scope and camera connected to a computer equipped with image analysis software. Viewing the tadpole from the side we digitized the length and depth of the body, maximum depth of the tail fin, maximum tail muscle depth at the base of the tail, and the tail length measured from the junction of the spinal column and the body. Viewing from the top we digitized body width and maximum width of the tail muscle at the base. To generate a measure of overall body size, we performed a principal component analysis on the correlation matrix constructed from five of these log-transformed linear measures (excluding width and depth of the tail muscle because they scale less reliably with body size) and the wet mass of the preserved tadpole, and kept the first component (which accounted for 90% of the total variance) as a measure of size for subsequent analyses.

Correlations among traits present a problem for interpreting estimates of plasticity and selection because they potentially obscure the traits which are actually exhibiting plasticity or undergoing selection. Phenotypic correlations can generate indirect selection, in which selection acting on one trait causes apparent selection on correlated traits (Falconer & Mackay, 1996). The ambiguity is less problematic in the context of plasticity, but knowledge of correlations among traits can indicate the extent to which changes in several traits actually represent the plastic response of a single multidimensional trait. We therefore began our analysis by testing for individual-level phenotypic correlations among all combinations of traits, separately for the two treatments (caged *Anax* and no-predators). We focused on four measures of tail shape and three measures of body shape, which were the residuals of log-transformed tail length, tail fin depth, tail muscle depth, tail muscle width, body length, body depth, and body width after regression against the first principal

component. Our method of standardizing measures of shape by regression against a single size component, termed shearing, is a common preliminary step in morphometric analysis (Bookstein, 1991).

We tested the significance of plasticity by comparing the morphology of tadpoles from the no-predator and caged-*Anax* tanks; phenotypes within each tank were estimated from the average of 10 tadpoles collected at day 18. First we performed a multivariate analysis of variance (MANOVA) testing for differences between treatments in the seven measures of shape. The dominant eigenvector for the tank treatment effect in the MANOVA represented the pattern of plasticity exhibited by all traits together, after accounting for correlations among traits. This vector is the linear combination of the seven response traits that distinguished most clearly between tadpoles originating from the two environments; thus it defines the principal phenotypic axis along which plasticity was expressed. We also inspected univariate ANOVAs to reveal how each trait responded to the difference between tank treatments when viewed in isolation.

#### *Predation trials: selection in the presence of Anax*

We measured selection imposed by dragonfly predation on *R. sylvatica* by comparing the morphology of tadpoles which survived exposure to free-ranging *Anax* in short-term predation trials with that of tadpoles exposed to no predators during the same time period. Eighteen days after starting the plasticity experiment, we removed 20 tadpoles from each tank, haphazardly divided them into two groups of 10, and placed each group into a plastic tub (10L water; 26 × 38 × 14 cm). The tubs contained a coil of plastic rope to provide perches for the predator, and were shelved in an indoor laboratory at a cycle of 14:10 hr light:dark. The experiment had two treatments (presence or absence of the predator, *Anax*), which were performed for tadpoles from each tank in the plasticity experiment, making 96 total tubs. A single final-instar *Anax longipes*, which had been starved for 2 days before the experiment, was introduced to the appropriate tubs at 17:00 on 24 May 1996. The predators were removed the following day at 12:00, and all surviving tadpoles were preserved in 10% formalin.

We tested for selection on morphology in the presence of predators by determining whether dragonflies killed tadpoles non-randomly with respect to body size and the seven measures of body shape. The average values of these traits for the 10 tadpoles that were not exposed to predators provided an estimate of phenotype prior to selection, and the averages for those tadpoles that survived *Anax* predation provided an estimate of phenotype after selection. For each cattle tank we determined the selection differential for each trait as the trait value after selection minus the trait value before selection. Selection differentials were standardized by dividing this difference by the phenotypic standard deviation before selection (Arnold & Wade, 1984). The resulting standardized selection differential is often called the 'selection intensity' (Falconer & Mackay, 1996).

We tested the significance of selection differentials imposed by *Anax* predation using a linear model that included tank treatment and intercept in the design matrix. The intercept tested whether the grand mean of the selection intensities was different from zero (that is, whether there was significant selection averaged across both phenotypes). The treatment effect tested whether the magnitude of the selection

intensities depended on prior exposure to either *Anax* or no predators in the tanks. We began with a multivariate analysis on selection intensities for all seven tail and body traits together, to determine whether there was significant selection on overall shape. As above, the dominant eigenvector for the intercept term in MANOVA reflected the pattern of selection acting on the entire suite of correlated traits. In this case, the eigenvector contained the linear combination of the seven response variables (selection intensities) that deviated most strongly from zero, which defined the phenotypic axis along which selection acted after accounting for phenotypic correlations. We also inspected univariate ANOVAs to reveal the apparent direction and magnitude of selection acting on each trait, disregarding the correlations among traits.

### *Selection in the absence of Anax*

The hypothesis of divergent selection across environments predicts that traits associated with higher performance in the absence of predators should be opposite to those that confer higher survival in the predation trials. We used two approaches to check for selection on tadpole morphology in the absence of predators. First, we performed a short-term laboratory experiment to determine if individual growth rate was related to morphology, in both the presence and absence of a competing species. Measuring selection under conditions of food limitation is realistic in light of the natural history of *Rana sylvatica*, since tadpoles in predator-free ponds occur very often at densities that are high enough for competition to be important (E.E. Werner *et al.*, unpublished).

The competition trial began 5 days after the predation trial, when the cattle tank experiment had been underway for 23 days. We collected 20 tadpoles from each tank, haphazardly divided them into two groups of 10 individuals each, and placed each group into an 8-L plastic tub on a laboratory shelf (96 tubs in total). Half of the tubs contained a single *Anax* larva, because the predator environment established in the cattle tank experiment was maintained during the competition trial. Dragonflies were confined within a small plastic cup with a screened lid, and were fed two *R. sylvatica* tadpoles (~300mg) every other day. The tadpoles from each tank were exposed to two treatments, consisting of the presence or absence of 3 second-year bullfrog tadpoles (*Rana catesbeiana*, average individual mass = 3.35g), chosen because they show no behavioural response to *Anax* at such large sizes (Peacor & Werner, 1997). We weighed the 96 sets of 10 tadpoles just before placing them in the tubs, and fed them every day during the trial. For the first 3 days we added 10% of the average body mass of the *R. sylvatica* tadpoles per day (3:1 mixture of rabbit chow: Tetramin fish flakes), but this ration proved to be too high, causing excessive bacterial growth. We therefore changed the water and reduced the daily ration to 5% of body mass. The experiment ended after 7 days, at which time all survivors were weighed. Our analysis compared the growth of the 10 tadpoles within each tub (proportional change in mass) with their average morphological trait values measured at 18 days. Multiple regressions were performed on growth in the presence and absence of competitors, as well as the difference in growth between the two competitor treatments, which we interpret as an estimate of the impact of competition.

