Predator-induced phenotypic plasticity in tadpoles: extension or innovation?

P. G. KRAFT, C. E. FRANKLIN & M. W. BLOWS

School of Integrative Biology, University of Queensland, Brisbane, Australia

Abstract

Phenotypic plasticity, the ability of a trait to change as a function of the environment, is central to many ideas in evolutionary biology. A special case of phenotypic plasticity observed in many organisms is mediated by their natural predators. Here, we used a predator–prey system of dragonfly larvae and tadpoles to determine if predator-mediated phenotypic plasticity provides a novel way of surviving in the presence of predators (an innovation) or if it represents a simple extension of the way noninduced tadpoles survive predation. Tadpoles of Limnodynastes peronii were raised in the presence and absence of predation, which then entered a survival experiment. Induced morphological traits, primarily tail height and tail muscle height, were found to be under selection, indicating that predator-mediated phenotypic plasticity may be adaptive. Although predator-induced animals survived better, the multivariate linear selection gradients were similar between the two tadpole groups, suggesting that predator-mediated phenotypic plasticity is an extension of existing survival strategies. In addition, nonlinear selection gradients indicated a cost of predator-induced plasticity that may limit the ability of phenotypic plasticity to enhance survival in the presence of predators.

Introduction

Phenotypic plasticity is an organism’s ability to modify its phenotype in response to an environmental change, and is a common mechanism among plants and animals that allows a high level of fitness to be maintained in a number of environments. The genetic basis of the relationship between plastic traits observed in different environments has been a primary focus of investigations into the evolution of phenotypic plasticity (Scheiner & Callahan, 1999). Genetic correlations have been used to investigate whether plastic traits, expressed in different environments, share the same genetic basis or whether they are genetically distinct from each other and are able to evolve independently (Falconer, 1952). Whether plasticity is a trait in itself (Schlichting & Levin, 1984, 1986; Schlichting, 1984; Scheiner & Lyman, 1989; Scheiner, 1993), or whether the same genes are involved in the expression of the trait in all environments (Lande & Arnold, 1985; Via & Lande, 1987; Van Tienderen, 1991; Gomulkiewicz & Kirkpatrick, 1992), remains controversial.

Although the extent of the shared genetic basis among environments for plastic traits is a key determinant of how plasticity may evolve, there has been less attention given to how selection on plastic phenotypes operates in different environments (Scheiner & Callahan, 1999). There are at least two important aspects of phenotypic plasticity that can be addressed by phenotypic analyses of selection on plastic phenotypes. Firstly, Scheiner & Callahan (1999) showed how selection for phenotypic plasticity itself could be directly measured. Selection on phenotypic plasticity may be measured by the difference in selection within each environment when individuals from the same families are grown in both environments. Secondly, rather than measuring the strength of selection on phenotypic plasticity directly, it is important to determine whether plastic responses represent novel solutions to the challenges posed by the exposure to multiple environments at the phenotypic level. That is, does phenotypic plasticity simply enhance how the organism would usually resist an environmental stress for example (a positive or negative extension of normal...
stress resistance), or does the plastic response represent an innovative solution to stress resistance that employs a different morphological, physiological, or biochemical (and ultimately genetic) set of traits? Genetic analysis can reveal if different genes contribute to the expression of the trait in different environments, therefore suggesting the plastic response is an innovation. A complementary view of an innovation at the phenotypic level can be defined by determining how the differential expression of the trait among environments interacts with fitness.

The formal analysis of selection (Lande & Arnold, 1983) provides a framework in which the comparison of how the differential expression of a trait among environments interacts with fitness can be achieved. Linear and nonlinear selection gradients can be used to quantify and visualize how traits affect fitness. Here, we show how selection gradients can be used to determine whether organisms use phenotypic plasticity as a means to deal with an alternate environment via a simple (positive or negative) shift in their phenotypic expression of the same trait (extension), or whether plasticity solves the challenge of maintaining fitness in alternate environments in a new (innovative) way. This can be accomplished by contrasting selection gradients that are obtained from a plastic trait expressed in different environments exposed to an identical bout of selection.

