

The heritability of inducible defenses in tadpoles

R. A. RELYEA

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA

Keywords:

ecological genetics;
genetic variation;
inducible defenses;
quantitative genetics.

Abstract

The evolution of plastic traits requires phenotypic trade-offs and heritable traits, yet the latter requirement has received little attention, especially for predator-induced traits. Using a half-sib design, I examined the narrow-sense heritability of predator-induced behaviour, morphology, and life history in larval wood frogs (*Rana sylvatica*). Many of the traits had significant additive genetic variation in predator (caged *Anax longipes*) and no-predator environments. Whereas most traits had moderate to high heritability across environments, tail depth exhibited high heritability with predators but low heritability without predators. In addition, several traits had significant heritability for plasticity, suggesting a potential for selection to act on plasticity *per se*. Genetic correlations confirmed known phenotypic relationships across environments and identified novel relationships within each environment. This appears to be the first investigation of narrow-sense heritabilities for predator-induced traits and confirms that inducible traits previously shown to be under selection also have a genetic basis and should be capable of exhibiting evolutionary responses.

Introduction

Being able to exhibit alternative phenotypes offers an individual the opportunity to optimize their fitness as they experience different environments over time and space. For the past century, ecologists and evolutionary biologists have documented the widespread occurrence of phenotypic plasticity, from protists and bacteria to plants and animals (Clausen *et al.*, 1941; Bradshaw, 1965; Harvell, 1990; Pigliucci, 2001). During this time, we have made outstanding progress in identifying the environmental cues that cause induction, the range of traits and trait types altered by different environments, and the impact of combined environmental factors on the magnitude of induction (Karban & Baldwin, 1997; Schlichting & Pigliucci, 1998; Tollrian & Harvell, 1999; Pigliucci, 2001; West-Eberhard, 2003).

From an evolutionary perspective, there has been a recent emphasis on documenting the costs and benefits associated with phenotypic plasticity. Although we cannot identify the original evolutionary cause of plasticity,

we can determine whether phenotypic plasticity is currently being maintained by divergent selection in different environments (Dudley & Schmitt, 1996; Van Buskirk & Relyea, 1998). However, for evolutionary responses to this divergent selection, the traits under consideration must have a heritable basis. The heritability of traits have been widely examined across taxa, with the general result that morphological and behavioural traits tend to have moderate to high heritabilities ($h^2 > 0.3$) while life history traits tend to have low heritabilities ($h^2 < 0.3$), either because life history traits are closely-related to reproductive fitness or because environmental contributions to phenotypic variation are high for life history traits (Roff & Mousseau, 1987; Price & Schluter, 1991). In contrast, the heritability of phenotypically plastic traits has received considerably less attention, with much of the existing work being carried out in model systems including *Drosophila* responses to different temperature environments and *Arabidopsis* responses to different light environments (reviewed in Roff, 1997). These studies have shown that most traits have a heritable basis within a given environment and the genetic variance-covariance matrix (the G-matrix) can differ substantially among environments (Stearns *et al.*, 1991), violating a basic (but simplifying) assumption of many theoretical models of

Correspondence: Rick A. Relyea, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA.
Tel.: (412) 624-4656; fax: (412) 624-4759;
e-mail: relyea@pitt.edu

plasticity evolution (Via & Lande, 1985, 1987). Moreover, the plasticity of the traits (the change in traits across environments) can also be heritable (Scheiner & Lyman, 1989), offering the possibility for selection on traits *and* trait plasticities. While existing data on the heritability of plastic traits has been insightful, work has been restricted to a relatively small set of taxa and relatively few environmental conditions (Roff, 1997).

Predator-induced plasticity provides an excellent case in point. In both plant and animal systems, we have amassed a tremendous quantity of literature on anti-herbivore and anti-predator defenses. We know how a wide variety of plants and animals respond to predators, the environmental cues that cause the induced defenses, and the costs and benefits of being induced (Karban & Baldwin, 1997; Tollrian & Harvell, 1999). However, we have few data on the genetic variation (and covariation) of the defensive traits. In plants, there are several studies documenting genetic variation for resistance to herbivory (James & Newcombe, 2000; Jones *et al.*, 2002; Fornoni *et al.*, 2003) and at least two studies that estimate the heritability of particular herbivore-induced defenses in plants (broad-sense heritability; Nicholsons *et al.*, 1993; narrow-sense heritability; Agrawal *et al.*, 2002). In animals, there appears to be a paucity of heritability studies, these being restricted to marine bryozoans (Cheetham *et al.*, 1995; Harvell, 1998) and *Daphnia* (Parejko & Dodson, 1991; Spitze, 1992). These animal studies offer valuable insights into the potential genetic underpinnings of predator-induced plasticity, but because bryozoans and *Daphnia* are clonal, the studies can provide only broad-sense heritability estimates (which can include dominance and maternal effects). Ideally, one would like to estimate the narrow-sense heritability (which contains only additive genetic variation) to examine potential signals of past selection and predict outcomes of future selection.

To investigate the heritability of inducible defenses, one would prefer to work with an organism that has well-documented predator-induced defenses and can also be bred in half-sib designs to provide powerful estimates of additive genetic variation. Larval anurans (tadpoles) are excellent candidates that fit these two criteria. Tadpoles are becoming well known for their inducible defenses which typically include a reduction in foraging activity, an increase in relative tail size, and a decrease in relative body size (Smith & Van Buskirk, 1995; McCollum & Van Buskirk, 1996; Relyea & Werner, 1999; Relyea, 2000, 2001a, 2002b, 2004). These phenotypic changes make it harder for predators to detect and capture tadpoles (Van Buskirk *et al.*, 1997; Van Buskirk & Relyea, 1998), but they come at the cost of slower growth and development in no-predator environments (Relyea, 2002a; Van Buskirk, 2002). In addition, many anurans are explosive breeders that come into ponds and deposit all of their eggs within a few days, allowing researchers to

collect large numbers of males and females and breed them in artificial, half-sib crosses (Berven, 1987; Emerson *et al.*, 1988; Newman, 1988; Laurila *et al.*, 2002). Hence, amphibians represent an excellent system for investigating the heritability of predator-induced traits.

