

PHENOTYPIC LABILITY AND THE EVOLUTION OF PREDATOR-INDUCED PLASTICITY IN TADPOLES

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Abstract.—The hypothesis that predator-induced defenses in anuran larvae are maintained by divergent selection across multiple predation environments has not been fully supported by empirical results. One reason may be that traits that respond slowly to environmental variation experience a fitness cost not incorporated in the standard adaptive model, due to a time lag between detecting the state of the environment and expressing the phenotypic response. I measured the rate at which behavior and morphology of *Rana temporaria* tadpoles change when confronted with a switch in the predation environment at two points in development. Hatchling tadpoles that had been exposed during the egg stage to *Aeshna* dragonfly larvae were not phenotypically different from those exposed as eggs to predator-free conditions, and both responded similarly to post-hatching predator treatments. When 25-day-old tadpoles from treatments with and without dragonflies were subjected to a switch in the environment, their activity budgets reversed completely within 24–36 h, and their body and tail shape began changing significantly within 4 days. The behavioral response was conservative: Tadpoles switched from high-risk to predator-free treatments were slower to adjust their activity. The study confirmed that behavioral traits are relatively labile and exhibit strong plasticity, but it did not reveal such a pattern at the level of individual traits: Morphological traits that developed slowly did not show the least plasticity. Thus, I found that differences in lability of traits were useful for predicting the magnitude of plasticity only for fundamentally different kinds of characters.

Key words.—Anura, behavior, development, induced defense, morphology, phenotypic plasticity, *Rana temporaria*.

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Phenotypic plasticity exists in a wide variety of traits and organisms, yet it is not ubiquitous and much evidence suggests that the extent of plasticity in specific traits is limited (DeWitt et al. 1998). One type of limit to plasticity can arise when developmental shifts in phenotypes involve time delays. This may restrict the range of phenotypic expression that is feasible, either because the time required to develop a phenotype imposes a constraint on the range of traits that can be produced or because delayed expression increases the likelihood of a mismatch between phenotype and environment. In this paper I estimate the magnitude of time delays in the expression of plasticity in anuran larvae, and ask whether they might act to restrict the extent of behavioral or morphological responses to predators.

Several models show that the time scale of response to environmental variation may influence the scope of plasticity that can evolve (Clark and Harvell 1992; Moran 1992a; Padilla and Adolph 1996; Gabriel 1999). Traits that can be adjusted rapidly to match environmental change are especially likely to show plasticity. For such traits the phenotypic response is produced in close temporal association with the cue that signals the state of the environment, increasing the probability that the phenotype will be appropriate for the environment in which it exists (West-Eberhard 1989). These models may not apply if a lag between the cue and the development of the phenotype does not affect the reliability of the cue, such as when seasonal polyphenism in insects is triggered by predictable changes in the environment (Brakefield 1987; Moran 1992b). But for many situations the models make a clear prediction: Traits that show the most rapid responses to changes in the environment are those that can most easily evolve plasticity.

In principle, a comparison between the amplitude of plasticity and the rate of phenotypic response to environmental

change could reject this prediction because the two are not necessarily related. In practice, though, a positive relationship is inevitable if trait values are measured before they have had sufficient time to develop their full response. The problem is illustrated in Figure 1, which depicts two patterns of response to a change in the environment, in two traits with different maximum extent of response. In Figure 1A the trait with the greatest response also shows the faster response, whereas the reverse is true in Figure 1B. If traits are sampled relatively soon after the environmental shift (at time b), the amplitude of plasticity will be underestimated for the trait with the slower response rate, so the more labile trait will show the greatest plasticity (solid line in Fig. 1A, dashed line in Fig. 1B). If traits have ample opportunity to produce their full response (time c) the bias in measuring plasticity disappears, and there remains no necessary relationship between the rate of response and the magnitude of plasticity. This suggests that the hypothesized relationship between the extent and rate of response is unlikely to be rejected unless plasticity is measured after lengthy exposure to divergent environments.

Predator-induced phenotypic plasticity in anuran larvae represents a good opportunity to test the prediction that highly labile traits evolve more extensive plasticity. Tadpoles raised in the presence of insect predators show many behavioral and morphological differences from those in predator-free ponds (Van Buskirk and McCollum 2000). The differences do not continually increase throughout the larval period (McCollum and Van Buskirk 1996), which is important for accurate measurement of the amplitude of plasticity (Fig. 1). The maintenance of predator-induced plasticity in tadpoles can be explained partly in terms of divergent natural selection between situations with and without predators (Van Buskirk et al. 1997), but purely adaptive explanations are not entirely

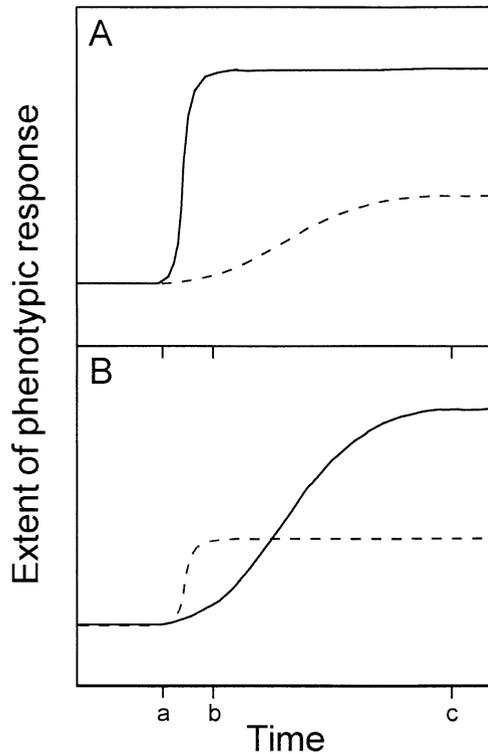


FIG. 1. Responses to a change in the environment in two traits that differ in the extent of phenotypic plasticity. The solid line represents the trait having greater plasticity. The environmental change occurs at time a, whereas b and c represent early and late samples of trait values. (A) The trait with greater plasticity also shows the faster rate of response to a shift in the environment; (B) the trait with a slower response eventually achieves a greater amplitude of response. If traits are sampled soon after the environmental change (at time b), there will be an inevitable positive correlation between rate of response and extent of plasticity. However, if there is sufficient time to develop the full response (sampling at time c), there is no necessary correlation between rate and amplitude of response.