Tadpoles of many frog species possess the ability to change their shape, behaviour and/or coloration when exposed to a natural predator (Hews, 1988; Lawler, 1989; Skelly & Werner, 1990; Laurila et al., 1997; McCollum & Leimberger, 1997; Chivers et al., 1999; Lardner, 2000; Laurila, 2000; Van Buskirk & Ripple, 2000; Relyea, 2001). In general, tail height increases and overall activity is drastically reduced in the presence of dragonfly larvae that typically prey upon tadpoles. Although tail modification was initially associated with an increase in escape performance (McCollum & Leimberger, 1997; Van Buskirk et al., 1997), the larger tail in predator-induced tadpoles may also serve as a deflection, drawing the lethal attention away from the more vulnerable head-body (Hoff & Wassersug, 2000; Van Buskirk et al., 2003). The predator-induced change in phenotype in tadpoles has become a model system for the study of phenotypic plasticity. It has been used to test several models and hypotheses about plasticity such as the prerequisites for the evolution of predator-induced phenotypic plasticity (Tollrian & Harvell, 1999).

This study employed tadpoles of the striped marsh frog, *Limnodonastes peronii*, to test whether the observed predator-induced traits are based on already existing, noninduced, tadpole morphology (positive or negative extension), or whether they are based on a morphological innovation. We exposed the tadpoles to their natural predator, a dragonfly larva (*Aeshna* spp.), and after rearing them in two different environments (predator/no predator) a survival experiment allowed a selection analysis for each group of tadpoles to be performed.

If phenotypic plasticity is an innovation, it may utilize a novel phenotype to increase its fitness in the environment it is expressed in. Consequently, we would expect that the induced traits of predator-exposed tadpoles are associated with fitness in a qualitatively different manner than noninduced forms. In other words, the way morphology influences survival (i.e. the selection gradients) would be significantly different in predator-exposed and nonexposed tadpoles. For example, if directional selection operates on different traits in induced and noninduced phenotypes, linear selection gradients will not be parallel. Similarly, if nonlinear selection differs among the two groups, nonlinear selection gradients will differ in curvature. To answer this question, we compare the linear and nonlinear selection gradients between predator-induced and noninduced tadpoles to determine if survival is related to morphology in the same way in induced and noninduced tadpoles.

**Methods**

**Animal collection and husbandry**

Ten freshly laid striped marsh frog (*L. peronii*) clutches (foam nests) were collected from a wetland near Brisbane, Australia (152°E, 27.5°S). To ensure that each clutch had a set of unique parents, clutches separated by >100 m were collected from a large pond over a 30-min period. After the foam nests were transported back to the laboratory, each nest was put into a black plastic tank (80 × 30 × 30 cm), containing 20 L of aged water with the base covered by a 1 cm layer of coarse pebbles. Sixty-four tadpoles from each of the ten tanks were taken at random 24 h post-hatching and split into two groups of 32 animals each (predator-exposed/nonexposed). Each animal was placed into its own rearing habitat, which consisted of a plastic container (diameter 10 cm × height 25 cm) filled with a 3-cm layer of gravel and approximately 800 mL of aged pond water. Tadpoles were collectively exposed to 12 : 12 photoperiod and a constant temperature of 24 °C.

Each day containers with nonexposed tadpoles received 15 mL of normal pond water, whereas predator-exposed animals received the same amount of ‘predatory pond water’. ‘Predatory pond water’ was obtained from 40 L tanks in which 15 dragonfly larvae (*Anax* spp.) were feeding on *L. peronii* conspecific tadpoles producing a concentration of about 0.1 g of tadpole tissue per litre of pond water. Nymphs of the dragonfly, *Aeshna brevistyla*, were used as predators and were collected from ponds that typically contained larval *L. peronii*.

To avoid pseudo replication, tadpoles were divided into four different blocks (80 control and 80 predator-exposed animals per block) whereby tadpoles of each block received water (predatory/normal) from one of four independent predation/pond tanks, respectively. Tadpoles were fed approx. 0.05 g of spinach every
48 h. To compensate for the daily (predatory/normal pond) water addition, about 60 mL of water was extracted every 4 days from each tadpole container, thereby keeping the amount of water in each rearing container constant.

