

The evolution of prey body size reaction norms in diverse communities

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Summary

1. Heterogeneous predation risks can select for predator-specific plastic defences in prey populations. However, diverse predation threats can generate diffuse selection, which, in turn, can lead to the evolution of more generalized reaction norms. Unreliable predator cues also can select for more generalized plasticity in prey.
2. Here, I evaluated the extent to which variation in risk from a focal predator vs. variation in risk from predator diversity and composition were associated with variation in body mass reaction norms in 18 prey populations. Toward this end, I assayed the body mass reaction norms in a common garden experiment for spotted salamander larvae *Ambystoma maculatum* in response to marbled salamander predators *Ambystoma opacum*, local predator richness and the densities of two auxiliary predator species.
3. When raised under controlled conditions, prey larvae generally were smaller when exposed to *A. opacum* kairomones. Among populations, the mean and slope of body mass variation was unrelated to *A. opacum*'s local density.
4. Predator richness and several key environmental factors were not associated with reaction norm variation. Instead, the density of an auxiliary newt predator species was correlated with reduced mass reaction norm slopes. Results suggest that diffuse selection from auxiliary predators can modify the evolution of life-history plasticity.

Key-words: common garden experiments, microgeographical adaptation, predator kairomones, prey size refuge, temporary pond amphibians.

Introduction

Geographic variation in predator composition supplies a common source of divergent selection on prey defences that can elicit different evolutionary trajectories among populations (Reznick, Bryga & Endler 1990; Parejko & Dodson 1991). Because predator selection regimes normally vary across space and time, prey often respond to reliable predator cues with species-specific plastic defences (Tollrian & Harvell 1999). What is less known is the degree to which a reaction norm – the specific relationship between phenotype and environment evaluated for genetically related individuals (Woltereck 1909) – evolves in response to selection by a focal predator species or, alternatively, evolves toward a more generalized response under diffuse selection by a diverse pool of potential predator species (Janzen 1980; Poitrineau, Brown & Hochberg 2003; Strauss & Irwin 2004).

Here I evaluate the body size reaction norms of spotted salamander *Ambystoma maculatum* (Shaw) larvae in response to the predatory marbled salamander's *Ambystoma opacum* (Gravenhorst) waterborne chemical cues emitted during the consumption and digestion of prey (hereafter kairomones). I assayed the reaction norms of 18 *A. maculatum* populations from three geographically separated regions distributed across a latitudinal gradient in predator composition. Physiological growth and its relation to body size plays a crucial role in shaping a population's demography and evolution because body size influences multiple fitness components such as future fecundity, survival under predation risk, and the ability to perform ontogenetic shifts (Werner & Gilliam 1984; Nylín & Gotthard 1998). In this system, larval body size mediates *A. maculatum*'s survival during encounters with *A. opacum* salamanders because these predators only can capture prey smaller than their maximum gape width (Stenhouse 1985). At the same time, foraging behaviour, which is often correlated with growth, also increases *A. maculatum*'s predation risk from *A. opacum* by elevating the probability of its detection by this movement-orientated predator (Urban 2006). Hence, prey foraging and growth determine a trade-off

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between a prey individual's probability of detection and its probability of capture (Lima & Dill 1990). Theory predicts that prey exposed to intense gape-limited predation will evolve either rapid or slow growth rates depending on the costs associated with rapid growth into a size refuge and the cumulative risks from other types of predators (Case 1978; Day, Abrams & Chase 2002; Urban 2007a). In particular, high predation risk from gape-limited predators combined with a low cost to risky foraging behaviour can select for rapid growth. On the other hand, high risk from gape-unconstrained predators or strong mortality risks due to risky foraging behaviour can select for cautious behaviour and slow growth.

In this study, I evaluated two key questions:

1. When reared under similar conditions, do *A. maculatum* larvae from ponds that vary in *A. opacum* density differ in their body mass reaction norms in response to *A. opacum* kairomones? Plastic growth offers a flexible solution to heterogeneous selection for it provides prey with the opportunity to alter their instantaneous growth rates depending on the current predator composition (Pigliucci 2001). The evolution of plastic defences requires substantial spatial or temporal heterogeneity in predation risks, predictable changes in predation threats (e.g. reliable predator kairomones), and the absence of strong costs and limits to plasticity, such as might characterize the maintenance of sensory systems, be associated with mistaken phenotypic expression, or describe organisms with minimal additive genetic variation for plasticity (DeWitt 1998; Tollrian & Harvell 1999). In the absence of a particular predator, costs to plasticity can lead to the loss of specific induced defences (e.g. Cousyn *et al.* 2001). In this system, predator composition changes dramatically across sites, across ponds and during the developmental season (Urban 2007b). Given this predator heterogeneity and assuming moderate costs to plasticity, I predicted that *A. maculatum* larvae from ponds with high densities of *A. opacum* predators would modify their growth in response to *A. opacum* kairomones while those from ponds lacking the predator would not.
2. Does predator richness or composition constrain the evolution of predator-specific defensive reaction norms? Most prey populations face diverse predator species (Sih, Englund & Wooster 1998), and this system is not an exception: *A. maculatum* larvae coexist with up to 14 predator species. Assuming interference among defences against different predators, the optimal investment in two predator-specific defences is expected to decline at high joint predator densities (Poitrineau *et al.* 2003). Empirical research has shown that the combination of induced prey traits that best defend against one predator can increase the mortality risk for prey from other predators (Kishida & Nishimura 2005; Benard 2006). More generally, the presence of multiple predators can alter prey fitness in nonadditive ways, thereby frustrating predator-specific prey evolution (Iwao & Rausher 1997; Strauss & Irwin 2004). Genetic correlations among defensive traits or