satisfying. Fitness costs that oppose the development of plasticity have been difficult to measure (Van Buskirk and Saxer 2001), and some traits that never exhibit plasticity are consistently under selection by predators (Van Buskirk and Reylea 1998). These observations suggest that measurements of selection are not sufficient to predict the observed extent of plasticity. It therefore seems worthwhile to evaluate alternative hypotheses, including West-Eberhard's (1989) proposal that a trait's lability may influence the evolution of plasticity in that trait. Here I compare the rate of response to a sudden change in the predation environment with the maximum extent of plasticity, for several behavioral and morphological traits in *Rana temporaria* tadpoles (Anura: Ranidae). I ask specifically whether traits that show the greatest response to predators are those that also respond most rapidly to an experimental switch in the predation environment.

MATERIALS AND METHODS

The study had three stages. First, I traced the developmental time-course for behavior and morphology of tadpoles reared in environments with and without predators. This pro-

vided data on growth and allometry during development, and ensured that plasticity was sampled after traits were fully expressed (i.e., time c in Fig. 1). Next, I switched tadpoles between predation treatments at two stages during the larval period and compared rates of response to shifts in the environment across different traits. Finally, I surveyed predators within a collection of natural ponds on two occasions separated by several weeks, in four different years; here the objective was to assess the extent to which tadpoles encounter temporal variation in the predation environment in nature.

Measuring the Time Course of Plasticity

I recorded morphological and behavioral responses of *R. temporaria* tadpoles to chemical cues signaling the proximity of dragonfly larvae in fiberglass tanks placed outdoors in a field on the campus of the University of Zurich, Switzerland. The experiment included five tanks with caged predators and five with no predators, for a total of 10 tanks. Each tank was 1.4 m², filled with water to a depth of 40 cm (560 L) on 6 March 2000 and stocked with dried leaf litter (0.5 kg), commercial rabbit chow (10 g), and mixtures of phytoplankton and zooplankton from a natural pond. All tanks contained three plastic cages with window screen covering the ends (1-L volume). The cages were left empty in the no-predator tanks; in the caged-predator tanks each cage contained one late-instar *Aeshna cyanea* larva (Odonata: Aeshnidae). Dragonflies were each fed 300 mg *R. temporaria* tadpoles every other day throughout the experiment.

Ten clutches of eggs were collected immediately after they were laid, from a large population 15 km north of Zurich. I added 80 tadpoles to each tank (57 tadpoles/m²) on 3 April 2000, when the tadpoles were 4 days old. Each clutch contributed eight individuals to all tanks.

I collected two measures of tadpole behavior (activity budgets and refuge use) at intervals of 5–6 days throughout the larval period, usually during midafternoon on sunny days. Activity budgets were estimated from five haphazardly chosen tadpoles within each tank, recording the proportion of time spent feeding, swimming, and resting inactively for a period of 60 sec each. The fraction of individuals hiding was estimated by counting the number visible outside the leaf litter. I repeated counts three times on each sampling day, and the mean number visible in each tank was divided by the estimated number alive on that date (calculated from the number remaining alive at the end of the experiment and assuming a constant mortality risk through time) to yield the proportion of individuals not hiding.

I measured the morphology, body size, and developmental stages of tadpoles at intervals of 5–7 days throughout the larval period. On each occasion I captured a sample of six individuals from each tank, photographed them in side and bottom view, weighed them, recorded their developmental stages following Gosner (1960), and immediately returned them to the tanks. Photographic images were imported into a computer for measurement of seven distances that together define the size and shape of a tadpole (Van Buskirk 2001). The distances were body length, width, and depth; tail length and maximum depth; and tail muscle width and depth at the base. Body size was defined as the first component derived

from principal components analysis on the covariance matrix of all seven distances. Shape was defined by the residuals of the distances after regression against body size. The regressions explained a high fraction of the variance in the original lengths (R^2 between 0.89 and 0.98), and the residuals showed no nonlinear trends when plotted against body size.

Measuring Lability of Morphology and Behavior

I performed two experiments, at different stages of development, in which tadpoles were switched between environments to compare the rates at which different traits responded to signals indicating predation risk.

The first experiment tested whether the environment experienced by eggs imposed a lasting effect on tadpole behavior or morphology. There were four treatments: presence and absence of caged *Aeshna* during the egg stage, crossed with presence and absence of *Aeshna* during the early tadpole stage. The egg portion of the experiment took place in plastic tubs (0.27 m²) filled with 65 L of water, including five replicates of each treatment arranged outdoors in blocks. Each tub contained a predator cage: half were left empty, and the other half enclosed a single dragonfly larva, which was fed 200 mg of tadpoles every other day. I introduced eggs to the experiment on 17 March 1999, when they were 1–2 days old (Gosner stages 8–11). Each tub received parts of 10 clutches collected in a pond 3 km north of Zurich, with the representation of clutches approximately equal in every tub. Hatching occurred after about 17 days (on 2–4 April), and on 6 April I preserved a sample of 10 hatchling tadpoles from each tub.

The tadpole portion of the experiment was performed in 20 outdoor tanks, following nearly identical procedures to those used in the first experiment (see Measuring the Time Course of Plasticity). Tanks were filled on 11 March 1999, inoculated shortly thereafter with the same recipe of ingredients, and stocked with 3-day-old tadpoles on 6 April. Each block in the tadpole experiment received hatchlings originating from one block of the egg plasticity experiment. I observed behavior on four occasions during the first 3 weeks, and sampled morphology of tadpoles when they were 25 days old, following procedures described above.