Predation trials

On day 20 of larval development, all animals were arranged for a predation trial, which was structured so that 20 tadpoles of each group (per block) were placed together into an arena consisting of a black plastic tank (80 x 30 x 30 cm), containing 40 L of aged water with the base covered with a 2-cm layer of fine pebbles. Before entering the arena, each of the 40 tadpoles per trial was photographed. Each tadpole was photographed twice (dorsal and lateral view) and nine linear measurements (total length, total tail height, tail muscle height, body length, body height, total tail area, tail muscle area, body width, tail muscle width) were taken using the software VIDEO TRACE (Leading Edge Pty. Ltd, Marion, South Australia). Four dragonfly larvae (A. brevistyla), which had been starved for 3 days prior to the trials, were then added to the arena. After the nymphs had preyed in the tanks freely over a period of 24–36 h, during which they reduced the tadpole population by approx. 50%, the surviving frog larvae were removed from the arenas and identified.

Tadpole marking

The design of the predation trials required the identification of 40 tadpoles within one tank. Each individual tadpole received a unique series of very small cuts to the fin perpendicular to the tail muscle. To test whether the cuts would affect tadpole survival when preyed upon by dragon fly larvae, 120 20-day old L. peronii larvae were chosen at random. The animals were split into three equal groups of 40 individuals for predation trials; half of the tadpoles in each trial received cuts to their fins with micro scissors. Each of the remaining 20 control animals were handled for the same amount of time as those receiving cuts, but without cutting their fins. After allowing recovery for 1 h, predation trials were conducted as above. A logistic analysis indicated that marking of the fins did not affect survival (Mantel–Haenszel $\chi^2 = 0.5239, P = 0.469$), permitting the use of this marking method for the predation trials.

Fitness surface estimation

All morphological measurements were log-transformed, and because of the relatively high correlation among the original traits, a principal component analysis (PCA; using the covariance matrix) on both treatment groups combined was performed. The PCA provided a new set of standardized uncorrelated morphological variables, ideal for calculating multivariate selection gradients (Lande & Arnold, 1983).

Linear selection gradients ($\beta$) were calculated for each treatment group separately using multiple regression. Estimates of selection gradients are not affected by the distribution of the (binomial) fitness measure (Brodie & Janzen, 1996), however significance testing of the gradients may be biased. With our data, the binomial distribution could be approximated by a normal distribution, as the sample size was large ($n = 560$) and frequencies were approximately equal, allowing the use of parametric tests. A separate quadratic regression was used to obtain nonlinear selection gradients ($\gamma$) (Lande & Arnold, 1983).

Fitness surface visualization

Although separate estimation of the fitness surfaces in each treatment group allowed direct tests of the differences in selection between the two groups, it is also informative to estimate a combined fitness surface to allow a visualization of how the two groups differed in the way morphology was associated with fitness. We, therefore, conducted a quadratic regression on the combined data. We then tested the presence of significant convex and concave selection along the major axes of the response surface after performing a canonical analysis of the $\gamma$ matrix (Phillips & Arnold, 1989). The resulting eigenvectors ($m_1$) that denoted the major axes constituted the $M$ matrix, which indicated how the original traits (PCs) contributed to the major axes of the response surface. To determine the significance of the quadratic terms in the model, new variables were created from the eigenvectors of $\gamma$ and a second quadratic regression was performed (Blows & Brooks, 2003).

To visualize the fitness surface, we employed a multivariate generalization of the cubic spline, the thin plate spline (Green & Silverman, 1994), as performed by the ‘TPSPLINE’ procedure in sas®. This method allows fitness values to be predicted without being constrained to a particular polynomial form (Blows et al., 2003). The eigenvectors of the response surface that presented the most curvature were identified by their absolute size of their associated eigenvalues ($\lambda_i$) from the canonical rotation. A thin-plate spline was applied to the two major axes identified by the diagonalization of the $\gamma$ matrix ($m_1$ and $m_3$). The lambda value that minimized the generalized cross-validation (GCV) score was taken as the value of the smoothing parameter. A surface and contour plot were generated by importing $18 \times 18$ grids of predicted values from the thin-plate splines into ‘R’ (The R Development Core Team®) for plotting. Predicted values of all individual values were placed on both plots, which highlighted the number of individuals that supported each feature of the surface.
Comparison of fitness surfaces of predator-exposed and nonexposed tadpoles