Our second approach to estimating selection without predators was a comparative analysis of tadpole performance in the absence of free-ranging predators within the

plasticity experiment. We began with a multivariate multiple regression testing for a relationship between performance and tadpole phenotypes within all 48 cattle tanks, since none contained free-ranging predators. The three performance measures were growth [ $\log(\text{mass on day 35}/\text{initial mass})$ ], survival to metamorphosis, and average time to metamorphosis; the model included the predator treatment (*Anax* present or absent) and the seven morphological traits (measured at day 18). We also examined univariate relationships between the three performance measures and phenotype. Tank means were the unit of analysis, because survival is a collective variable and we have no data on individual phenotypes late in the experiment. Predator treatment was included in the model to account for variation in growth or survival due to plastic responses to predators, before asking whether populations of tadpoles having different average phenotypes performed differently.

Both of our tests for selection in predator-free environments assume that survival, body size, and timing of metamorphosis are correlated with fitness, which is supported by field data from several amphibian species (Smith, 1987; Semlitsch, Scott & Pechmann, 1988; Berven 1990; Scott, 1994).

## RESULTS

### *Phenotypic correlations*

There were strong phenotypic correlations among measures of body shape and size of *Rana sylvatica* tadpoles in both environments after 18 days (Table 1). All four measures of tail shape were positively correlated with one another, the three measures of body shape were positively correlated, and all body traits were negatively correlated with tail traits. The congruence between matrices measured in the two environments was highly significant ( $P < 0.0001$ , Mantel randomization test; Mantel, 1967), indicating that the pattern of phenotypic correlation was similar in the presence and absence of dragonflies. These results indicate that our measures of body and tail shape do not represent independent traits, presumably because they share some level of underlying genetic or developmental causation. This confirms that multivariate measures of plasticity and selection are appropriate.

### *Phenotypic plasticity*

*Rana sylvatica* displayed dramatic plasticity in body and tail shape at 18 days. Tadpoles from the two environments differed most strongly in their body length and tail fin depth, relative to overall body size, according to the dominant eigenvector derived from MANOVA on all seven traits (Table 2A). In comparison with tadpoles reared in predator-free ponds, those exposed to *Anax* had relatively short bodies and long, deep tails. Univariate analyses suggested that predator-induced tadpoles were smaller, had shorter and narrower bodies, deeper tail fins, and marginally longer tails (Fig. 1A, B, Table 2B), and variation in tail muscle shape across predator environments was not significant (Fig. 1C). There were minor differences between the multivariate and univariate depictions of plasticity, resulting from correlations among the traits that responded to predators. For example, when viewed in isolation,



TABLE 1. Phenotypic correlations among measures of size and shape in *Rana sylvatica* tadpoles. Estimates are based on 233 individuals sampled in the no-predator environment (above the diagonal) and 230 sampled in the *Anax* environment (below the diagonal), on day 18. Body size was the first component from a PCA; all other traits are residuals on PC1. Boldfaced values are significant:  $P < 0.05$  if  $r > 0.121$ ,  $P < 0.01$  if  $r > 0.150$

Trait	Trait							
	Body size	Body length	Body depth	Body width	Tail length	Tail fin depth	Muscle depth	Muscle width
Body size	–	<b>–0.486*</b>	<b>–0.177</b>	<b>–0.307*</b>	<b>0.200</b>	<b>0.540*</b>	<b>0.253</b>	<b>0.158</b>
Body length	<b>–0.719*</b>	–	<b>0.164</b>	<b>0.212*</b>	<b>–0.402*</b>	<b>–0.541*</b>	<b>–0.320*</b>	<b>–0.228*</b>
Body depth	<b>–0.132</b>	0.051	–	<b>0.420*</b>	<b>–0.600*</b>	<b>–0.537</b>	<b>–0.462*</b>	<b>–0.133</b>
Body width	<b>–0.215*</b>	0.104	<b>0.473*</b>	–	<b>–0.646*</b>	<b>–0.577*</b>	<b>–0.287*</b>	<b>–0.215*</b>
Tail length	–0.120	<b>–0.227*</b>	<b>–0.580*</b>	<b>–0.705*</b>	–	<b>0.235*</b>	<b>0.282*</b>	0.020
Tail fin depth	<b>0.676*</b>	<b>–0.627*</b>	<b>–0.471*</b>	<b>–0.456*</b>	<b>0.136</b>	–	<b>0.386*</b>	0.105
Tail muscle depth	0.101	<b>–0.212*</b>	<b>–0.401*</b>	<b>–0.285*</b>	<b>0.305*</b>	<b>0.423*</b>	–	<b>0.289*</b>
Tail muscle width	–0.045	–0.005	<b>–0.133</b>	–0.097	0.095	<b>0.276*</b>	<b>0.322</b>	–

\*Significant at  $\alpha = 0.00183$  (Bonferroni adjustment for 28 tests).

TABLE 2. Multivariate and univariate analyses of plasticity in *Rana sylvatica* tadpoles. Morphology was measured after 18 days exposure to environments having either caged *Anax* larvae or no predators. The dominant eigenvector represents the linear combination of traits that differed most strongly between predator treatments; traits with negative coefficients were larger in the *Anax* treatment

A. MANOVA: plasticity in seven measures of tadpole shape

Source	df	Wilks' <i>F</i>	<i>P</i>	Coefficients of the dominant eigenvector						
				Body length	Body depth	Body width	Tail length	Tail fin depth	Muscle depth	Muscle width
Tank Environment	7,40	11.69	0.0001	3.420	-1.100	-1.249	-1.636	-3.676	0.757	0.601

B. Univariate ANOVA: plasticity in separate traits

Response	Model SS (1 df)	Error SS (46 df)	<i>F</i>	<i>P</i>
Size (PC1)	95.3	71.73	61.1	0.0001
Body length	0.029	0.018	75.18	0.0001
Body depth	0.003	0.017	7.48	0.0088
Body width	0.001	0.011	3.63	0.0631
Tail length	0.003	0.032	3.97	0.0524
Tail fin depth	0.058	0.042	63.29	0.0001
Muscle depth	0.002	0.049	2.34	0.1329
Muscle width	0.0005	0.085	0.29	0.5959