The second experiment tested how quickly a tadpole could modify its phenotype after it had been exposed to a constant predation environment for 3 weeks. The design had five replicates of four treatments: two levels of the previous environment (no predator and caged *Aeshna*) crossed with two levels of the current environment (no predator and caged *Aeshna*). Five spatial blocks each corresponded to a block from the foregoing experiment on eggs. The experiment occurred outdoors in plastic tubs (65 L, 0.27 m²). Each tub contained a single floating cage, which held either a single late-instar *Aeshna* larva or no predator, depending on the treatment. The *Aeshna* larvae were each fed one 100-mg tadpole every day.

The experiment lasted for 10 days, and began when the tadpoles were 25 days old and weighed on average 85 mg and 160 mg in the presence and absence of *Aeshna*, respectively. On 28 April I removed 10 tadpoles from each cattle tank, photographed them, and placed them immediately into the tubs. Each tub received 10 tadpoles, five each from the

two tanks having the same predator treatment during the preceding tadpole phase (there was no phenotypic difference between the egg treatments at this stage). I fed tadpoles daily with rabbit food totaling 10% of their estimated mass.

I sampled behavior and morphology at regular intervals after the environmental switch. Morphology was measured from photographs of tadpoles collected from each tub after 4 days (five tadpoles/tub) and 10 days (all 10 tadpoles). Size and shape were defined as described above. Behavior was sampled at 1-h intervals for 6–8 h after starting the experiment, during the morning and afternoon of days 2–4, and on day 8. Each hourly sample of activity was averaged from three separate visits to the tub, 5 min apart, during which I scored the number of tadpoles that were swimming, feeding, or resting inactively. I made between 16 and 22 of these samples on each tub during the course of the experiment. For analyses and figures the samples were grouped into eight time intervals, delineated partly by the temporal availability of observations and partly with the intent of increasing resolution of responses early in the experiment. The time intervals were 0–2.5 h, 2.5–5 h, 5–10 h, 17–24 h, 24–33 h, 45–55 h, 67–79 h, and 190–220 h.

In this and all other experiments, I randomized the locations of treatments and the distribution of animals and materials, and I collected behavioral and morphological data without knowledge of the treatments.

Changes in Predation Risk within Natural Ponds

I made quantitative surveys of predator densities in approximately 35 ponds near Zurich, Switzerland, to assess the frequency with which predation risk within ponds changes during the season. West-Eberhard's (1989) proposal that phenotypic lability may limit the evolution of plasticity is only relevant here if tadpoles regularly encounter temporal changes in predator density. The ponds were sampled 5–18 May and 4–18 July 1997–2000. I estimated the densities of common predators based on 20–40 samples collected in each pond with a hollow pipe (35-cm diameter), and noted the presence of rarer taxa by dipnetting for 10–20 min per pond. Predators were identified and measured to the nearest millimeter. Analysis was restricted to individuals that were at least 15 mm long, because I was interested only in exposure to predators that represent an appreciable mortality threat to medium-sized tadpoles. The analysis asked whether the density of predators in a pond during July could be predicted by the density in May. Although the July sample occurred after many *R. temporaria* had metamorphosed, the data are nevertheless useful for revealing the frequency and magnitude of seasonal changes in predator composition within natural ponds.

RESULTS

Developmental Time Course of Plasticity

Rana temporaria tadpoles responded to predators by growing and developing more slowly, hiding, decreasing their activity, and developing shorter bodies and deeper tail fins (Fig. 2). Differences in growth and behavior between treatments first appeared by about 10 days of age and continued

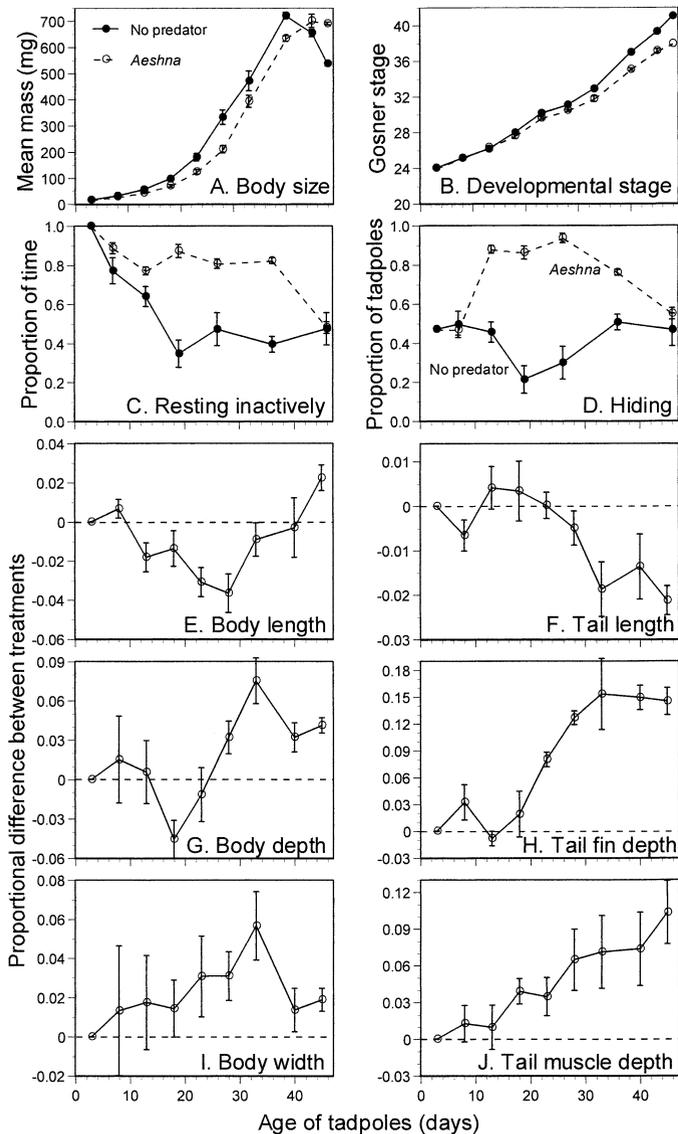


FIG. 2. Temporal changes in body size, developmental stage, behavior, and morphological plasticity of *Rana temporaria* tadpoles reared in ponds with and without caged dragonflies. Symbols show mean \pm 1 SE of five replicate tanks. Plasticity (E–J) is the difference between predator-induced and no-predator phenotypes, expressed as a proportion of the trait value in the no-predator treatment. Tadpoles exposed to predators grew and developed relatively slowly, became less active and spent more time hiding, and had shorter bodies, shorter and deeper tails, and larger tail muscles. Behavioral responses were apparent earlier than morphological responses.