We used a sequential model building approach based on the employment of a dummy variable to determine if selection gradients differed between the two treatments (Draper & John, 1988), an approach which has been presented in detail with regard to its application to fitness surfaces in Chenoweth & Blows (2005). Briefly, the interaction between the dummy variable of treatment and each of the traits (PCs in this instance) was determined to test three hypotheses (Chenoweth & Blows, 2005); (1) the two fitness surfaces do not differ in linear selection; (2) the two fitness surfaces share the same curvature along the axes of the original traits (quadratic selection); (3) the two fitness surfaces share the same curvature along axes not parallel to the original traits (correlational selection). In each case, the complete model that includes terms for the interaction between the dummy variable and the traits was compared with the reduced model that did not have interaction terms of interest using a partial test (Chenoweth & Blows, 2005).

Results

Induction and survival

The PCA matrix of trait loading (Table 1) shows how the original traits contribute to the new PCs. PC1 consists exclusively of high positive loadings from each original variable (0.92–0.98) and can therefore be considered a measure of size, while the remaining PCs can be interpreted as various representations of shape morphology. Phenotypic means for control and induced tadpoles are displayed in Fig. 1. When compared with their nonexposed counterparts, predator-exposed tadpoles of L. peronii differed significantly in their morphology. Examination of univariate nested ANOVA indicated that exposed animals possessed deeper tails with wider tail muscles (PC4; $F_{1,6.01} = 7.49; P = < 0.05$) and deeper tail muscles, with a narrower body and tail muscle (PC5; $F_{1,6.02} = 11.98; P < 0.05$), but did not differ from nonexposed tadpoles in either overall size (PC1) or head-body height (PC2).

When both groups were preyed upon by A. brevistyla simultaneously, 63% of predator-exposed tadpoles survived predation, compared with only 38% of nonexposed tadpoles. A logistic model, which included block, treatment, survival and the interaction between treatment and survival, was employed to test whether tadpoles that were exposed to predator water survived better than nonexposed animals in the predation trials. The increase in survival of predator exposed tadpoles was significant as indicated by the treatment by survival interaction term ($\chi^2 = 39.41$, d.f. = 1, $P < 0.001$).

Table 1 Principal components analysis of the nine linear morphological measurements.

<table>
<thead>
<tr>
<th>Principal component</th>
<th>PC 1</th>
<th>PC 2</th>
<th>PC 3</th>
<th>PC 4</th>
<th>PC 5</th>
<th>PC 6</th>
<th>PC 7</th>
<th>PC 8</th>
<th>PC 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of overall variance</td>
<td>91.4</td>
<td>3.1</td>
<td>1.7</td>
<td>1.3</td>
<td>0.9</td>
<td>0.6</td>
<td>0.5</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Total length</td>
<td>0.975</td>
<td>-0.093</td>
<td>-0.079</td>
<td>-0.144</td>
<td>-0.049</td>
<td>0.027</td>
<td>-0.001</td>
<td>-0.001</td>
<td>-0.085</td>
</tr>
<tr>
<td>Tail height</td>
<td>0.943</td>
<td>0.135</td>
<td>-0.210</td>
<td>0.210</td>
<td>-0.002</td>
<td>-0.031</td>
<td>-0.041</td>
<td>0.023</td>
<td>0.0297</td>
</tr>
<tr>
<td>Tail muscle height</td>
<td>0.952</td>
<td>-0.162</td>
<td>0.102</td>
<td>0.046</td>
<td>0.222</td>
<td>-0.065</td>
<td>-0.021</td>
<td>-0.031</td>
<td>-4.6 E-05</td>
</tr>
<tr>
<td>Body length</td>
<td>0.970</td>
<td>0.110</td>
<td>0.043</td>
<td>-0.121</td>
<td>-0.006</td>
<td>0.034</td>
<td>-0.167</td>
<td>0.039</td>
<td>-0.017</td>
</tr>
<tr>
<td>Body height</td>
<td>0.920</td>
<td>0.353</td>
<td>0.088</td>
<td>-0.012</td>
<td>0.071</td>
<td>0.093</td>
<td>0.091</td>
<td>-0.010</td>
<td>7.4 E-04</td>
</tr>
<tr>
<td>Tail area</td>
<td>0.977</td>
<td>-0.053</td>
<td>-0.182</td>
<td>-0.013</td>
<td>-0.024</td>
<td>0.001</td>
<td>0.032</td>
<td>-0.049</td>
<td>-0.070</td>
</tr>
<tr>
<td>Tail muscle area</td>
<td>0.965</td>
<td>-0.191</td>
<td>-0.069</td>
<td>-0.090</td>
<td>0.020</td>
<td>0.022</td>
<td>0.079</td>
<td>0.113</td>
<td>0.009</td>
</tr>
<tr>
<td>Body width</td>
<td>0.962</td>
<td>0.105</td>
<td>0.134</td>
<td>-0.030</td>
<td>-0.115</td>
<td>-0.171</td>
<td>0.039</td>
<td>0.007</td>
<td>-0.002</td>
</tr>
<tr>
<td>Tail muscle width</td>
<td>0.939</td>
<td>-0.192</td>
<td>0.182</td>
<td>0.164</td>
<td>-0.114</td>
<td>0.095</td>
<td>-0.006</td>
<td>-0.008</td>
<td>-0.004</td>
</tr>
</tbody>
</table>