traits used to recognize kairomones from different predator species also can constrain predator-specific responses in a given environment (Tollrian & Harvell 1999). However, the dependence of genetic correlations on specific environmental contexts may lessen the role of this constraint in heterogeneous environments (Sgro & Hoffmann 2004). General adaptations also might evolve when predator cues are unreliable or a sufficient lag time exists between sensing a threat and developing a response (DeWitt 1998). Thus, in many cases, the optimal defence may be to adapt to a more diffuse pattern of selection by employing a general or average defensive phenotype (Van Buskirk 2001). This leads to the prediction that specific induced responses to focal predators will be reduced in high-diversity predator communities and replaced by general responses (Langerhans & DeWitt 2002; Poitrineau *et al.* 2003; Relyea 2003; Strauss & Irwin 2004). To date, most studies have evaluated either the genetic integration of defences or changes in selection attributed to auxiliary predators rather than variation in the outcome of diffuse selection, the diffuse evolution of responses to predator diversity and composition (Strauss & Irwin 2004; Lau 2006). Here I tested whether the diversity and composition of auxiliary predators has altered the evolution of prey life histories by comparing body mass reaction norm slopes in response to a focal predator among prey populations distributed across a natural gradient in mean annual predator richness and predator community composition.

Materials and methods

NATURAL HISTORY

The spotted salamander *A. maculatum* is a relatively large terrestrial salamander (up to 33 g) that inhabits eastern North America. Each spring, adults move from uplands into temporary ponds to mate and to lay eggs. Larvae hatch after 8–10 weeks and emerge as small (c. 15 mg) aquatic larvae that are vulnerable to diverse vertebrate and invertebrate predators. Predatory marbled salamander larvae *A. opacum* substantially reduce the survival of early stage *A. maculatum* larvae in the field (Stenhouse 1985; Urban 2007b). *A. opacum* are gape-limited predators that, on average, feed on prey items with a maximum body width less than 37% of their gape width (Urban 2006). As a consequence, *A. opacum* larvae induce selection for larger body size in developing *A. maculatum* larvae (Urban 2006). At the same time, selection may act to reduce growth-correlated foraging activity in *Ambystoma* larvae because foraging usually is associated with an elevated predation risk (Storfer & Sih 1998). Previous research on the same *A. maculatum* populations has demonstrated a significant positive phenotypic correlation between foraging behaviour (measured as movement rate, feeding frequency, and refuge microhabitat use) and larval mass. Together, these behaviours explain 73% of the variation in larval mass (Urban 2006). Other research has shown that *A. maculatum* populations that forage frequently encounter higher risks of *A. opacum* predation in prey population choice experiments before they have entered a size refuge (Urban 2007). Hence, larval growth affects predation risk both through its correlation with risky foraging behaviours and by providing a size refuge from gape-limited predators.



Fig. 1. Map of study sites in southern New England, USA. From south to north, study sites were located in Northford (CT), Union (CT) and Chesterfield (NH). *A. opacum* salamanders are common at the southernmost site, less abundant at the intermediate site and do not occur at the northern site because this site is located beyond their northern range limit.

In addition to predator species richness, the densities of two key auxiliary predators were evaluated for their potential effects on the evolution of *A. maculatum* growth responses to *A. opacum*. Red-spotted newt adults *Notophthalmus viridescens* (Rafinesque) and diving beetle larvae *Dytiscus* spp. impose significant mortality on *A. maculatum* larvae. These two predators often co-occur with *A. opacum*: 42% and 84% of prey populations that were collected with *A. opacum* also were collected with newts and *Dytiscus*, respectively. Newt adults are strongly gape-limited (Wilbur & Fauth 1990) and likely impose selection for large body size in *A. maculatum* hatchlings. Under common garden conditions, *A. maculatum* larvae grow 23% more rapidly when exposed to kairomones from *N. viridescens* adults compared with individuals raised under control conditions (Urban 2006). In contrast to the other two predators, *Dytiscus* larvae are gape-unconstrained and preferentially prey upon larger bodied *A. maculatum* larvae (Urban 2006).

STUDY SITES AND PREDATOR DISTRIBUTIONS

Salamander populations were studied at three sites along a latitudinal gradient in southern New England, USA (Fig. 1). Each of these sites encompasses a 2 km² area of undisturbed deciduous forest at altitudes of < 300 m. *A. opacum* varies in its distribution across sites, such that at the southern site (Northford, CT) *A. opacum* occurs at high densities, at the intermediate site (Union, CT) *A. opacum* occurs less regularly, and at the northern site (Chesterfield, NH) *A. opacum* does not occur at all. At each site, 12–14 populations of *A. maculatum* were identified. A total of 18 study populations (six populations from each of three sites) were assayed for their mass reaction norms for this study. I stratified sampling across overall mean predator richness so as to represent the full natural gradient in predator richness at each site. Field predator densities were sampled by dip net at least every 2 weeks during *A. maculatum*'s development over the course of 3 years and predators were identified as described in Urban (2006). Predator diversity was estimated as the mean species richness of potential *A. maculatum* predators averaged over 3 years. The mean predator richness of sampled populations varied from 1.3 to 11.0.

