



## Microgeographic adaptations of spotted salamander morphological defenses in response to a predaceous salamander and beetle

Mark C. Urban

M. C. Urban ([mark.urban@uconn.edu](mailto:mark.urban@uconn.edu)), Dept of Ecology and Evolutionary Biology, Univ. of Connecticut, 75 North Eagleville Rd., Unit 3043 Storrs, CT 06269, USA.

Spatial heterogeneity in the selection imposed by different predator species could promote the adaptive diversification of local prey populations. However, high gene flow might swamp local adaptations at limited spatial scales or generalized phenotypic plasticity might evolve in place of local diversification. Spotted salamander larvae *Ambystoma maculatum* face strongly varying risks from gape-limited marbled salamander larvae *Ambystoma opacum* and gape-unconstrained diving beetle larvae *Dytiscus* spp. across natural landscapes. To evaluate if *A. maculatum* adapts to these predation risk across microgeographic scales, I measured selection gradients in response to the two focal predators and then assayed the defensive morphologies of ten populations in a common garden experiment. I found that *A. opacum* induced selection on *A. maculatum* for larger tailfins and bodies whereas beetles induced selection for larger tail muscles and smaller bodies. In accordance with the local adaptation hypothesis, *A. maculatum* populations inhabiting ponds with high beetle densities grew larger tail muscles relative to other populations when raised in a common environment. However, populations exposed to strong *A. opacum* selection did not evolve larger tailfins as predicted. High gene flow or morphological plasticity could explain the absence of this morphological response to *A. opacum*. Overall, results suggest that populations can sometimes evolve adaptive traits in response to locally variable selection regimes even across the very limited distances that separate populations in this study. If prey populations often differ in their defenses against local predators, then this variation could affect the outcome of species interactions in local communities.

Species often confront a spatial mosaic of varying natural selection across natural landscapes (Wright 1931, Thompson 2005). Given sufficient time and genetic variation, each population might adapt to their specific local selection regime. High gene flow can surpass the strength of divergent selection and thereby prevent local adaptive evolution across the limited distances at which individual habitat patches occur (Wright 1931, Slatkin 1985, Garant et al. 2007). However, complete local adaptation and maladaptation represent two extremes of a continuum of potential evolutionary endpoints (Hendry and Gonzalez 2008). Often, populations demonstrate some degree of suboptimal local adaptation even as maladapted immigrants prevent the mean phenotype from reaching the optimal state (Crespi 2000, Moore and Hendry 2005, Bolnick and Nosil 2007, Urban 2007c). Along these lines, multiple studies now suggest that populations can adapt across the same distances at which organisms normally disperse (Jain and Bradshaw 1966, Sork et al. 1993, Storfer and Sih 1998, Skelly 2004). Such microgeographic evolutionary studies provide critical information about the joint importance of gene flow, natural selection heterogeneity, and neutral drift in determining evolutionary dynamics across the fine spatial scales which often separate natural populations (Schemske and Bierzychudek 2001, Urban and Skelly 2006, Garant et al. 2007).

Aquatic predators often differ in composition across regional landscapes, and these predators can impose strong natural selection on prey morphologies in local populations (Van Buskirk et al. 1997, Johnson et al. 2008). Aquatic prey can defend against predators through morphologies that improve swimming performance, frustrate capture by gape-limited predators with large size or appendages, or present a non-lethal target for predatory attacks (Van Buskirk and McCollum 2000b, Fitzpatrick et al. 2003, Van Buskirk et al. 2003, Kishida and Nishimura 2004). Broadly, evidence suggests that morphological traits frequently are under stronger selection (Kingsolver et al. 2001) and have higher heritabilities (Mousseau and Roff 1987) than life history traits. Therefore, we might expect that locally adapted morphological defenses often will evolve across natural mosaics of varying predation threats. However, remarkably few studies of aquatic organisms have experimentally demonstrated genetic differences in morphological defenses among populations in response to specific predation threats (exceptions include Parejko and Dodson 1991, Endler 1995, Trussell 2000, Langerhans et al. 2004, Reimchen and Nosil 2004). Moreover, no study to date has examined if salamander populations evolve different morphologies to defend against local predation threats (but see Storfer 1999 for a study on overall escape performance).

Here, I studied ten *A. maculatum* populations, located on an isolated ridge in southern New England (Fig. 1), that coexist with varying densities of predaceous marbled salamanders *Ambystoma opacum* and diving beetles *Dytiscus* spp. Genetic studies suggest high gene flow among *A. maculatum* populations (Zamudio and Wicczorek 2007, Purrenhage et al. 2009). Zamudio and Wicczorek (2007) found that *A. maculatum* populations separated by less than 4.8 km shared similar neutral genetic markers. Purrenhage et al. (2009) additionally found no evidence for isolation by distance among *A. maculatum* populations separated by as much as 55 km. Therefore, gene flow has the potential to curb local adaptation within the  $1 \times 2$  km study region if natural selection is weak. Previous research suggests that these populations have adapted different foraging behaviors in response to varying predation risks (Urban 2007c). In this research, the populations that experienced the highest risk from gape-limited *A. opacum* predation foraged most intensely when raised under common conditions (Urban 2007c). A demographic model suggests that this intense foraging by young *A. maculatum* larvae could enhance their fitness by permitting them to grow into a size refuge from gape-limited predators (Urban 2007a). However, to date, morphological adaptations to predator regimes have not been evaluated in this system. Morphological traits could reduce the risk of intense foraging in *A. maculatum* by aiding their escape following detection by predators.

I concentrated on four specific traits that frequently have been found to determine larval amphibian escape performance in previous research: overall body size and three size-independent shape variables, including relative head width, relative tail muscle cross-sectional area, and relative tailfin area (Van Buskirk and Schmidt 2000, Van Buskirk et al. 2003, Kishida and Nishimura 2004, Teplitsky et al. 2005, Johnson et al. 2008). I first evaluated selection on these

traits originating from *A. opacum* and *Dytiscus*. I predicted that these two predators would impose divergent selection on *A. maculatum* larval morphology because they differ in their hunting strategies and gape-limitation. Second, I tested if morphological traits measured in a common garden covaried with the predator densities found in each population's habitat in a manner consistent with local adaptation. Such common garden experiments provide important information about the genetic contributions to trait variation by raising individuals in a controlled environment and thereby reducing environmental influences on the phenotype. I expected that populations that live with either high *A. opacum* or *Dytiscus* densities would evolve larger tailfins and tail muscles as a generalized means to improve their escape performance from predators. Moreover, I expected that populations exposed to high *A. opacum* predation would evolve either wider heads or more rapid growth into a larger size to defend against the gape-limited *A. opacum*. Alternatively, high gene flow among populations and/or limited genetic variation might prevent the evolution of morphological defenses against these predators across microgeographic distances.

## Material and methods

### Natural history

I studied ten *Ambystoma maculatum* larval populations inhabiting temporary ponds situated on an isolated forested ridge (area = 2 km<sup>2</sup>) on Totoket Mountain near Northford, CT, USA (Fig. 1). The spotted salamander *A. maculatum* is a large terrestrial salamander (up to 22 g) that inhabits the eastern US and Canada. Each spring, adults move from upland terrestrial habitat into temporary ponds to mate and

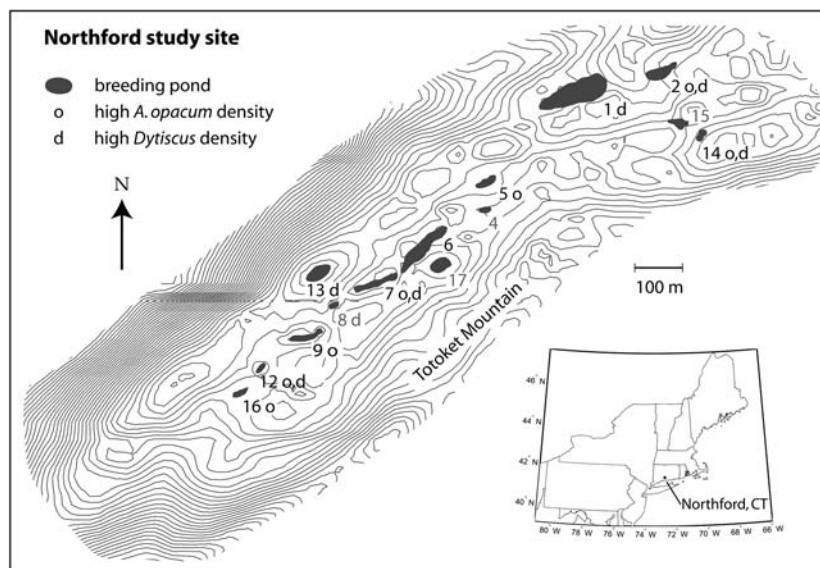


Figure 1. Map of study site in Northford, CT. The 14 temporary ponds inhabited by *A. maculatum* populations are indicated by their approximate shapes on a 2-m contour map of Totoket Mountain. Numbers identify each pond and these numbers correspond to Appendix 5. Bold text indicates ponds evaluated in the selection or common garden experiments. Gray-numbered ponds were not evaluated in this study but were included to give a sense of the landscape configuration of populations. In general, ponds excluded from the study were small, possibly sink, populations. Letters indicate ponds with higher than average *A. opacum* (o) and *Dytiscus* (d) densities.

to lay eggs. Small (~15 mg) aquatic larvae emerge from eggs after 8–10 weeks. *Ambystoma maculatum* larvae must survive a gauntlet of diverse predators before metamorphosing into terrestrial juveniles by late summer.