Fig. 1 Means of the nine morphological principal components for induced and control tadpoles. Control tadpoles closed squares, closed circles induced tadpoles. Error bars are 95% confidence intervals.
Fitness surface estimation

In nonexposed tadpoles, directional selection was significant for PC2, PC4 and PC5 and significant quadratic selection was detected on PC2 (Table 2). Tadpoles with smaller head-body height (PC2) tended to survive predatory attacks better. Both, tail height and tail muscle height, were positively associated with survival. Nonexposed animals also displayed a significant convex selection gradient with respect to head-body height, indicating that tadpoles with very shallow and very deep head-bodies survive well. No correlational selection was found in nonexposed tadpoles (Table 2). The morphological traits that were under significant directional selection in predator-exposed tadpoles were very similar in direction and strength when compared with gradients of nonexposed animals. In contrast to nonexposed tadpoles, predator-exposed tadpoles exhibited no significant quadratic gradients, but instead displayed a significant correlational gradient between the two traits most affected by the induction, PC4 and PC5 (Table 2).

The normalized eigenvectors ($m_i$) of the response surface and their associated eigenvalues ($\lambda_i$) with respect to the PCs are shown in Table 3. Diagonalization of the $\gamma$ matrix revealed the two eigenvectors with the largest eigenvalues of the response surface ($m_1 = 0.05$, concave selection; $m_9 = -0.07$, convex selection), both displayed significant quadratic selection. As both positive and negative eigenvalues are associated with this surface, significant quadratic selection. As both positive and negative eigenvalues are associated with this surface, no stationary optimum existed, and the surface is described by a saddle. The loadings of the $M$ matrix (Table 3) help interpret how the PCs (and hence the original traits) contribute to the major axes of the response surface. The main traits contributing to $m_1$ are PC9 (contrasting total length and tail area), PC7 (contrasting body length with body height) and PC5 (tail muscle height contrasted with body and tail muscle width). The main traits contributing to $m_9$ were PC9 (contrasting total length and tail area) and PC5 (tail muscle height contrasted with body and tail muscle width).

Fitness surface visualization

Figure 2 depicts the fitness surface and its associated contour plot, defined by the two significant major axes of nonlinear selection ($m_1$; $m_9$) and their corresponding survival (fitness). In relation to the axis $m_1$, the surface follows a concave shape with a plateau towards positive values of $m_1$, indicating that, in general, tadpoles with larger tail muscle height survive better than tadpoles with intermediate values of the same trait. Along the major axis of $m_9$, the surface displays an asymmetrical convex shape: animals with intermediate to high values in tail height and tail muscle height survive better in the presence of predators than tadpoles with small values of these two traits.

Comparison of fitness surfaces of induced and noninduced tadpoles

When constrained to a linear gradient, tadpole morphology relates to survival similarly in the two tadpole groups. In other words, sequential model fitting and

Table 2  Vector of standardized directional selection gradients ($b_i$), and the matrix of standardized quadratic ($\gamma_{ik}$) and correlational selection gradients ($\gamma_{ik}$).

