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A trade-off between prey- and predator-induced polyphenisms in larvae of the salamander *Hynobius retardatus*

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Abstract Organisms in natural habitats participate in complex ecological interactions that include competition, predation, and foraging. Under natural aquatic environmental conditions, amphibian larvae can simultaneously receive multiple signals from conspecifics, predators, and prey, implying that predator-induced morphological defenses can occur in prey and that prey-induced offensive morphological traits may develop in predators. Although multiple adaptive plasticity, such as inducible defenses and inducible offensive traits, can be expected to have not only ecological but also evolutionary implications, few empirical studies report on species having such plasticity. The broad-headed larval morph of *Hynobius retardatus*, which is induced by crowding with heterospecific anuran (*Rana pirica*) larvae, is a representative example of prey-induced polyphenism. The morph is one of two distinct morphs that have been identified in this species; the other is the typical morph. Here, we report that typical larval morphs of *Hynobius* can respond rapidly to a predatory environment and show conspicuous predator-induced plasticity of larval tail depth, but that broad-headed morphs cannot respond similarly to a predation threat. Our findings support the hypothesis that induction or maintenance of adaptive plasticity (e.g., predator-induced polyphenism) trades off against other adaptive plastic responses (e.g., prey-induced polyphenism). For a species to retain both an ability to forage for larger prey and an ability to more effectively resist predation makes sense in light of the range of environments that many salamander larvae experience in nature. Our results suggest that the salamander larvae clearly discriminate between cues from prey and those from predators and accurately respond to each cue; that is, they adjust their phenotype to the current environment.

Keywords Broad-headed morph · Predator · Prey · Phenotypic plasticity · Salamander

Introduction

Traditional studies of phenotypic plasticity have focused on adaptive behavioral or morphological plasticity in organisms of diverse taxa, and on the benefits and costs of phenotypic plasticity across experimental or natural environments; thus, the comparative fitness of individuals with and without such plasticity has been examined (Schlichting and Pigliucci 1998; Pigliucci 2001; West-Eberhard 2003). Although the benefits of being induced to display an adaptive morph have been frequently identified, determination of the costs of being induced can be more elusive (Tollrian and Harvell 1999). Diverse prey taxa construct inducible morphological defenses to escape predators, but they share in common the cost of slower growth (e.g., fish, salamanders, snails, and *Daphnia*; Bronmark and Pettersson 1994; Agrawal et al. 1999; Van Buskirk and Schmidt 2000; Trussell and Nicklin 2002). A representative example, the prey rotifer species *Keratella slacki* has been well characterized for its ability to develop a larger body and longer anterior spines in the presence of the predatory rotifer *Asplanchna*, making it less vulnerable to predation but at the cost of a drastically decreased growth rate (Abrusán 2003). Although it is still important to extend traditional research on adaptive phenotypic plasticity in a specific environmental setting, the recognition that plasticity can be adaptive has stimulated a wealth of studies on less understood aspects of the relationships among different adaptive phenotypic plasticities induced in an individual by more complex environmental conditions (Relyea 2002).

Predator-induced morphological defenses occur in prey (Tollrian and Harvell 1999), prey-induced offensive morphological traits may develop in predators (e.g., cannibal or carnivorous morphs; Elgar and Crespi 1992), and competitor-induced morphology can

also be induced in conspecifics and related species (Relyea 2002). Each altered phenotype affects in turn subsequent interactions between species in ecological communities (Agrawal 2001; Miner et al. 2005). However, an organism in its natural habitat is presumed to participate in complex ecological interactions that include competition, predation, and foraging. Thus, under realistic environmental conditions, an organism can simultaneously receive compound stimulations (cues) from predators, conspecifics, and prey. Although multiple adaptive plasticity such as inducible defenses and inducible offensive traits are expected to have not only ecological but also evolutionary implications, few empirical studies report on species with such plasticity.