In this study, I examined the heritability of predator-induced traits in larval wood frogs (*Rana sylvatica*). Wood frogs possess well-documented anti-predator defenses, they are explosive spring breeders, and they have rapid growth (Relyea, 2000, 2001a, 2003a, 2004). Collectively, these attributes enable highly replicated studies. To assess the heritability of traits and trait plasticities, I conducted artificial crosses of adults, reared their progeny in predator and no-predator environments, and then quantified tadpole behaviour (activity), morphology (body and tail shape), and life history (mass and development). This study was part of a larger experiment in which I previously quantified the costs of plasticity *per se* by regressing half-sib family means against tadpole fitness (Relyea, 2002b) to determine if the most plastic sibships experienced a fitness cost. In the current study, I used full-sib family data from the same parental crosses to examine the genetic basis of the phenotypic changes and test the following hypotheses: (1) there is heritable variation in the traits within each environment; (2) there is heritable variation in the plasticity of the traits; (3) within each environment, heritability will be high for behavioural traits, moderate for morphological traits, and low for life history traits.

Methods

To determine the heritability of tadpole traits, I collected adult wood frogs and conducted controlled crosses. Wood frogs can be effectively bred in large numbers because all adults migrate into a pond during a few days in the spring. On 31 March to 5 April 1997, I collected adult wood frogs as they congregated in Buffer Zone Marsh on the E.S. George Reserve in Michigan. I brought the adult frogs back to the laboratory and randomly assigned three females to one male and placed the group of four into a 10-L tub filled with 8 L of aged well water. In wood frogs, each female lays a single egg mass (~650 eggs) that is easily identified and separate from egg masses laid by other females. This protocol was repeated for 22 males, each crossed with three unique females, for a total of 66 crosses. Of these 22 half-sib families and 66 full-sib families, 12 crosses produced an insufficient numbers of eggs, leaving 21 half-sib families and 54 full-sib families (a mean of 2.6 dams per sire).

The experiment used a completely randomized design in which 50 tadpoles from each full-sib family were raised in both no-predator and caged-predator environments. Thus, the 54 full-sib families (mean mass \pm SE = 16.2 \pm 0.6 mg) were raised in two environments for a total of 108 experimental units (100-L pond wading pools placed in a field). While it would have been preferable to

independently replicate the progeny of each dam, the experimental design was a necessary compromise to conduct the study under semi-natural conditions with pseudoreplication at the level of progeny (independently replicating the progeny of each dam would have required ≥ 216 mesocosms). As a result, the among-progeny variance should be decreased while the among-dam variance should be increased. Assuming that this transfer of environmental variance from progeny to dams does not alter the total phenotypic variance, then the proportion of total variance due to sires should remain the same. Hence, estimates of heritability based on among-sire variance should be unaffected by this design.

The wading pool mesocosms were set up on 23–24 April. They contained 100 g of leaf litter (primarily *Quercus* spp.), 5 g of rabbit chow, and water from a local pond to serve as a source of algae and zooplankton. Tadpoles were added on 1 May. All pools were covered with 60% shade cloth to prevent colonization by other organisms. Each pool was equipped with a single predator cage constructed of 10 × 10 cm sewer pipe with nylon window screen on each end. In pools assigned the no-predator treatment, the cages remained empty. In pools assigned the predator treatment, each cage housed a single late-instar dragonfly nymph (*Anax longipes*). The dragonflies were fed approximately 300 mg of wood frog tadpoles three times per week. During the feeding of the dragonflies, I lifted the empty predator cages to equalize disturbance across both treatments. Midway through the experiment, I observed tadpole behaviour. I quietly approached each pool and counted the number of tadpoles that could be observed and the number of observable tadpoles that were moving. Dividing the latter by the former provided a measure of proportion of tadpoles that were active. Activity was measured 20 times during 13–16 May and I used the mean of the 20 observations on each pool as the response variable.

On 4 June, I removed 10 tadpoles from each experimental unit and preserved them for subsequent morphological analysis. Each tadpole was individually weighed and developmentally staged (Gosner, 1960) and its morphology was measured using a digital camera and digital analysis software (Optimas, Bothell, WA, USA). I measured five dimensions from the lateral view (the maximum length and depth of the tail, the depth of the tail muscle, and the maximum length and depth of the body) and two dimensions from the dorsal view (tail muscle width and body width; for a photo of tadpole dimensions, see Fig. 1 in Relyea, 2000).

Quantifying relative morphology

In assessing changes in morphology, it is important that one removes the effect of overall size to capture changes in relative (size-independent) shape. I regressed the seven linear dimensions of the tadpoles against mass for all individuals in the experiment. All variables were log-

transformed to improve the linearity of the regression. From this regression, I saved the residuals for each tadpole which served as size-independent measures of tadpole shape. This protocol was been successfully used in previous experiments (Relyea, 2002a–d).

Estimating the heritability of traits

The first objective was to quantify the genetic variation for the traits in each of the two predator environments. Due to the experimental design, there were 10 individuals from each full-sib family for quantifying morphology and life history traits, but there was only a single estimate of behaviour for each full-sib family (the proportion of tadpoles active in a pool). Thus, the two types of data had to be analysed in slightly different ways.

For the morphology and life history data, I analysed the half-sib families using nested analyses of variance (ANOVAs) for each environment. For each trait, I examined the impact of predator environment (as a fixed factor), and the impacts of sires and dams nested within sires (as random factors). To estimate the additive genetic variance for each trait within each environment, I calculated the variance terms using restricted maximum likelihood protocols in the VARCOMP function of SPSS statistical software (SPSS, Chicago, IL, USA). I then followed the protocols of Roff (1997) for half-sib designs in which:

$$h^2 = \frac{4V_{AS}}{V_P} = \frac{4V_{AS}}{V_{AS} + V_{AD} + V_{AP}}$$

where h^2 is the heritability, V_P the total phenotypic variance, V_{AS} the variance among sires, V_{AD} the variance among dams and V_{AP} the variance among progeny. Because all progeny from each dam were reared in a common environment, I had an unbiased estimate of additive genetic variation from the sires but a potentially biased estimate from the dams (due to the progenies' common environment). In most cases, the estimated variance based on the dams exceeded that based on sires, suggesting that the dam estimate contained additional sources of variance including dominance and common environment. Thus, all reported variance estimates are based only on the analysis of sires. Standard errors for heritability estimates follow the protocols of Roff (1997). To analyse the heritability of the behavioural data within each environment (in which there was only a single measure from each dam), I used a modification of the above analysis as recommended by Roff (1997). In this case, one can examine only the effect of the predator environments, sires, and their interaction (with each dam within a given sire serving as a replicate of that sire). Significant heritabilities were tested using analyses of variance and variance components were again calculated from the VARCOMP analysis of SPSS. In estimating the standard error of activity heritability, I used the standard equation in Roff (1997). However, because the mean number of tadpoles being observed in each pool was

10.1, I used this number as an estimate of the number of actual progeny that provided the behavioural data.