<table>
<thead>
<tr>
<th>$b_i$</th>
<th>PC 1</th>
<th>PC 2</th>
<th>PC 3</th>
<th>PC 4</th>
<th>PC 5</th>
<th>PC 6</th>
<th>PC 7</th>
<th>PC 8</th>
<th>PC 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC 1</td>
<td>-0.016</td>
<td>-0.012</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC 2</td>
<td>-0.069*</td>
<td>0.042</td>
<td>-0.046*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC 3</td>
<td>0.021</td>
<td>-0.044</td>
<td>-0.002</td>
<td>0.027</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC 4</td>
<td>0.105**</td>
<td>-0.02</td>
<td>0.022</td>
<td>-0.002</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC 5</td>
<td>0.144**</td>
<td>-0.035</td>
<td>-0.067</td>
<td>-0.005</td>
<td>0.024</td>
<td>0.019</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC 6</td>
<td>-0.007</td>
<td>-0.035</td>
<td>0.022</td>
<td>-0.018</td>
<td>0.016</td>
<td>0.007</td>
<td>-0.020</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC 7</td>
<td>0.021</td>
<td>0.030</td>
<td>0.011</td>
<td>0.019</td>
<td>0.010</td>
<td>0.006</td>
<td>0.055</td>
<td>-0.004</td>
<td></td>
</tr>
<tr>
<td>PC 8</td>
<td>0.031</td>
<td>-0.005</td>
<td>0.021</td>
<td>-0.011</td>
<td>0.017</td>
<td>-0.005</td>
<td>-0.011</td>
<td>0.008</td>
<td>0.009</td>
</tr>
<tr>
<td>PC 9</td>
<td>-0.009</td>
<td>0.015</td>
<td>0.011</td>
<td>-0.016</td>
<td>-0.060</td>
<td>-0.080</td>
<td>-0.067</td>
<td>-0.064</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Separate regressions were utilized to estimate the linear and quadratic selection gradients. *$P < 0.05$; **$P < 0.005$. 

partial $F$-tests, which were applied to investigate the differences in the response surface between the nonexposed and predator-exposed tadpoles, showed that both surfaces were parallel to each other ($\text{Partial } F$-test; $F_{9,55} = 0.57; P = 0.818$). Although the quadratic selection gradient in nonexposed tadpoles was significant, quadratic selection along individual traits (convex/concave selection) exhibited no difference between the two planes ($\text{Partial } F$-test; $F_{9,64} = 0.979; P = 0.466$). However, the off-diagonal curvature (correlational selection) differed significantly ($\text{Partial } F$-test; $F_{18,73} = 2.55; P < 0.01$) between the two treatments. The difference in correlational selection suggests that individual traits interacted in a different manner between the tadpole groups to facilitate the survival of predatory attacks by dragonfly larvae.

To explore the difference in correlational selection further, we examined the form of selection along $m_9$ (Fig. 3). The positive shift in the mean of $m_9$ resulting from predator exposure produces the increased rate of mean survival. When the underlying distribution of $m_9$ in both treatment groups is considered, only a small fraction of nonexposed tadpoles (but the majority of exposed animals) show optimum survival. Furthermore, maximum values of $m_9$, which were almost entirely represented by predator-exposed tadpoles, displayed a decreased chance of survival (broken line). This suggests that tadpoles can display an induced morphology that is too extreme, and begin to be more susceptible again to predation.

**Discussion**

Tadpoles of *L. peronii*, like many other anuran species (Lawler, 1989; McCollum & Leimberger, 1997; Chivers et al., 1999; Lardner, 2000; Van Buskirk & Ripple, 2000; Relyea, 2001), exhibit morphological phenotypic plasticity in the presence of predators. In total, traits associated

---

**Table 3** The $M$ matrix showing the eigenvectors from the canonical analysis of $\gamma$. The first column represents the eigenvalues ($\lambda_i$) of each eigenvector ($m_i$); *$P < 0.05$.

