Functional mechanisms of an inducible defence in tadpoles: morphology and behaviour influence mortality risk from predation

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Abstract

In many amphibian larvae a suite of morphological and behavioural characters varies together in an induced defence against predators, but it remains unclear which features are functionally related to defence. We independently manipulated behaviour and morphology in tadpoles of *Hyla versicolor* and assessed their consequences for swimming performance and predator escape. Data on burst swimming showed that tadpoles which accelerated rapidly were elongate, with shallow bodies and tails. Predator escape was measured by exposing tadpoles to predators (larval *Anax* dragonflies or larval *Ambystoma* salamanders) and recording time until death. Tadpoles were first reared for 30 days in ponds containing either caged *Anax* or no predators; individuals responded to predators by developing large brightly coloured tails and short bodies. We placed tadpoles of both morphological phenotypes into plastic tubs, and manipulated their behaviour using food and chemical cues from predators. Mortality risk experienced by the predator-induced phenotype was about half that of the no-predator phenotype, and risk increased with time spent swimming. An interaction between morphology and behaviour arose because increasing activity caused higher risk for tadpoles with deep tail fins but not shallow tail fins.

Introduction

Measuring the fitness costs and benefits of environmentally induced phenotypes is critical for understanding the evolution of plasticity. In theory, plasticity is maintained by trade-offs arising because environments vary in space and time, causing the fitness ranks of alternative phenotypes to vary across environments (Via & Lande, 1985; van Tienderen, 1991). Estimates of many such trade-offs are now available for a variety of phenotypically plastic animals and plants (Karban & Baldwin, 1997; Tollrian & Harvell, 1999). These studies demonstrate the adaptive basis of entire phenotypes in different environments, but they do not show how particular traits affect individual performance. Because the development of different traits is often correlated, some traits exhibiting plasticity may have little effect on fitness, even if the overall induced phenotype is maintained by selection. Functional studies connecting different components of the phenotype to performance can help decide which traits directly contribute to fitness. Here we ask how selection maintains predator-induced plasticity in anuran larvae by determining how different aspects of the phenotype influence swimming and susceptibility to predators.

Anuran larvae are well-suited for studying the functional consequences of plasticity for several reasons. When exposed to odonate predators, tadpoles of many species exhibit a phenotypic response that reduces their risk of predation and yet is costly to build or maintain (Smith & Van Buskirk, 1995; McCollum & Van Buskirk, 1996; Van Buskirk, 2000). It is therefore plausible that a fitness trade-off maintains plasticity. However, the induced response includes many kinds of traits, and the functional consequences of each are unclear. Tadpoles that co-occur with predators have decreased activity and growth, deep tail fins, large tail muscles and conspicuous

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coloration in the tail. Some of these traits apparently undergo selection by predators, at least within experimental settings, because survival is frequently biased with respect to phenotype (Avezedo-Ramos *et al.*, 1992; Skelly, 1994; Watkins, 1996; Van Buskirk *et al.*, 1997; Van Buskirk & Relyea, 1998). Predators kill proportionately more tadpoles that have relatively shallow tail fins and active behaviour, but the targets of selection are not obvious because these traits covary strongly. What is needed are experiments that disentangle behaviour and morphology, and then connect them to risk of predation.

Determining the targets of selection is a general problem in evolutionary biology. Even when the putative agent of selection is experimentally manipulated and resulting selection differentials are measured for specific traits, it can be difficult to decide whether the traits in question are causally connected with fitness. Indirect selection is no more easily detectable with such experiments than with any other approach. Measures of individual fitness and phenotype illustrate the form of the selection gradient resulting from a combination of direct selection on the trait itself and indirect selection acting on phenotypically correlated traits. It is therefore unclear whether the traits associated with a selection gradient are themselves under selection, or are only correlated with the traits that actually affect fitness.

This problem can be partially resolved with information on phenotypic function. Functional studies measure the use or mechanical role of a trait, without any necessary relationship between a trait's function and its impact on individual fitness or selection (Lauder, 1996; Wainwright, 1996). If the functions of traits are related to survival, growth or fecundity, then functional studies may address the causal links between an individual's phenotype and its fitness, and the results can provide mechanistic detail on how selection operates. Functional information can thus help decide whether specific traits experience direct selection by demonstrating whether they are involved in intermediate measures of performance.

Here we report an experiment in which we independently manipulated the morphology and behaviour of tadpoles, and assessed consequences for two aspects of individual fitness: burst swimming performance and survival when exposed to predators. The results offer mechanistic insight into predator-induced defences in amphibian larvae, and provide an example of how functional information can clarify the results of selection studies.

Methods

Tadpoles of *Hyla versicolor* (Anura: Hylidae; grey treefrog) are abundant and inhabit most aquatic habitats at the University of Michigan's E. S. George Reserve in southern Michigan, USA (Van Buskirk & McCollum, 1999). They are ideal for this work because they show dramatic phenotypic responses to predators, and because the naturally occurring correlation between behaviour and morphology can be broken by careful manipulation of food level and chemical signals associated with predators. *Hyla* tadpoles alter the shape, musculature and colour of their tails in response to long-term exposure to chemical byproducts of predation. They alter their activity in response to short-term exposure to predator cues and to food availability. Thus, in this species we can independently manipulate morphology (using long-term exposure to predator cues) and behaviour (using short-term variation in food level and predator cues).

Our study included three phases. First we exposed tadpoles to treatments with and without caged predators to produce distinct morphological phenotypes. Next, we manipulated the tadpoles' hunger level and chemical environment to produce individuals of each morphological phenotype with distinct behavioural phenotypes. Finally, we exposed tadpoles to predation risk in order to estimate the association between individual phenotypes and susceptibility to predators.