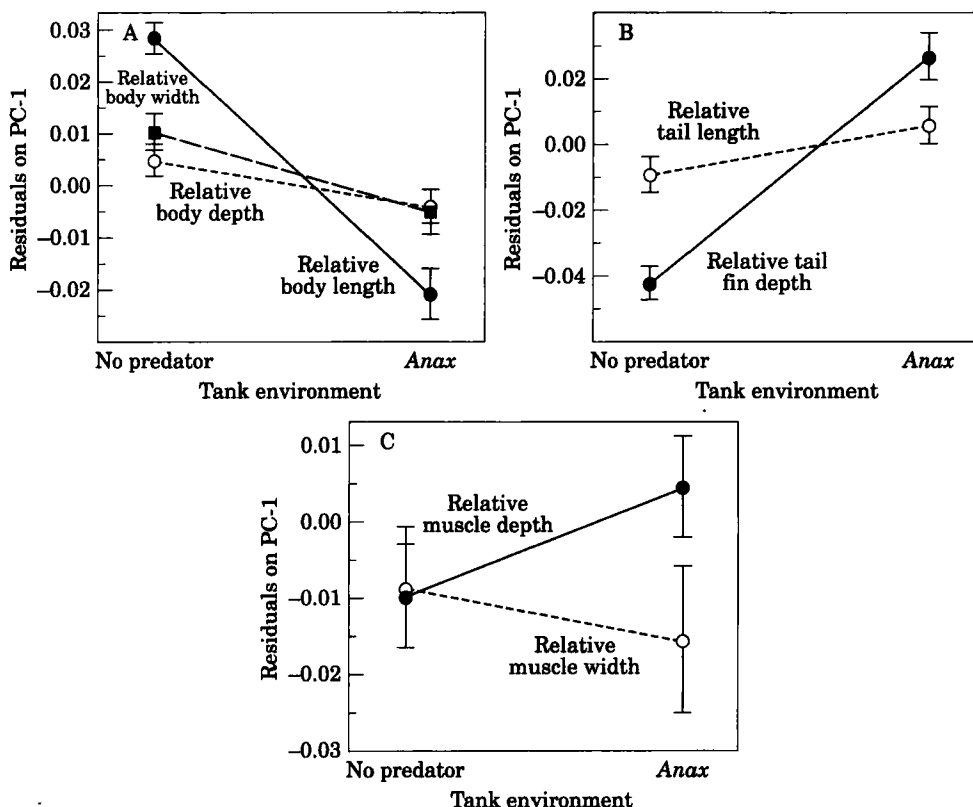


Figure 1. Plastic morphological responses of *Rana sylvatica* tadpoles after 18 days in artificial ponds containing either caged *Anax* dragonflies or no predators. All responses are corrected for body size by first regressing against PC1 and retaining the residuals for subsequent analysis. Shown are means  $\pm 1$  SE, calculated from univariate comparisons between treatments, with 24 replicates. (A) body shape; (B) tail fin shape; (C) tail muscle shape.

relative tail muscle depth increased slightly when *Anax* was present (Fig. 1A), whereas the same trait showed a decrease in the multivariate analysis (Table 2A). The increase in muscle depth apparent in the univariate comparison may have arisen because of its negative correlation with body length (Table 1).

#### *Estimates of selection*

In the overnight predation trials, dragonflies killed an average of  $3.0 \pm 0.4$  tadpoles from the *Anax* treatment and  $2.0 \pm 0.3$  tadpoles from the no-predator treatment (mean  $\pm 1$  SE;  $P=0.029$ , *t*-test). The fact that tadpoles having the predator-induced phenotype did not experience enhanced survival in this experiment, as in some others (e.g. McCollum & Van Buskirk, 1996), presumably resulted from their smaller body size (Table 2B).

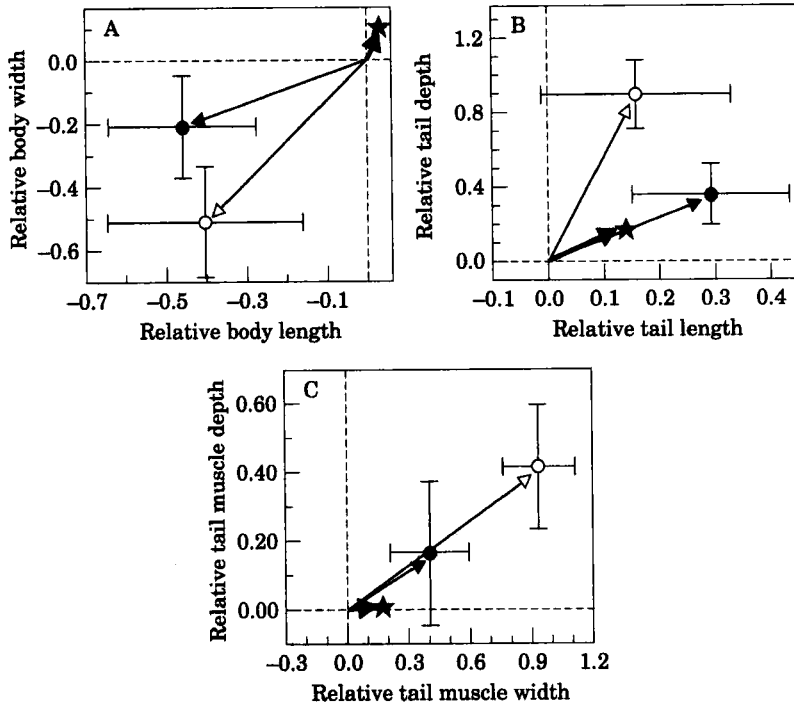


Figure 2. Standardized selection differentials (in units of standard deviation of the trait prior to selection) on (A) body, (B) tail fin and (C) tail muscle shape imposed by *Anax* preying on *Rana sylvatica* tadpoles. The two phenotypes were induced by exposure to caged dragonflies or no predators in artificial ponds for 18 days. The heavy arrows and the ★ depict the direction and magnitude of direct selection acting on both phenotypes, estimated by the coefficients of the dominant eigenvector in MANOVA on seven traits. Circular symbols ( $\pm 1$  SE) represent selection coefficients estimated from univariate analyses, and therefore result in part from indirect selection acting on other traits. Dashed lines indicate selection differentials of zero, as expected if no selection is acting on the trait. (●) no-predator phenotype; (○) predator-induced phenotype.

Predation by *Anax* imposed strong selection on tadpole shape, and the magnitude of selection was similar for both the predator-induced phenotype and the no-predator phenotype (Fig. 2, Table 3). Multivariate and univariate estimates of selection intensity were quite different, as expected when selection acts on highly correlated traits. The significant intercept term in the MANOVA (which tests for selection across both tank treatments) resulted from selection favouring tadpoles with relatively deep tail fins and large tail muscles in the presence of predators, although all seven traits were under positive selection to some degree (Table 3A). In contrast, standardized selection differentials calculated from univariate comparisons revealed selection for smaller body dimensions and larger tail dimensions (Table 3B, Fig. 2). There was no selection on body size (PC1). Figure 2 makes clear that the univariate and multivariate approaches yielded similar estimates for selection on the tail, but very different results for body length and body width. These results suggest that the tail fin and tail muscle were under direct selection for increasing values, while the body measures were under indirect selection mediated through their negative correlation with tail traits.