The marbled salamander larva *A. opacum* and diving beetle larva *Dytiscus* spp. are two of the most important predators of *A. maculatum* larvae in the study region based on field densities and feeding trials (Urban 2007b, 2008). The two predators differ in their ability to attack large prey. *Ambystoma opacum* are strongly gape-limited and cannot consume large *A. maculatum* larvae (Urban 2008) whereas *Dytiscus* attack *A. maculatum* larvae of most size classes (Urban unpubl.). However, *Dytiscus* sometimes fail to capture the largest pre-metamorphic *A. maculatum* larvae.

Although both predators vary in their regional annual absolute abundances, their local abundances remained relatively similar for the years during which I studied the pond. *Ambystoma opacum* local abundances in each of the 14 studied ponds were correlated across years (mean abundance correlation ( $\pm$  SE) among four years for which samples were available:  $0.54 \pm 0.09$ ; Appendix). The consistent differences in *A. opacum* densities among ponds likely reflects landscape variation in the probabilities that ponds freeze solid and kill overwintering *A. opacum* larvae (Urban unpubl.). *Dytiscus* densities also remained consistent from year to year (e.g. mean correlation ( $\pm$  SE) among three years:  $0.47 \pm 0.18$ ). The specific environmental determinants of *Dytiscus* density are not known. However, data suggests that *Dytiscus* reach their highest densities in semi-permanent ponds with dense emergent vegetation (Urban unpubl.). These consistent differences in predator densities among local ponds could impose differential selection on prey phenotypes.

The two predators differ in densities across the landscape such that *A. opacum* dominates the predator community of some ponds, *Dytiscus* dominates in other ponds, and high densities of both predators co-occur in still others (Fig. 1). The mean field densities of *Dytiscus* and *A. opacum* among ponds were not significantly correlated ( $\rho = -0.35$ ,  $r_{12} = -1.31$ ;  $p = 0.215$ ), suggesting that the two predation gradients occur relatively independent of each other.

## Field surveys

To estimate predator densities in the field, I performed standardized dip-net surveys in all ponds at the field site every two weeks during *A. maculatum*'s annual development for three years (Urban 2007b). I estimated predation threat as mean annual density and mean spring density of *Dytiscus* and *A. opacum*, respectively. I estimated *A. opacum* spring densities as the density of *A. opacum* larvae during *A. maculatum* hatching (Urban 2007b). I used spring densities of *A. opacum* to estimate their predation threat because these gape-limited predators do not feed on large *A. maculatum* larvae later in the season (Urban 2007b). Unfortunately, *Dytiscus* larvae cannot yet be identified to species (Larson et al. 2000), and thus I pool species in my estimates of their density. The few adults collected from the region suggest that larvae of both *D. verticalis* and *D. marginalis* can occur in study ponds.

## Traits

I evaluated morphological variation among the ten *A. maculatum* populations after they were raised in a common garden experiment. I selected four morphological traits expected to defend *A. maculatum* against predators based on previous research on aquatic amphibians (Van Buskirk and Schmidt 2000, Fitzpatrick et al. 2003, Van Buskirk et al. 2003, Kishida and Nishimura 2004, Teplitsky et al. 2005, Johnson et al. 2008). These traits included total body size and three size-independent morphological variables: relative head width, tailfin area, and tail muscle area. Hereafter, I apply the term 'relative' to a trait if I corrected it for overall animal size by using the residuals of a regression between the trait and body size (centroid size). A larger individual might escape from gape-limited predators like *A. opacum* by entering a size refuge (Urban 2007c) or because burst swimming speed increases with salamander body size (Fitzpatrick et al. 2003). A relatively wider head might prevent capture by gape-limited predators (Kishida and Nishimura 2004) and stabilize the body during rapid swimming (Fitzpatrick et al. 2003). Relatively deep tailfins can quicken an amphibian larva's acceleration away from predators or provide a nonlethal target for predator attacks (Fitzpatrick et al. 2003, Van Buskirk et al. 2003, Johnson et al. 2008). A relatively large tail muscle can provide greater or more enduring thrust away from predators that make repeated attacks (Teplitsky et al. 2005, Wilson et al. 2005).

I measured body size as centroid size or the square root of the sum of squared distances between 28 morphological landmarks and their centroid (Bookstein 1991). Morphological landmarks were measured from lateral and dorsal photographs of each larva in a program that I wrote in Matlab using the Image Analysis Toolbox. See Fig. 2 for individual landmark locations and Appendix 2 in the online supplementary materials for details about measurements. Tailfin area was estimated as the area of a parabola circumscribed within a rectangle that has tailfin depth and tail length as sides (Fitzpatrick et al. 2003). I estimated tail muscle size as the cross-sectional area of an ellipse delimited by tail muscle depth and width at the midpoint of the tail. The three morphological variables were first ln-transformed and then residuals were calculated from each variable's regression on ln centroid size to make them size-independent variables. I found no significant evidence for any remaining allometric relationships between these variables and ln centroid size in both selection and common garden experiments ( $p > 0.10$ ). I also examined the relationship between shape residuals and ln centroid size and found no evidence for any remaining non-linear relationships. Finally, I found no evidence that interactions between ln centroid size and population of origin explained significant variation in any of the shape variables ( $p > 0.10$ ), which would indicate a different allometrical relationship for each population and the need to apply population-specific size corrections.

## Prey selection experiment

To evaluate natural selection, I re-analyzed morphological results from a mesocosm selection experiment performed as

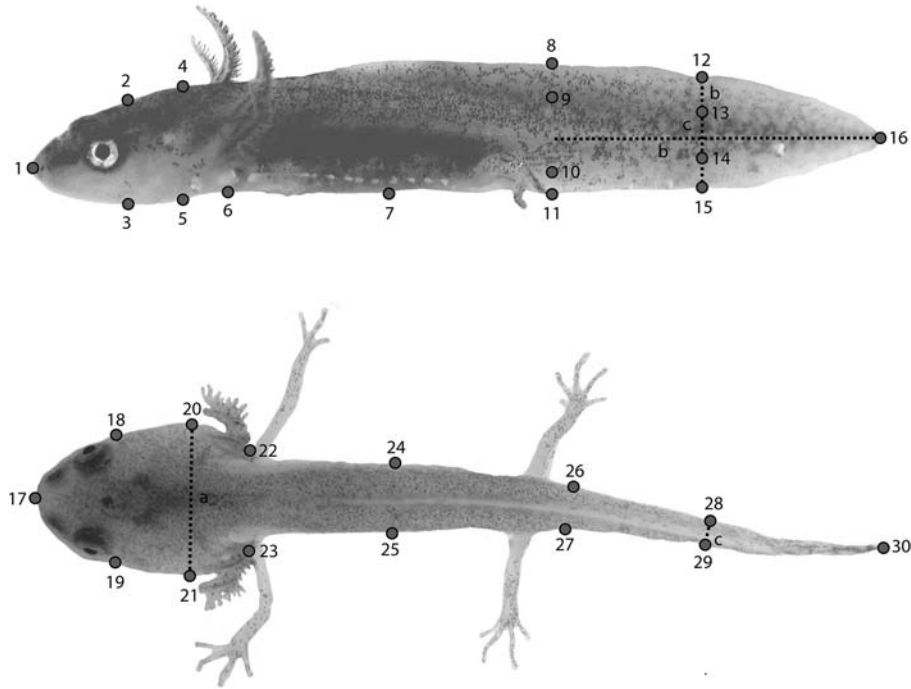


Figure 2. Morphological landmarks used to define the body shapes of *A. maculatum* larvae. Numbers indicate landmarks, and lettered lines indicate measurements used to calculate traits used in analyses. Lateral and dorsal views were integrated by normalizing and centering coordinates relative to the longitudinal axis of the larva to create a three-dimensional set of points to calculate centroid body size. The landmarks were designed to line up between views (e.g. points 24 and 25 in the dorsal view line up with point 7 in the lateral view). See Appendix 2 for detailed descriptions of landmarks.

part of a broader geographic survey about size-selection by *A. opacum* and *Dytiscus* larvae on *A. maculatum* size (Urban 2008). This study revealed that *A. opacum* predation induced selection for larger *A. maculatum* head widths (raw sizes not corrected for body size) whereas *Dytiscus* induced selection for smaller head widths. The four morphological traits analyzed here were not evaluated in this earlier analysis. Details of the experimental design can be found in Urban (2008). Briefly, I raised larvae in a common garden after collecting them from four egg masses from each of four ponds from each of three geographically divergent regions in New England (48 families total), including four ponds (Fig. 1; no. 2, 7, 13, 14) from the specific region studied here. Two predator chemical cue treatments (*A. opacum* and *Dytiscus*) and a control treatment were applied during larval development to broaden the scope of phenotypic variation available for selection (i.e. phenotypic engineering) to act on and to simulate the range of phenotypes found in nature. Previous work indicates that these cue treatments induce changes in salamander growth and refuge use (Urban 2008) and also tail muscle depth (Urban unpubl.).