Manipulating morphology

In the first phase of the study, we took advantage of the anuran response to chemical cues associated with predation to produce large numbers of morphologically distinct tadpoles (e.g. McCollum & Van Buskirk, 1996). This involved rearing tadpoles in outdoor artificial ponds in the presence or absence of predatory dragonfly larvae. The ponds were plastic stock tanks, placed in an open field at the George Reserve and filled with well water to a depth of 38 cm (2.6 m^2 surface area, 1000 L volume). We filled the tanks on 19 June 1996 and stocked them with a standard recipe of leaf litter, pond water and zooplankton to establish aquatic communities typical of ponds in the surrounding area. Each tank contained three floating cages, constructed of a 12-cm-length of plastic tube (1 L volume) capped at both ends with fibreglass window-screen. We manipulated predator composition by enclosing three late-instar dragonfly larvae (*Anax longipes*) inside the cages within half the tanks; cages in the other tanks were left empty. The *Anax* were fed ~1 g *Hyla* tadpoles every other day throughout the experiment to ensure that the experimental tadpoles within the caged-*Anax* treatment were exposed to chemical evidence of predation. There were 10 tanks, arranged into five spatial blocks, with treatments assigned to tanks within blocks by a coin toss.

We gathered amplectant pairs of *Hyla versicolor* on the night of 18 June from a large breeding aggregation in Cornfield Pond, just outside the George Reserve. The experiment began on 26 June, when we added to every tank 25 newly hatched tadpoles from each of 10 clutches (250 tadpoles per tank, or 95 m^{-2}). Over the next several weeks, the tadpoles grew rapidly and developed distinct morphology and tail coloration in the two treatments (see below).

Measuring morphology, colour and swimming performance

We sampled tadpoles from the artificial ponds and measured several components of the phenotype for each individual. On 10 different days between 18 July and 8 August we collected 4–6 tadpoles from each tank for assessing morphology, swimming performance and predator escape. We measured morphology by photographing tadpoles within a small water-filled plexiglas chamber, using a 35-mm camera equipped with a 50-mm macro lens and Kodak TMAX professional black-and-white film. We later digitized five linear measurements from negatives projected onto a computer video monitor via a digital camera. The measurements were body length, body depth, tail length, maximum tail fin depth and tail muscle depth at the base. We chose these traits because earlier studies suggest that they exhibit predator-induced plasticity, they may affect swimming performance and they undergo selection in the presence of predators (McCollum & Leimberger, 1997; Van Buskirk et al., 1997).

We did not weigh the tadpoles, so body size was defined as the first component derived from a principal components analysis on the correlation matrix of all five measurements. The first axis explained 94% of the variance in the original data, and was positively and equally correlated with all five traits. Body and tail shape were described by the residuals of original variables after regression on body size. These regressions were highly significant (r^2 ranging from 0.929 to 0.954); transformation was unnecessary because all traits showed nearly perfect linear relationships with size. Defined in this way, any single measure of shape is a linear combination of the other four and body size, so we excluded one trait, tail muscle depth, from all subsequent analyses. We selected this trait because it shows relatively little plasticity or selection (Van Buskirk et al., 1997), and because it has more measurement error than any of the other four traits (J. Van Buskirk, unpublished).

We scored the tail colour of every tadpole by visual comparison with a colour chart (Kornerup & Wanscher, 1963; hue 6, tone A).

We also measured two components of swimming performance. After photographing and scoring the tail colour of each tadpole, we videotaped it swimming at 30 frames s^{-1} in a glass-bottomed tray (25 × 25 cm), filled 1.8 cm deep with water from the tank from which the tadpole originated. The camera viewed tadpoles from below, through a glass shelf and reflected off a mirror, to avoid the distorting influence of waves at the water's surface. We waited for tadpoles to come to a rest, then motivated them to swim suddenly by prodding them near the base of the tail. Every tadpole was filmed until it made at least three attempts to swim rapidly; in most cases this occurred within just a few seconds.

Our analyses focused on the first 10 frames of the three fastest swimming records for each tadpole, beginning when the animal was completely immobile. During the first 10 frames (333 ms) the tadpole usually executed a C-start (Webb, 1986; Frith & Blake, 1991), reached a maximum velocity, and began to slow down. For each frame we digitized the locations of the base of the tail and tip of the snout, and calculated the position of the tadpole as the midpoint between these two landmarks. To reduce error we used lowess smoothing on the raw position data and estimated all subsequent values from the smoothed results (TriMetrix, 1993). We recorded two measures of swimming performance: maximum velocity estimated between successive video frames, and elapsed time until the tadpole moved 2.5 cm radially from its point of origin. The latter measure reflects the time it takes an individual to move a safe distance from a sit-and-wait predator, which we consider to be of ecological importance. While this measure is closely related to speed and acceleration, it differentiates between a tadpole that swims in a circle and thus gets nowhere and one that swims in a straight line, thus putting distance between itself and a predator. For every tadpole we retained from its fastest swimming record the maximum velocity and minimum time to travel 2.5 cm, under the assumption that slower records reflected events in which the tadpole was not fully motivated.