COMMON GARDEN EXPERIMENT

In spring 2005, I collected six egg mass samples from each pond within 2 days of egg laying (as determined by visual surveys every 2 days). Egg masses were sampled from divergent locations within each pond. Although the extent of within-pond movement by males during the breeding season is unknown, this design likely samples different fathers as well as mothers because courtship takes place over limited spatial scales and adults usually enter and leave the pond at the same location (Petranka 1998). Divergent breeding dates among ponds within sites and among sites meant that egg masses were collected over a period of 2½ weeks. Upon collection, egg masses were immediately transported in ice-cooled, aerated containers to Greeley Laboratory (New Haven, CT, USA) where they were maintained in an incubator (Precision model 818, Winchester, VA, USA) at 8.0 °C, a temperature that slows further development, so that all eggs would be exposed to the same outdoor temperatures at similar stages and on the same date. The mean stage (Harrison 1969) of egg sections used in the experiment was not significantly related to site of origin upon outdoor initiation of this experiment ($F_{2,15} = 0.3$, $P = 0.734$) in a mixed effects model with site as a fixed factor and population as a random effect.

Once all egg masses were collected, two sections of six eggs were separated from each egg mass with a sterilized scalpel. Each egg mass section was placed in a 19-L container under 50% shade cloth in an outdoor enclosure and exposed to either water conditioned by live *A. opacum* or control water handled in the same way but without predators. Hence, kairomone treatment was nested within full-sib family. Each site was represented by six ponds, and each pond by six families (2 treatments × 6 families × 6 ponds × 3 sites = 216 replicates). Containers were distributed among six blocks orientated three to a side along an east–west axis (perpendicular to southern sunlight exposure and potential spatial heterogeneities in temperature). Each population was assigned to each block such that treatments applied to the same family were randomly distributed within the same block and a family from each population was found in each block. The effect of the block on larval mass was not significant

in a mixed effects model with the random effects population, family and container (likelihood ratio = 0.2, d.f. = 1, $P = 0.620$) and was not analysed further in subsequent models.

Each egg section was censused every 2 days until hatching occurred. For the small number of replicates in which some egg mortality was observed (5% of replicates), dead eggs were removed and replaced by eggs held in the laboratory for this purpose. No trends were observed in the distribution of arcsin-transformed egg mortality at the container level between treatments ($F_{1,105} = 0.0$, $P = 1.000$), sites ($F_{2,15} = 0.6$, $P = 0.551$), or their interaction ($F_{2,105} = 0.4$, $P = 0.687$) in a mixed-model with population and family as nested random effects. Replacement of eggs did not affect hatching date ($F_{1,107} = 0.1$, $P = 0.798$) or final larval mass ($F_{1,107} = 0.0$, $P = 0.891$) in a similarly constructed mixed model. Once all eggs had hatched, the number of surviving larvae was reduced to four in each container; this yielded an initial larval density (62 per m²) that was within the range of natural *A. maculatum* field densities (Brodman 1996). All remaining laboratory-raised reserve hatchlings (90% of families represented) were weighed after all experimental eggs had hatched in order to obtain an estimate of family-level initial hatchling mass. I estimated initial mass from these surplus individuals because weighing hatchlings directly involved in the experiment would have caused significant mortality.

Three times each week, I added either 1 L of water conditioned by *A. opacum* larvae or 1 L of control water to each container depending on randomly assigned treatments. The *A. opacum* kairomone treatment consisted of water from nine 68-L containers in which *A. opacum* were fed *A. maculatum* larvae *ad libitum*. Control water was maintained in the same way except that it lacked predators. A small overflow hole in each *A. maculatum* container allowed for a gradual reduction in volume to a standardized level. Conditioned and control water treatments were filtered with 70 µm Nitex screen to prevent the introduction of different food resources. In previous experiments, *A. maculatum* larvae exposed to *A. opacum* kairomones obtained in the same way and under similar common garden conditions induced significantly lower activity outside of structural refuge habitat, which was expected to lead to lower foraging opportunities (Urban 2006). However, final mass was not significantly lower in the kairomone treatment compared with the control in this previous experiment. One explanation for this pattern is that final masses were measured long after the natural period of *A. opacum* risk in the wild and thus compensatory prey growth late in the season could have occurred. Second, food was provisioned equally among containers, and thus growth rates in containers were confounded with differential survival. In the experiment presented here, I evaluated larval mass during the natural period of *A. opacum* risk. I also provided homogenized aliquots of cultured *Daphnia magna* on a per-capita basis following twice-weekly assays of container survival.

I evaluated the final body mass of larval salamanders after 4 weeks of accumulated growth under controlled conditions. I was interested in final body size at 4 weeks post-hatching because field observations indicate that *A. maculatum* vulnerability to *A. opacum* decreases rapidly following this critical developmental period (Urban 2007b). The wet mass of each larva was measured to the nearest 0.1 mg on a Mettler AE 100 balance (Mettler-Toledo Inc., Columbus, OH, USA). Following detection of strong right-skew with the K^2 -statistic, the final mass of each larva was ln-transformed. Several observations suggested that final larval mass was correlated with growth rate. Larvae were raised under standardized feeding conditions and temperatures for the same time period to minimize external environmental influences on final mass to the greatest

extent possible. For those samples for which I had estimates of initial hatchling mass from reserve family means, final mass was not significantly correlated with initial mass ($\rho = 0.08$; $F_{1,88} = 1.0$; $P = 0.322$) in a mixed effects model with a random effects population and family. Initial masses also did not differ significantly among sites (fixed effect: $F_{2,15} = 1.8$; $P = 0.203$). However, population and family means of initial mass were significantly different (random effect tested using likelihood ratio tests (LR_{df}) (populations: $LR_1 = 9.9$, $P = 0.002$; families: $LR_1 = 122.2$; $P < 0.001$). Therefore, extrapolation of results obtained with final masses to population and family growth rates should be treated with caution. However, I re-did the analyses in the manuscript using the sparser size-specific growth data [(ln final mass – ln initial mass)/time] and found qualitatively similar patterns as those obtained using the complete final mass data.