Practical limitations to marking and recapturing individual *A. maculatum* larvae in natural ponds make measurement of natural selection in the wild difficult in this system. Instead, I replicated the substrate and predation conditions as closely as possible in outdoor mesocosms. I conducted selection trials in 60-l wading pools (area = 0.80 m<sup>2</sup>) to which I added a standardized mixture of substrate leaf litter and zooplankton food for prey. Twelve *A. maculatum* larvae were selected by randomly stratifying samples among

populations and predator cue treatments. Thus, in each experiment I exposed a randomly selected larva from each of the three treatments from each of four populations per region.

Eighteen replicated selection trials were evaluated for each of the two predator species for a total of 36 trials. Selection experiments were performed over the course of two years (*Dytiscus*, followed by *A. opacum*). I applied the same procedure in each year. Each set of larvae was exposed to either two field-collected *A. opacum* larvae or one *Dytiscus* larva. These mesocosm densities corresponded to mean field densities. I selected *A. opacum* with gape sizes within  $\pm 1$  SD of those observed in the field. Prior to selection trials, I photographed each *A. maculatum* larva dorsally and laterally so that surviving individuals could be re-identified after selection by comparing individual spotting patterns (Urban 2007c). A selection trial ended when half the larvae remained or 10 days had passed.

I evaluated linear and quadratic selection gradients using standard parametric approaches because these measures relate to commonly used quantitative genetic models of evolution (Lande and Arnold 1983) and allow comparison with other studies (Kingsolver et al. 2001). I estimated linear and non-linear (quadratic) selection gradients on all traits in a multiple logistic regression. The initial means and variances of each trait before selection are given in Appendix 3. All variables were standardized to a mean of zero and variance of one before analysis. Data were pooled across replicate selection arenas after finding that the interaction between each trait and mesocosm identity did not significantly explain

survival (Sokal and Rohlf 1995). Logistic coefficients were converted into linear gradients using standard methods (Janzen and Stern 1998).

I also estimated correlational selection which occurs when two or more traits interact in their effects on fitness (Lande and Arnold 1983, Schluter and Nychka 1994). I estimated correlational selection via projection pursuit regression to estimate and to visualize selection on the multivariate larval morphology. Projection pursuit regression addresses correlations between traits by estimating the axes of strongest selection as linear combinations of each trait in the directions of greatest fitness variance (Schluter and Nychka 1994). This method also has the advantage that it does not require that fitness surfaces conform to a specific (e.g. quadratic) functional relationship (Schluter and Nychka 1994, Brodie et al. 1995). Instead, projection pursuit regression uses cubic splines to estimate fitness surfaces across multivariate trait axes. The method indicates strong correlational selection when two or more fitness maxima or minima describe the fitness-trait relationship across an axis comprised of two or more traits (Schluter and Nychka 1994). For each analysis, I found the value of the smoothing function that minimized the general cross-validation score, which describes how well data fit the regression. In both predator selection experiments, an additional projection pursuit axis did not significantly explain fitness variation ( $p > 0.2$ ). I then bootstrapped data 1000 times to evaluate the significance of individual traits to the major axis of fitness variation. I applied a bias-correction to the percentile method to calculate confidence intervals because median bootstrapped trait loadings diverged strongly from estimated parameter values (Manly 2001).

### Common garden experiment

I analyzed the morphological traits of salamander larvae from ten different populations raised in a common garden experiment. In another manuscript (Urban 2007c), I explored behavioral and life history traits (foraging intensity and final weight) in animals from this experiment but did not analyze morphological traits. For experimental details see Urban (2007c). Briefly, I examined morphological traits from five full-sibs from each of five families from each of ten ponds except for one pond for which only four egg masses were collected before it dried prematurely for a total of 245 individuals.

Egg masses were collected from ponds within two days of egg-laying in spring 2004 to minimize environmental conditioning. I placed each larva in a separate 1-l clear plastic container and randomly assigned them to a location in a temperature-controlled room with full-spectrum lighting. Larvae were exposed to temperatures ( $14.3^{\circ}\text{C} \pm 0.5$  SD) and photoperiods that matched natural conditions in the region. Every two days, I fed larvae with equal numbers of size-matched cultured *Daphnia magna* and added 25-ml of water conditioned by *A. opacum* fed *A. maculatum* larvae ad libitum. These proportional volumes of *A. opacum* cues induced significant behavioral phenotypic plasticity in prior research (Urban 2008). I chose to expose all populations to *A. opacum* cues instead of no cues because few natural populations live in environments completely devoid of predators. I could not expose individuals to both *A. opacum* and *Dytiscus* cues and

still ensure substantial replication of genetically variable individuals (among and within families) given spatial limitations. Therefore, I chose to evaluate responses to *A. opacum*, rather than *Dytiscus*, because preliminary research suggested that these populations differed in survival when exposed to *A. opacum*. Under this experimental design, I could not assess variation in the evolution of predator-specific reaction norms in this experiment. However, I could assay variation in fixed traits and variation in plastic responses to general predation threats elicited by *A. opacum* cues (but not a genotype  $\times$  environment interaction). In this experiment, any significant variation in body size would originate from differences in body structure (i.e. a wider body) or increased allocation of energy to growth as opposed to activity because all individuals were fed the same amount of food and hatchlings did not differ significantly in size upon hatching (Urban 2007c).

I used mixed-effects restricted error maximum likelihood (REML) methods to estimate the relationship between field predator densities and trait variation. I included maternal family nested within population with both factors entered as random effects. I first evaluated a multivariate regression (the continuous version of a MANOVA) of the four morphological variables in relation to *Dytiscus* and *A. opacum* predator densities in source ponds to test for overall morphological differentiation across selection regimes. Such multivariate approaches should be used to analyze correlated traits (Scheiner 2001) like morphological traits. I performed univariate mixed-effects regressions for each response trait after finding that the multivariate model was significant (Scheiner 2001). All mixed-effects models were calculated in Genstat ver. 11. Probabilities were calculated based on the Wald-statistic using a method that approximates the denominator degrees of freedom for an unbalanced design (Kenward and Roger 1997). Note that these approximated degrees of freedom often are not integers.

## Results

### Prey selection experiment

*Ambystoma opacum* predation resulted in selection for *A. maculatum* larvae with larger bodies and tailfins as determined by linear selection gradients (Table 1; body size:  $B_{\text{avggrad}}' \pm \text{SE} = + 0.115 \pm 0.035$ ;  $\chi^2_{1,214} = 11.9$ ,  $p = 0.001$ ; relative tailfin size:  $B_{\text{avggrad}}' \pm \text{SE} = + 0.089 \pm 0.040$ ;  $\chi^2_{1,212} = 4.4$ ,  $p = 0.036$ ). Quadratic terms did not differ significantly from zero ( $p > 0.54$ ). Multivariate results corroborated parametric analyses by suggesting that larger larvae with larger tailfins survived better under attack by *A. opacum* (Fig. 3A). Projection pursuit regression did not suggest correlational selection among any traits, i.e. no multiple minima or maxima along a multi-trait axis. Instead, strong directional selection was detected along a trait axis dominated by larger tailfin sizes and body size, although the latter was not significant (Table 1).

*Dytiscus* caused significant linear selection for smaller bodied *A. maculatum* larvae (Table 1;  $B_{\text{avggrad}}' \pm \text{SE} = - 0.134 \pm 0.036$ ;  $\chi^2_{1,214} = 15.5$ ,  $p < 0.001$ ). Projection pursuit regression showed that survival changed most along a phenotypic axis dominated by both body size and relative

Table 1. Estimates of natural selection on *A. maculatum* morphology under predation by either *A. opacum* or *Dytiscus* larvae based on univariate logistic multiple regressions and multivariate projection pursuit regressions.