TABLE 3. Analyses of selection by dragonflies on *Rana sylvatica* morphology. Selection differentials were divided by the SD of the trait prior to selection. The intercept tests whether selection intensity differed from zero (i.e. tests for selection); the tank environment term tests whether selection intensities for the two phenotypes differed in magnitude. The dominant eigenvector represents the linear combination of traits most strongly aligned with the source of variation; traits with negative values (for the intercept) underwent increasing selection, or (for tank environment) showed stronger increasing selection in the no-predator treatment. Entries for univariate analyses give *F* (above) and *P*-value (below)

A. MANOVA: selection on seven measures of tadpole shape

Source	df	Wilks' <i>F</i>	<i>P</i>	Coefficients of the dominant eigenvector						
				Body length	Body depth	Body width	Tail length	Tail fin depth	Muscle depth	Muscle width
Selection (intercept)	7,33	7.58	0.0001	-0.002	-0.023	-0.103	-0.141	-0.166	-0.004	-0.157
Tank environment	7,33	1.41	0.2333	0.092	0.027	-0.007	-0.053	-0.188	0.028	0.102

B. Univariate ANOVA: selection on separate traits

Source (df)		Response							
		Body size	Body length	Body depth	Body width	Tail length	Tail fin depth	Muscle depth	Muscle width
Selection (intercept) (1)	<i>F</i>	1.00	7.23	3.78	8.78	4.37	25.99	3.76	24.73
	<i>P</i>	0.324	0.011	0.059	0.005	0.043	0.000	0.060	0.000
Tank environment (1)	<i>F</i>	0.00	0.03	0.52	1.53	0.81	4.21	0.32	4.42
	<i>P</i>	0.964	0.858	0.475	0.224	0.374	0.047	0.572	0.042

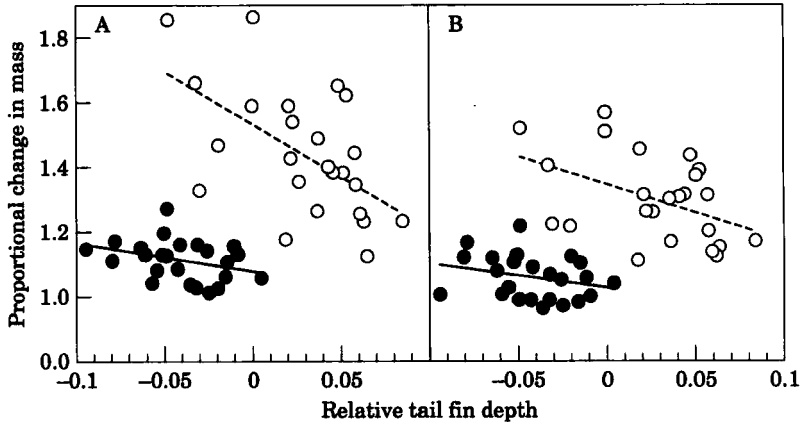


Figure 3. Growth rates of groups of 10 *Rana sylvatica* tadpoles, measured over 7 days in the (A) absence and (B) presence of competing *R. catesbeiana*. Growth rate declined with increasing tail fin depth, especially in the treatment without competitors and for tadpoles originating from tanks with caged dragonflies. Predator-induced tadpoles grew relatively more during the experiment, probably because of their small initial size. (●) no-predator phenotype; (○) predator-induced phenotype.

Our two tests for selection in the absence of predators yielded mixed results. The competition trials provided convincing evidence for selection acting against tadpoles with deep tail fins. Size-specific growth rate was higher for the predator-induced tadpoles than for no-predator tadpoles (Fig. 3, Table 4), probably because they were smaller at the beginning of the trials and therefore received more food in proportion to their mass. Tail fin depth (Fig. 3) and body depth were negatively related to growth in both the presence and absence of predators: tadpoles with deep bodies and deep tails grew relatively slowly. Also, introducing competitors reduced the growth rate of all tadpoles, but the reduction was significantly greater for the *Anax* treatment than for the no-predator treatment (Table 4, 9.1% decrease in growth for predator-induced tadpoles versus 4.4% decrease for no-predator tadpoles). Although the impact of competition was not related to any morphological trait within tank treatments (Table 4), the experiment as a whole suggests that some traits favoured in environments with predators lead to reduced growth rates, and that predator-induced tadpoles may be more sensitive to competition.

In contrast with the results of the competition trials, there was no consistent evidence of selection on tadpole morphology in the absence of free predators in the outdoor tanks. None of the measures of shape at day 18 was significant in a multivariate analysis on survival, growth to 35 days, and average time to metamorphosis (all  $P > 0.15$ ). The predator treatment strongly influenced performance because tadpoles exposed to caged *Anax* survived slightly worse (83.6% versus 85.2%) and had a longer larval period (57.0 d versus 50.8) than did tadpoles in predator-free tanks (Wilks'  $F_{3,37} = 19.5$ ,  $P = 0.0001$ ). Separate multiple regressions on the three measures of performance confirmed the weak relationship between phenotypic traits and the three correlates of fitness (Table 5). We conclude that the predator-induced phenotype performs more poorly than the no-predator phenotype in ponds without free-ranging predators, but that selection on tadpole morphology in these ponds may be weak or difficult to detect using the aggregate measures of performance and phenotype available here.

TABLE 4. Multiple regressions for the relationship between tadpole morphology and growth in the competition trials. Growth [ $\log(\text{final mass}/\text{initial mass})$ ] of *R. sylvatica* tadpoles from tanks with and without predators was measured in the presence and absence of 3 *Rana catesbeiana* tadpoles (competitors). The impact of competitors is the proportional decline in growth in the presence of *R. catesbeiana*. Entries in the table are the regression coefficient (top), *F*-value (middle), and *P*-value (bottom). A positive coefficient indicates that tadpoles which possessed a relatively large value of the trait grew fast or were less negatively affected by competition