In the common garden experiment, I recovered larvae from all replicates. Mean survival was 86% within containers and was not affected significantly by site, treatment or their interaction (mixed effects model with population and family as random effects; site: $F_{2,15} = 0.3$; $P = 0.770$; treatment: $F_{1,105} = 1.7$; $P = 0.199$; interaction: $F_{2,105} = 0.8$; $P = 0.433$). Container survival was not a significant covariate in determining eventual mass (random effects as in model 5, Table 1: $F_{1,104} = 1.4$; $P = 0.233$). Median hatching date did not differ significantly among sites ($F_{2,15} = 0.9$; $P = 0.431$), treatments ($F_{1,105} = 1.2$; $P = 0.279$), or as an interaction between the two factors ($F_{2,105} = 0.3$; $P = 0.762$).

STATISTICAL ANALYSES

Hierarchical models of phenotypic variation

I used a series of multilevel mixed effects models to estimate the factors underlying phenotypic variation across hierarchical levels of site, population and family. Final mass was evaluated with respect to the fixed factors treatment, site and their interaction. Site was analysed as a fixed effect because specific locations were chosen to represent the heterogeneous distribution of *A. opacum* across the study region (Bennington & Thayne 1994). Random effects included container nested within full-sibs family (clutch) which, in turn, was nested within population (population/family/container). All analyses were performed in S-Plus v. 6.1 (Insightful Corp., Seattle, WA, USA). I applied orthogonal a priori contrasts to examine the differences between the southern and northern sites (high *A. opacum* density site and no-*A. opacum* site) and between both the southern and intermediate sites and the northern site (both *A. opacum* sites vs. no-*A. opacum* site).

The contributions of random effects to explanations of phenotypic variation were evaluated through a set of nested models of differing complexity. All models were parameterized with the two fixed effects and their interaction. The minimal model lacked random error structure. Subsequent models were considered by adding each nested random term in turn and applying the likelihood ratio test to evaluate if the introduction of each term explained significant additional phenotypic variation (Pinheiro & Bates 2000). Standardized residuals were plotted across each hierarchical level to assess variance heterogeneity. Where heteroscedasticity was detected, a model of nonconstant error variance was applied and tested for fit using a likelihood ratio test (Pinheiro & Bates 2000). The interaction between kairomone treatment and random effects was omitted in initial models, which amounts to assuming heterogeneous elevations of reaction norms, but parallel reaction norm slopes. More sophisticated models were then fit with heterogeneous reaction

Table 1. Comparisons of hierarchical mixed effects models. Model 1 is the minimal (no random effects) model. Each successive model includes additional nested random effects. The *P*-value associated with a particular model represents the likelihood ratio test of the particular model compared with the model indicated in the column labelled 'Test'. Random treatment interactions incorporate both variation in slopes and intercepts. These were tested against a model with similar structure but lacking slope heterogeneity to test for an effect of variable reaction norm slopes. Models 5 and 9 include a different variance structure between populations exposed to control or *A. opacum* kairomones. The overall best model as described by the minimal Akaike Information Criterion (AIC) is in **bold**. Note that no model exists for the treatment–container interaction because reaction norms were only estimated across containers

	AIC	Log(L)	d.f.	Test	Likelihood ratio	Δd.f.	<i>P</i>
<i>Nested model</i>							
Random intercepts							
1. mass = treatment × site	331.2	−157.6	7				
2. mass = treatment × site, random = population	318.2	−151.1	8	1 vs. 2	13.0	1	< 0.001
3. mass = treatment × site, random = population/family	245.6	−113.8	9	2 vs. 3	74.6	1	< 0.001
4. mass = treatment × site, random = population/family/container	70.0	−25.0	10	3 vs. 4	177.6	1	< 0.001
5. Model 4 with heteroscedastic variance among ponds across treatment	48.7	20.7	45	4 vs. 5	91.3	35	< 0.001
Random intercepts and slopes							
6. mass = treatment × site, random = treatment × population	307.8	−143.9	10	2 vs. 6	14.4	2	< 0.001
7. mass = treatment × site, random = treatment × population/family	73.9	−23.9	13	3 vs. 7	179.7	4	< 0.001
				6 vs. 7	239.9	3	< 0.001
8. mass = treatment × site, random = treatment × population/family/container*	75.9	−23.9	14	4 vs. 8	2.1	4	0.723
				7 vs. 8	0.0	1	0.993
9. Model 8 with heteroscedastic variance among ponds across treatment	54.7	21.7	49	5 vs. 9	2.0	4	0.733
				8 vs. 9	41.2	35	< 0.001

*Container is nested within family but does not interact with treatment.

norm slopes and their significance was assessed with a likelihood ratio test comparing models with and without random reaction norm slopes at each hierarchical level (Brommer *et al.* 2005). All models also were assessed by the parameter-penalized log-likelihood statistic, the Akaike information criterion (AIC).

The phenotypic correlation between larval mass under *A. opacum* kairomones and control conditions for individuals within each full-sib family was calculated to determine if it differed significantly from absolute one. Rejecting an absolute correlation of one suggests the potential for genotype × environment interactions and that trait variation is determined, at least in part, by different genes (Falconer 1952). I estimated confidence intervals around the mean phenotypic correlation from the distribution of mean correlations obtained from 10 000 bootstrapped samples (Lynch & Walsh 1998).