In general, because many studies have reported the existence of heterochronically or heterotopically observed developmental trade-offs between different traits in diverse taxa (Gilbert 2003; West-Eberhard 2003), when predator- and prey-induced responses are considered simultaneously, it becomes apparent that these two types of plasticity might be intricately linked and traded off. To test this hypothesis, one should search for predator-induced morphological plasticity in a species known to exhibit prey-induced morphological plasticity. Larvae of the salamander, *Hynobius retardatus*, have a well-documented ability to exhibit prey-induced plastic morphology, namely, the broad-headed morph (Michimae and Wakahara 2002). Under conditions of crowding with conspecifics or heterospecific anuran (*Rana pirica*) larvae, these salamander larvae frequently develop wider heads and larger mouths compared with conspecific larvae reared in conditions of lower larval density of conspecifics or heterospecifics (Michimae and Wakahara 2002). The prey-induced broad-headed larvae are better able to survive starvation conditions during their larval stage, spent in ponds created by melting snow, by eating larger prey items (conspecific and heterospecific larvae). *H.*

retardatus larvae co-occur also with the dragonfly (*Aeschna juncea*) larvae in many natural ponds (Kishida and Nishimura 2005; Michimae unpublished data) as well as *R. pirica* larvae (Michimae 2006). Little is known about changes to larvae morphology of *H. retardatus* in the presence of aquatic predators such as dragonfly larvae, but a few salamander and many amphibian larvae develop relatively large tails and small bodies in the presence of dragonfly larvae (e.g. Van Buskirk and Schmidt 2000; Relyea 2004). Therefore, *H. retardatus* larvae are also expected to develop a predator-induced defensive morphology. It is also expected that prey-induced offensive traits and predator-induced defensive traits are being traded off in this phenotypically plastic species.

In the natural environment, both predation and foraging are probably important ecological factors affecting morphology, and it is likely that the morphological response in a particular environment is affected by a trade-off between foraging success and predator avoidance. Here, we hypothesize that prey- and predator-induced phenotypes are intricately linked owing to a trade-off between foraging ability and the ability to resist predators, because while prey-induced traits may give individuals increased foraging ability, they may decrease their resistance to predators. This trade-off makes sense in light of the range of environments, along a continuum of densities of predators (Kishida and Nishimura 2005) and larger prey (Michimae 2006), in which *H. retardatus* lives.

Methods

Experiment

We collected fertilized eggs of the salamander *Hynobius retardatus* and the brown frog *Rana pirica* in 2005 in the vicinity of Sapporo, Japan, during the breeding season (from early April to late May). Eggs of each species were separately placed in stock tanks filled with 1.6 L of dechlorinated tap water at room temperature (20–21 °C) until hatching. After five clutches of salamander had simultaneously hatched, all newly hatched larvae were collected and reared in a tank (30 × 25 × 17.5 cm) filled with 5 L of dechlorinated tap water for 4 days. Then, we collected 30 typical morphs and 30 of the broad-headed morphs that were induced under the crowded conditions of the tank during the rearing (Michimae and Wakahara 2001) at the pre-feeding stage to use as the two types of morph in the following experiments.

We designed two distinct experimental conditions, with and without predation threat (from larvae of the dragonfly *Aeschna juncea*). Fifteen larvae of each morph were exposed to each of the two conditions. Thus, the experiments examined two factors, morphotype (typical and broad-headed morphs) and predation threat (presence or absence). Each of the 60 salamander larvae was placed individually in a cage (5 × 5 × 8 cm) made of plastic mesh (mesh size, 3 mm) in an experimental tank (8 × 8 × 8 cm) containing 300 ml of either dechlorinated tap water or dragonfly larvae rearing water. We substituted rearing water of dragonfly larvae for the presence of the predators themselves because many amphibian larvae can develop predator-induced morphology just by receiving chemical cues from predators. The dechlorinated tap water or rearing water of dragonfly larvae was exchanged every day during the experiment. The dragonfly larvae rearing water was prepared by placing three dragonfly larvae in a tank (22 × 15 × 12.5 cm) for 1 day before using the water from the tank in the experiment.

To maintain the broad-headed morph during the experiment, ten *R. pirica* tadpoles

were placed in the open space between each experimental tank and cage containing a broad-headed morph, whether with or without exposure to predation threat, because a previous study (Michimae and Wakahara 2002) showed that a larva with an induced broad-headed morphology reverts to the typical morph when placed by itself and because hydraulic vibration originating from the flapping tails of anuran tadpoles or conspecific larvae is a key cue for the induction or maintenance of the broad-headed morph (Michimae et al. 2005). All ten tadpoles were replaced twice each day during the experiments because anuran tadpoles in the presence of predators become less active than conspecifics reared without predators.