Estimating the heritability of trait plasticities

I estimated the heritability of trait plasticities by first calculating the environment \times sire variance and total variance (using VARCOMP) and then estimating the heritability of plasticity using the following equation:

$$h^2 = \frac{4V_{E \times S}}{V_p}$$

Standard errors were estimated using the standard equations (setting the number of progeny per dam at 9.85). I also estimated standard errors using bootstrap sampling and arrived at nearly identical results (results not shown). I examined an alternative approach suggested by Gavrillets & Scheiner (1993) in which one calculates the difference in trait values in the two environments (i.e. the magnitude of plasticity) for each full-sib family based on the mean progeny values in each environment. One can then apply a full-sib design and replace the among-family mean squares with the among-sires mean squares and replace the among-progeny mean squares with the among-dam mean squares. This approach provided nearly identical heritability estimates.

Correlations among traits

In addition to estimating the genetic variation of the traits, I also estimated the genetic correlations among traits within each environment and within traits across environments. To estimate the genetic correlations among traits within each environment, I examined the correlations among the behavioural, morphological, and life history traits using sire means and Pearson's product-moment correlations. Correlations among the 10 traits were conducted within each environment. Because there were 45 possible pairwise comparisons, the Bonferroni significance value for each test was $P < 0.0011$. To

estimate the genetic correlations within traits across environments, I examined the correlations of each trait between the two environments using sire means and Pearson's product-moment correlations. Because there were 10 correlations, the Bonferroni significance value for each test was $P < 0.005$.

Results

Heritability of the traits

The analyses of wood frog traits demonstrated that most traits responded to the predator environments and that many of these traits possessed significant additive genetic variation (Table 1, Fig. 1). In general, predators induced lower activity, relatively large tails, relatively small bodies, slower growth, and slower development. There were significant sire effects for tadpole activity, tail length, body width, mass, and development. Additionally, there were significant ($P < 0.05$) or nearly significant ($P = 0.083$) predator-by-sire interactions for three of the 10 traits examined (tail depth, body length, and body width), suggesting that the wood frogs also possess significant additive genetic variation for trait plasticity. For these three traits, within-environment ANOVAs indicated significant (or nearly significant) sire effects in predator and no-predator environments for body length ($P = 0.058$ and $P < 0.05$, respectively), and body width ($P < 0.01$ and $P < 0.05$, respectively). However, for tail depth, the sire effect was significant in the predator environment ($P < 0.05$) but nonsignificant in the no-predator environment ($P = 0.516$).

Based on the estimated variance components, I was able to estimate the heritability of the traits in each environment (Table 2). I first estimated the heritability of the traits in the absence of predators. The two life history traits (mass and development) exhibited high heritabilities ($h^2 \pm SE$ for each trait was 1.0 ± 0.4). In contrast, the heritabilities of the morphological traits were more moderate ($h^2 \leq 0.6$). The most striking result was that tail

Table 1 Results (P -values) from analyses of variance to test for significant effects of predators, sires, and dams in the morphology and life history traits of wood frogs.

Trait	Predator (d.f. = 1)	Sire (d.f. = 20)	Dam (sire) (d.f. = 33)	Predator \times sire (d.f. = 20)	Predator \times dam (sire) (d.f. = 33)
Activity	<0.001	0.002	–	0.656	–
Tail length	<0.001	0.012	0.002	0.619	0.618
Tail depth	<0.001	0.155	0.005	0.083	<0.001
Muscle depth	0.115	0.066	0.174	0.176	<0.001
Muscle width	0.034	0.260	0.159	0.138	<0.001
Body length	<0.001	0.457	0.057	0.014	<0.001
Body depth	0.006	0.142	0.325	0.482	<0.001
Body width	0.106	0.147	0.045	0.009	0.001
Mass	<0.001	0.014	<0.001	0.785	<0.001
Development	<0.001	0.002	0.023	0.904	<0.001

In these analyses, the response variables were individual tadpoles. For the heritability of activity, the response variables were pool means for tadpole activity.