Environmental selection regimes

To test whether *A. opacum* densities in natal habitats were associated with variation in reaction norms to *A. opacum* kairomones, a mixed effects analysis of covariance (ANCOVA) model was analysed with the fixed factors, *A. opacum* mean density in natal ponds, treatment, and site and the random effects structure of the minimum-AIC model determined in initial analyses (model 5, Table 1). I applied the same approach to understand the potential effects of selection from different predator species on prey evolution (i.e. diffuse evolution) and from alternative environmental selection regimes. In this model, I jointly analysed the relationships between *A. maculatum* mass reaction norms and predator richness, the density of two auxiliary predators of *A. maculatum* (*N. viridescens* and *Dytiscus* spp.), intraspecific density (*A. maculatum* mean egg density), and pond permanence (mean number of days from breeding date until the pond dried or the end of the developmental season). In this model, all variables and their interactions with treatment were entered initially as dependent variables. Interactions were removed if they did

not contribute to variance explained in a stepwise fashion. At each step, the interaction term with the highest *P*-value was removed and the model was re-estimated. The interaction was removed if the likelihood ratio test between the models with and without this term suggested that including the term did not result in a better model fit (Crawley 2002). To allow comparisons among models with different fixed effects structures, I estimated models using unrestricted maximum likelihood (Pinheiro & Bates 2000).

Lastly, I explored how newt densities affected mass reaction norms in more detail. I parameterized a mixed linear regression model where the split-family reaction norm slopes were entered as response variables and newt density was entered as an explanatory variable. Pond was introduced as a random effect. Initial analyses of standardized residuals suggested strong heteroscedasticity with respect to newt densities. To address this nonconstant variance, I modelled the variance–newt density relationship using a power relationship (Pinheiro & Bates 2000). Incorporating heterogeneous reaction norm slope variance was supported by likelihood ratio tests ($LR_1 = 5.1$, $P = 0.024$).

Results

ESTIMATES OF VARIATION AMONG PONDS, FAMILIES AND INDIVIDUALS

In models with random intercepts, population, family and container each explained significant variation in larval mass (Table 1, $P < 0.001$). By far the most variation characterized individuals within a container followed by variation among populations (Table 2). The least variation occurred among families. Plots of the mean population reaction norms suggested that populations varied more under control, rather than *A. opacum* kairomone conditions (Figs 2 and 3). I explicitly

Table 2. Estimates of parameter values and variance components for the minimum-AIC model 5 (see Table 1) and the most sophisticated model (no. 9)

Fixed effects	Estimate (SE)	d.f.	<i>F</i>	<i>P</i>
Model 5				
Treatment	-0.104 (0.017)	1,105	35.8	< 0.001
Site				
S vs. N	0.021 (0.028)	2,15	1.7	0.217
S & I vs. N	0.030 (0.028)			
Site × treatment				
S vs. N	-0.019 (0.025)	2,105	0.3	0.739
S & I vs. N	0.012 (0.024)			
Random effects	Variance component			
	(intercept)			
Pond	0.0015			
Family	0.0003			
Container	0.0550			
Residual	0.0958			
Model 9				
Treatment	-0.104 (0.019)	1,105	30.3	< 0.001
Site				
S vs. N	0.021 (0.029)	2,15	1.5	0.246
S & I vs. N	0.030 (0.029)			
Site × treatment				
S vs. N	-0.020 (0.027)	2,105	0.3	0.748
S & I vs. N	0.011 (0.027)			
Random effects	Variance component	Variance		
	(intercept)	component (slope)		
Pond	0.0026	0.0013		
Family	0.0052	0.0050		
Container	0.0429			
Residual	0.0955			

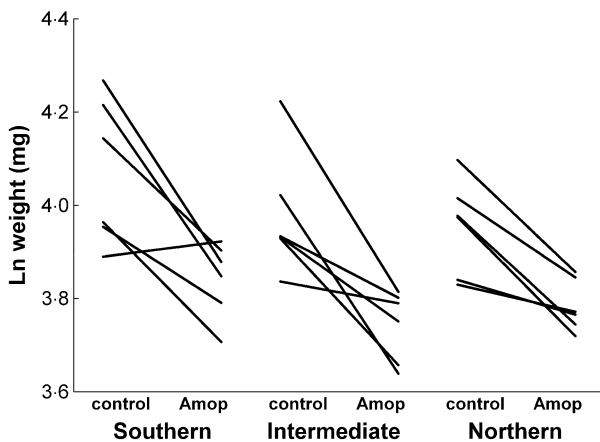


Fig. 2. Mean prey size reaction norms (final mass in mg, ln-transformed) of *A. maculatum* populations in response to no predators (control) and predator kairomones from *A. opacum* (Amop) larvae organized by site.

tested whether this was the case by modelling the variation among ponds depending on treatment type. This model of heteroscedastic variation (model 5, Table 1) explained significantly more of the mass variation than the model with homogeneous variation, indicating that mass variation differed among treatments within ponds.

Prior analyses assumed homogeneity of reaction norm slopes. I next evaluated this assumption by modelling both

the intercepts and slopes of reaction norms in nested random groups. Results suggested that reaction norms significantly varied among populations and among families (Table 1, $P < 0.001$). However, variation within containers (intercepts only) no longer contributed significantly to explanations of mass variance (Table 1, $P > 0.7$). Heteroscedastic variance across ponds within each treatment again provided a significant model improvement over models assuming homogeneous variation ($P < 0.001$). Model 5 without random slopes was the overall best model according to minimum AIC. However, models with interactions and heteroscedastic variation (e.g. model 9) had similar AIC values to this model, suggesting that the more complex model with heterogeneous reaction norms offers a viable alternative model (Burnham & Anderson 2002). In the minimum-AIC model with variable reaction norm slopes (model 9, Table 1), individuals within containers again were the most variable component (Table 2). In this model, both the intercepts and reaction norm slopes for families also were highly variable.