to increase in magnitude over the first 3 weeks of the larval period. Differences in developmental stage appeared somewhat later, when tadpoles were more than 25 days old, and by the end of the experiment the predator-induced tadpoles were delayed by 5–6 days relative to the no-predator tadpoles. At this point, 31.5% had reached metamorphosis in the absence of predators, whereas none had metamorphosed in the caged-*Aeshna* treatment. Morphological responses appeared within 20–30 days, depending on the trait, and reached their maximal extent when the tadpoles were about 35 days old (Gosner stages 30–33). Body shape showed a more complex

development than tail shape: The predator-induced phenotype had small body dimensions between days 15–25, but was relatively large bodied by late in the larval period.

The temporal changes in behavior and morphology and effects of the predator treatment, were confirmed by repeated measures analyses (Table 1). All responses showed significant date and predator effects. Significant effects of the date-by-predator interaction indicated that for most traits the two treatments showed different temporal trajectories.

Environmental Switch at Hatching

There was no influence of predator environment during the egg stage on the developmental rate of eggs or the size and morphology of tadpoles. Hatching occurred at the same time in all tubs, over a 3-day period between 2–4 April. At the time when the first environmental switch was performed (age 3 days), the mean mass of hatchlings was 10.5 mg in both treatments, and none of the size-corrected measures of body and tail shape showed a treatment effect (all $P > 0.36$).

There were no lasting or delayed effects of exposure to predators during the egg stage on behavior or morphology after 3 weeks of development. Multivariate analyses indicated that all aspects of the phenotype at 25 days were influenced only by the tadpole treatment and not by the previous egg environment (Table 2). Univariate tests on the 13 response variables (daily growth rate, developmental stage, seven size-corrected measures of body and tail shape, and four behavioral responses) revealed no effects of egg treatment, 10 effects of the tadpole treatment significant at $\alpha = 0.05$, and no interactions between egg and tadpole treatments (not shown).

Environmental Switch after Three Weeks

The previous results show that exposure to dragonfly predators during the egg stage had no detectable influence on tadpoles, and that strong phenotypic distinctions had developed by the time of the second switching experiment (age 25 days). Individuals in the caged-*Aeshna* treatment were less active, and had relatively short bodies, deep tail fins, and shallow and wide tail muscles (Fig. 2).

Tadpoles that remained within the same environment as they had previously experienced showed little change in behavior during the experiment (Fig. 3A). Those that were switched to the other environment showed an immediate but incomplete behavioral response to the environmental change, after which activity gradually adjusted to the current environment over a period of several days. The pattern is especially clear in a diagram depicting temporal changes in the proportions of variance explained by the previous and current environments (Fig. 3B). The variance component associated with the previous tank treatment remained important and significant over the first 20 h and thereafter was nonsignificant. The current tub treatment was important and significant immediately after the tadpoles were placed into the tubs, and grew increasingly important over about 3–4 days.

The temporal trajectories visible in Figure 3A suggest that the behavioral response to a switch in the predation environment was asymmetric. Tadpoles that moved from a high-risk to a low-risk environment decreased their resting behavior rather little within the first few hours and required

TABLE 1. Repeated measures analyses on body size, developmental stage, behavior, and morphology of *Rana temporaria* tadpoles reared in cattle tanks with and without caged dragonfly larvae, with five replicates of each treatment. Entries in the table are F-ratios, with P-values in parentheses. Directions and magnitudes of the effects are visible in Figure 2. Between-tank effects are tested over the block-by-treatment interaction. Degrees of freedom for the within-tank effects are deflated according to the Greenhouse-Geisser method, to adjust for unequal correlations among pairs of repeated measures (Littell et al. 1991).

Response	No. of dates	Between-tank effects		Within-tank effects	
		Block	Predator treatment	Date	Date × predator
Mass	8	1.3 (0.4090)	8.3 (0.0454)	1254.6 (0.0001)	33.5 (0.0001)
Developmental stage	8	1.2 (0.4429)	77.4 (0.0009)	1474.3 (0.0001)	12.7 (0.0001)
Proportion inactive	6	0.2 (0.9359)	104.2 (0.0005)	9.0 (0.0036)	6.8 (0.0094)
Proportion hiding	6	1.9 (0.2684)	902.6 (0.0001)	28.8 (0.0001)	102.9 (0.0001)
Relative body length	8	0.2 (0.9154)	11.0 (0.0295)	13.5 (0.0041)	3.7 (0.0812)
Relative body depth	8	2.8 (0.1737)	42.0 (0.0029)	35.4 (0.0001)	6.6 (0.0082)
Relative body width	8	5.7 (0.0597)	50.3 (0.0021)	19.3 (0.0003)	1.4 (0.2954)
Relative tail length	8	0.7 (0.6300)	90.8 (0.0007)	19.4 (0.0004)	4.8 (0.0334)
Relative tail fin depth	8	1.5 (0.3419)	602.6 (0.0001)	10.6 (0.0090)	14.6 (0.0038)
Relative tail muscle depth	8	5.4 (0.0663)	244.4 (0.0001)	3.3 (0.0131)	3.7 (0.0863)
Relative tail muscle width	8	2.9 (0.1663)	21.2 (0.0100)	23.9 (0.0001)	2.1 (0.1771)

30–40 h to converge on the activity level of tadpoles that had always inhabited predator-free ponds. Individuals that were switched into the high-risk environment decreased their activity immediately and converged within 24 h on the tadpoles that had spent their entire lives with caged predators.