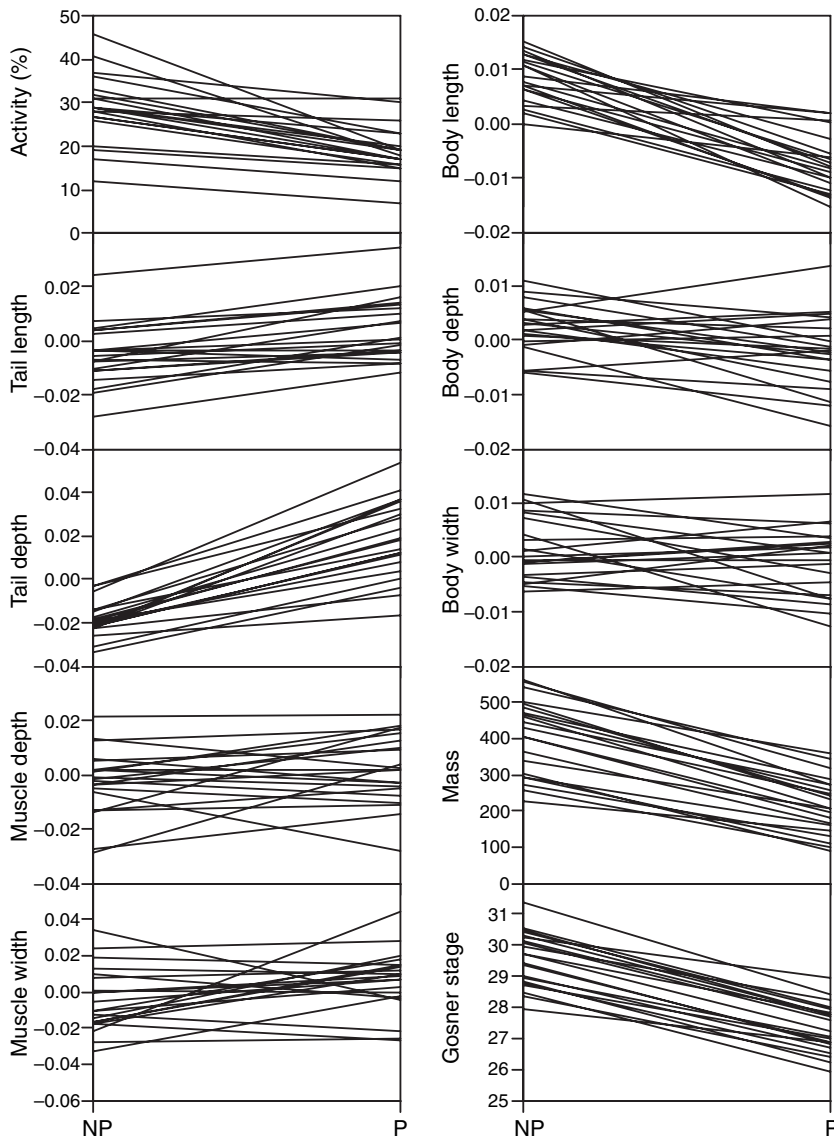


Fig. 1 Reaction norms of tadpole behaviour, relative morphology, and life history across 21 half-sib families (21 sires each bred to three unique females). The progeny from each family were reared under two different environments (NP = no-predator environment, P = predator environment). Morphological dimensions were made size-independent by regressing the dimensions against tadpole mass and saving the residuals. Data are sire means.

depth and body depth had very low heritabilities ($h^2 \leq 0.1$), consistent with their nonsignificant sire effects. In the separate analysis of behaviour, tadpole activity differed significantly among sires and the estimate of heritability was high ($h^2 = 1.1 \pm 0.2$).

In the presence of predators, the heritability estimates exhibited some different patterns (Table 2). The heritability of the life history traits (mass and development) tended to be lower (0.4–0.5) than in the absence of predators. The morphological traits generally continued to have moderate levels of heritability (0.2–0.7). Surprisingly, the heritability of tail depth in the presence of predators was high (0.7 ± 0.3) compared with the same trait in the absence of predators (0.00 ± 0.06). In the analysis of behaviour, tadpole activity differed among sires and the estimated heritability was high (1.4 ± 0.2).

Heritability of the trait plasticities

The analyses of variance indicated that three of the 10 traits (tail depth, body length, and body width) exhibited significant or nearly significant environment-by-sire interactions (Table 1). As expected, these same traits had the highest estimated heritability values (Table 3). The estimates of plasticity heritability ranged from low heritabilities of plasticity in life history and behaviour traits (0.00) to high heritabilities of plasticity in tail depth, body length and width plasticity (0.2–0.4).

Genetic correlations within and across environments

The first analysis examined genetic correlations among traits within the no-predator environment (Table 4,

Table 2 The estimated variance components among sires, dams, and progeny for larval wood frog morphology and life history analysed within predator and no-predator environments.

Trait	Environment	Among-sire variance	Among-dam variance	Among-progeny variance	Total variance	$h^2 \pm SE$
Activity	No-predator	0.003328	0.008993	–	0.012321	1.08 \pm 0.22
	Predator	0.001823	0.003524	–	0.005347	1.36 \pm 0.18
Tail length	No-predator	0.000064	0.000056	0.000776	0.000896	0.29 \pm 0.17
	Predator	0.000063	0.000070	0.000905	0.001038	0.24 \pm 0.16
Tail depth	No-predator	0.000000	0.000114	0.000325	0.000496	0.00 \pm 0.06
	Predator	0.000168	0.000306	0.000522	0.000996	0.67 \pm 0.30
Muscle depth	No-predator	0.000084	0.000117	0.000496	0.000697	0.48 \pm 0.24
	Predator	0.000080	0.000116	0.000681	0.000877	0.36 \pm 0.20
Muscle width	No-predator	0.000119	0.000351	0.001060	0.001530	0.31 \pm 0.18
	Predator	0.000131	0.000215	0.001991	0.002337	0.22 \pm 0.15
Body length	No-predator	0.000010	0.000019	0.000098	0.000127	0.31 \pm 0.18
	Predator	0.000017	0.000024	0.000109	0.000150	0.45 \pm 0.23
Body depth	No-predator	0.000005	0.000032	0.000135	0.000172	0.12 \pm 0.11
	Predator	0.000019	0.000050	0.000221	0.000141	0.26 \pm 0.16
Body width	No-predator	0.000023	0.000016	0.000111	0.000150	0.61 \pm .028
	Predator	0.000023	0.000027	0.000141	0.000191	0.48 \pm 0.24
Mass	No-predator	6840.9	8803.6	11110.4	26754.9	1.02 \pm 0.39
	Predator	1832.8	10532.1	5323.5	17688.3	0.41 \pm 0.22
Development	No-predator	0.566	0.382	1.228	2.177	1.04 \pm 0.39
	Predator	0.237	0.791	0.854	1.882	0.50 \pm 0.25

Based on these variance estimates, I estimated the heritability of each trait (h^2 , based on sires). The heritability of activity was determined in a separate analysis (see Methods).

Table 3 The estimated components for sire \times environment variance and total variance for larval wood frog behaviour, morphology, and life history analysed within predator and no-predator environments.

Trait	Sire \times predator variance	Total variance	$h^2 \pm SE$
Activity	0.000000	0.008761	0.00 \pm 0.08
Tail length	0.000000	0.000966	0.00 \pm 0.06
Tail depth	0.000039	0.000716	0.22 \pm 0.15
Muscle depth	0.000024	0.000787	0.12 \pm 0.11
Muscle width	0.000060	0.001932	0.12 \pm 0.11
Body length	0.000013	0.000138	0.38 \pm 0.20
Body depth	0.000001	0.000232	0.02 \pm 0.07
Body width	0.000014	0.000170	0.33 \pm 0.19
Mass	0.000000	22246.5	0.00 \pm 0.06
Development	0.000000	2.027	0.00 \pm 0.06

Based on these variance estimates, I estimated the heritability of plasticity for each trait. The heritability of activity was determined in a separate analysis (see Methods).

above diagonal). In no-predator environments, higher activity was correlated with greater mass and development (although only the latter was significant after Bonferroni adjustment). There were negative relationships between tail and body size (although all were nonsignificant after Bonferroni adjustment). Tail size had no association with mass or development and only one of the body dimensions (body depth) was associated (negatively) with mass and development. When correlating activity with morphology, six of the seven morphological

traits were uncorrelated; body depth had a significant negative correlation with activity level. Finally, mass and development demonstrated a significant positive correlation.