The masses of *A. maculatum* larvae in *A. opacum* treatments were relatively uncorrelated with their sibling counterparts' body sizes in control treatments ($\rho = 0.08$), suggesting potentially low genetic correlations underlying these traits for these populations and under these experimental conditions. Based on bootstrapped confidence intervals, phenotypic correlations were not significantly different from zero, and correlations did not overlap with absolute one (95% confidence interval: $-0.08, 0.24$).

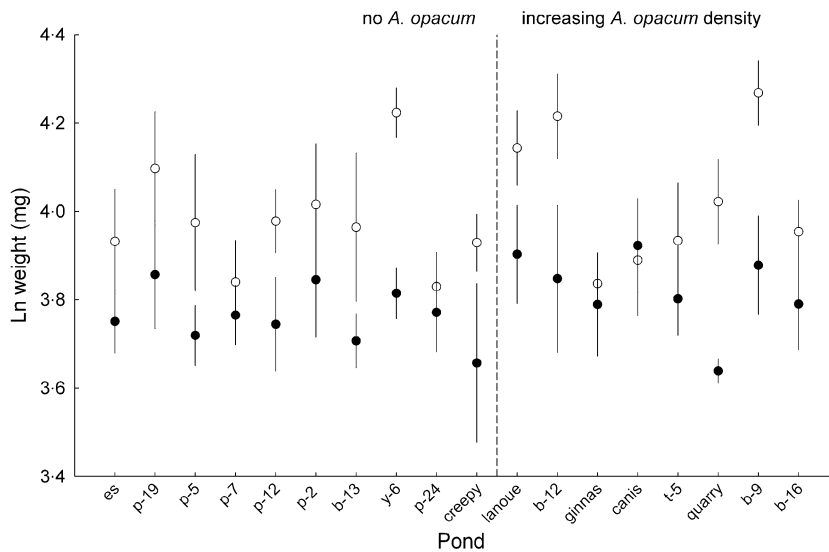


Fig. 3. Mean ln-transformed masses of *A. maculatum* larvae exposed to control conditions (open symbols) or to *A. opacum* kairomones (closed) by pond. Ponds are organized along an axis of increasing mean *A. opacum* predator densities to the right of the dotted vertical line and randomly to the left of this line where *A. opacum* field densities were zero. Bars represent standard errors.

SITE, TREATMENT AND FOCAL PREDATOR DENSITIES

When exposed to *A. opacum* kairomones, *A. maculatum* larvae were 20% smaller, on average, than larvae raised under control conditions (Table 1: model 5, Fig. 2; $F_{1,105} = 35.8$, $P < 0.001$). Overall body size did not differ significantly across sites ($F_{2,15} = 1.7$, $P = 0.217$). The interaction between site and treatment was not significant ($P = 0.739$). Contrary to predictions, mean *A. opacum* density in natal pond and its interaction with treatment did not explain a significant amount of variation in larval final body size (Fig. 3; $P > 0.87$).

PREDATOR RICHNESS AND ALTERNATIVE PREDATION AND ABIOTIC SELECTION REGIMES

Prey mass was not explained significantly by predator richness, the densities of an auxiliary gape-unconstrained predator (*Dytiscus*), pond permanence, intraspecific *A. maculatum* densities, or any of their interactions with treatment (Table 3). However, newt *N. viridescens* densities were associated with reduced larval masses (Table 3, Fig. 4; $F_{1,10} = 8.0$, $P = 0.018$). In addition, the reaction norm slope between control and *A. opacum* kairomone treatments decreased with increasing newt densities (Fig. 4, inset; $F_{1,16} = 7.5$, $P = 0.015$).

Discussion

Predator-specific defensive traits allow prey to respond with an adaptive solution to predation risks that vary in space and time (Tollrian & Harvell 1999). However, predator-specific responses may not evolve when environmental cues of potential predator species are unreliable (DeWitt 1998) or natural selection is diffuse due to the co-occurrence of multiple predators (Strauss & Irwin 2004). Therefore, a generalized plastic or fixed response may be favoured in species-rich predator communities where diverse mixtures of kairomones

Table 3. Mixed effects ANCOVA results for prey growth under control and *A. opacum* kairomone conditions (treatment) in relation to predator species richness, the densities of two auxiliary predator species, pond permanence, and *A. maculatum* densities in prey natal ponds. Random effects are the same as for the best model (no. 5) determined in Table 1. The final model was chosen based on a stepwise process of eliminating interaction terms between each variable and treatment based on the significance of the additional variation explained by their inclusion. Significant results are in **bold**

Source	Estimate (SE)	d.f.	F	P
Site				
Southern vs. northern	0.041 (0.031)	2,10	0.6	0.575
Southern and intermediate vs. northern	0.023 (0.041)			
Treatment	-0.104 (0.017)	1,107	35.9	< 0.001
Predator richness	-0.041 (0.069)	1,10	0.2	0.672
Dytiscus density	-0.242 (0.162)	1,10	1.7	0.226
Newt density	-1.156 (0.541)	1,10	8.0	0.018
Pond permanence	0.003 (0.002)	1,10	1.6	0.229
Intraspecific density	0.000 (0.004)	1,10	0.0	0.922

lessen the information content of individual kairomones or increase the likelihood of diffuse selection. In this study, patterns of prey body size plasticity were shared generally across populations, except in those that co-occurred with an auxiliary predator that induces a different plastic growth response in prey.