Trait	<i>A. opacum</i>			<i>Dytiscus</i>		
	$\beta$	$\gamma_{1,1}$	Non-parametric correlational estimates	$\beta$	$\gamma_{1,1}$	Non-parametric correlational estimates
Centroid body size	<b>0.115 ± 0.035</b>	0.000 ± 0.023	0.45 (-0.37, 0.93)	<b>-0.134 ± 0.036</b>	0.061 ± 0.034	<b>-0.66</b> (-0.99, -0.12)
Relative head width	-0.037 ± 0.040	0.004 ± 0.017	-0.39 (-0.94, 0.40)	-0.052 ± 0.034	0.010 ± 0.021	-0.23 (-0.93, 0.61)
Relative tailfin size	<b>0.089 ± 0.040</b>	0.005 ± 0.020	<b>0.78</b> (0.39, 0.99)	0.025 ± 0.033	0.007 ± 0.021	0.21 (-0.59, 0.94)
Relative tail muscle size	-0.039 ± 0.034	-0.018 ± 0.020	-0.19 (-0.82, 0.48)	0.017 ± 0.033	-0.035 ± 0.024	<b>0.68</b> (0.23, 0.99)

Note. – Univariate linear ( $\beta$ ) and non-linear selection ( $\gamma_{1,1}$ ) gradients reflect the average gradient ( $\pm 1$  SE) calculated from logistic multiple regression results according to the method of Janzen and Stern (1998). Non-parametric multivariate correlational estimates describe the relative contributions of each trait (with bootstrapped confidence intervals) to the multi-trait axis that explains the direction of strongest selection. Values in bold indicate statistically significant coefficients from least-squares regression or based on bias-corrected percentile bootstrapped confidence intervals for projection pursuit regression.

tail muscle size (Fig. 3B; Table 1). The fitness curve was characterized by two maxima at either end of the phenotypic axis, a pattern which indicates negative correlational selection. To visualize the joint contributions of body size and relative tail muscle size to survival better, I performed a second projection pursuit regression evaluating only these two significant traits along two axes (Appendix 4). In this more restricted analysis, survival increased strongly in the direction of smaller body sizes and increased rapidly but then flattened in the direction of larger relative tail muscle sizes. Hence, the combined analyses indicate that small-bodied larvae with large tail muscles survive *Dytiscus* predation best.

### Common garden experiment

Next, I evaluated morphological variation among natural populations raised in a common garden (trait means and standard deviations listed by population in Appendix 5). I first performed a mixed-effect multivariate regression to see if, when analyzed together, morphological traits varied with a population's historical exposure to *A. opacum* and *Dytiscus* predation threats. Multivariate results suggested that only *Dytiscus* density was marginally related to overall morphological variation (Table 2;  $F_{4,32.6} = 2.6$ ,  $p = 0.052$ ). Relative tail muscle size dominated the multivariate regression with *Dytiscus* density (Table 2). Contrary to results from selection experiments, relative tailfin size was not significantly related to *A. opacum* density in multivariate or univariate tests (Fig. 4A; Table 2;  $p > 0.5$ ). Given the marginal significance of at least one multivariate result, I next evaluated univariate relationships for each trait. In line with multivariate results, relative tail muscle size, a trait demonstrated to be under correlational selection by *Dytiscus*, increased significantly in *A. maculatum* populations that co-occur with high densities of *Dytiscus* (Table 2; Fig. 4B;  $F_{1,6.4} = 6.4$ ,  $p = 0.013$ ). *Dytiscus* density predicted 57% of the variation in mean tail muscle sizes among ponds. All other traits were not significantly associated with either *Dytiscus* or *A. opacum* densities or their interaction (Table 2;  $p > 0.08$ ), and I found little evidence for significant variation among populations or families ( $p > 0.07$ ).

## Discussion

Assuming adequate genetic variation and low gene flow, strong natural selection is expected to generate adaptive differentiation among populations (Endler 1986, Lynch and Walsh 1998). Aquatic predators often impose selection for prey with morphological traits that allow them to accelerate away from attacks or provide non-lethal targets (Van Buskirk and McCollum 2000a, Fitzpatrick et al. 2003, Van Buskirk et al. 2003, Teplitsky et al. 2005, Wilson et al. 2005) or for prey with larger or wider bodies that protect them against gape-limited predators (Kishida and Nishimura 2004). *Ambystoma maculatum* encounter varying predation risks from gape-limited *A. opacum* and gape-unconstrained *Dytiscus* across natural landscapes (Urban 2007b). Selection experiments suggested that these two predators impose divergent selection on overall prey body size and relative tailfin and tail muscle sizes, which could lead to adaptive morphological divergence across populations. I found a significant correlation between relative tail muscle size in populations and the local density of *Dytiscus*. However, *A. maculatum* larvae did not grow the larger tailfins predicted to protect against *A. opacum* predation in habitats dominated by *A. opacum*.

### Natural selection and adaptive differentiation: *A. opacum*

*Ambystoma opacum* preyed selectively on small larvae with relatively smaller tailfins (Table 1, Fig. 3A). *Ambystoma opacum* likely prey on small larvae owing to their strong gape-limitation (Urban 2007b, 2008) or because large amphibian larvae swim faster (Fitzpatrick et al. 2003, Johnson et al. 2008). Selection for larger tailfins might occur if a larger tailfin allows *A. maculatum* larvae to accelerate rapidly away from *A. opacum* as has been found for the congener *A. tigrinum* (Fitzpatrick et al. 2003). However, support for large tailfins contributing to swimming acceleration has been mixed for other amphibians (Van Buskirk and McCollum 2000a, Teplitsky et al. 2005). Alternatively, large tailfins might provide a region for non-lethal predatory attacks through the so-called lure effect (Van Buskirk et al. 2003). I

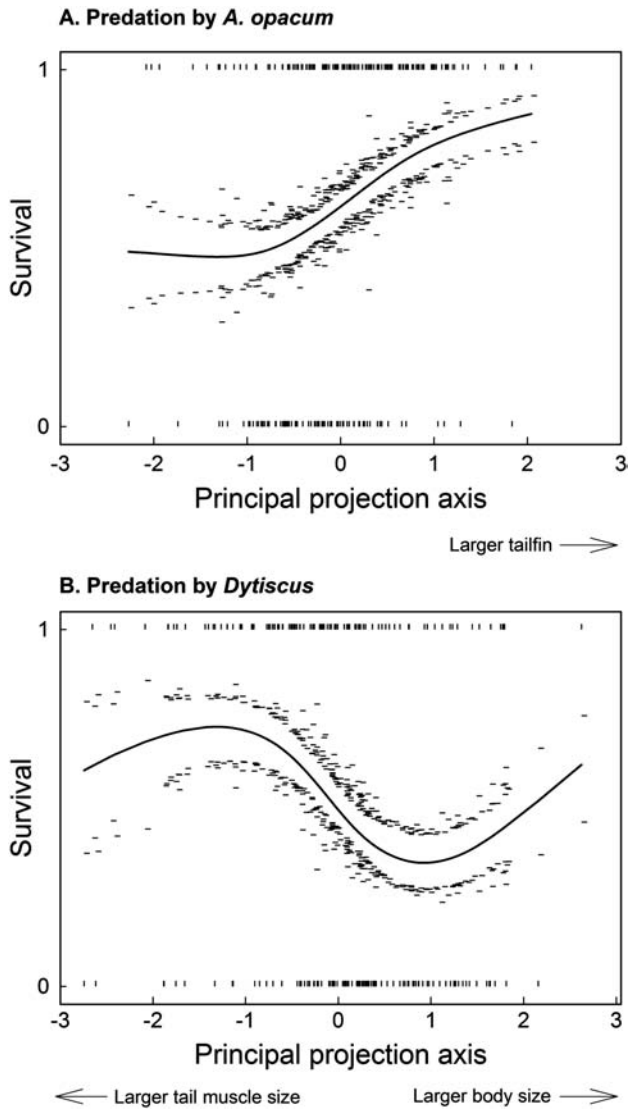


Figure 3. The relationship between mortality selection by *A. opacum* (A) and *Dytiscus* (B) in relation to the direction of strongest selection on *A. maculatum* body size, relative head width, tailfin area, and tail muscle cross-sectional area as determined by projection pursuit regression. The principal trait axis for each selection experiment represents the linear combination of each trait in the direction of greatest survival variation. I only list the traits that contributed significantly to the trait axis in each selection experiment. However, other traits also likely contributed to survival patterns in the directions indicated in Table 1. In (A), relative tailfin size contributed significantly and positively to the major trait axis. In (B), both relative muscle size and centroid body size contributed significantly to the trait axis. Relative tail muscle size increased to the left and centroid body size increased to the right on the axis. Horizontal dashes indicate bootstrapped standard errors for the estimated spline regression. Vertical marks indicate the actual data where a one indicates survival and a zero indicates death.

find this explanation unlikely in this case because *A. opacum* almost always attack *A. maculatum* head-first. Relative tail muscle size did not affect survival as expected, even though larger tail muscles increase swimming acceleration in other amphibians (Teplitsky et al. 2005, Johnson et al. 2008). In addition, relative head width did not mediate *A. maculatum*'s

survival under *A. opacum* gape-limited predation even though a wider head would allow them to enter a size refuge sooner (Kishida and Nishimura 2004).