Manipulating activity level

After photographing and videotaping each individual, we created groups of tadpoles that differed in behaviour. To do this, we placed pairs of tadpoles, one of each morphological type, into plastic tubs (26 × 38 × 14 cm, filled with 10 L water) and exposed them to one of two sets of conditions, which differed in predator cues and the timing of food. To render tadpoles inactive, we added rabbit chow (~10% of the wet mass of the tadpoles) to their tanks on the same afternoon that we placed them in the tubs. The following morning, we added 600 mL of water containing chemical cues of predation. The predator water was drawn from a single container in which six Anax had just killed and eaten one Hyla tadpole each; each tub therefore received about 20% of the alarm substance produced by a single Anax-killed tadpole. To render tadpoles active, we added no food on the afternoon we placed them into the tanks. The following morning we added the food (again, 10% of the tadpoles' wet mass) and 600 mL of aged tapwater. These treatments produced tadpoles of both morphological types with both high and low levels of activity.

We observed behaviour after the morning additions of food and water-borne cues to the tubs. Tadpoles were clearly visible to the observer, and were always distinguishable from their partners by external morphology and colour. Beginning 15 min after the water addition (predator water or aged water, depending on the treatment),

we watched each tadpole for three 45-s periods spread over a 3-h session between 06:30 and 10:30. We recorded the proportion of time spent swimming, feeding and resting inactively, using a laptop computer programmed to convert sequences of keystrokes into elapsed time during each activity. The combined effect of food and predators on tadpole behaviour was dramatic (see Results).

Susceptibility to predation

We assessed the vulnerability to predation of tadpoles taken from the cattle tanks to determine the contributions of morphological and behavioural traits to predator escape. The predation experiment was carried out in 10 rounds between 18 July and 8 August. For each round, we collected an equal number of tadpoles from the caged-*Anax* and no-predator treatments, photographed them, videotaped them and manipulated their behaviour as described above. On each round there were 24–28 tubs; tadpoles remained within the same tubs in which we had manipulated activity. Each tub contained a standard quantity of loosely knotted plastic rope strands to provide structural heterogeneity and perches for the predators. Within a tub, predators were presented with a choice between two morphologically distinct prey individuals, which enabled us to ask whether vulnerability depended on relative or absolute phenotypic values.

Each tub was assigned to one of four treatments: two species of predator crossed with the two behavioural treatments (active and inactive). The predators were *Anax longipes* and larval *Ambystoma tigrinum* (tiger salamander). The goal was to discover whether the predator-induced phenotype is a generalized defence or is specific to particular predator species: do traits induced by exposure to *Anax* protect tadpoles against *Ambystoma*? Like *Anax*, *Ambystoma* is an ambush predator, but it captures prey by suddenly engulfing them (Reilly & Lauder, 1992), a quite different mechanism than the labial strike of an odonate (Tanaka & Hisada, 1980). Aeshnid dragonflies and *Ambystoma* salamanders are both common in natural ponds on our study area in southern Michigan (Van Buskirk & McCollum, 1999).

Predators were introduced to the tubs immediately after the behavioural observations were complete (~11:00), and we checked the tubs at 10- to 15-min intervals thereafter for at least 500 min. A trial was terminated when the predator killed one of the two tadpoles, at which point we recorded which tadpole died and the time at which it died (to the nearest 5–15 min). In ambiguous cases we photographed the survivor and identified it by comparison with the two original photos. We did not have enough predators to use naive individuals in every round, so we rotated them through the experiment so that each rested a week between successive rounds.

Statistical analyses

There were 264 separate trials (tubs) over the 10 rounds of the experiment, involving 528 tadpoles and comprising 66 replicates of each treatment. However, none of the analyses employs the full sample because of various oversights and accidents. For example, tail colour scores were inadvertently lost for five tadpoles, and observations of swimming performance were not made for 11 tadpoles. In 19 tubs neither tadpole was killed during the full 500 min. A film developing accident destroyed the images of 109 tadpoles, and prevented us from identifying which tadpole survived in seven tubs. This accident affected trials conducted early in the experiment, from 19 to 25 July, but they were distributed evenly with respect to treatment and outcome. Analyses incorporate every record for which all the required variables are available, and therefore sample sizes differ from one analysis to the next.

We tested whether swimming was associated with tadpole morphology using canonical correlation analysis on two sets of variables representing swimming performance (maximum speed and time to travel 2.5 cm) and morphology (size and size-corrected body length, body depth, tail length and tail fin depth). The method, a multivariate extension of standard bivariate correlation analysis, finds the linear combination of each set of variables, called a canonical variate, that maximizes the correlation between the two canonical variates (SAS, 1990). An *F* approximation tests the null hypothesis that the correlation among canonical variates representing swimming performance and morphology is zero.

Vulnerability to predation was analysed in two steps. First, we estimated the effects of experimental treatments and phenotypic traits on the risk of mortality using Cox proportional hazards regression models ('phreg' procedure in PC SAS version 6.12). This method is termed 'semiparametric' because it requires no choice of probability distribution for survival times, and it estimates the effects of covariates by maximum partial likelihood (Cox, 1972; Allison, 1995). Our analysis included both completed observations and right-censored data: a tadpole that was killed during the experiment represented a complete observation, whereas a tadpole that was not killed represented an observation that was right-censored at the time when its trial was halted. Surviving tadpoles contribute information to all earlier survival estimates because we know that they lived at least until the end of the trial. We estimated the relationship between mortality risk and the experimental treatments, along with a list of phenotypic traits that included body size, the four measures of shape, tail colour, and the proportions of time spent feeding and swimming, all of which may influence vulnerability to predation. Proportion of time spent inactive was not included because the three behaviours together sum to one. Tied mortality events were resolved using the 'exact' method, which explores

all possible orderings under the assumption that ties result from imprecise measurement (Allison, 1995).