Common garden experiments revealed significant genetically determined size variation among populations and families. Reaction norms also varied significantly across populations and families (Table 1). This variation within and among populations suggests the potential for genotypic \times environment variation that could lead to the future evolution of these plastic responses. However, results are based on phenotypic variation; the estimation of genetic heritabilities and correlations requires future experiments with greater replication within populations. Additional research shows that foraging

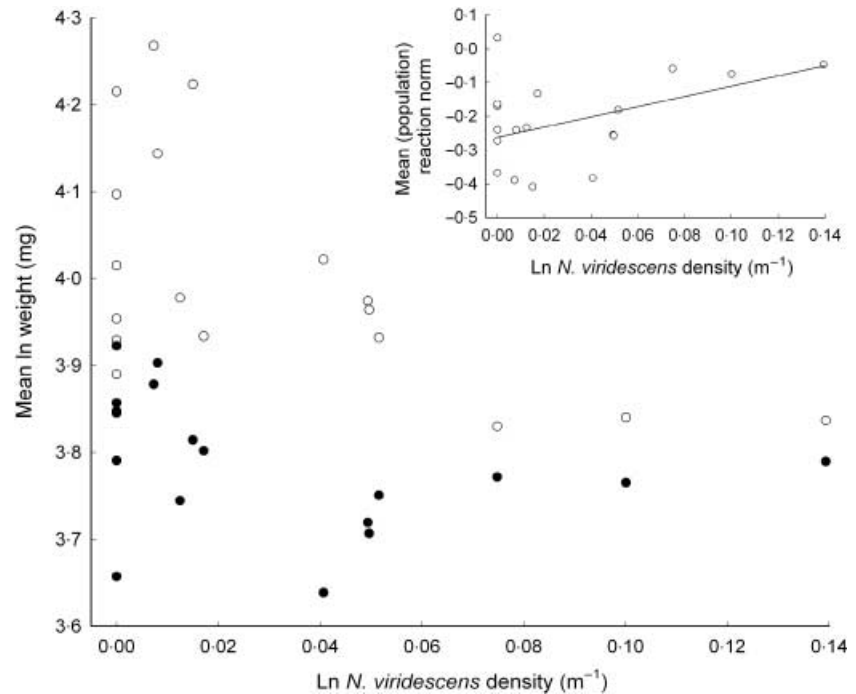


Fig. 4. Mean ln-transformed masses of *A. maculatum* larvae exposed to control conditions (open symbols) or to *A. opacum* kairomones (black) by pond in relation to ln-transformed newt densities in natal pond habitats. Inset: family reaction norm slopes vs. ln-transformed newt density. Line represents the best fit of a mixed effects model with pond treated as a random effect. Heteroscedasticity among ponds across newt density was accounted for in the model.

rate, a trait correlated with growth, under *A. opacum* predation risk is heritable in some of the populations analysed in this study (Urban 2007), suggesting that growth rate also may be heritable to some degree.

Mass reaction norms did not vary significantly across sites as expected. Instead, *A. maculatum* from most sites reduced growth when exposed to *A. opacum* kairomones. This reduced growth probably reflects the outcome of reduced foraging activity in risky, but resource-rich, habitats. Behavioural plasticity allows *A. maculatum* larvae to alter their growth rates depending on specific threats from *A. opacum* larvae distributed heterogeneously across natural landscapes and over time. *A. opacum* selectively capture *A. maculatum* larvae from populations with high genetically determined foraging rates (Urban 2007). This strong growth cost imposed by mortality risk, in turn, probably negates some of the potential fitness advantage of rapid growth into a size refuge from this gape-limited predator. Previous work confirms that *A. maculatum* larvae spend more time in refuge habitats when exposed to *A. opacum* kairomones, which can diminish their growth rates by limiting their foraging time in resource-rich habitats (Walls & Williams 2001; Urban 2006).

Contrary to predictions, reaction norms were not associated with differences in *A. opacum* density. This outcome applied to larvae originating from ponds that did not encounter *A. opacum* predators during the 3-year survey period and to larvae from ponds beyond the northern range limit *A. opacum* (Figs 2 and 3). These results suggest a weak fitness cost to plasticity *per se* and conjure up the 'ghost of predators past' hypothesis whereby prey defences that evolved under past selection can be retained when maintenance costs are low (Peckarsky & Penton 1988). Differences in plasticity did exist among populations, just not along a gradient in *A. opacum*

selection intensity. In fact, some populations exposed to high *A. opacum* predator densities in the field demonstrated little body size plasticity in common garden experiments. For instance, populations from Ginna's and Canis ponds regularly co-occurred with *A. opacum* yet displayed little plasticity in body size (Fig. 3). This result does not appear to be rare. Other studies show that plasticity can vary substantially among populations and that plastic defences may be absent despite intense predation risk (Parejko & Dodson 1991; De Meester 1993; Lardner 1998). Diffuse evolution of defensive plasticity offers one potentially general explanation for variable plastic responses in diverse predator communities (Iwao & Rausher 1997; Strauss & Irwin 2004).