<table>
<thead>
<tr>
<th>$\lambda_i$</th>
<th>PC 1</th>
<th>PC 2</th>
<th>PC 3</th>
<th>PC 4</th>
<th>PC 5</th>
<th>PC 6</th>
<th>PC 7</th>
<th>PC 8</th>
<th>PC 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m_1$</td>
<td>0.050*</td>
<td>-0.150</td>
<td>-0.059</td>
<td>-0.201</td>
<td>0.095</td>
<td>0.359</td>
<td>0.330</td>
<td>0.603</td>
<td>0.029</td>
</tr>
<tr>
<td>$m_2$</td>
<td>0.028</td>
<td>0.406</td>
<td>0.236</td>
<td>-0.847</td>
<td>-0.010</td>
<td>-0.338</td>
<td>0.193</td>
<td>0.143</td>
<td>0.404</td>
</tr>
<tr>
<td>$m_3$</td>
<td>0.017</td>
<td>-0.047</td>
<td>0.049</td>
<td>0.237</td>
<td>0.831</td>
<td>-0.309</td>
<td>0.192</td>
<td>-0.178</td>
<td>0.212</td>
</tr>
<tr>
<td>$m_4$</td>
<td>0.011</td>
<td>-0.297</td>
<td>-0.075</td>
<td>0.448</td>
<td>-0.279</td>
<td>-0.425</td>
<td>0.053</td>
<td>0.475</td>
<td>0.435</td>
</tr>
<tr>
<td>$m_5$</td>
<td>0.010</td>
<td>0.535</td>
<td>0.209</td>
<td>0.282</td>
<td>0.040</td>
<td>-0.312</td>
<td>-0.014</td>
<td>0.397</td>
<td>-0.574</td>
</tr>
<tr>
<td>$m_6$</td>
<td>-0.008</td>
<td>0.122</td>
<td>0.695</td>
<td>0.345</td>
<td>-0.256</td>
<td>0.264</td>
<td>0.351</td>
<td>-0.241</td>
<td>0.216</td>
</tr>
<tr>
<td>$m_7$</td>
<td>-0.026</td>
<td>-0.387</td>
<td>0.042</td>
<td>-0.135</td>
<td>0.005</td>
<td>-0.129</td>
<td>0.684</td>
<td>-0.041</td>
<td>-0.434</td>
</tr>
<tr>
<td>$m_8$</td>
<td>-0.031</td>
<td>-0.431</td>
<td>0.625</td>
<td>-0.171</td>
<td>0.244</td>
<td>0.057</td>
<td>-0.465</td>
<td>0.283</td>
<td>-0.102</td>
</tr>
<tr>
<td>$m_9$</td>
<td>-0.074*</td>
<td>0.295</td>
<td>-0.119</td>
<td>0.197</td>
<td>0.309</td>
<td>0.543</td>
<td>0.088</td>
<td>0.269</td>
<td>0.166</td>
</tr>
</tbody>
</table>

**Fig. 2** Fitness surface (a) based on the major response surface axes $m_1$ and $m_9$. The fitness values have been generated using the thin-plate spline procedure. The topographic contour plot (b) provides an unobstructed view from above onto the surface. Both plots contain the original predicted points of each tadpole that were used to calculate the entire surface (open circles: nonexposed tadpoles; closed circles: predator-exposed animals).
with only 2.2% of the phenotypic variance in our linear morphological measurements displayed phenotypic plasticity. Although the inducible morphological changes observed in tadpoles of *L. peronii* in the presence of predators were relatively minor, they play a significant role in surviving predation by dragonfly larvae. When predator exposed and nonexposed tadpoles were simultaneously preyed upon by *A. brevistyla*, 25% more predator-exposed tadpoles survived predation than nonexposed tadpoles.

Changes in tail shape and tail muscle shape of *L. peronii* larvae were most affected by the presence of predators, whereas overall body size did not display any phenotypic plasticity. Crucially, the traits that displayed the most phenotypic plasticity were those that were under selection when tadpoles were preyed upon by dragonfly larvae. Linear selection gradients of morphological traits were found to be of the same direction and similar strength on the inducible traits in predator exposed and nonexposed tadpoles. These results suggest that tadpoles, whether predator-exposed or not, use their tail height and tail muscle height in a similar way to evade attacks by larval dragonflies. Thus, certain morphological traits important to the survival of induced tadpoles appear to be also responsible for the survival of noninduced animals, indicating that the observed phenotypic plasticity is an extension of the noninduced phenotype. That is, the inducible morphological defence of the tadpoles simply enhances the way they survive attacks by dragonfly larvae under nonexposed conditions.