We measured interactions among multiple traits to determine whether selection imposed by predators favours particular combinations of traits. The prohibitive number of potential interactions prevented us from exploring all experimental treatments and traits, so we focused on three traits and their two–way interactions. The traits (body size, proportion of time spent swimming and relative tail fin depth) were selected because they varied independently from one other and were tightly correlated with other traits not included in the analysis (see Results). We used analysis of deviance to compare the fit of proportional hazards models with and without interactions (Weisberg, 1985).

Hazard models employ data for individuals, thereby disregarding the fact that tadpoles occurred in tubs together as pairs. We also asked whether it was possible to predict which of the two tadpoles was killed from the differences between their traits, using linear logistic regression. This analysis recognized that the chance of being killed may depend not only on a tadpole's own phenotype but also on the phenotype of the other individual in the tub.

Results

Effects of experimental treatments on tadpole phenotype

Manipulation of predator-exposure and food availability strongly influenced tadpole phenotype. MANOVA on the seven traits depicted in Fig. 1 (excluding body size) showed highly significant effects of both the morphological
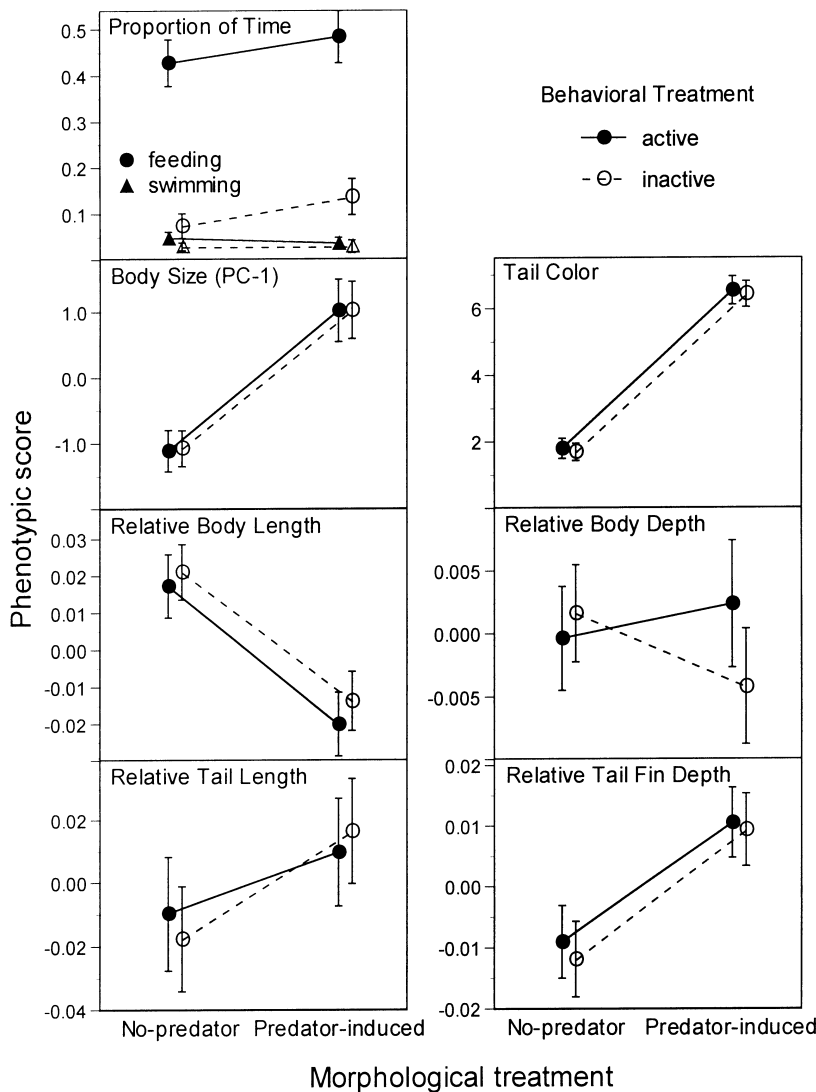

Fig. 1 Means (±95% CI) of phenotypic values of *Hyla versicolor* tadpoles in the four treatments just before the predation experiment began. The morphological treatment consisted of long-term exposure to either caged *Anax* or empty cages: predator-induced individuals were larger with shorter bodies and deeper, longer, and more brightly coloured tails. The behavioural treatment consisted of short-term exposure of either satiated tadpoles to high food availability and predator water or of hungry tadpoles to little food and plain water: satiated tadpoles spent less time active (especially feeding). Behaviour and morphology varied independently because the morphological treatment had no lasting effect on behaviour.

Table 1 Phenotypic correlations among traits for *Hyla versicolor* tadpoles used in the predation trials. The five measures of morphological shape were highly correlated with one another, but the two measures of behaviour were mostly unrelated to morphology. Morphological traits are residuals after regression against overall body size (PC-1). Sample size was 494 for behaviour, 489 for colour and 385 for morphology. Values shown in bold are significant at $P < 0.05$; *significant after Bonferroni adjustment for $\alpha = 0.05$ across 36 tests ($P = 0.00142$).