Despite evidence for selection on body size and relative tailfin size, populations from ponds with high densities of *A. opacum* did not differ significantly in these traits (Fig. 4A). Differences in body size in the common garden experiment only could develop through differential allocation or a structural increase in volume because all salamanders began at roughly the same size and were fed the same food rations. The failure of populations to evolve greater allocation to growth or an increase in volume might indicate genetic constraints, a reduced need for this trait given that increased foraging has already evolved in populations exposed to high *A. opacum* predation risk, or that this trait already has reached its maximum, considering the many benefits to growth for temporary pond amphibians once resources have been obtained.

Potential explanations for why larger tailfins did not evolve in response to *A. opacum* include low statistical power, strong negative genetic correlations with other traits, and phenotypic plasticity. The statistical power to substantiate a correlation between selection regimes and a trait was low even though the number of experimental units and populations examined was high relative to other common garden examinations of local adaptation in vertebrates. However, even accounting for low statistical power, additional replication would have been unlikely to uncover a significant result here given that the estimated relationship was opposite that predicted and almost significantly so ( $p = 0.08$ ). The study design did not allow a sufficient number of families to estimate genetic correlations accurately for each population. However, preliminary estimates suggested little evidence for strong (near  $\pm 1.0$ ) correlations between tailfin size and other traits that would slow evolutionary rates (average correlations ranging from  $-0.30$  to  $+0.51$ ). A more likely explanation is that predator cues induce larger tailfins and that this environment-specific trait obviates the need for locally adapted fixed traits. Yurewicz (2004) found that *A. maculatum* larvae grow larger tailfins in response to dragonfly predators. Future experiments should determine if chemical cues from *A. opacum* affect *A. maculatum* larval morphology.

### Natural selection and adaptive differentiation: *Dytiscus*

*Dytiscus* preferentially preyed on larger bodied *A. maculatum* larvae with smaller tail muscles (Table 1). Larger relative tail muscles likely sustain a faster or more enduring escape response from an attacking predator (Dayton et al. 2005, Teplitsky et al. 2005). The fitness advantage for small prey probably occurs either because *Dytiscus* actively prefer larger and thus more energetically valuable prey items or because they cannot catch small larvae (Urban 2008). Multivariate analyses also suggested that larger larvae with smaller tail muscles survived better than intermediate phenotypes when attacked by *Dytiscus* (Fig. 3B). However, a projection pursuit regression analysis focused on just body size and relative tail muscle size showed no evidence for any survival advantage to large larvae with small tail muscles (Appendix 4). The second survival optimum at larger body size might reflect an

Table 2. Mixed-effects multivariate and univariate regressions of morphological variation versus *A. opacum* and *Dytiscus* densities.

Response	Source of variation				
	<i>A. opacum</i>	<i>Dytiscus</i>	<i>A. opacum</i> × <i>Dytiscus</i>	Population	Family
Multivariate	$F_{4,31.5} = 1.0$ (0.415)	$F_{4,32.6} = 2.6$ (0.052)	$F_{4,32.4} = 0.9$ (0.499)	$LR_4 = 2.0$ (1.000)	$LR_4 = 6.0$ (0.201)
Estimated coefficients					
Centroid body size	-0.003	0.033	-0.197		
Relative head width	0.003	0.044	0.156		
Relative tailfin area	-0.036	-0.024	0.068		
Relative tail muscle area	0.072	0.401	0.204		
Univariate centroid body size	$F_{1,5.9} = 0.1$ (0.748)	$F_{1,6.1} = 2.2$ (0.189)	$F_{1,6.1} = 2.3$ (0.181)	$LR_1 = 2.0$ (0.162)	$LR_1 = 0.1$ (0.708)
Relative head width	$F_{1,5.9} = 0.2$ (0.640)	$F_{1,6.4} = 0.8$ (0.416)	$F_{1,6.3} = 1.5$ (0.266)	$LR_1 = 0.0$ (0.841)	$LR_1 = 2.1$ (0.151)
Relative tailfin size	$F_{1,5.9} = 4.5$ (0.080)	$F_{1,6.4} = 0.5$ (0.499)	$F_{1,6.3} = 0.2$ (0.687)	$LR_1 = 0.0$ (0.862)	$LR_1 = 1.5$ (0.215)
Relative tail muscle size	$F_{1,5.8} = 0.0$ (0.795)	$F_{1,6.4} = 6.4$ (0.013)	$F_{1,6.2} = 0.2$ (0.660)	$LR_1 = 0.1$ (0.823)	$LR_1 = 3.2$ (0.074)

Note. – Multivariate mixed-effects restricted error maximum likelihood (REML) results are listed first, followed by univariate mixed-effects results. The first three columns convey the F-statistic, subscripted degrees of freedom and parenthesized p-values. Denominator degrees of freedom are approximated (Kenward and Roger 1997). The last two columns give the likelihood ratio tests for the random effects, family and population, and their associated  $\chi^2$ -square probabilities.

advantage to large size independent of tail muscle size if *Dytiscus* cannot capture the largest larvae (Urban 2008) or might indicate the complex contributions of other traits. Regardless, larvae that are large enough to experience this divergent selection are nearing metamorphosis and thus long-term investment in morphological alterations would be unlikely at this stage. I did not find any fitness advantage to larger tailfins in selection analyses even though relative tailfin size was expected to facilitate larval escape from *Dytiscus* either by increasing swimming acceleration or by providing a lure for non-lethal attacks.

*Ambystoma maculatum* from populations that inhabit ponds with high *Dytiscus* densities had larger tail muscles compared to those that inhabit ponds with low *Dytiscus* densities when raised in a common garden (Fig. 4B). This finding matches the predicted adaptive response to positive selection on tail muscle size from *Dytiscus* indicated by selection experiments (Fig. 3B). Genetic differences likely underlie the significant differentiation among populations because traits were measured under controlled conditions. Furthermore, I removed eggs from natural environments within two days of laying at a time when *Dytiscus* larvae generally have not yet recruited (Urban unpubl.), suggesting that observed effects do not constitute induced reactions to *Dytiscus* cues. However, the role of maternal effects remains unknown and should be explored in future studies. Given that larvae were only exposed to *A. opacum* and not *Dytiscus* cues, tail muscle differences among populations must represent either variation in fixed traits or variation in generalized plasticity to *A. opacum* cues. In previous research, *A. opacum* cues did not significantly induce larger tail muscles in *A. maculatum* larvae (Urban unpubl.), providing evidence against the latter explanation. Also, cues from predaceous dragonflies did not induce differences in tail muscle depth in *A. maculatum* in another study (Yurewicz 2004). However, plasticity could still augment morphological variation in natural populations. Future research should assess variation in gene-by-environment responses.

### Microgeographic adaptations and gene flow

Evidence now suggests that both relative tail muscle size and foraging behavior (Urban 2007c) have evolved in *A. maculatum* populations in response to two distinct predation threats. Each population lives, on average, within 116 m of its nearest neighbor at the study site (Fig. 1). Two studies of neutral microsatellite markers in *A. maculatum* suggest high gene flow across these spatial scales. A New York study determined that populations separated by less than 4.8 km were effectively integrated by gene flow (Zamudio and Wieczorek 2007). An Ohio study found no evidence for isolation by distance among ponds separated from 0.02 to 55 km and estimated the number of migrants per generation at 6.9 (Purrenhage et al. 2009). If the populations studied here disperse similarly, then adaptations appear to have evolved despite a high potential for genetic exchange among populations.