In predator environments, higher activity was again correlated with greater mass and development (Table 4, below diagonal). As in the no-predator environment, the tail dimensions were generally negatively correlated to the body dimensions. However, in contrast to the no-predator environment, tadpoles with deeper tails exhibited greater mass and development while tadpoles with longer bodies exhibited lower mass and development. In correlations between activity and the morphological traits, higher activity was correlated with deeper tails and shorter bodies. Once again, mass and development demonstrated a significant positive correlation. In general, there were major differences in the among-trait correlations within each environment (Table 4). Of the 11 significant correlations in the no-predator environment, only five were significant in the predator environment (prior to Bonferroni correction). Of the 17 significant correlations in the predator environment, only five were significant in the no-predator environment (prior to Bonferroni correction).

There also were significant correlations of each trait across the two environments (Table 5). For the behavioural trait (activity), there was a significant positive correlation between environments, suggesting that the half-sib families with relatively high activity in the no-predator environment also exhibit relatively high

Table 4 Genetic correlations (Pearson product-moment correlations) among traits of tadpoles reared within a given environment.

	Activity	Tail length	Tail depth	Muscle depth	Muscle width	Body length	Body depth	Body width	Mass	Development
Activity	–	0.346	0.343	0.190	0.342	0.117	–0.792*	–0.422	0.592	0.666*
Tail length	–0.055	–	0.256	–0.065	–0.048	0.180	–0.470	–0.102	0.060	0.084
Tail depth	0.654	0.065	–	0.650	0.291	–0.343	–0.435	–0.448	0.244	0.401
Muscle depth	0.390	0.098	0.443	–	0.376	–0.277	–0.368	–0.263	0.033	0.182
Muscle width	0.159	0.060	–0.053	0.388	–	–0.144	–0.360	–0.082	0.394	0.482
Body length	–0.650	–0.090	–0.727*	–0.156	0.218	–	–0.041	–0.163	–0.382	–0.404
Body depth	–0.047	–0.441	–0.363	–0.284	0.080	0.336	–	0.403	–0.489	–0.547*
Body width	–0.352	–0.699*	–0.423	–0.425	–0.336	0.223	0.572	–	0.202	0.080
Mass	0.838*	0.028	0.760*	0.447	0.028	–0.674*	–0.332	–0.345	–	0.962*
Development	0.824*	0.131	0.808*	0.498	0.124	–0.652	–0.309	–0.452	0.965*	–

Values above the diagonal come from tadpoles reared without predator cues whereas values below the diagonal come from tadpoles reared with predator cues. The correlations are calculated from sire means.

Coefficients in boldface font indicate significance prior to Bonferroni adjustment ($P < 0.05$).

*Significance after Bonferroni adjustment for 45 tests ($P < 0.001$).

Table 5 Genetic correlations of behavioural, morphological, and life history traits across predator and no-predator environments.

Trait	Cross-environment correlation
Activity	0.580
Tail length	0.806*
Tail depth	0.703*
Muscle depth	0.431
Muscle width	0.162
Body length	0.191
Body depth	0.365
Body width	0.332
Mass	0.877*
Development	0.842*

The correlations are calculated from sire means.

Coefficients in boldface font indicate significance prior to Bonferroni adjustment ($P < 0.05$).

*Significance after Bonferroni adjustment for 10 tests ($P < 0.0051$).

All correlations are positive.

activity in the predator environment (although this correlation was not significant after Bonferroni correction). A similar pattern was observed for some of the morphological traits. Half-sib families with relatively larger tails in the no-predator environment also had relatively larger tails in the predator environment. Interestingly, none of the muscle dimensions or body dimensions exhibited any significant cross-environment correlations (even prior to Bonferroni correction). The two life history traits, mass and development, both exhibited positive correlations across environments, suggesting that half-sib families with more rapid growth and development in the no-predator environment also had more rapid growth and development in the predator environment. In short, there were cross-environment correlations for behaviour, life history, and some of the morphological traits and all correlations were in the positive direction.

Discussion

The analysis of tadpole families reared in predator and no-predator environments demonstrated that there is additive genetic variation in larval wood frogs. Although life history traits typically have low heritabilities across a wide range of taxa (Roff & Mousseau, 1987; Price & Schluter, 1991), wood frog growth and development exhibited relatively high heritabilities (0.4–1.0). However, the wood frog result is consistent with other studies of anurans. In wood frogs, Berven (1987) found moderate heritabilities for development (0.35) and variable heritability for growth (0.07–0.66). Spadefoot toads (*Scaphiopus couchii*) have high heritabilities of development (0.87), but no significant heritability for tadpole length (a surrogate measure of growth; Newman, 1988). Common frog tadpoles (*R. temporaria*) have significant (or nearly significant) heritability of development (0.1–0.3) and low heritability of growth (0–0.3; Laurila *et al.*, 2002; Sommer & Pearman, 2003). Finally, spring peeper tadpoles (*Pseudacris crucifer*) appear to possess no significant heritability for either development or growth (less than 0.3; Travis *et al.*, 1987; Emerson *et al.*, 1988). Thus, the heritability of life history traits can be quite variable in anurans.

The heritability of tadpole behaviour and morphology was generally moderate to high in both environments. There are few comparative data on the heritability of tadpole behaviour and morphology within any environmental context. Common frog tadpoles have low additive genetic variation for tadpole morphology (Pakkasmaa *et al.*, 2003) and post-metamorphic spring peepers have moderate to high heritability (0.4–0.9) of morphological traits (Emerson *et al.*, 1988). In other aquatic organisms, there is some evidence of heritable variation in behavioural defenses in *Daphnia* (Stirling & Roff, 2000) and good evidence of heritable variation in morphological defenses in marine bryozoans and cladocerans (Parejko & Dodson, 1991; Cheetham *et al.*, 1995; Spitze & Sadler,

1996; Harvell, 1998). Although bryozoans and cladocerans can only provide broad-sense heritabilities, the results are consistent with my study. The results are also consistent with studies across a range of taxa that demonstrate heritabilities of morphological traits (reviewed in Roff, 1997). Thus, there is the potential for selection to act on morphological traits and cause an evolutionary response in the next generation.