Results for morphology were similar and revealed a surprisingly rapid adjustment to the current environment (Fig. 4). Responses in many traits were complex, but relative body length and tail fin depth showed clear reversals within only 4 days. The variance components explained by current and previous environments exhibited the same pattern as that seen in the behavioral data (Fig. 5): At the beginning of the experiment, most traits showed large and significant effects of the tank treatment, but these grew smaller within 4 days. The variance component associated with the current predator environment usually grew larger over the first 4 days, and was often significant after 10 days. This pattern was especially obvious for body shape and tail fin depth (Fig. 5).

There was no evidence for asymmetric morphological responses to the environmental switch in tadpoles originating from the two tank treatments, as there was for behavior. Tad-

TABLE 2. Multivariate analyses of variance testing for the effect of predators during the egg and early tadpole stages on behavior and morphology of *Rana temporaria* tadpoles. Responses for the analysis of behavior (A) were the proportions of time spent swimming and feeding at 24 days of age and the proportion of tadpoles that were not hiding at 33 days. Responses for the analysis of growth and shape (B) were daily growth rate and the seven size-corrected measures of body and tail shape, all measured at 25 days of age (22 days after the reversal of the predation environment). In both cases the tadpole treatment had a much greater impact on phenotypes than did the egg treatment.

Source of variation	df	Wilks' F	P
A. Behavior			
Block	12,29.4	1.97	0.0666
Egg treatment	3,2	0.65	0.6515
Tadpole treatment	3,2	126.04	0.0079
Egg treatment × tadpole treatment	6,18	0.68	0.6679
B. Growth and morphology			
Block	32,27.4	0.99	0.5159
Egg treatment	8,1	4.93	0.3356
Tadpole treatment	8,1	377.83	0.0398
Egg treatment × tadpole treatment	8,9	0.36	0.9166

poles moved from the no-predator treatment to the caged *Aeshna* treatment did not show an especially rapid shift in morphology compared with those switched in the other direction.

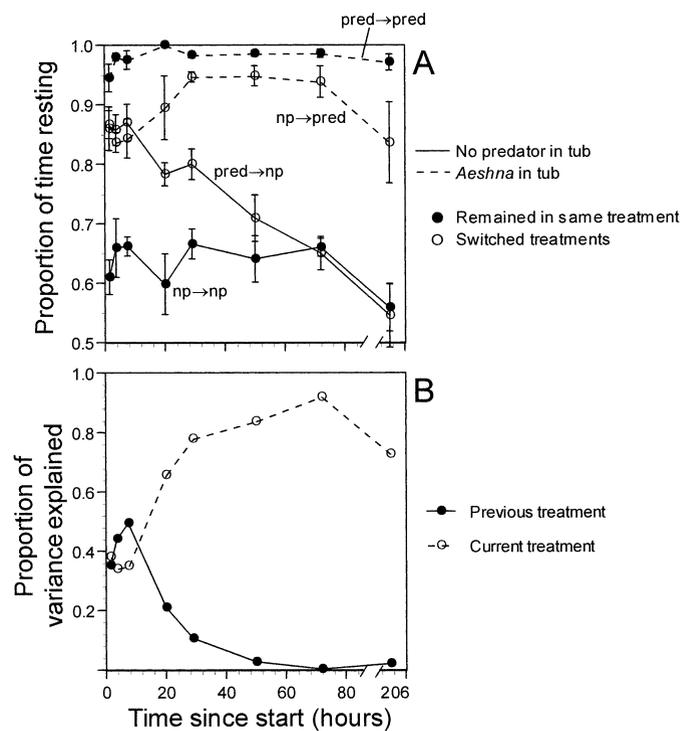


FIG. 3. Activity of *Rana temporaria* tadpoles during the first 8 days following an experimental reversal of the predator environment conducted after tadpoles had been held for 22 days in tanks with either no predators (np) or caged *Aeshna* dragonflies. Symbols in panel A depict means ± 1 SE, and labels indicate the predation environment before and after the switch. (B) The change in the proportion of among-tub variance in time resting explained by the previous tank treatment and current tub treatment. The previous predation environment was as important as the current environment during the first 10 h after tadpoles were introduced to the tubs, but within 20 h tadpoles adjusted their activity to current predation risk. Results for time spent feeding and swimming were similar.