Evidence has been mixed for the adaptive morphological divergence of aquatic amphibians across microgeographic scales in response to varying predation risks. Van Buskirk and Arioli (2005) found that the *Rana temporaria* tadpole morphologies did not differ significantly among populations encountering different overall predator densities that were separated from nearest neighbors by a mean minimum distance of 1.5 km. In a study among oceanic islands separated by 100–1000 m, Lardner (1998) found that size at metamorphosis increased in response to a generalized predation risk gradient but found no evidence that tail shape, the trait expected to affect predator escape performance, increased along this same gradient. Relyea (2002) found evidence for morphological differentiation among *Rana sylvatica* populations that differed in both generalized predation and competition environments across a mean minimum distance of 652 m. None of these studies examined how morphologies changed according to specific predator selection regimes. If specific predators select for different prey traits, then an aggregated predation risk gradient might obscure adaptive



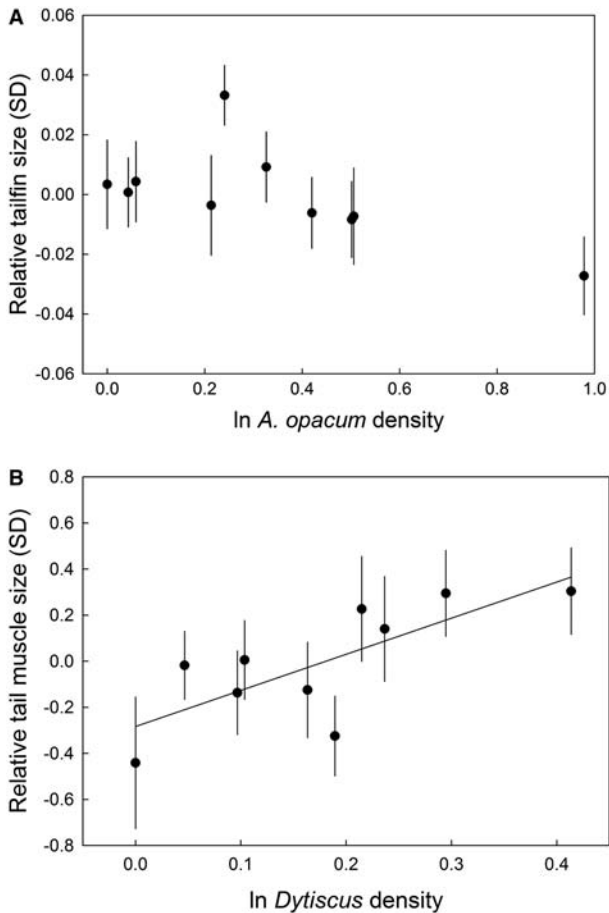


Figure 4. (A) the mean relative tailfin area of each population of *A. maculatum* larvae ( $\pm$  SE) relative to the  $\ln(x + 1)$  density of *A. opacum* larvae in its natural environment. (B) the mean relative tail muscle cross-sectional area of each population of *A. maculatum* larvae ( $\pm$  SE) relative to the  $\ln(x + 1)$  density of *Dytiscus* larvae in its natural environment. Tailfin area did not increase with *A. opacum* density whereas tail muscle size increased with *Dytiscus* density (indicated with a least-squares regression line).

clines in predator-specific traits. In an exceptional study analyzing a specific sunfish predation gradient, Storfer (1999) found that *Ambystoma barbouri* populations differed in maximum swimming acceleration depending on exposure to sunfish predation when separated by 14 km, but not when separated by 1 km. Although not established for *A. barbouri*, morphological variation probably underlies some variation in its maximum swimming acceleration. These studies suggest that amphibian populations sometimes evolve morphological adaptations across hundreds of meters to several kilometers. *Ambystoma maculatum* populations, meanwhile, appear to adapt to local predator assemblages across some of the shortest distances reported for an amphibian.

The degree to which populations become adapted or maladapted to local selection regimes depends on the balance between local selection and gene flow (Wright 1931, Slatkin 1985). Although moderate gene flow can introduce genetic variation to fuel an adaptive response (Gomulkiewicz et al. 1999), high gene flow can swamp out the evolution of locally adapted phenotypes or, in less extreme cases, greatly restrict a population from evolving an optimal mean phenotype

(Bolnick and Nosil 2007, Garant et al. 2007, Hendry and Gonzalez 2008). This critical balance between gene flow and selection becomes even more important at the microgeographic scales that often separate habitat patches. A growing number of empirical studies demonstrate microgeographic adaptation across finer scales than expected (Jain and Bradshaw 1966, Sork et al. 1993, Storfer and Sih 1998, Skelly 2004). These results indicate lower gene flow and stronger divergent selection than assumed based on prior considerations. Gene flow might be lower than expected if migrants seldom reproduce successfully in novel habitats because they cannot mate or their maladapted offspring survive poorly (De Meester et al. 2002, Nosil et al. 2005). Also, biased mate choice could sometimes lead to outbreeding avoidance. Interestingly, *A. maculatum* females prefer related males (Chandler and Zamudio 2008), which could mean that outbreeding avoidance in this species facilitates its microgeographic adaptation. Alternatively, natural selection might be stronger than expected in natural habitats because our estimates often do not incorporate all components of fitness or do not cover an appropriately long time scale (Endler 1986). In general, we need more research on how populations adapt to heterogeneous selection over microgeographic scales in the wild.

The temporal consistency of selection environments also will dictate how well local populations become adapted to currently observed selection. Biotic selection might vary more than abiotic selection if the species responsible for natural selection frequently colonize new habitats or become locally extirpated from habitats. If selection regimes shift rapidly relative to the generation length of a focal species, then non-differentiated generalists (e.g. fixed plasticity) should evolve. However, stable abiotic environments might often determine the distribution of biotic selection regimes (Reznick et al. 1990), thereby guaranteeing their stability. Unfortunately, researchers seldom know the historical dynamics of biotic selection (exceptions include Grant et al. 1996, Cousyn et al. 2001). In this study, I recorded *A. opacum* and *Dytiscus* distributions for 3–4 years. This short-term data does not cover the many generations over which we expect evolution to occur (*A. maculatum* generation time  $\sim$  seven years). Given the observed response to selection and the underlying abiotic determinants of *A. opacum* and *Dytiscus* distributions, I believe that these distributions have remained relatively constant over time. However, only long-term historical data would confirm this supposition. One goal of long-term ecological research should be to establish the dynamics of selection regimes over time.

### Caveats and additional considerations

Results from selection analyses should be viewed cautiously because I only evaluated the mortality component of fitness. Size at metamorphosis also affects future survival and fecundity in *Ambystoma* salamanders (Scott 1994). Incorporating fecundity into fitness calculations likely would strengthen existing selection for large size in larvae faced with both *A. opacum* and *Dytiscus* predation. Maternal effects could affect observed genetic variation among populations and families. One of the most important maternal effects in amphibians, egg provisioning, did not differ

significantly among populations (Urban 2007c). Practical limitations prevented me from performing a third experiment that examined trait selection from both predators. Future work will be needed to address if selection from the two predator species is interactive or additive when both species co-occur. Also, all larvae in the experiment were exposed only to *A. opacum* cues. This design did not allow me to evaluate plastic reactions to *Dytiscus* cues. The significant gradient in tail muscle shape with *Dytiscus* density found in this study warrants future experiments that examine if trait variation arises as different fixed (non-plastic) traits or as different levels of induced reactions (genotype by environment interactions) to predator cues. In particular, the degree to which different levels of induced reactions evolve under relatively strong gene flow would be an interesting future direction. Finally, correlations between genetically determined population traits and natural selection regimes do not demonstrate causation and therefore should be interpreted cautiously. Clearly, field manipulations of natural selection or genetic variation followed by long-term genetic monitoring would be desirable (Reznick and Ghalambor 2005), although logistically challenging, for this long-lived species.

## Conclusions

Despite much research documenting the relationship between morphology and predator escape in aquatic animals, we have few experimental demonstrations of inter-population differences in defensive morphology consistent with adaptations to different predator communities. A few exceptional studies suggest evolved differences in adaptive morphologies in aquatic organisms (Parejko and Dodson 1991, Endler 1995, Trussell 2000, Langerhans et al. 2004, Reimchen and Nosil 2004) or escape performance suspected to be underlain, at least in part, by morphological differences (Storfer 1999, Ghalambor et al. 2004). This study demonstrates the first case of adaptive morphological differentiation among salamander populations in response to varying predation threats. Relative tail muscle size increased among populations by 0.7 standard deviations along a natural gradient of increasing *Dytiscus* density. In contrast, *A. maculatum* populations did not differ in relative tailfin size across a gradient in *A. opacum* densities despite selection for this trait. I suggest that this lack of an adaptive response might result from a plastic response shared by all populations or lower selection for this trait relative to local gene flow. Overall, results indicate that *A. maculatum* populations likely have evolved at least one morphological trait in response to varying predation risks over microgeographic spatial scales, but gene flow might swamp other traits at these microgeographic scales. If the relative roles of gene flow and selection frequently determine traits involved in species interactions, then we will often need to understand spatial evolutionary dynamics in order to accurately predict the ecological dynamics and structure of natural communities.