	Swim	Feed	Tail colour	Body length	Body depth	Tail length	Tail depth	Muscle depth
Size (PC-1)	**0.161**	**0.142**	**0.717***	0.008	−0.013	0.002	−0.009	0.007
Time spent swimming	–	**0.101**	0.032	0.083	−0.046	**−0.114**	0.007	0.050
Time spent feeding		–	**0.138**	0.009	−0.037	**−0.113**	0.050	0.085
Tail colour			–	**−0.222***	−0.070	**0.110**	**0.207***	0.022
Relative body length				–	−0.003	**−0.328***	**−0.455***	**−0.374***
Relative body depth					–	**−0.393***	−0.042	**−0.440***
Relative tail length						–	−0.153	−0.122
Relative tail fin depth							–	−0.158

treatment ($F_{7,375} = 135.8$, $P = 0.0001$) and the behavioural treatment ($F_{7,375} = 39.5$, $P = 0.0001$), but no interaction between them ($F_{7,375} = 0.64$, $P = 0.72$). Univariate tests indicated that the two kinds of treatments affected different traits. Long-term exposure to dragonflies altered morphology and tail colour, whereas behaviour was affected only by the short-term manipulation of food and predator water (Fig. 1). *Hyla* from tanks with caged predators had more brightly coloured tails, shorter bodies and tails, and deeper tail fins than those from no-predator tanks (all $P < 0.0015$). Predator-induced tadpoles were also larger, partly due to the contribution of their large tails to the estimate of body size. Manipulating food and predator-chemicals in the indoor tubs strongly modified behaviour: satiated tadpoles that detected predators spent little time feeding ($P < 0.0001$), and this was equally true for both morphological phenotypes (Fig. 1). Time spent swimming was only weakly affected by the behavioural treatment ($P = 0.0409$). Thus, behaviour was influenced mostly by the immediate environment, and showed no 'memory' effect.

Behaviour and morphology are usually highly correlated: tadpoles having the predator-induced morphology also show reduced activity. Our experiment broke this correlation (Table 1). Body size was positively correlated with tail colour and activity, and uncorrelated with five measures of shape (as expected since shape is defined relative to size). Some shape measures were correlated with one another, but they were unrelated to behaviour. This implies that the experiment was well-suited for estimating the independent effects of behaviour and morphology on predator escape. The correlations also suggest that a multivariate approach to the performance consequences of phenotypic variation is appropriate.

Phenotypic effects on performance: swimming

Both measures of swimming performance improved with body size. Across the two-fold range in body length present within our sample, the minimum time to swim 2.5 cm declined from 201 ms on average (for tadpoles with a 7.5-mm body length) to 127 ms (15-mm body length). Over the same size range the maximum velocity increased from 14 cm s^{-1} to 37 cm s^{-1}. Hereafter we confine analysis to measures of swimming performance relative to body size, which are residuals after regression against PC-1.

Canonical correlation analysis demonstrated a strong relationship between morphology and the two measures of swimming performance, size-corrected maximum velocity and minimum time required to travel 2.5 cm (Table 2). The linear combination of swimming variables that was most closely related to morphology was dominated by time to swim 2.5 cm: tadpoles with large scores on this axis accelerated slowly relative to their body size

Table 2 Canonical correlation analysis testing the relationship between two measures of swimming performance and four measures of shape in *Hyla versicolor* tadpoles. Body size was not included because both swimming performance and morphology were residuals after regression on body size. The significance test is a maximum likelihood estimate of the probability that the correlation between that pair of canonical variates, and between all subsequent pairs of variates, is equal to zero. $N = 378$ tadpoles for which the full set of variables was measured.

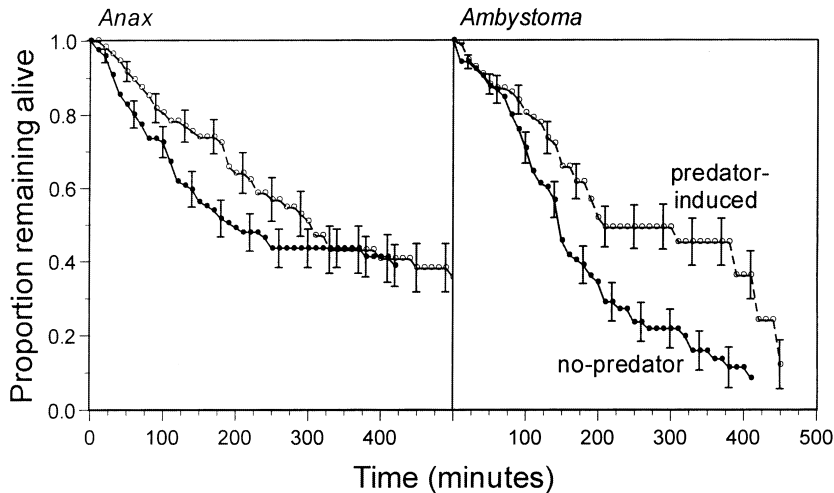| Standardized coefficients of the variate					Swimming variate		Morphology variate			
Canonical variate	Canonical correlation	r^2	Approximate F	P	Time to 2.5 cm	Maximum velocity	Body length	Body depth	Tail length	Tail fin depth
1	0.275	0.075	3.85	0.0002	0.982	0.302	−0.200	0.737	−0.156	0.493
2	0.053	0.003	0.27	0.7876	−0.212	0.958	−0.134	0.256	−0.567	−0.878

Fig. 2 Survivorship curves for the two morphological phenotypes of *Hyla versicolor* tadpoles when exposed to *Ambystoma* and *Anax* in laboratory predation trials. Vertical bars show ±1 SE of the mean proportion surviving. Predator-induced tadpoles survived significantly better with both predator species.

and had somewhat fast maximum swimming velocity. The morphological axis that correlated most closely with swimming performance was a contrast between overall depth and length: tadpoles with large scores on this axis had relatively deep bodies with short, deep tails. Tadpoles with elongate bodies and tails reached 2.5 cm rapidly.