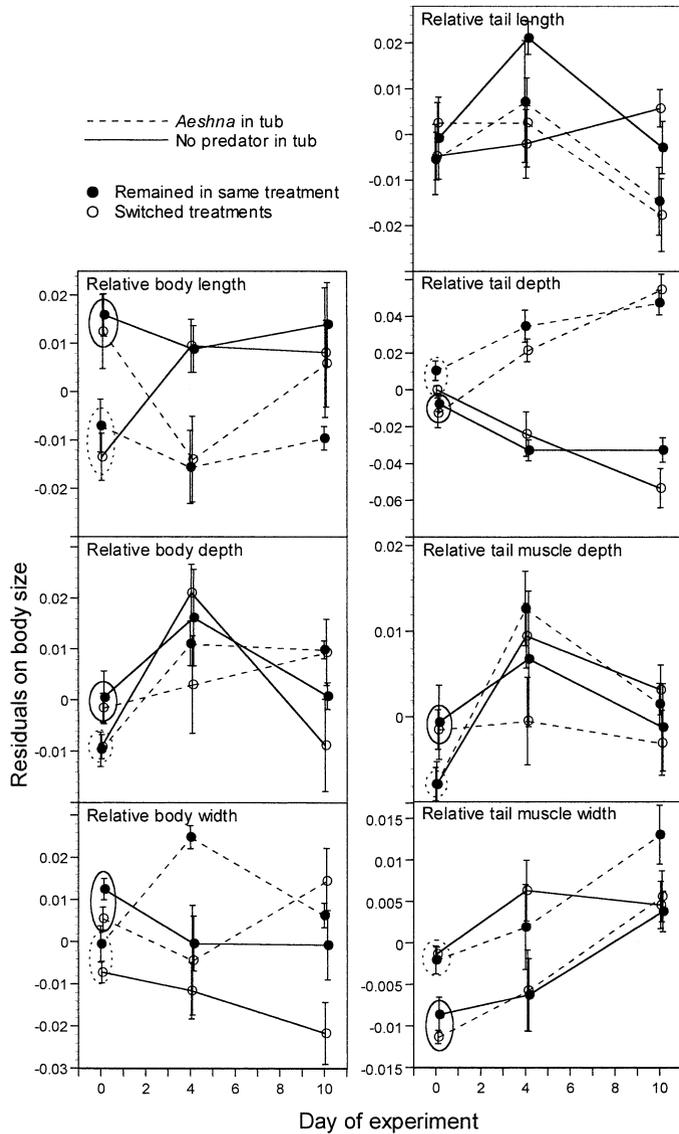


FIG. 4. Body and tail shape of *Rana temporaria* tadpoles after an experimental switch of the predation environment. The experiment was conducted after tadpoles had been held for 22 days in tanks with either no predators or caged *Aeshna* dragonflies. Morphology was sampled immediately after tadpoles were collected from the tanks, and again 4 and 10 days after the experiment began. Symbols depict mean \pm 1 SE. The solid and dashed ellipses indicate the no-predator and caged-*Aeshna* treatments at the beginning of the experiment, in cases where the two treatments were significantly different at $\alpha = 0.05$.

Relationship between Plasticity and the Rate of Response

There was little support for the prediction that plasticity is most extensive in highly labile traits. I measured the extent of plasticity for each trait as the response to caged predators in the 2000 tank experiment expressed as a proportion of the phenotypic value in the no-predator treatment. The rate of response was measured from the 1999 reversal experiment, by regressing the proportion of variance explained by the current environment on the time since the environmental switch ($\ln[\text{no. of hours}]$). For all traits this relationship is

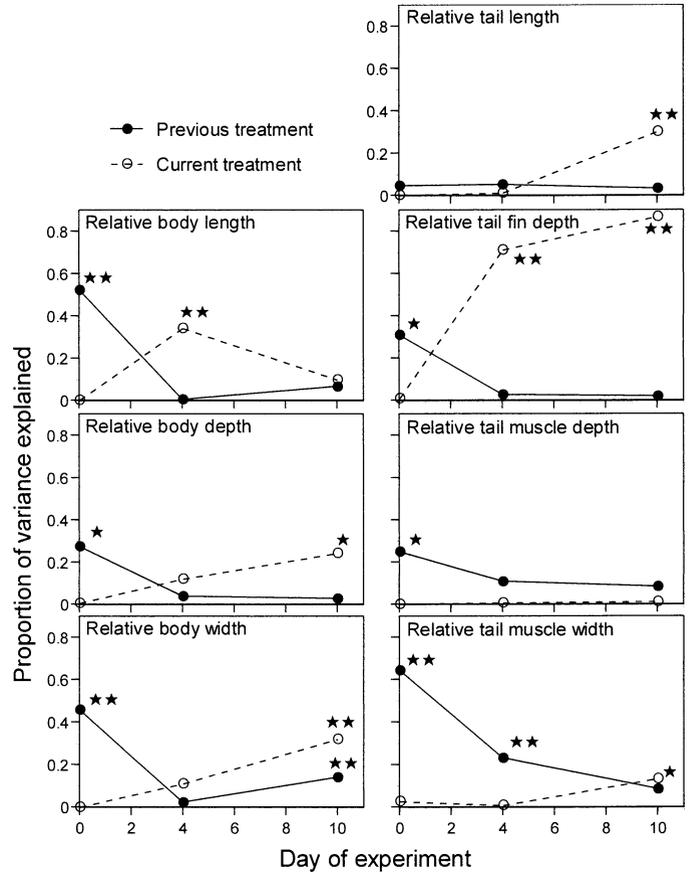


FIG. 5. Change in the proportion of among-tub variance in morphology explained by the tank treatment and tub treatment. The previous predation environment (tank treatment) was important when the experiment began, but the current environment (tub treatment) was often more important after only 4 days, suggesting that tadpoles rapidly adjusted their morphology to current predation risk. ★ indicates that the effect is significant at $P < 0.05$ (ANOVA); ★★ indicates $P < 0.01$.

positive and begins at about zero (Figs. 3B and 5), and it estimates within a single measure the rate of response to switches into both environments.

Behavioral and morphological traits differed in their extent of plasticity and their rate of response (Fig. 6). The three behavioral traits (proportion of time resting, swimming, and feeding) responded rapidly to the environmental switch and showed strong plasticity. The seven morphological traits were generally slower to respond to the reversal, with the exception of tail fin depth, and they all showed less extensive plasticity.

Variation in Predator Numbers in Natural Ponds

The field survey showed that tadpoles regularly experience temporal variation in the density of predators within ponds. There was in all years a positive relationship between the May and July densities of dangerous predators, because ponds with many predators in spring were likely to have many predators 2 months later (Fig. 7). Extensive scatter around the positive relationship reflects seasonal changes in predator composition, and in four cases the density of predators varied

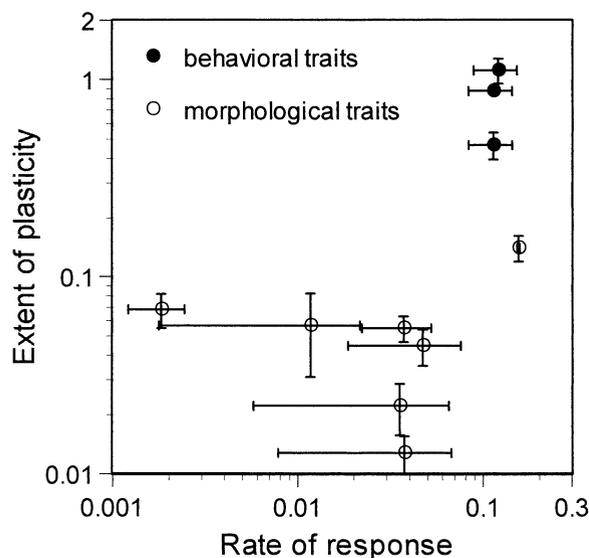


FIG. 6. Comparison between the extent of predator-induced behavioral and morphological plasticity of *Rana temporaria* tadpoles and the rate at which those traits can respond to a sudden change in the predator environment. The extent of plasticity is the proportional change in the caged-*Aeshma* environment relative to the no-predator environment in samples taken during the middle of the larval period. The rate of response is estimated from the 1999 tadpole reversal experiment as the slope of the regression of variance explained by the tub treatment on time (see Figs. 3B and 5). Each point represents a single trait (± 1 SE).

by more than an order of magnitude between May and July. Some variation between sampling dates arose from sampling error, but seasonal changes in predator numbers were outside the 95% confidence intervals of the estimates in 62% of the cases. This indicates that tadpoles in natural ponds are often faced with real changes in predation risk, at least over a period of weeks. It therefore seems reasonable to ask whether trait lability influences the evolution of plasticity.