*Acknowledgements* – D. Skelly, M. McPeck, O. Schmitz and S. Stearns contributed greatly to the development of this work. S. Bolden and K. Freidenburg provided lab assistance. I thank J. Shurin for comments that improved the manuscript. Research was

supported by generous grants from the John F. Enders and Perry Miller foundations and from the National Center for Ecological Analysis and Synthesis, a Center funded by the NSF (Grant no. DEB-0553768), the Univ. of California, Santa Barbara, and the State of California.

## References

- Bolnick, D. I. and Nosil, P. 2007. Natural selection in populations subject to a migration load. – *Evolution* 61: 2229–2243.
- Bookstein, F. L. 1991. Morphometric tools for landmark data: geometry and biology. – Cambridge Univ. Press.
- Brodie, E. D., III et al. 1995. Visualizing and quantifying natural selection. – *Trends Ecol. Evol.* 10: 313–318.
- Chandler, C. H. and Zamudio, K. R. 2008. Reproductive success by large, closely related males facilitated by sperm storage in an aggregate breeding amphibian. – *Mol. Ecol.* 17: 1564–1576.
- Cousyn, C. et al. 2001. Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. – *Proc. Natl Acad. Sci. USA* 98: 6256–6260.
- Crespi, B. J. 2000. The evolution of maladaptation. – *Heredity* 84: 623–629.
- Dayton, G. H. et al. 2005. Body shape, burst speed and escape behavior of larval anurans. – *Oikos* 111: 582–591.
- De Meester, L. et al. 2002. The monopolization hypothesis and the dispersal-gene flow paradox in aquatic organisms. – *Acta Oecol.* 23: 121–135.
- Endler, J. A. 1986. Natural selection in the wild. – Princeton Univ. Press.
- Endler, J. A. 1995. Multiple-trait coevolution and environmental gradients in guppies. – *Trends Ecol. Evol.* 10: 22–29.
- Fitzpatrick, B. M. et al. 2003. Morphology and escape performance of tiger salamander larvae (*Ambystoma tigrinum mavortium*). – *J. Exp. Biol.* 297A: 147–159.
- Garant, D. et al. 2007. The multifarious effects of dispersal and gene flow on contemporary adaptation. – *Funct. Ecol.* 21: 434–443.
- Ghalambor, C. K. et al. 2004. Constraints on adaptive evolution: the functional tradeoff between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). – *Am. Nat.* 164: 38–50.
- Gomulkiewicz, R. et al. 1999. The effects of density dependence and immigration on local adaptation and niche evolution in a black-hole sink environment. – *Theor. Popul. Biol.* 55: 283–296.
- Grant, B. S. et al. 1996. Parallel rise and fall of melanic peppered moths in America and Britain. – *J. Hered.* 87: 351–357.
- Hendry, A. P. and Gonzalez, A. 2008. Whither adaptation? – *Biol. Philos.* 23: 673–699.
- Jain, S. K. and Bradshaw, A. D. 1966. Evolutionary divergence among adjacent plant populations. I. The evidence and its theoretical analysis. – *Heredity* 21: 407–441.
- Janzen, F. J. and Stern, H. S. 1998. Logistic regression for empirical studies of multivariate selection. – *Evolution* 52: 1564–1571.
- Johnson, J. B. et al. 2008. Form, function and fitness: pathways to survival. – *Evolution* 62: 1243–1251.
- Kenward, M. G. and Roger, J. H. 1997. Small sample inference for fixed effects from restricted maximum likelihood. – *Biometrics* 53: 983–997.
- Kingsolver, J. G. et al. 2001. The strength of phenotypic selection in natural populations. – *Am. Nat.* 157: 245–261.
- Kishida, O. and Nishimura, K. 2004. Bulgy tadpoles: inducible defense morph. – *Oecologia* 140: 414–421.
- Lande, R. and Arnold, S. J. 1983. The measurement of selection on correlated characters. – *Evolution* 37: 1210–1226.

- Langerhans, R. B. et al. 2004. Predator-driven phenotypic diversification in *Gambusia affinis*. – *Evolution* 58: 2305–2318.
- Lardner, B. 1998. Plasticity or fixed adaptive traits? strategies for predator avoidance in *Rana arvalis* tadpoles. – *Oecologia* 117: 119–126.
- Larson, D. J. et al. 2000. Predaceous diving beetles (Coleoptera: Dytiscidae) of the Nearctic region with emphasis on the fauna of Canada and Alaska. – NRC Research Press.
- Lynch, M. and Walsh, B. 1998. Genetics and analysis of quantitative traits. – Sinauer.
- Manly, B. F. J. 2001. Randomization, bootstrap and Monte Carlo methods in biology. – Chapman and Hall/CRC.
- Moore, J.-S. and Hendry, A. P. 2005. Both selection and gene flow are necessary to explain adaptive divergence: evidence from clinal variation in stream stickleback. – *Evol. Ecol. Res.* 7: 871–886.
- Mousseau, T. A. and Roff, D. A. 1987. Natural selection and the heritability of fitness components. – *Heredity* 59: 181–197.
- Nosil, P. et al. 2005. Reproductive isolation caused by natural selection against immigrants from divergent habitats. – *Evolution* 59: 705–719.
- Parejko, K. and Dodson, S. I. 1991. The evolutionary ecology of an antipredator reaction norm: *Daphnia pulex* and *Chaoborus americanus*. – *Evolution* 45: 1665–1674.
- Purrenhage, J. L. et al. 2009. Population structure of spotted salamanders (*Ambystoma maculatum*) in a fragmented landscape. – *Mol. Ecol.* 18: 235–247.
- Reimchen, T. E. and Nosil, P. 2004. Variable predation regimes predict the evolution of sexual dimorphism in a population of threespine sticklebacks. – *Evolution* 58: 1274–1281.
- Relyea, R. A. 2002. Local population differences in phenotypic plasticity: predator-induced changes in wood frog tadpoles. – *Ecol. Monogr.* 72: 77–93.
- Reznick, D. N. and Ghalambor, C. K. 2005. Selection in nature: experimental manipulations of natural populations. – *Integr. Comp. Biol.* 45: 456–462.
- Reznick, D. N. et al. 1990. Experimentally induced life-history evolution in a natural population. – *Nature* 346: 357–359.
- Scheiner, S. M. 2001. MANOVA: multiple response variables and multispecies interactions. – In: Scheiner, S. M. and Gurevitch, J. (eds), Design and analysis of ecological experiments. Oxford Univ. Press, pp. 99–115.
- Schemske, D. W. and Bierzychudek, P. 2001. Evolution of flower color in the desert annual *Linanthus parryae*: Wright revisited. – *Evolution* 55: 1269–1282.
- Schluter, D. and Nychka, D. 1994. Exploring fitness surfaces. – *Am. Nat.* 143: 597–616.
- Scott, D. E. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum*. – *Ecology* 75: 1383–1396.
- Skelly, D. K. 2004. Microgeographic countergradient variation in the wood frog, *Rana sylvatica*. – *Evolution* 58: 160–165.
- Slatkin, M. 1985. Gene flow in natural populations. – *Annu. Rev. Ecol. Syst.* 16: 393–430.
- Sokal, R. R. and Rohlf, F. J. 1995. Biometry: the principles and practice of statistics in biological research. – W. H. Freeman and Co.
- Sork, V. L. et al. 1993. Evidence for local adaptation in closely adjacent subpopulations of northern red oak (*Quercus rubra* L.) expressed as resistance to leaf herbivores. – *Am. Nat.* 142: 928–936.
- Storfer, A. 1999. Gene flow and local adaptation in a sunfish–salamander system. – *Behav. Ecol. Sociobiol.* 46: 273–279.
- Storfer, A. and Sih, A. 1998. Gene flow and ineffective antipredator behavior in a stream-breeding salamander. – *Evolution* 52: 558–565.
- Teplitsky, C. et al. 2005. Escape behaviour and ultimate causes of specific induced defences in an anuran tadpole. – *J. Evol. Biol.* 18: 180–190.
- Thompson, J. N. 2005. The geographic mosaic of coevolution. – Univ. of Chicago Press.
- Trussell, G. C. 2000. Phenotypic clines, plasticity, and morphological tradeoffs in an intertidal snail. – *Evolution* 54: 151–166.
- Urban, M. C. 2007a. The growth–predation risk tradeoff under a growing gape-limited predation threat. – *Ecology* 88: 2587–2597.
- Urban, M. C. 2007b. Predator size and phenology shape prey survival in temporary ponds. – *Oecologia* 154: 571–580.
- Urban, M. C. 2007c. Risky prey behavior evolves in risky habitats. – *Proc. Natl Acad. Sci. USA* 104: 14377–14382.
- Urban, M. C. 2008. Salamander evolution across a latitudinal cline in gape-limited predation risk. – *Oikos* 117: 1037–1049.
- Urban, M. C. and Skelly, D. K. 2006. Evolving metacommunities: toward an evolutionary perspective on metacommunities. – *Ecology* 87: 1616–1626.
- Van Buskirk, J. and McCollum, S. A. 2000a. Functional mechanisms of an inducible defence in tadpoles: morphology and behaviour influence mortality risk from predation. – *J. Evol. Biol.* 13: 336–347.
- Van Buskirk, J. and McCollum, S. A. 2000b. Influence of tail shape on tadpole swimming performance. – *J. Exp. Biol.* 203: 2149–2158.
- Van Buskirk, J. and Schmidt, B. R. 2000. Predator-induced phenotypic plasticity in larval newts: trade-offs, selection, and variation in nature. – *Ecology* 81: 3009–3028.
- Van Buskirk, J. and Arioli, M. 2005. Habitat specialization and adaptive phenotypic divergence of anuran populations. – *J. Evol. Biol.* 18: 596–608.
- Van Buskirk, J. et al. 1997. Natural selection for environmentally induced phenotypes in tadpoles. – *Evolution* 51: 1983–1992.
- Van Buskirk, J. et al. 2003. The lure effect, tadpole tail shape and the target of dragonfly strikes. – *J. Herpetol.* 37: 420–424.
- Wilson, R. S. et al. 2005. Predator-specific changes in the morphology and swimming performance of larval *Rana lessonae*. – *Funct. Ecol.* 19: 238–244.
- Wright, S. 1931. Evolution in Mendelian populations. – *Genetics* 16: 97–159.
- Yurewicz, K. L. 2004. A growth/mortality tradeoff in larval salamanders and the coexistence of intraguild predators and prey. – *Oecologia* 138: 102–111.
- Zamudio, K. R. and Wieczorek, A. M. 2007. Fine-scale spatial genetic structure and dispersal among spotted salamander (*Ambystoma maculatum*) breeding populations. – *Mol. Ecol.* 16: 257–274.