Diverse predation threats can constrain the evolution of defensive plasticity by modifying selection regimes or by increasing the probability that defences are genetically correlated (Stinchcombe & Rausher 2001). In my study, mean predator species richness was unrelated to variation in reaction norms as originally supposed. Instead, the density of one predator, a newt, was associated with reduced body size and body size plasticity in prey populations. Hence, selection on *A. maculatum* body size appears to be modified by another species, thereby signalling the potential for diffuse, rather than pairwise, evolution (Janzen 1980; Iwao & Rausher 1997; Strauss & Irwin 2004). I excluded the densities of another common predator (*Dytiscus*), intraspecific density, and pond permanence as potential explanations for this pattern. Other unmeasured environmental variables (e.g. prey resources) could be correlated with newt density and thus explain observed relationships. Long-term manipulations of natural selection in the wild are needed to tease apart the selective factors responsible for the evolved responses of long-lived species in natural landscapes (Reznick & Ghalambor 2005).

A genetic correlation between prey responses to newts and *A. opacum* offers one explanation for reduced growth plasticity at high newt densities. For instance, a trait expressed in response to newt kairomones might be negatively correlated with growth under control conditions and positively correlated with growth under *A. opacum* conditions. However, the masses of *A. maculatum* larvae exposed to newt kairomones were positively (but not significantly) correlated with masses under both control and *A. opacum* kairomones in another experiment (Urban 2006). Also, we might expect that masses under control and *A. opacum* kairomones would be correlated themselves. This was not the case here. If genetic correlations reflect their phenotypic counterparts, then body size in these two environments can be treated essentially as independent traits that should evolve to a separate optimum in each environment (Via & Lande 1985).

A more likely explanation is that natural selection by *A. opacum* is modified by the presence of newts. *A. maculatum* larvae respond to *A. opacum* kairomones with a greater reduction in growth than they do in response to newt kairomones, most likely because a size refuge from newts is easier to reach (Urban 2006). Hence, induced prey foraging activity and growth into large body size likely is under differing selection from these two predators. This situation could generate 'ecological pleiotropy' whereby multiple predation risks are mediated by the same trait (Strauss & Irwin 2004). While selection may favour the strong growth reductions of prey response to *A. opacum* in most community compositions, a high density of newts may shift selection on prey body size toward a more muted response. Moreover, the two salamander predators likely convey kairomones that are more similar to each other than those elicited from less phylogenetically related predator species. These similarities could reduce kairomone reliability in multispecies communities and thereby increase the induction of inappropriate phenotypes (Getty 1996; Langerhans & DeWitt 2002). In this multispecies 'olfactory sea' (Tollrian & Harvell 1999), a generalized constitutive strategy can minimize the potential that combined salamander kairomones would mistakenly produce too rapid or too slow a growth rate relative to the actual selection regime. Although diffuse evolution is consistent with observed phenotypic patterns, comparing selection gradients on larval growth measured during single and multispecies predation events will be necessary to provide a more conclusive test (Iwao & Rausher 1997; Strauss & Irwin 2004).

Lastly, this experiment revealed interesting differences in the variance structure of larval body size in control and predator kairomone environments. Larval masses under control conditions varied almost three times more than larval masses under exposure to *A. opacum*. This pattern runs counter to studies that show higher variation in body size under predator-induced conditions (Kraft *et al.* 2006). One interpretation of this heterogeneous variance is that body size in control environments is seldom under direct selection in this system. Only three ponds in this study ever lacked predators, and then only for a single season. If no-predator conditions rarely occur, then noninduced prey growth may undergo genetic

drift or become a by-product of weak correlated selection on other traits. This explanation appeals for greater caution when attributing the shape or slope of reaction norms to variable selection regimes. Clearly this is the case if no-predator experimental treatments have little biological relevance, such as for prey never found in predator-free habitats.

I evaluated larval mass as a surrogate index of multiple intercorrelated traits, including foraging behaviours, growth and underlying physiological factors. In this system, body size is strongly correlated with risky foraging behaviours that include feeding frequency, movement rate and refuge use. Growth likely mediates a trade-off between predation threats due to risky foraging strategies and predation threats due to being too small. However, because multiple intercorrelated traits are involved and some of the potentially important traits have not been measured in this or previous research, it cannot be said with certainty which traits are under direct selection and which ones are under correlated selection. In addition, without explicit data on neutral genetic variation, I cannot address other causes of phenotypic variation among populations, including shared ancestry or gene flow.

An important goal of ecological and evolutionary research is to generalize predictions about species interactions and their evolution across multiple spatial scales (Thompson 2005). Because prey generally encounter multiple predator species, responses to selection may not reflect tightly coevolved responses, but rather more generalized responses dependent on the interactive effects of multipredator selection and genetically integrated defensive responses (Stinchcombe & Rausher 2001; Strauss & Irwin 2004). The combined perspectives of geographical variation in the evolution of species interactions and diffuse selection in diverse communities are likely to be particularly informative for explaining species interactions across environmental gradients (Agrawal & Van Zandt 2003). Although predator diversity *per se* did not significantly affect the nature of specific induced defences, the occurrence of a particular newt species was associated with less plastic growth responses to the focal predator. From an applied perspective, this means that alterations in the composition of predator species through anthropogenic or natural perturbations could result in substantial maladaptation in prey populations as the selection regime shifts from one favouring generalized defences to one favouring specific defences or vice versa. The population and community repercussions of adaptive phenotypic plasticity remain unexplored but are promising avenues for future exploration (Tollrian & Harvell 1999).

Predictions in community ecology frequently originate from experiments performed on single populations and therefore do not adequately represent the full extent of genetic variation present over regional or geographical scales. In this study, explanations for the highly variable reaction norms were evident only after evaluating phenotypic assays from a large number of populations distributed across different predator communities. Additional multipopulation studies are needed in order to predict the effect of multispecies selection on the outcome of species interactions and to support a broader integration of ecology and evolutionary biology (Urban & Skelly 2006).

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