Response		Source of variation							
		Tank treatment	Relative body length	Relative body depth	Relative body width	Relative tail length	Relative tail fin depth	Relative tail muscle depth	Relative tail muscle width
Growth with no competitors	$\beta$	–	–1.27	–2.69	–1.72	–1.68	–2.62	0.15	0.21
	<i>F</i>	92.66	1.31	4.32	2.11	2.96	7.58	0.19	1.02
	<i>P</i>	0.0001	0.2598	0.0442	0.1544	0.0935	0.0089	0.6643	0.3184
Growth with competitors	$\beta$	–	–0.91	–1.96	–0.58	–0.87	–1.52	–0.23	0.35
	<i>F</i>	62.44	0.97	3.25	0.34	1.13	3.63	0.64	3.98
	<i>P</i>	0.0001	0.3310	0.0790	0.5647	0.2944	0.0640	0.4292	0.0532
Proportional impact of competitors	$\beta$	–	0.47	1.04	1.71	1.27	1.58	0.60	0.26
	<i>F</i>	18.62	0.18	0.65	2.09	1.70	2.74	3.18	1.54
	<i>P</i>	0.0001	0.6746	0.4267	0.1563	0.2005	0.1061	0.0825	0.2216

TABLE 5 Multiple regressions for the relationship between tadpole morphology and performance in the plasticity experiment. Entries in the table are the regression coefficient (top), *F*-value (middle), and *P*-value (bottom). A positive coefficient indicates that tanks in which tadpoles possessed a relatively large value of the trait also scored high values on the performance measure. There is no consistent evidence for a relationship between phenotype and performance in tanks with no free predators

Response		Source of variation							
		Tank treatment	Relative body length	Relative body depth	Relative body width	Relative tail length	Relative tail fin depth	Relative tail muscle depth	Relative tail muscle width
Growth to 35 days	$\beta$	–	0.171	0.099	0.089	0.061	0.041	0.022	–0.004
	<i>F</i>	2.74	2.76	0.69	0.66	0.44	0.21	0.49	0.04
	<i>P</i>	0.1059	0.1047	0.4127	0.4224	0.5093	0.6462	0.4885	0.8400
Survival to metamorphosis	$\beta$	–	–0.142	1.843	–0.346	0.286	1.765	0.277	–0.072
	<i>F</i>	4.91	0.00	0.25	0.01	0.01	0.42	0.08	0.02
	<i>P</i>	0.0327	0.9645	0.6210	0.9189	0.9189	0.5201	0.7759	0.9029
Time to metamorphosis	$\beta$	–	–0.304	–1.284	0.063	0.044	0.789	0.080	0.386
	<i>F</i>	58.09	0.12	1.61	0.00	0.00	1.12	0.09	5.69
	<i>P</i>	0.0001	0.7273	0.2127	0.9460	0.9547	0.2961	0.7654	0.0220



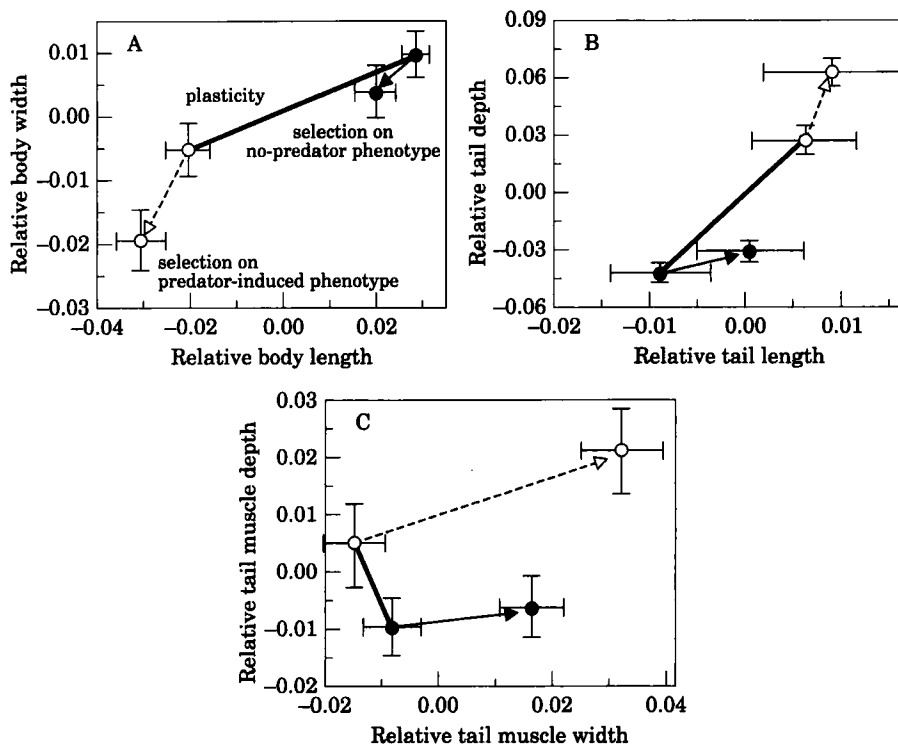


Figure 4. Phenotypic plasticity and selection on (A) body (B) tail fin and (C) tail muscle shape of *Rana sylvatica* tadpoles. The heavy line represents plasticity, which was measured by exposing tadpoles to environments with either caged *Anax* or no predators for 18 days. The arrows represent selection differentials, measured as the change in mean phenotype during overnight predation trials with free *Anax* (dashed arrow = predator-induced tadpoles; solid arrow = no-predator tadpoles). Both plasticity and selection values were taken from univariate comparisons. Selection on body shape and tail fin shape was consistent with the adaptive plasticity hypothesis. (●) no-predator phenotype; (○) predator-induced phenotype.

#### Comparison of selection and plasticity

The hypothesis that plasticity represents an adaptation to environmental variability predicts that traits which show the greatest plasticity are also under the strongest selection. Univariate estimates from our predation trials support this prediction. Bivariate plots of body and tail shape measurements illustrate that plastic responses to predators in cattle tanks were closely aligned with natural selection imposed by dragonfly predation (Fig. 4A, B), but this was not true for tail muscle shape (Fig. 4C). Body length and tail depth showed the strongest plasticity, and these two traits also underwent strong selection in these univariate contrasts. The only variable that departed strongly from the expected pattern was tail muscle width, which was slightly smaller in the presence of *Anax*, but was the target of strong positive selection in the predation trials.