One of the most striking results was that while most traits had similar magnitudes of heritability in both environments, tail depth exhibited a pronounced difference in heritability between environments. The heritability of tail depth was high with predators (0.7) but quite low without predators (0). The lack of variation in tail depth in the no-predator environment may simply reflect some unknown constraint. However, low heritability values often can reflect the impact of past selection that removed genetic variation (Price & Schluter, 1991). The zero heritability of tail depth in the no-predator environment might reflect selection for an optimal shallow tail without predators. For example, increased competition can induce shallower tails when predators are present (counteracting the predator-induced response). However, when predators are absent, increased competition cannot make the already shallow tail any shallower (Relyea, 2002d, 2004; Relyea & Hoverman, 2003). Thus, in predator-free environments, the tadpole tail may reach its minimal depth. Past experiments have demonstrated that tadpoles with relatively smaller tails and larger bodies grow and develop faster (Van Buskirk & Relyea, 1998; Relyea, 2002a). Therefore, selection for growth in no-predator environments may be the cause of the low genetic variation in body length, body depth, and tail depth.

If this scenario is correct, we can ask why there is so much variation in tail depth when predators are present. Tadpole tails are under strong selection by predators, with deeper-tailed tadpoles escaping larval dragonflies better than shallow-tailed tadpoles (Van Buskirk *et al.*, 1997; Van Buskirk & Relyea, 1998). If the tadpole's world consisted of only predator and no-predator environments, predator-induced tails might be under strong selection in predator environments and, as a result, have low heritability. However, wood frogs (and many other species of tadpoles) live with a wide array of predators, and the particular composition can vary across both time and space. In response to predators, the tadpoles produce predator-specific changes in tail size (Relyea, 2000, 2003a; Van Buskirk & Arioli, 2002). All of this suggests that there is no single optimal tail depth in predator environments because there is no single predator environment. Selection is likely favouring different tail depths in different predator environments and this process, in turn, maintains a large amount of genetic variation in predator-induced tails.

Wood frogs not only had significant heritability for most traits, they also had significant (or nearly significant) heritability for the plasticity of three traits (tail

depth, body length and width). There are few data on the heritability of plasticity in amphibians (in response to any environment). In what appears to be the only example, Laurila *et al.* (2002) found no significant heritability for the plasticity of growth and development in tadpoles of the common frog, which is consistent with the current study. *Daphnia* exhibit among-clone variation in the plasticity of *Chaoborus*-induced morphology and life history traits (although heritabilities of plasticity were not estimated; Parejko & Dodson, 1991; Spitze, 1992; Weber & Declerck, 1997) and they have significant broad-sense heritability of behavioural plasticity ($H^2 = 0.2$; Stirling & Roff, 2000). Given the low number of studies, it is too early to draw any generalizations for the heritability of plasticity in predator-induced traits. However, for traits induced by other types of environments, there is widespread evidence for genetic variation in plasticity among clones, strains, and populations of plants and animals (Roff, 1997). These studies suggest that heritable plasticity might be common (e.g. Scheiner & Lyman, 1989).

The analysis of trait correlations within each environment identified a number of correlations that were opposite those observed across environments. As mentioned above, when we examine trait changes in wood frog tadpoles across environments, we typically find that predators induce lower activity, deeper tails and smaller bodies and these changes are associated with slower growth and development (Relyea & Werner, 1999, 2000; Relyea, 2000, 2002a; Relyea & Auld, 2004). Within the no-predator environment, activity was correlated with greater growth and development, but the morphological dimensions were largely uncorrelated with growth. The only morphological trait correlated with growth and development was body depth, but the correlation was opposite of that expected from previous work; shallower bodies were correlated with faster growth. Thus, within the no-predator environment, the more active tadpoles (which possessed shallower bodies) were growing and developing faster. Shallower bodies are likely spuriously correlated with greater mass and development simply because shallower bodies are correlated with higher activity. Therefore, while both activity and morphology are important in determining growth and development across predator and no-predator environments, differences in growth and development *within* a no-predator environment appears to be driven primarily by activity.

Within the predator environment, the pattern of trait correlations was somewhat different. High activity levels still had a positive impact on growth and development. However, both tail depth and body length were now positively associated with tadpole growth and development, despite the fact that this is the combination of morphological traits that has been associated with slower growth when we compare tadpoles across predator and no-predator environments (Relyea, 2000, 2002b, 2004). There are two possible mechanisms to explain this

contradiction. First, it is possible that the benefits of having higher foraging activity outweigh the costs of having a morphological phenotype that does not promote efficient digestion. A related, and more intriguing, possibility is that among tadpoles in a predator environment, those with greater morphological defenses (deeper tails and smaller bodies) are free to increase their activity to a higher level (although still less active than tadpoles reared without predators) and garner additional food resources. Similar trade-offs among behavioural and morphological defenses have been observed in other prey (Rundle & Brönmark, 2001; Relyea, 2003b). All of this suggests that we can observe qualitatively different trade-offs for organisms within environments vs. between environments with both having potentially adaptive explanations.

Correlating each trait across the two environments indicated that several of the traits were positively correlated. Behaviour, life history, and two of the morphological traits (tail depth and length) were positively correlated. When traits exhibit genetic correlations across environments, it suggests that any selection that occurs in one environment can indirectly cause selection on the trait in the other environment (Falconer, 1952; Via, 1984). In the current study, this suggests that tadpole activity, tail size, growth, and development cannot independently evolve in predator and no-predator environments because directional selection on the traits in one environment will indirectly cause the same directional change on the trait when expressed in the other environment. In contrast, the body and tail muscle dimensions are uncorrelated across the two environments, suggesting that selection can act more independently on these traits.

The heritability results of the current study can be combined with past work on selection intensities, cost and benefits of alternative phenotypes, and costs of plasticity *per se*. If we consider activity, we see that high activity is favoured in a no-predator environment (Relyea & Werner, 1999) whereas low activity is favoured in a predator environment (Skelly, 1994; Relyea, 2001b). Given that activity in each environment is heritable, the divergent selection pressures should favour the maintenance of a plastic response. However, the high cost of behavioural plasticity *per se* (Relyea, 2002b) and the lack of heritability for plasticity *per se* (this study) suggests that there is a potential constraint on the maximal amount of behavioural plasticity that can evolve.