Phenotypic effects on performance: predator escape

The two morphological phenotypes differed in their susceptibility to predation. *Ambystoma* and *Anax* both killed somewhat more of the no-predator *Hyla* than the predator-induced *Hyla*, although the bias was significant only for *Ambystoma* (*Ambystoma*: 74 no-predator tadpoles killed out of 115 total, $P = 0.001$, binomial test; *Anax*: 58 no-predator tadpoles killed out of 102 total, $P = 0.099$). Cox regression indicated that the risk of mortality was significantly influenced by predator species, morphological treatment and time spent swimming, but not by the behavioural treatment (Fig. 2, Table 3). The mortality hazard faced by tadpoles having the no-predator phenotype was 2.3 times higher than that experienced by predator-induced tadpoles (hazard ratio = 0.44), and tadpoles exposed to *Ambystoma* faced a hazard that was 1.4 times higher than that of tadpoles exposed to *Anax* (Table 3). Although tadpoles in the 'active' treatment faced no significant increase in risk, the proportion of time spent swimming was significantly related to mortality. The hazard ratio of 25.2 for swimming means that a tadpole that spent 10% of its time swimming faced a 2.4-fold increase in mortality risk compared with one that did not swim at all. This result suggests that our manipulation of activity had much less influence on mortality hazard than did swimming *per se*, probably because the manipulation did not alter swimming (Fig. 1).

Mortality risk was significantly affected by interactions among traits, suggesting that specific relationships among traits confer enhanced ability to escape predation. A proportional hazards model with predator species, body size, time spent swimming and relative tail fin depth gave an improved fit relative to a model with only predator species ($\chi_3^2 = 11.35$, $P = 0.01$). A model including predator species, the same three traits and all interactions among traits was a further improvement over the model without interactions ($\chi_3^2 = 8.01$, $P = 0.047$). The improvement in the fit of the model occurred because the effect of swimming depended on both body size and tail depth, although neither interaction is highly

Table 3 Cox Proportional Hazards model testing for effects of experimental treatments and phenotypic traits on mortality risk in *Hyla versicolor* tadpoles. Significant effects are shown in bold type: the mortality risk from *Ambystoma* was greater than that from *Anax*, the no-predator phenotype had a higher risk than the predator-induced phenotype, and tadpoles faced a higher risk if they spent much time swimming. The overall model was significant (likelihood ratio $\chi_{11}^2 = 31.8$, $P = 0.0008$).

| Variable	d.f.	Wald χ^2	P	Hazard ratio
Experimental design				
Predator species	1	**4.44**	**0.0352**	**0.721**
Morphological phenotype	1	**7.96**	**0.0048**	**0.437**
Behavioural phenotype	1	0.08	0.7769	0.943
Phenotypic covariates				
Body size (PC1)	1	2.49	0.1149	0.919
Tail colour	1	1.11	0.2927	1.062
Time spent swimming	1	**6.72**	**0.0095**	**25.181**
Time spent feeding	1	0.00	0.9478	1.024
Relative body length	1	0.64	0.4241	0.151
Relative body depth	1	0.67	0.4113	0.035
Relative tail length	1	1.09	0.2965	3.512
Relative tail fin depth	1	3.17	0.0750	239.741
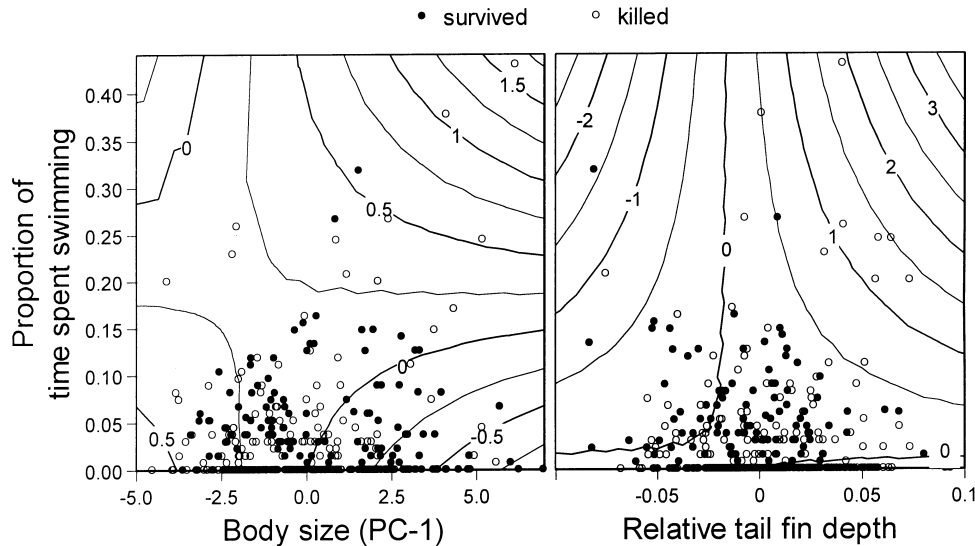
Fig. 3 Contour plots of mortality risk for *Hyla versicolor* tadpoles in relation to their body size, proportion of time spent swimming and relative tail fin depth. The surfaces were fit by a Cox proportional hazards regression model that included predator species in addition to the three traits depicted here. Contours represent the change in hazard relative to an individual with values of zero for all traits, and points depict the fates of 385 individual tadpoles. The interaction between tail fin depth and swimming is significant (Wald $\chi^2 = 4.55$, $P = 0.0329$); that between body size and swimming is nearly significant (Wald $\chi^2 = 3.58$, $P = 0.0585$).

significant and both are strongly influenced by a relatively small number of tadpoles which swam frequently (Fig. 3). Both interactions showed a saddle-shaped hazard surface, indicating that opposite combinations of traits conferred maximal risk. Mortality risk was highest for large active tadpoles and small inactive tadpoles, and least for large inactive tadpoles (Fig. 3, left). Likewise, mortality risk of deep-tailed active tadpoles was relatively high, while that of shallow-finned active tadpoles was low (Fig. 3, right).