Each focal larva was fed with frozen Chironomidae of the same wet weight every other day during the experimental period (4 weeks). The wet weight of each food item was measured to the nearest 0.01 g on an electronic balance. The experiment was conducted in the laboratory at room temperature (20–21 °C) and with a natural light/dark schedule.

Five salamander larvae from each treatment were removed and fixed in 4% paraformaldehyde at 7, 14, and 28 days after the beginning of the experiments. Then, we measured the body length, tail length, snout–vent length (SVL), and maximum tail depth in lateral view, maximum head width in dorsal view, and maximum mouth width in ventral view, to the nearest 0.005 mm with calipers. We chose an experimental period of 4 weeks because the experimental cage was too small for rearing salamander larvae more than 4 weeks old.

Statistical analysis

Differences in morphology can be caused by differences in overall size as well as by differences in shape. Therefore, to examine relative differences in morphology, we regressed the five log-transformed linear measurements against the log-transformed body length of each individual. We obtained regression lines for larvae at 7, 14, and 28 days, and calculated the values of the residuals for each salamander larva from these lines. These size-independent measures served as our primary response variables. The effects of morphotype (prey), predation threat, developmental changes, and various interactions among the factors on salamander larvae were analyzed by using multivariate analysis of variance (MANOVA). After MANOVA, we assessed which variables were responsible for the significant main effects by a univariate analysis of variance (one-way ANOVA) of each response variable.

Results

There were significant multivariate effects associated with all three factors (day of experiment, predation threat, and morphotype) and with interactions among the factors, except the interactions day of experiment by predation threat and day of experiment by predation threat by morphotype (Table 1a). Subsequent ANOVA detected significant effects of morphotype on larval SVL, tail length, head width, and mouth width, and significant interactive effects between day of experiment and morphotype on head width and mouth width (Table 1b, Figure 1). We also detected significant effects of predation threat and of the interaction of morphotype and predation threat on tail depth (Table 1b, Figure 1). In the absence of a predation threat, typical morphs had longer tail length, shorter SVL, and narrower head and mouth widths than broad-headed morphs over the

experimental period (Table 1b, Figure 1). In the presence of a predation threat, however, typical morphs responded by developing deeper tail fins than were developed in the absence of a predation threat and, furthermore, than were developed by the broad-headed morphs (Figure 2), but the remaining morphological measurements were not significantly altered by the presence of a predation threat (Table 1b, Figure 1). On the other hand, the relative width of the head and mouth gradually decreased in all larvae with the broad-headed morph, as indicated by the interaction between day of experiment and morphotype (Table 1b, Figure 1); this narrowing of the head and mouth was attributed to the broad-headed morphs' approaching metamorphosis more quickly than the typical morphs (Michimae and Wakahara 2002).

Differences in tail depth between predation threat- and no-predation threat treatments first appeared by 7 days, they maintained their magnitude over the 4 weeks (Figure 1). Significant effects of the interaction morphotype by predation threat on tail depth indicated that typical morphs responded rapidly to the predatory environment and showed strong predation threat-induced plasticity with respect to tail depth, but that broad-headed morphs did not respond similarly to the predation threat during the experimental period (Figure 2).

Discussion

The specific morphology of the broad-headed morph is advantageous for the consumption of large prey such as conspecific larvae or heterospecific anuran (*Rana Pirica*) larvae (Michimae and Wakahara 2002). Such specialization on certain resources may have strong implications for morphological adaptations because the foraging

efficiency of individuals is strongly related to individual morphology (Pfennig 1990; Stephen et al. 1992; Day et al. 1994; Michimae and Wakahara 2002). Cannibalism can greatly enhance an individual's growth or developmental rate (Elgar and Crespi 1992), and, consequently, broad-headed morphs that prefer to consume conspecific larvae develop a bigger body size than typical morphs that feed on typical prey items (Wakahara 1995).