Selection tended to favour opposite values of traits in the predation and competition trials. For this comparison we used coefficients derived from multivariate analyses of plasticity and selection, because they incorporate information about correlations

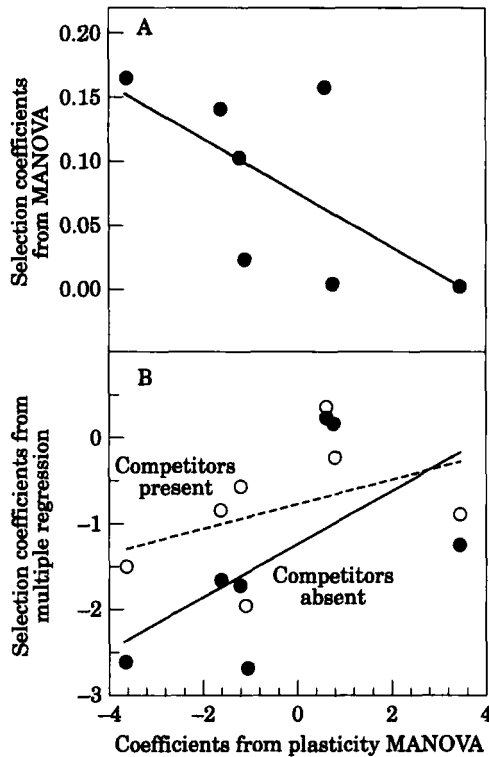


Figure 5. Relationship between morphological plasticity and selection in experiments with predators and competitors, for seven measures of shape in *Rana sylvatica* tadpoles. Coefficients reflect the strength of plasticity or selection on each trait, and are derived from multivariate analyses that adjust for phenotypic correlations among traits. Plasticity coefficients are the elements of the dominant eigenvector from MANOVA on phenotypes in the cattle tank experiment (Table 2A); traits with positive values were larger in the no-predator environment. Selection coefficients in the presence of *Anax* (A) are the elements of the dominant eigenvector from MANOVA testing for selection in predation trials (Table 3A; coefficients multiplied by  $-1$  so that positive values indicate selection favouring large trait values). There was a significant correlation between selection by dragonflies and plasticity ( $r_s = -0.79$ ,  $P = 0.036$ ). For the growth experiment (B), coefficients are from multiple regression predicting growth rate from morphology (Table 4), shown separately for tadpoles in the presence and absence of competing *R. catesbeiana*. Traits that were relatively large in the presence of *Anax* were associated with reduced growth in the lab, although these correlations were not significant ( $P > 0.1$  in both cases).

among the seven traits. The same traits that were under selection for increasing values in the presence of *Anax* were exhibited by tadpoles in cattle tanks with caged *Anax* (Fig. 5A;  $r_s = 0.79$ ,  $P = 0.036$ ), with the single exception of tail muscle width. Likewise, trait values that were correlated with enhanced growth in the competition experiment tended to be exhibited by tadpoles in predator-free ponds (Fig. 5B), although this pattern was not significant.

#### DISCUSSION

We have shown that dragonfly predation imposes selection on tail shape of *Rana sylvatica* tadpoles, and that the targets of selection are generally the same traits that

display phenotypic plasticity in response to predators. At the same time, tadpoles exhibiting opposite combinations of traits grow faster in the absence of predators. We argue that plasticity could be maintained in natural populations by this kind of selection, but to make our argument plausible we must first address three issues. First, what sort of environmental variability do tadpoles experience in nature, and what does the nature of environmental variability imply about selection for plasticity? Second, what are the functional bases of selection favouring different traits in environments having different predation regimes? Finally, how can this argument be reconciled with two of our findings that contradict predictions of the adaptive plasticity hypothesis: weak or inconsistent selection in the absence of predators, and the lack of plasticity in one trait that is under strong selection by dragonflies?

#### *Natural variation in predation regime*

The natural distribution of predators among ponds creates a coarse-grained environment for anuran larvae, in which the predator regime varies among ponds and among years within ponds, but is relatively consistent within ponds over a single season. Lentic freshwater habitats can be arranged along a gradient defined by persistence, extending from ephemeral pools to permanent lakes (Wiggins, Mackay & Smith, 1980; Wellborn, Skelly & Werner, 1996). The persistence gradient is critically important for anuran larvae and many other aquatic organisms because it determines the distribution of predators. Invertebrate and vertebrate predators tend to be larger, more numerous, and more predictable in occurrence in more permanent ponds (Smith, 1983; Woodward, 1983; Skelly, 1996; Wellborn *et al.*, 1996). As a group, these predators exact a heavy toll on tadpoles (Heyer, McDiarmid & Weigman, 1975; Caldwell, Thorpe & Jervey, 1980; Azevedo-Ramos *et al.*, 1992; Gascon, 1992), and aeshnid dragonflies in particular can eliminate all tadpoles from natural ponds (Smith, 1983).

Within relatively temporary ponds, predators show a characteristic pattern of temporal abundance. Individual ponds contain highly variable numbers of predators, depending on recent history of drying and refilling (Harris, Alford & Wilbur, 1988; Jefferies, 1994; Skelly, 1996). Following wet years, most ponds within a region contain high numbers of predators, whereas few predators are present immediately after an extended dry period. These large-scale rainfall patterns can dramatically affect the exposure of tadpoles to predators. Even though a few anurans display some habitat selection at the time of oviposition (Resetarits & Wilbur, 1989; Hopey & Petranka, 1994), the regional scale of hydroperiod cycles probably prevents frogs from entirely avoiding predators in some years. While temporary ponds may show some temporal variation in predators within seasons, they seem likely to contain a consistent predator fauna after an initial colonization phase. As a result, temporary pond anurans are exposed to a range of predation regimes over generations, but a more stable collection of predators during the larval period of any individual tadpole (Smith, 1983; Skelly, 1996).

Quantitative samples collected from natural ponds on our study area partially support this characterization of the predator environment for larval *Rana sylvatica* (Fig. 6). In both 1996 and 1997, many populations of *R. sylvatica* occurred in ponds

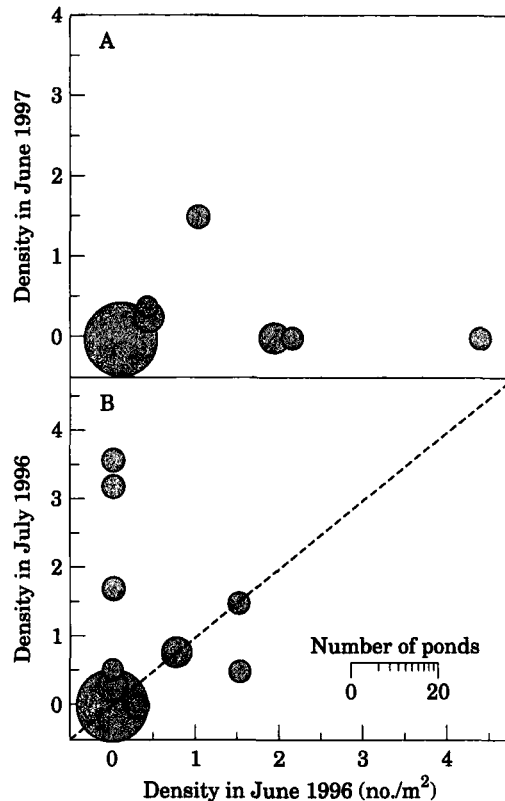


Figure 6. Densities of aeshnid dragonflies (*Aeshna* and *Anax*) in pipe-samples taken in natural ponds on the E.S. George Reserve in southern Michigan. The diameter of the bubbles represents the number of ponds having each value. Samples collected in June during both 1996 and 1997 for the 21 ponds that contained *Rana sylvatica* tadpoles in both years (A) illustrated variation within and between years in the density of dragonflies. In both years there were ponds with and without aeshnids, and the density within some ponds changed considerably between years. Samples collected for 25 ponds during both June and July in 1996 (B) illustrated that aeshnid density generally increased or stayed the same within that year. The dashed line corresponds to identical densities in the June and July samples. These data were collected in collaboration with S.A. McCollum, D.K. Skelly, E.E. Werner, and K.L. Yurewicz.