Finally, we asked whether the identity of the tadpole that was killed within a tub (predator-induced or no-predator) depended on phenotypic differences between the two. This method has the conceptual advantage of asking whether or not the predators chose prey based on their relative phenotypes, and it explicitly recognizes the potential statistical relationship between the fates of the two tadpoles in a tub. We performed logistic regressions on subsets of traits and all traits together, and found no significant effects of differences between traits on the identity of the unlucky tadpole. These results suggest that predators did not choose between tadpoles, but instead captured one tadpole or the other (or neither) on the basis of the individual's attributes.

We constructed similar models to explore the relationship between swimming performance and mortality risk. Maximum velocity and minimum time to swim 2.5 cm were never significant in any model, either alone or in combination with morphological traits.

Discussion

Studies of performance in ecologically realistic settings are crucial for understanding how selection acts and which traits directly influence fitness. Our results address this issue for *Hyla* tadpoles exposed to two kinds of predators, confirming some known relationships and casting doubt upon others. We have unambiguously identified predation as an agent that imposes selection on behaviour, and we have shown that behavioural and morphological traits can interact to influence predation risk.

Phenotypic effects on swimming

Size-specific swimming performance was significantly related to body and tail shape: long and shallow tadpoles swam relatively rapidly. These results are as predicted for tail length, but not for tail depth. Kinematic studies of tadpoles suggest that the long trailing tip acts as a foil to decrease turbulence or as ballast to dampen oscillations of the mid-tail (Wassersug & Hoff, 1985). Thus, *Hyla* tadpoles having relatively long tails may swim faster because they generate less turbulence, although turbulence is less important during sudden starts than during sustained swimming.

There are two reasons to expect a deep tail fin to enhance acceleration and turning ability. First, modelling and kinematic studies of both fish and tadpoles suggest that the large lateral surface area afforded by a deep tail

should improve swimming (Webb, 1984; Wassersug & Hoff, 1985; Wassersug, 1989; Weihs, 1989; Liu *et al.*, 1996). A tadpole achieves peak thrust when the tail tip reaches its maximal amplitude, strongly arched to one side or the other (Liu *et al.*, 1996). At this point the greatest amount of tail surface area is orientated properly for producing the pressure differences (positive on the outside of the tail and negative on the inside) that combine to generate thrust. A second line of evidence supporting the expectation that tail depth is positively related to swimming comes from selection studies, which find that amphibian larvae with relatively deep tail fins are effective at escaping predators (Van Buskirk *et al.*, 1997; Van Buskirk & Schmidt, 2000). It has been assumed that reduced vulnerability stems from enhanced acceleration during predator escape.

While modelling, kinematic and selection studies all suggest that the deep tail should be associated with rapid swimming, our results instead revealed a negative correlation between tail fin depth and swimming performance. This suggests that the large tail is involved in predator escape for reasons other than its influence on sudden acceleration. Tail depth could improve manoeuverability (Wassersug, 1989; Weihs, 1989), which is often involved in escaping predators (Weihs & Webb, 1984). Alternatively, large tails may have little positive effect on locomotion but may attract the attention of the predator, thereby deflecting attacks away from the body and onto the tail (Caldwell, 1982). Tadpoles captured by the tail are probably more likely to wriggle free than are those struck on the body itself, because tail tissue is easily torn (Doherty *et al.*, 1998). This explanation predicts that tadpoles exhibiting the predator-induced phenotype should suffer increased rates of tail damage, and this was confirmed in our experiment. We witnessed 27 cases of tail damage, indicating that a tadpole had sustained an attack but escaped; in 67% of these cases (18/27) the damaged tadpole was the one with the predator-induced phenotype ($P = 0.030$, one-tailed sign test). One interpretation of this result is that predator-induced *Hyla* experienced twice as many strikes on the tail from which they escaped, perhaps because of their large and brightly coloured tails. Still another explanation for plasticity in tail depth is that it evolved in response to indirect selection on some other trait, but is not adaptive in itself. This seems unlikely in part because plasticity in tail depth is widespread in amphibian larvae, and is generally more extensive than plasticity in other traits (Van Buskirk & McCollum, unpublished).

The data on swimming performance can be used to pose a series of testable predictions about the importance of variation in tail shape. If the correlations found in Table 2 reflect causation, manipulations of the length and depth of the tail should have opposite effects on acceleration: individuals with artificially shortened tails should accelerate slowly, those with reduced tail depth should accelerate more rapidly, while maximum speed should be relatively unaffected by changes in tail shape.

Phenotypic effects on predator escape

Which traits account for variation among tadpoles in mortality risk? Our results suggest that behavioural traits are important, and unknown morphological traits may be involved. The strong negative effect of time spent swimming on mortality hazard reflects a clear functional connection between movement and detection by predators: animals that move frequently are highly visible to predators and more likely to encounter them (Lima & Dill, 1990; Werner & Anholt, 1993). The activity treatment itself was unrelated to mortality risk because it primarily affected feeding behaviour. Feeding may be so inconspicuous that it does not increase tadpole visibility, or perhaps tadpoles stopped feeding as soon as the predator was introduced. We were able to detect an impact of swimming in spite of the nonsignificant activity treatment because the amount of time spent swimming was only weakly correlated with other behavioural and morphological traits (Table 1).