If we next consider the morphological traits, we see that large tails and small bodies are favoured in predator environments (Van Buskirk *et al.*, 1997; Van Buskirk & Relyea, 1998) whereas small tails and large bodies are favoured in no-predator environments (Relyea, 2002a,b). Given that the morphological traits are heritable in both environments and given that body length and width are heritable for plasticity *per se*, this divergent selection should favour the maintenance of

plastic morphology. However, we must also consider the fitness effects of plasticity *per se*. In no-predator environments, wood frog sibships with greater plasticity for tail depth, muscle depth, body length, and body width experience more rapid growth and development; in predator environments, wood frog sibships with greater plasticity for muscle width, body length, and body width experience less rapid growth and development (Relyea, 2002b). Thus, the selection for plastic morphological traits may be constrained by the fitness effects of plasticity *per se* in predator environments but be unconstrained by the fitness effects of plasticity *per se* in no-predator environments. Collectively, the data suggest that we should see the continued maintenance of predator-induced defenses in wood frog tadpoles and that these behavioural and morphological defenses will, in turn, affect the growth and development of tadpoles. Because predator and no-predator environments can vary spatially, it also suggests that we should observe population-level differences in tadpole plasticity (Relyea, 2002c).

Conclusions

This study appears to be the first to examine the narrow-sense heritability of predator-induced defenses. Because the heritabilities in this experiment were observed under relatively natural conditions, the results likely apply to heritabilities in nature (Weigensberg & Roff, 1996). Combined with previous studies of broad-sense heritabilities, it is becoming clear that predator-induced traits can frequently be heritable, although the magnitude of heritability can be wide ranging across environments. Moreover, the plasticity of these defenses also can be heritable, offering the possibility for natural selection to act on both the trait values expressed in each environment as well as the magnitude of plasticity *per se*. Examining the heritability of inducible defenses is only beginning to receive attention in plant and animal taxa (e.g. Nicholsons *et al.*, 1993; James & Newcombe, 2000; Agrawal *et al.*, 2002; Jones *et al.*, 2002; Fornoni *et al.*, 2003), yet it is critical that we conduct such studies when considering the adaptive nature of predator-induced defenses. While heritability studies require large experiments, the insights into genetic variation and trait correlations within and across environments can lead to powerful insights concerning past and future selection as well as phenotypic trade-offs within and across environments. As we continue to amass data on the heritability of plastic traits, we will continue to make substantial inroads to understanding the evolution of phenotypic plasticity.

Acknowledgments

Many thanks to Jason Moll and Keith Wittkopp for the great field assistance and to Earl Werner for the use of the

Experimental Pond Facility at the E.S. George Reserve. My thanks to Steve Tonsor for statistical advice and to Josh Auld, Jason Hoverman, and Nancy Schoeppner for reviewing the manuscript. The research was supported by the National Science Foundation.

References

- Agrawal, A.A., Conner, J.K., Johnson, M.T.J. & Wallsgrove, R. 2002. Ecological genetics of an induced plant defense against herbivores: additive genetic variance and costs of phenotypic plasticity. *Evolution* **56**: 2206–2213.
- Berven, K.A. 1987. The heritable basis of variation in larval developmental patterns within populations of wood frog (*Rana sylvatica*). *Evolution* **41**: 1088–1097.
- Bradshaw, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet.* **13**: 115–155.
- Cheetham, A.H., Jackson, J.B.C. & Hayek, L.-A.C. 1995. Quantitative genetics of bryozoan phenotypic evolution. III. Phenotypic plasticity and the maintenance of genetic variation. *Evolution* **49**: 290–296.
- Clausen, J., Heck, D.D. & Hiesey, W. 1941. *Experimental Studies on the Nature of Plant Species. I. The Effect of Varied Environments on Western North American Plants*. Carnegie Institute of Washington Publication, no. 520, Washington, DC.
- Dudley, S.A. & Schmitt, J. 1996. Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *Am. Nat.* **147**: 445–465.
- Emerson, S.B., Travis, J. & Blouin, M. 1988. Evaluating a hypothesis about heterochrony: larval life-history traits and juvenile hind-limb morphology in *Hyla crucifer*. *Evolution* **42**: 68–78.
- Falconer, D.S. 1952. The problem of environment and selection. *Am. Nat.* **86**: 293–298.
- Fornoni, J., Valverde, P.L. & Nunez-Farfan, J. 2003. Quantitative genetics of plant tolerance and resistance against natural enemies of two natural populations of *Datura stramonium*. *Evol. Ecol. Res.* **5**: 1049–1065.
- Gavrilets, S. & Scheiner, S.M. 1993. The genetics of phenotypic plasticity. V. Evolution of reaction norm shape. *J. Evol. Biol.* **6**: 31–48.
- Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**: 183–190.
- Harvell, C.D. 1990. The ecology and evolution of inducible defenses. *Q. Rev. Biol.* **65**: 323–340.
- Harvell, C.D. 1998. Genetic variation and polymorphism in the inducible spines of a marine bryozoan. *Evolution* **52**: 80–86.
- James, R.R. & Newcombe, G. 2000. Defoliation patterns and genetics of insect resistance in cottonwoods. *Can. J. For. Res.* **30**: 85–90.
- Jones, T.H., Potts, B.M., Vaillancourt, R.E. & Davies, N.W. 2002. Genetic resistance of *Eucalyptus globulus* to autumn gum moth defoliation and the role of cuticular waxes. *Can. J. For. Res.* **32**: 1961–1969.
- Karban, R. & Baldwin, I.T. 1997. *Induced Responses to Herbivory*. University Chicago Press, Chicago, IL.
- Laurila, A., Karttunen, S. & Merila, J. 2002. Adaptive phenotypic plasticity and genetics of larval life histories in two *Rana temporaria* populations. *Evolution* **56**: 617–627.
- McCollum, S.A. & Van Buskirk, J. 1996. Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis*. *Evolution* **50**: 583–593.
- Newman, R.A. 1988. Genetic variation for larval anuran (*Scaphiopus couchii*) developmental time in an uncertain environment. *Evolution* **42**: 763–773.
- Nicholsorians, C.M., Fritz, R.S. & Clausen, T.P. 1993. The genetic basis for variation in the concentration of phenolic glycosides in *Salix sericea*: Clonal variation and sex-based differences. *Biochem. Syst. Ecol.* **21**: 535–542.
- Pakkasmaa, S., Merila, J. & O'Hara, R.B. 2003. Genetic and maternal effect influences on viability of common frog tadpoles under different environmental conditions. *Heredity* **91**: 117–124.
- Parejko, K. & Dodson, S.I. 1991. The evolutionary ecology of an antipredator reaction norm: *Daphnia pulex* and *Chaoborus americanus*. *Evolution* **45**: 1665–1674.
- Pigliucci, M. 2001. *Phenotypic Plasticity: Beyond Nature and Nurture*. Johns Hopkins University Press, Baltimore, MD.
- Price, T. & Schluter, D. 1991. On low heritability of life-history traits. *Evolution* **45**: 853–861.
- Relyea, R.A. 2000. Trait-mediated effects in larval anurans: reversing competition with the threat of predation. *Ecology* **81**: 2278–2289.
- Relyea, R.A. 2001a. Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology* **82**: 523–540.
- Relyea, R.A. 2001b. The relationship between predation risk and anti-predator responses in larval anurans. *Ecology* **82**: 541–554.
- Relyea, R.A. 2002a. Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. *Ecol. Monogr.* **72**: 523–540.
- Relyea, R.A. 2002b. Costs of phenotypic plasticity. *Am. Nat.* **159**: 272–282.
- Relyea, R.A. 2002c. Local population differences in phenotypic plasticity: predator-induced changes in wood frog tadpoles. *Ecol. Monogr.* **72**: 77–93.
- Relyea, R.A. 2002d. The many faces of predation: how selection, induction, and thinning combine to alter prey phenotypes. *Ecology* **83**: 1953–1964.
- Relyea, R.A. 2003a. How prey respond to combined predators: a review and an empirical test. *Ecology* **84**: 1827–1839.
- Relyea, R.A. 2003b. Predators come and predators go: the reversibility of predator-induced traits. *Ecology* **84**: 1840–1848.
- Relyea, R.A. 2004. Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. *Ecology* **85**: 172–179.
- Relyea, R.A. & Auld, J.R. 2004. Having the guts to compete: how intestinal plasticity explains costs of inducible defenses. *Ecol. Lett.* **7**: 869–875.
- Relyea, R.A. & Hoverman, J.T. 2003. The impact of larval predators and competitors on the morphology and fitness of juvenile tree frogs. *Oecologia* **134**: 596–604.
- Relyea, R.A. & Werner, E.E. 1999. Quantifying the relation between predator-induced behavior and growth performance in larval anurans. *Ecology* **80**: 2117–2124.
- Relyea, R.A. & Werner, E.E. 2000. Morphological plasticity of four larval anurans distributed along an environmental gradient. *Copeia* **2000**: 178–190.
- Roff, D.A. 1997. *Evolutionary Quantitative Genetics*. Chapman and Hall, New York: 493 pp.