with no aeshnid larvae, and a few populations were exposed to moderate densities of aeshnids. Predator numbers generally declined in 1997 as a result of a late-season drought in 1996, but there were examples of populations with both increasing and decreasing aeshnid density between the two years (Fig. 6A). Within 1996, the density of aeshnids remained consistent between the June and July samples in 22 of 25 ponds, primarily because many ponds had no dragonflies in either census (Fig. 6B). For these ponds, the environment was temporally autocorrelated within a season. But three other ponds had increasing dragonfly densities during the summer, resulting from growth of one-year-old *Aeshna* which were too small to be counted in the early June census. In terms of large dragonflies, then, the environment experienced by *R. sylvatica* can vary within ponds among years, and among ponds within years. For most populations the environment remains fairly consistent within years.

Theory suggests that coarse-grained environmental variation of this sort is conducive to the evolution of phenotypic plasticity (Via & Lande, 1985; Gomulkiewicz & Kirkpatrick, 1992; Moran, 1992; Via *et al.*, 1995). Models show that selection operating only within environments can favour genotypes that express the appropriate reaction norm across environments (Moran, 1992; Via *et al.*, 1995). Such genotypes have phenotypes that are close to the optimum across a range of environments, and therefore experience relatively high fitness in all years and will increase in frequency relative to genotypes that possess optimal phenotypes only during some years.

With respect to predator density, the temporary pond system exhibits a combination of environmental variation (among years) and moderate autocorrelation (within years), both of which may be necessary for plasticity to evolve (Travis, 1994). Individual anuran larvae may be exposed to only a single environment or a slowly changing environment within their lives, but populations experience different numbers of predators in different years, and therefore undergo selection for plasticity. The widespread occurrence of predator-induced defences in organisms inhabiting temporary ponds (Dodson, 1989; McCollum & Leimberger, 1997; DeWitt, 1998) strengthens our conclusion that plasticity is a plausible and likely evolutionary response to variation in predation regime in these habitats. Other features of aquatic habitats thought to promote the evolution of plasticity, such as the presence of reliable cues indicating the condition of the environment, are discussed elsewhere (Dodson, 1989; McCollum & Leimberger, 1997).

#### *The functional causes of selection*

One reason we feel confident that selection by predators acts to maintain plasticity in *Rana sylvatica* is that the connection between trait variation and fitness variation has a clear causal basis. Functional relationships between predator escape and morphology are fairly well established in tadpoles, although based primarily on theoretical rather than empirical results. Basic locomotory performance features such as swimming speed, acceleration, and maneuverability are thought to depend on the same morphological traits that are targets of selection by feeding *Anax*. Because these measures of swimming performance are of general importance in predator escape (Feder, 1983; Weihs & Webb, 1984; Watkins, 1996), we suspect that the facultative response to *Anax* found in this and other studies (McCollum & Van Buskirk, 1996; Van Buskirk *et al.*, 1997) may have evolved as a generalized induced response to variation in the overall predation regime.

Structures involved in acceleration, burst speed, and perhaps rapid turning are important for escaping sit-and-wait predators (Howland, 1974; Weihs & Webb, 1984). Hydrodynamic analyses of swimming vertebrates show that the tapering muscle of the tail and the deepest part of the tail fin are used in sudden starts and turns (Weihs, 1972; Webb, 1984; Wassersug & Hoff, 1985; Wassersug, 1989), although these features are apparently not critical for maintaining steady swimming (Blight, 1977; Wassersug, 1989). These results suggest that the anuran tail fin and tail muscle are important for escaping predators, and our data show that they are targets of selection imposed by predation. There are indications of a trade-off between swimming speed and maneuverability (Webb, 1984; Brown & Taylor, 1995), which might constrain the responses that tadpoles could potentially evolve to multiple predators that hunt in different ways.

Activity level and habitat selection also affect vulnerability to predators in anurans and other organisms (Wright & O'Brien, 1982; McPeck, 1990; Anholt & Werner, 1995), and many taxa show apparently adaptive behavioural responses to predators (Sih, 1987; Lima & Dill, 1990; Werner & Anholt, 1993). In this study we could not associate behavioural phenotypes with individuals, and thereby test for selection imposed by predators. This would be a worthwhile exercise, because there are good reasons for expecting behaviour and morphology to interact non-additively to influence individual fitness (Christiansen, 1965; Kingsolver, 1988; Brodie, 1992; McPeck, 1995). Such tests would have to be conducted in more natural settings, in which behavioural mechanisms of predator avoidance have the opportunity to be expressed.

### *Results that contradict the adaptive plasticity hypothesis*

We obtained two results that conflict with expectations stemming from the adaptive plasticity hypothesis. The first of these was that tail muscle width underwent strong selection by *Anax* but displayed no plasticity (Fig. 4C). We are confident of this result, because previous work confirms that *Rana sylvatica* shows no flexibility in tail muscle width (R. A. Relyea, unpublished) and that predators select for wide tail muscles in other anurans (Van Buskirk *et al.*, 1997). There may be a constraint that prevents tail muscle from widening during development, or a trade-off preventing the tail from evolving increased width. The possibility of such a constraint is supported by the observation that no anuran yet studied exhibits significant plasticity in tail muscle width. A plausible explanation is that increased tail muscle width can be obtained only at the cost of decreased tail flexibility, which is itself critical for sudden acceleration (Wassersug, 1989; Andraso, 1997).

A second result that contradicts the adaptive plasticity hypothesis is the inconsistent selection on plastic morphological traits in the absence of free-ranging predators. If plasticity is adaptive we expect to see different combinations of traits conferring the highest relative fitness in the presence and absence of predators. Both the competition trials and plasticity experiment demonstrated that the predator-induced phenotype was costly when free predators were absent, but the evidence for selection on particular morphological traits was weak. For example, predator-induced tadpoles were affected more severely than no-predator tadpoles by competing *Rana catesbeiana*, and in the plasticity experiment tadpoles in caged-*Anax* tanks survived more poorly and metamorphosed later than tadpoles in ponds without caged predators. Because predators were not actually killing tadpoles in either of these experiments, reduced rates of survival, growth, and development must have arisen from the physiological costs of responding to *Anax*, or of maintaining the predator-induced phenotype. In spite of costs associated with the entire suite of traits, though, we found selection on particular morphological traits only in the competition trials. In that experiment, reduced growth rates of deep-tailed tadpoles supported the adaptive plasticity hypothesis, but other traits did not so clearly affect growth, and there was no evidence that the response to competitors depended on a tadpole's morphology. In the plasticity experiment, our analyses were unable to associate specific traits with the reduced performance of the predator-induced phenotype. The targets of selection in the absence of predators may well include behavioural traits related to foraging activity and intake rates, which were not measured in this experiment. Taken

together, our results support earlier findings that the predator-induced phenotype is costly (McCollum & Van Buskirk, 1996), and they suggest that selection in the predator-free environment for morphological traits induced by that environment is measurable but relatively weak.