Tadpoles originating from tanks with caged predators faced a mortality risk only half as great as those from predator-free tanks. Because tadpoles from the two treatments differed dramatically in colour and several morphological traits, but not in behaviour, we attribute this difference in risk to the morphological manipulation. However, none of the shape variables significantly predicted risk of mortality, so we conclude that they are either not responsible for the survival advantage of the predator-induced phenotype, or they act only in combination to affect survival. The first hypothesis suggests that tadpoles developed unmeasured phenotypic differences during the long-term rearing treatments, most likely involving physiology or behaviour (other than activity). Although no data are presently available to assess this possibility, our informal observations suggest that tadpoles are shy and relatively difficult to catch when exposed to predators, quite apart from their swimming or feeding time budgets.

The second hypothesis to explain the lack of a relationship between morphological traits and predation risk is that these traits function properly only when they occur in particular combinations. If this is the case, univariate tests of single traits may not reveal functional relationships (Lande & Arnold, 1983; Brodie, 1992). Several of the shape and colour variables covary strongly (Table 1) and our study was not designed to disentangle them, so this hypothesis cannot be evaluated directly.

However, some of the traits that we manipulated interacted to influence mortality risk, suggesting a possible role for correlational selection. The relationship between tail fin depth and risk depended significantly on swimming activity: tail fin depth had no effect for inactive tadpoles, but deeper fins were associated with increased risk for tadpoles that spent much time swimming. Perhaps large tails become a liability for very active tadpoles, because they increase visibility to the predator

and thereby attract more attacks. The interaction between body size and time spent swimming, while not significant, could be interpreted in the same way: large body size serves as a partial refuge from predation for inactive tadpoles but increases the apparency of active tadpoles. In summary, these results confirm that traits interact to affect mortality risk; similar interactions may operate among the shape variables and explain why none is significant by itself.

These findings help clarify interpretations of the mechanistic basis of selection measured in previous studies. We documented prominent roles for swimming activity and morphology in determining vulnerability to predators, although isolated morphological traits were not associated with mortality risk. The activity results agree with several earlier studies suggesting that predators impose selection on tadpole behaviour (Azevedo-Ramos *et al.*, 1992; Werner & McPeek, 1994; Watkins, 1996). The morphology results cast doubt on a primary role for tail fin depth. Most anurans respond to predators by developing a deep tail, and tail depth (along with several other tail traits) is under increasing selection when dragonflies are present (Van Buskirk *et al.*, 1997), yet the present study showed that tail depth was not directly associated with predation risk. Our preferred explanations for this, mentioned above, are that co-linearity among traits may preclude an adequate test for effects of specific traits, and that a large and brightly coloured tail may attract a disproportionate number of strikes within small experimental tubs.

Specificity of responses

If different predators select for different phenotypes there can result a trade-off between susceptibility to one predator and susceptibility to another. We included two predator species in the experiment to test for such trade-offs. If different combinations of traits improved survival with *Anax* and *Ambystoma*, we would conclude that the appropriate response to one predator can only be produced at the expense of increased vulnerability to the second species. The existence of such trade-offs might impose constraints on plasticity, because specialized responses to different predators may be difficult to support if chemical alarm substances are produced by the tadpole rather than the predator (Wilson & Lefcort, 1993; Roedel & Linsenmair, 1997), and they may be costly if they involve the maintenance of separate receptors specifically for alarm substances from each predator species. Under these conditions a more likely outcome is evolutionary specialization to the most common predator (van Tienderen, 1991).

Instead of a trade-off, we found that the phenotype induced by exposure to *Anax* protects tadpoles against both *Anax* and *Ambystoma*. This implies that the induced response is a generalized rather than specific defence, and that ponds containing either *Anax* or *Ambystoma* may

represent essentially the same environment from a tadpole's perspective. This result is perhaps not surprising, even though the two predators consume prey in different ways. Both species hunt similarly, moving slowly through ponds, with frequent stops to watch for movement by potential prey. When a potential victim is encountered, *Ambystoma* rapidly engulfs and swallows prey whereas *Anax* grasps the prey with a protractile labium and uses its mandibles to tear off pieces of the prey. Presumably, traits that reduce encounter rates, improve a sudden escape response or redirect strikes toward the tail would defend effectively against both predators. According to this explanation, the generality or specificity of antipredator responses will depend more strongly on the predators' searching behaviour than their mode of prey capture.

Conclusions

Behaviour, colour and morphology are highly correlated in naturally occurring tadpoles. The strength of our approach is that it broke the correlation between behaviour and other traits, and permitted us to estimate the relative contributions of morphology, feeding and swimming to risk of mortality from predators. While these strong correlations present a methodological and statistical challenge, they also suggest an answer to one of the larger questions that motivates our work. Correlations among traits reflect the characters that occur together and presumably function together, as suggested by models for the evolution of pleiotropy and phenotypic integration (Endler, 1995; Cheverud, 1996; Wagner, 1996). Under some conditions, correlated selection acting on sets of functionally related traits may promote genetic correlations among those traits, and cause the suppression of pleiotropic effects between groups of traits that perform different functions (Cheverud, 1982, 1988a; Lande, 1984; Wagner, 1996). If the data on phenotypic correlations reflect underlying genetic correlations, which is unknown but not impossible (e.g. Cheverud, 1988b; Roff, 1995; Koots & Gibson, 1996), then our results may provide indirect evidence for functional integration of the correlated traits. The overall phenotype of a tadpole, characterized by its behaviour, colour, and the relative length and depth of its body and tail, *varies* as an integrated unit and may *function* as a unit as well. If this is the case, phenotypic patterns will reflect an underlying functional coherence imposed by correlational selection, and their functional interdependence will assure that they continue to evolve as an integrated unit (Wagner, 1996).

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