## Appendix 1

### *Predator density correlations across years*

The mean densities of each predator from each of the 14 ponds studied were correlated across each of the years for which data was available. Note that spring densities were used for *A. opacum* as this predator leaves the pond in mid-summer. *Dytiscus*, in contrast, usually do not recruit until later in the spring and often remains in ponds until late summer/early fall. These inter-year correlations were then averaged to obtain the average temporal correlation in predator densities for the system. Note that I had data on *A. opacum* densities for 2009, when I resumed sampling after returning to this research, but not for *Dytiscus*.

Pearson's correlations across years for *A. opacum* spring densities among ponds.

	2002	2003	2004
2002			
2003	0.37		
2004	0.24	0.55	
2009	0.49	0.85	0.71

Mean correlation ( $\pm$  SE) =  $0.54 \pm 0.09$

Pearson's correlations across years for *Dytiscus* overall densities among ponds.

	2002	2003
2002		
2003	0.80	
2004	0.44	0.18

Mean correlation ( $\pm$  SE) =  $0.47 \pm 0.18$

## Appendix 2

### *Description of morphological landmarks used in analyses*

See Fig. 2 in text for numbered locations. Landmarks: 1 – nose; 2 – top of head at posterior of eye; 3 – bottom of head at posterior of eye; 4 – top of head at maximum head depth; 5 – bottom of head at maximum head depth; 6 – front of forelimb; 7 – body midpoint between 6 and 11; 8 – top of tailfin at posterior end of vent; 9 – top of trunk at vent; 10 – bottom of trunk at vent; 11 – bottom of tailfin at vent; 12 – top of tailfin at midpoint of tail (between points 11 and 16); 13 – top of tailfin at midpoint; 14 – top of tail at midpoint; 15 – bottom of tail at midpoint; 16 – tail tip; 17 – nose; 18, 19 – head width at posterior edge of eyes; 20, 21 – maximum head width; 22, 23 – neck width at front of forelimbs; 24, 25 midpoint body width; 26, 27 – trunk width at posterior end of vent; 28, 29 – tail muscle width at tail midpoint; 30 tail tip. Lengths used in measurements: a – maximum head width; b – tailfin area approximated as the area of a parabola circumscribed by a rectangle with base equal to tailfin height at tail midpoint and height equal to tail length; c – tail muscle cross-sectional area estimated from the ellipse connecting points 13, 14, 28 and 29.

## Appendix 3

Initial means and standard deviations for traits evaluated in selection experiments.

Traits	Predator	
	<i>A. opacum</i>	<i>Dytiscus</i>
Centroid body size	$2.02 \pm 0.12$	$2.35 \pm 0.14$
Relative head width	$0.00 \pm 0.07$	$0.00 \pm 0.07$
Relative tailfin size	$0.00 \pm 0.12$	$0.00 \pm 0.08$
Relative tail muscle size	$0.00 \pm 0.19$	$0.00 \pm 0.18$

Note. – Relative morphological measures were calculated as the residuals of each ln-transformed trait on ln-transformed centroid body size.

## Appendix 4

A bi-projection pursuit regression of survival under *Dytiscus* attack in relation to the two significant variables, centroid body size and relative tail muscle size, in the full analysis.

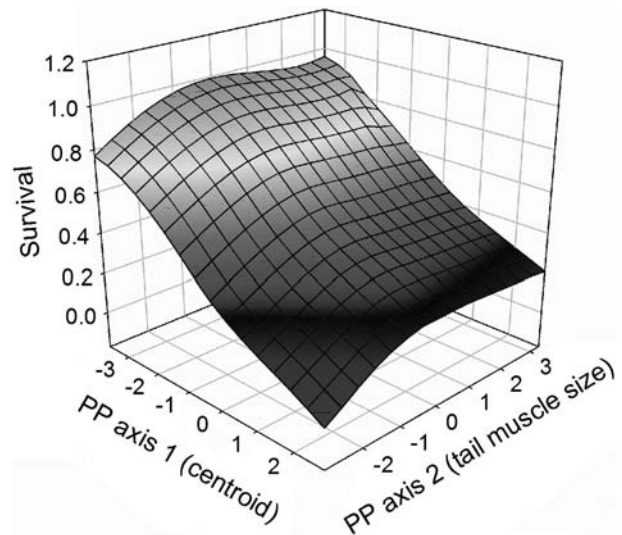


Figure A1. Results from a bi-projection pursuit regression of survival under *Dytiscus* attack in relation to the two significant variables, centroid body size and relative tail muscle size, in the full analysis. Centroid body size dominates the first projection pursuit axis (centroid: 1.00; relative tail muscle size: 0.49) whereas tail muscle size dominates the second axis (centroid: 0.10; relative tail muscle size: 0.87). Excluding relative tail width and tailfin size, this analysis suggests that selection by *Dytiscus* would favor small *A. maculatum* larvae with larger relative tail muscles. This analysis does not suggest any advantage to being a large larva with smaller tail muscles.

## Appendix 5

Predator densities in ponds and *A. maculatum* trait means and standard deviations from common garden experiment.

Population*	<i>A. opacum</i> mean ln density (no. m <sup>-2</sup> )	<i>Dytiscus</i> mean ln density (no. m <sup>-2</sup> )	Body size (SD)	Relative head width (SD)	Relative tailfin size (SD)	Relative tail muscle size (SD)
1	0.04	0.19	1.962 (0.042)	0.010 (0.081)	-0.002 (0.058)	-0.064 (0.170)
2	0.42	0.24	1.975 (0.043)	0.010 (0.045)	-0.006 (0.059)	0.028 (0.223)
5	0.51	0.00	1.981 (0.038)	-0.010 (0.046)	-0.007 (0.072)	-0.087 (0.250)
6	0.06	0.10	1.964 (0.041)	-0.008 (0.071)	0.006 (0.067)	-0.027 (0.178)
7	0.24	0.16	1.961 (0.045)	0.026 (0.061)	0.036 (0.050)	-0.025 (0.203)
9	0.33	0.05	1.982 (0.027)	-0.014 (0.054)	0.007 (0.059)	-0.004 (0.145)
12	0.50	0.29	1.979 (0.041)	0.003 (0.054)	-0.008 (0.062)	0.058 (0.179)
13	0.00	0.41	2.001 (0.040)	0.000 (0.056)	0.007 (0.074)	0.059 (0.184)
14	0.21	0.21	1.970 (0.036)	-0.002 (0.056)	-0.004 (0.083)	0.044 (0.223)
16	0.98	0.10	1.968 (0.037)	-0.011 (0.053)	-0.029 (0.065)	0.051 (0.168)

Note. – \* see Fig. 1 in main text for map of locations corresponding to these numbers.