Theory states clearly that selection must be operating in both environments to maintain plasticity (Via & Lande, 1985; Gomulkiewicz & Kirkpatrick, 1992; Moran, 1992). If relatively large tail fins and tail muscles have no effect on fitness in the absence of predators, then selection imposed only in the presence of predators would produce tadpoles that have large tails in all environments (assuming that tail shape is genetically correlated across environments). The fact that tail shape is plastic in *R. sylvatica* and several other anurans implies that there must be at least occasional selection favouring individuals with small tails in predator-free ponds.

Results of numerous studies suggest that selection against individuals exhibiting induced responses to predators in predator-free environments may be weak and inconsistent (O'Brien *et al.*, 1980; Lively, 1986b; Baldwin *et al.*, 1990; Spitze, 1992). One reason for the variability among results may be that costs are often expressed as a decline in growth or reproduction (Harvell, 1990; Spitze, 1992), which are likely to depend on availability of resources or energy (Appleton & Palmer, 1988; Stemberger, 1988; Pettersson & Brönmark, 1997). Thus, the magnitude of costs (and presumably the importance of selection in predator-free environments) may be highly sensitive to resource levels. This might explain why studies of anuran larvae have obtained different results: Van Buskirk *et al.* (1997) detected weak selection against predator-induced traits in ponds without free predators, while this study found selection against just a single trait and for only one measure of performance. We suspect that selection in predator-free ponds is irregular in occurrence, is probably rather context-dependent, and may target different fitness components under different conditions. So far, theory is not informative on the issue of how often divergent selection across environments must be in place in order to maintain plasticity.

It is possible that selection favouring shallow-tailed tadpoles in predator-free ponds is in fact quite rare. Several mechanisms could account for the existence of plasticity in the absence of divergent selection. Plasticity may have originally evolved when there was strong divergent selection across environments, but subsequent evolutionary changes have reduced the cost of anti-predator responses (Simms, 1992). Also, selection against tadpoles having deep tail fins may be acting during other stages of the life cycle or on components of fitness that we did not measure. Simultaneous exposure to multiple species of predators or competitors in natural ponds might alter the combinations of traits that are favoured, as well as their reaction norms across environments. Finally, traits expressed in predator-free ponds may evolve as correlated responses to selection acting in other environments. If alleles favoured by selection in other environments have pleiotropic effects in predator-free environments, then non-adaptive phenotypes could be maintained in predator-free environments. The existence of consistent genetic correlations among trait values across environments in other organisms makes this mechanism appear at least plausible (Via, 1984; Trexler & Travis 1990; Semlitsch, 1993; Andersson & Shaw, 1994).

*Adaptive plasticity in anurans*

We believe the evidence supports the hypothesis that plasticity in the morphology and behaviour of larval anurans represents an adaptation to environmental heterogeneity. There is now support for both assumptions of the adaptive plasticity hypothesis: that existing reaction norms are beneficial, and that they have been produced by natural selection. Studies of numerous anurans show that behavioural responses to predators improve individual survival (Hews, 1988; Lawler, 1989; Werner & Anholt, 1993; Skelly, 1994). The plastic morphological shift in *Hyla chrysoscelis* is clearly beneficial, because the predator-induced phenotype survives better with predators and the no-predator phenotype survives better in ponds without free predators (McCollum & Van Buskirk, 1996). The functional connections between tail morphology, swimming performance, and predator escape have been worked out in theory (Wassersug, 1989), and results from a flowtank experiment suggest that tadpoles with deep tail fins have higher swimming speed and stamina (McCollum & Leimberger, 1997).

The present study indirectly supports the second assumption of the adaptive plasticity hypothesis by demonstrating that traits which show the greatest plasticity also undergo the strongest selection (Fig. 5). This does not prove that the current reaction norm in *Rana sylvatica* was produced by selection acting in the past, but it does support the general case that reaction norms of this type are produced by selection (Brandon & Rausher, 1996).

*Plasticity and species diversity in anurans*

The role of species interactions in either promoting or limiting species diversity is an issue of long-standing interest. Comparative studies suggest that selection imposed by predators, such as we have measured in *R. sylvatica*, may be involved in divergence and speciation within anuran assemblages. The main evidence for this is that groups of closely-related species sort out such that the average phenotype of each species is appropriate for the predators in its environment. For example, the tadpoles of two *Pseudacris* species that partition a predation gradient in eastern North America exhibit fixed differences in behaviour and morphology that are consistent with selective differences between their habitats (Skelly, 1995; Smith & Van Buskirk, 1995). *Pseudacris triseriata* occurs in temporary ponds with few predators, feeds actively, and has a shallow tail fin and tail muscle. *Pseudacris crucifer* occurs in more permanent ponds with higher numbers of predators, is inactive, and has a relatively large tail. Thus, phenotypic differences between the two species are exactly congruent with divergent selection on behaviour and morphology in environments with and without predators (Skelly, 1994; Anholt & Werner, 1995; McCollum & Van Buskirk, 1996; Van Buskirk *et al.*, 1997; this study). In this case, then, and in others (McPeck, 1995, 1997), it appears that differences among species have evolved in response to variation in selection along a predation gradient, which highlights a positive role for species interactions in directing and probably promoting diversification.

To some extent, plasticity might serve to reduce species diversity as well. In the anuran system, for example, it is possible that plastic responses of tadpoles to the presence or absence of other species in the community act to limit diversity by reducing the number of potential species that can co-occur. Plasticity may allow a



single species to occupy a broad range of environments, thus preventing new species from invading or evolving within some of those environments. In certain cases divergent selection regimes are hypothesized to promote plasticity (e.g. Via *et al.*, 1995), whereas in other situations divergent selection leads to ecological separation, reproductive isolation, and eventual speciation (Futuyma & Moreno, 1988; West-Eberhard, 1986, 1989; Thompson, 1994; Schluter, 1996; Mopper, 1996). The circumstances that affect the likelihood of these alternative outcomes remain an important area for future research.

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