- Roff, D.A. & Mousseau, T.A. 1987. Quantitative genetics and fitness: lessons from *Drosophila*. *Heredity* **58**: 103–118.
- Rundle, S.D. & Brönmark, C. 2001. Inter- and intraspecific trait compensation of defence mechanisms in freshwater snails. *Proc. R. Soc. London Ser. B* **268**: 1463–1468.
- Scheiner, S.M. & Lyman, R.F. 1989. The genetics of phenotypic plasticity. I. Heritability. *J. Evol. Biol.* **2**: 95–107.
- Schlichting, C.D. & Pigliucci, M. 1998. *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer, Sunderland, MA.
- Skelly, D.K. 1994. Activity level and susceptibility of anuran larvae to predation. *Anim. Behav.* **47**: 465–468.
- Smith, D.C. & Van Buskirk, J. 1995. Phenotypic design, plasticity, and ecological performance in two tadpole species. *Am. Nat.* **145**: 211–233.
- Sommer, S. & Pearman, P.B. 2003. Quantitative genetic analysis of larval life history traits in two alpine populations of *Rana temporaria*. *Genetica* **118**: 1–10.
- Spitze, K. 1992. Predator-mediated plasticity of prey life-history and morphology: *Chaoborus americanus* predation on *Daphnia pulex*. *Am. Nat.* **139**: 229–247.
- Spitze, K. & Sadler, T.D. 1996. Evolution of a generalist genotype: multivariate analysis of adaptiveness of phenotypic plasticity. *Am. Nat.* **148**: S108–S123.
- Stearns, S., de Jong, G. & Newman, B. 1991. The effects of phenotypic plasticity on genetic correlations. *Trends Ecol. Evol.* **6**: 122–126.
- Stirling, G. & Roff, D.A. 2000. Behaviour plasticity without learning: phenotypic and genetic variation of naïve *Daphnia* in an ecological trade-off. *Anim. Behav.* **59**: 929–941.
- Tollrian, R. & Harvell, D. 1999. *The Ecology and Evolution of Inducible Defenses*. Princeton University Press, Princeton, NJ.
- Travis, J., Emerson, S.B. & Blouin, M. 1987. A quantitative-genetic analysis of larval life-history traits in *Hyla crucifer*. *Evolution* **41**: 145–156.
- Van Buskirk, J. 2002. A comparative test of the adaptive plasticity hypothesis: relationships between habitat and phenotype in anuran larvae. *Am. Nat.* **160**: 87–102.
- Van Buskirk, J. & Arioli, M. 2002. Dosage response of an induced defense: how sensitive are tadpoles to predation risk? *Ecology* **83**: 1580–1585.
- Van Buskirk, J. & Relyea, R.A. 1998. Natural selection for phenotypic plasticity: predator-induced morphological responses in tadpoles. *Biol. J. Linn. Soc.* **65**: 301–328.
- Van Buskirk, J., McCollum, S.A. & Werner, E.E. 1997. Natural selection for environmentally-induced phenotypes in tadpoles. *Evolution* **52**: 1983–1992.
- Via, S. 1984. Quantitative genetics of polyphagy in an insect herbivore. II. Genetic correlations in larval performance within and among host plants. *Evolution* **38**: 896–905.
- Via, S. & Lande, R. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**: 502–522.
- Via, S. & Lande, R. 1987. Evolution of genetic variability in a spatially heterogeneous environment: effects of genotype-environment interaction. *Genet. Res.* **49**: 147–156.
- Weber, A. & Declerck, S. 1997. Phenotypic plasticity of *Daphnia* life history traits in response to predator kairomones: genetic variability and evolutionary potential. *Hydrobiologia* **360**: 89–99.
- Weigensberg, I. & Roff, D.A. 1996. Natural heritabilities: can they be reliably estimated in laboratory? *Evolution* **50**: 2149–2157.
- West-Eberhard, M.J. 2003. *Developmental Plasticity and Evolution*. Oxford University Press, Oxford.

Received 11 August 2004; revised 22 November 2004; accepted 3 December 2004