DISCUSSION

Does Phenotypic Lability Limit the Evolution of Plasticity?

These results do not support the hypothesis that the evolution of predator-induced phenotypic plasticity is constrained by the time needed for traits to develop. This hypothesis is suggested by persuasive intuitive arguments (West-Eberhard 1989) and supported by models (Clark and Harvell 1992; Padilla and Adolph 1996). On one level, my findings agree with earlier work comparing plasticity in behavior with plasticity in traits that require more time to modify. West-Eberhard's (1989) review concluded that behavior exhibits the most immediate response to an environmental switch, and as a consequence behavioral phenotypic plasticity shows the broadest taxonomic distribution. My study confirms that behavioral traits respond comparatively quickly and strongly to predators, but on the finer scale of specific components of behavior and morphology the data do not support West-Eberhard's proposal. With the exception of tail fin depth, it was not true that the morphological traits having the most rapid response to predators were the same traits that exhibited the most extreme plasticity. The three behavioral

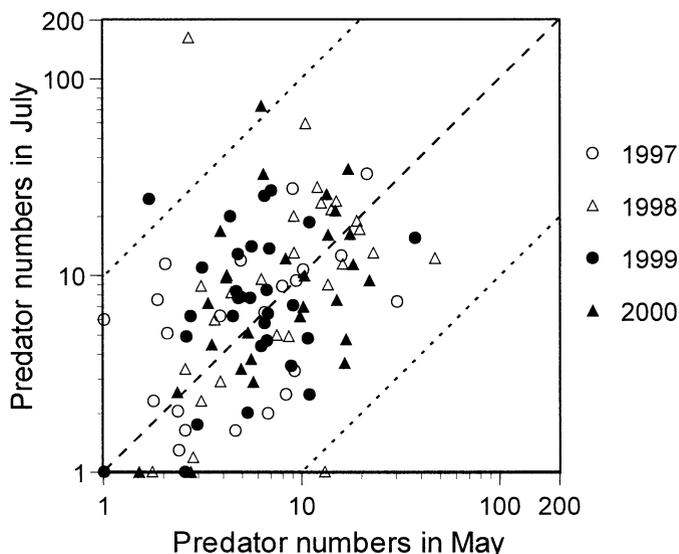


FIG. 7. Predator density in May and July within ponds near Zurich, Switzerland, including anisopteran dragonflies, dytiscid beetles, larval hydrophilid beetles, nepid bugs, and adult *Notonecta*. The figure shows the combined densities of all predators > 15 mm in body length ($1 + \text{number per m}^2$), with larval dytiscids and aeshnids weighted twice as heavily as other taxa. The dashed line represents the case in which predator densities were identical in May and July. Ponds falling above or below the two fine dotted lines experienced more than a 10-fold change in predator numbers within the 8-week period. The 95% confidence intervals of the estimates averaged 50% of the mean. Many of the same ponds were sampled in more than one year and therefore appear more than once in the figure ($r = 0.35$, $P = 0.0002$).

traits showed so little variation in plasticity or rate of response that such a comparison was not possible. In general, then, this study suggests that if trait lability affects the evolution of plasticity, it does so only at the scale of groups of traits that differ fundamentally from one another. The scope of plasticity in specific traits cannot be understood as constrained by inability to respond rapidly to environmental change.

A possible objection to West-Eberhard's (1989) hypothesis is that selection for plasticity may increase simultaneously both the extent of the response and the rate at which traits can be modified. Trait-modulation that confers an especially strong effect on fitness may be under selection to take place rapidly. In this case one would expect to often find a positive correlation between the extent and rate of plasticity. This may account for the contrast between behavior and morphology noted by West-Eberhard: Behavioral responses to predators may be pervasive not because of the extreme lability of behavior, but because behavioral defenses are very effective. This possibility is difficult to discount, but a comparison between data on natural selection (Van Buskirk et al. 1997; Van Buskirk and Relyea 1998; Van Buskirk and McCollum 2000) and the results of the present study indicates that traits that most strongly improve predator escape are not necessarily those that respond most rapidly to the proximity of predators.

A more philosophical objection to my approach is that the traits under consideration are artificial constructs produced

by atomizing a complex organism into components (Wagner and Laubichler 2000). Some pairs of morphological traits are highly correlated (Van Buskirk and Relyea 1998), and some measure different dimensions of the same larger structures. Others may share a common genetic basis because they function together as an integrated unit and have presumably evolved together (Lande 1984; Wagner 1996). Some of the behavioral traits are correlated if only because they sum to unity. Statistical inferences drawn from univariate tests of the separate traits would be open to question, and high correlations among traits may make it difficult to test whether labile traits are relatively plastic. Nevertheless, I maintain that West-Eberhard's proposal remains plausible because at least some of the traits are sufficiently independent to exhibit different temporal responses to environmental change, and because behavioral and morphological responses are triggered by different cues (Van Buskirk and Arioli 2002). These observations imply that some traits may be sufficiently independent to evolve plasticity separately, in which case non-independence of traits would not seriously weaken my main conclusions.