*Limnodynastes peronii* larvae with deeper tails experienced an increased rate of survival, which has also been reported for a number of other species of tadpoles (Fauth et al., 1996; McCollum & Leimberger, 1997; Lardner, 2000; Van Buskirk & McCollum, 2000b). Several studies have investigated the functional role of the tadpole tail. Tail shape (Hoff & Wassersug, 2000), but not tail size (Van Buskirk & McCollum, 2000a) may affect swimming performance in tadpoles, suggesting that enhanced swimming performance may be one mechanism by which a change in the tail shape seen in *L. peronii* may result in higher survival. However, an alternative functional consequence of the deeper tails found in predator-induced animals may be to attract predatory strikes away from the more vulnerable head-body region (Caldwell, 1982; Doherty et al., 1998). This notion is supported by an experiment that examined the frequency and target of predatory strikes on tadpoles of *Rana temporaria*, in which a greater proportion of strikes were aimed at the head-body area of the prey (Van Buskirk et al., 2003). Functional studies are required to determine how changes in tail shape affect survival in the presence of dragonfly larvae. In addition, our study cannot rule out an important contribution from induced behavioural changes that may also contribute to survival.

Although no difference was detected in linear selection among predator-exposed and nonexposed tadpoles, correlational selection did differ between the two groups. In contrast to nonexposed tadpoles, predator-exposed animals displayed a significant correlational selection gradient. Importantly, the correlational selection gradients appeared between the two traits that were induced in the presence of the predator. Tadpoles that survive predator attacks share a particular combination of the two traits in question, such that an increase (or decrease) in tail height is always associated with a decrease (or increase

---

Fig. 3 Graph showing the selection gradient of the combined treatment groups based on the nonconstrained regression (spline) of $m_9$ and survival. Broken line of the selection gradient displays decrease in survival for increases in $m_9$.  

**Morphology ($m_9$)**

\[
\begin{align*}
\bar{f}_{\text{Induced}} &= 0.54 \\
\bar{f}_{\text{Control}} &= 0.46 \\
\Delta m_9 &= -0.187 \\
\bar{m}_{\text{Induced}} &= 0.196 \\
\bar{m}_{\text{Control}} &= -0.187
\end{align*}
\]

For example, such that an increase (or decrease) in tail height is always associated with a decrease (or increase in $m_9$).
respectively) in tail muscle height. Thus, a predator-induced tadpole that possesses a deep tail and a deep tail muscle (or vice versa) will have a reduced chance of survival.

Although one of the key questions in the evolution of predator-induced phenotypic plasticity has been what costs the plastic phenotype may exhibit in the absence of predators (Skelly & Werner, 1990; Smith & Van Buskirk, 1995; McCollum & Van Buskirk, 1996; Relyea & Werner, 1999; Relyea, 2002), little attention has been given costs of the inducible defence in the presence of predators. The negative correlational selection gradient between tail height (PC4) and tail muscle height (PC5) suggests a limit to the benefits of the inducible morphological defence in this species. One consequence of the positive shift in the mean of $m_p$ traits (see Fig. 2) is that extreme values of induced tadpoles are ‘pushed’ over the maximum and placed on the downward part of the convex selection gradient, thereby experiencing a lower survival rate. This indicates that the response to predation may be costly because of potential ‘over-induction’. Without an understanding of the functional consequences of the change in tail shape seen here, it is not possible to determine why such a limit may exist. For example, if swimming performance is enhanced by the change in tail shape, the animals may experience deterioration of escape performance because of increased viscosity and the associated drag on the tail as the tail reaches a certain extreme shape.

In conclusion, predator-induced morphological plasticity in $L$. peronii simply enlarges the morphological characteristics that noninduced tadpoles use to survive predation. Not only did our analysis of linear selection gradients allow us to determine that the induction was an extension of how morphology influences survival in noninduced tadpoles, but also the analysis of nonlinear selection suggested that there is a limit to the extent to which induced morphological changes can enhance survival. The presence of fitness costs (in environments without predators) to induced plasticity is often of interest in studies of the evolution of phenotypic plasticity, and determining why the induced morphology in this tadpole is not present in the absence of predators will be an important next step in understanding predator-induced plasticity in this system. In addition, determining how the induced morphological phenotype enhances survival, either by increasing performance or by acting as a shield, would contribute to a more complete understanding of the evolution of phenotypic plasticity in anuran tadpoles.

References


Received 3 April 2005; revised 3 August 2005; accepted 3 August 2005