We are left finally with the question of what, if anything, constrains the evolution of plasticity in anuran larvae. I have argued that neither the intensity of selection imposed by predators nor the time required to respond to an environmental switch can fully explain differences among traits in the extent of plasticity. The answer may be that fitness costs more strongly oppose the evolution of plasticity in some traits than they do in others. Amphibian studies have measured mostly natural selection imposed by hunting predators over short periods of time, but only rarely selection against plasticity imposed by other agents (e.g., Van Buskirk and Saxer 2001). The costs associated with producing traits may differ, and the cost of plasticity itself may differ from one trait to another. Behavioral traits might be particularly inexpensive, because they can be altered without developing or maintaining new structures (but see Barnea and Nottebohm 1994). Morphological responses are perhaps more costly overall, but the costs may differ in degree. For example, a relatively deep tail fin may be less expensive to possess than a relatively large muscle, simply because the metabolic demands of muscle tissue are larger than those of skin and connective tissue (Slama 1984). Shifts in body shape could be costly because they require a rearrangement of the body cavity, containing organs involved in feeding and digestion (Nodzenski et al. 1989). Thus, differences among traits in the cost of responding to predators, rather than differences in lability, may explain why plasticity in the shape of the tail fin is more extensive than that in most other traits.

The Ontogeny of Behavioral and Morphological Plasticity

Ontogenetic differences in the ability to exhibit phenotypic plasticity seem likely, if only because patterns of gene regulation and expression are highly dependent on developmental stage (Raff 1996). The kind of tissue- and stage-specific gene activity described in *Xenopus* tadpoles (Brown et al. 1996; Valverde et al. 2001) could someday provide a proximate explanation for differences among traits and stages in the degree of predator-induced plasticity (Fig. 2). But even

without knowledge of genetic mechanisms, we may be able to predict stage-dependent plasticity using evolutionary models. For example, the absence of predator-induced plasticity in eggs can be understood in terms of life-history theory. Amphibians are capable of detecting predators while still in the egg stage: Egg predators can cause accelerated hatching and tadpole predators cause delayed hatching (Sih and Moore 1993; Warkentin 1995). But an impact of predators on the timing of a life-history transition is not predicted when mortality targets the stages both before and after the switch to a similar degree (Werner 1986; Rowe and Ludwig 1991), and this is often the situation for predators of eggs and hatchling tadpoles in freshwater ponds (Henrikson 1990; Miaud 1993). Thus, a shift in the timing of hatching may not alter mortality from predators in pond-breeding amphibians. The lack of behavioral or morphological response of tadpoles to predators experienced during the egg stage is presumably explained by the ability of tadpoles to quickly adjust their phenotypes after hatching to match their current environment.

Delay and asymmetry were two prominent features of the phenotypic response to sudden change in the predation environment. The causes for the delay may be straightforward. In the case of morphology, reorganization of shape can only be accomplished by differential growth of structures, which requires time and tissue growth. Developmental time courses in the presence and absence of predators confirm that shape does not diverge until tadpoles have grown appreciably, to perhaps 20–30% of their mass at metamorphosis. In contrast, the delay in behavioral responses to novel environments cannot be blamed on time required to produce the phenotype, because tadpoles show an instantaneous behavioral response when suddenly exposed to predator chemicals (Petranka et al. 1987). Instead, delayed responses probably reflect a tadpole's memory of previous experiences, at least for a few days (Semlitsch and Reyer 1992). In both cases, the delay may be thought of as an opportunity for natural selection to oppose the evolution of plasticity (DeWitt et al. 1998), because it makes it more difficult for an individual to ensure that its current phenotype is suitable for the current environment.

The asymmetry in response to environmental switches may have a more direct adaptive interpretation. In my experiment, there was an immediate and strong reaction to a transfer into tubs with predators, whereas a transfer in the reverse direction caused a more gradual behavioral response. The reaction is conservative, in the sense that the effect of the predator environment on behavior persists longer than that of the predator-free environment. When assessment of predation risk is imperfect, asymmetry of response in this direction may be adaptive because the animal reduces the probability that the more costly error will occur. Clearly, it would be more costly to conclude inaccurately that a dangerous environment is predator-free than to make the opposite mistake. These and other results on systems in which mortality risk differs among environments (Etter 1988; Kotler 1992; Sih 1992) suggest that animals cannot only assess the immediate risk, but can also weigh the consequences of mistaken assessments.

One idea discussed in the literature is that induced defenses appear at times when they are most needed, when exposure to predators is maximal (Harvell 1990; Tollrian 1993; Arnq-

vist and Johansson 1998). This is not true for larval anurans. Small tadpoles are more susceptible to predation than large tadpoles, which achieve some measure of safety due to their larger body size (Travis et al. 1985; Semlitsch 1990). The tadpole behavioral response to predators, reaching a maximum after about 30% of the larval period, therefore appears at a time when vulnerability to predators is high. But the morphological response reaches its maximum only late in the larval period, and therefore appears later than when it should be most needed. This suggests that tadpoles produce rapid behavioral defenses simply because they can, and they would probably produce morphological defenses earlier if they could. The timing of the maximal production of defenses in anurans is therefore probably best interpreted as a reflection of developmental capabilities, rather than as an adaptation per se. Knowledge of developmental changes in the activity of genes involved in growth and stress responses could help evaluate this idea (e.g., Denver 1999; Valverde et al. 2001).

This study has implications for understanding the scope and limitations of adaptation. The adaptive plasticity hypothesis asserts that the extent of plasticity can be understood in terms of divergent selection across different environments (Schmitt et al. 1995). In the case of tadpoles, this involves selection for different trait combinations imposed by hunting predators and in predator-free habitats (Van Buskirk and Relyea 1998). West-Eberhard's (1989) model modifies the purely adaptive hypothesis by recognizing that delays in the production of traits increase the likelihood of erroneous trait expression, making it difficult to ensure that traits will appear simultaneously with the environment for which they are suited. Although my work fails to identify an important role of delayed trait expression, I find that support for the purely adaptive model is incomplete as well (Van Buskirk and Relyea 1998). The results therefore focus attention on more complex models of the processes governing phenotypic expression. Some of these may invoke limits to adaptation, imposed perhaps by the genetic basis of the phenotype. Others may simply refine our model of the net effects of selection by incorporating selection acting in other life stages or on functionally interconnected characters. In either case, this study contributes to a picture of the evolution of phenotypic plasticity that is decidedly more complex than that embodied by the adaptive plasticity hypothesis.

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