However, in addition to the bigger body size of the broad-headed morph, its dumpy morphology, due to its shorter tail length, longer SVL, and wider head and mouth widths compared with the typical morph (Figure 1), may be disadvantageous for the avoidance of attack from predators. Moreover, prey-induced morphological changes or their maintenance suppressed the expression of plasticity of tail depth in response to apparent predator risk (Table 1b, Figure 2). In animals, differences in vulnerability to predators during larval stages are caused by differences in size and shape. Many amphibian larvae develop a relatively smaller body and deeper tail as an induced response to predators (Van Buskirk 2002; Relyea 2002). The broad-headed morphs that cannot respond to predators are more vulnerable to predation than the typical larval morph (Figure 2). In contrast, the plastic response of typical larvae to the presence of a predation threat (deeper depth of tail) was qualitatively similar to that previously found in anuran tadpoles and salamander larvae in response to chemical cues from a predatory dragonfly (Figure 2; Van Buskirk and Schmidt 2000; Van Buskirk 2002; Relyea 2002; Kishida and Nishimura 2005). Larvae of the induced morph presumably survive better when exposed to predation risk. Our findings support the hypothesis that induction or maintenance of an adaptive plasticity (predator-induced polyphenism) is traded off against another adaptive plastic response (prey-induced polyphenism).

The four morphological traits of tail length, SVL, head width, and mouth width, all showed less evidence of plasticity in response to the predation threat than tail fin depth (Figures 1 and 2). However, those four traits have plastic potential because their morphology shows extensive plastic changes in response to the presence of larger prey items such as conspecifics and *R. pirica* larvae, resulting in significant differences in the four traits between two morphs (Table 1b). The retention by a species of both the ability to forage for larger prey and the ability to better resist predation makes sense in light of the range of environments that many salamander larvae experience in nature. In natural ponds, salamander larvae live along a continuum of predator and prey environments (Kishida and Nishimura 2005; Michimae 2006). In ponds that contain few or no predators, the broad-headed morph may be more advantageous than the typical morph because the morphs can consume large prey such as conspecific and heterospecific larvae that they encounter. In ponds with high densities of predators, the typical morph may be more advantageous, because its smaller body size and ability to acquire a deeper tail depth may enhance larval survival in the face of predation risk. Our results suggest that the salamander larvae clearly discriminate between cues from prey (Michimae et al. 2005) and predators (this study) and respond accurately to each cue; that is, they adjust their phenotype to the current environment.

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Figure legends

Figure 1. Developmental changes in relative morphology, tail length (a), SVL (b), head width (c), mouth width (d), and tail depth (e), of larval *Hynobius retardatus* in the experiment under a factorial combination of larval morphs (typical or broad-headed morph) and apparent predation risk (no risk or risk), at 7 days, 14 days, and 28 days. Open circles, typical morph; open squares, broad-headed morph; dotted lines, no predation risk; solid lines, predation risk. Data are means \pm SD.

Figure 2. Relative tail depth of larval *Hynobius retardatus* when reared with either apparent predation risk or no predation risk. Typical or broad-headed morphs at 7 days (a), 14 days (b), and 28 days (c) of experiment. Open circles, typical morph; open squares, broad-headed morph. Data are means \pm SD.

Table 1. Results of MANOVA for effects of day of experiment, predation threat and morphotype on five morphological traits (SVL, tail length, tail depth, head width and mouth width). ANOVA results for each response variable are also shown.

a) MANOVA				
Factor	Wilks' lambda	d.f.	<i>F</i>	<i>P</i>
Day of experiment	0.644	10, 88	2.169	0.0271
Predation threat	0.581	5, 44	6.352	0.0002
Morphotype	0.366	5, 44	15.239	<0.0001
Day of experiment x Predation threat	0.859	10, 88	0.697	0.7246
Day of experiment x Morphotype	0.617	10, 88	2.399	0.0144
Predation threat x Morphotype	0.660	5, 44	4.538	0.0020
Day of experiment x Predation threat x Morphotype	0.676	10, 88	1.902	0.0553
b) ANOVAs				
Variables	MS	d.f.	<i>F</i>	<i>P</i>
SVL				
Day of experiment	3.652E-5	2, 48	0.024	0.9766
Predation threat	1.416E-5	1, 48	0.009	0.9241
Morphotype	0.027	1, 48	17.266	0.0001
Day of experiment x Morphotype	0.003	2, 48	1.878	0.1639
Predation threat x Morphotype	1.636E-5	1, 48	0.011	0.9184
Tail length				
Day of experiment	4.155E-5	2, 48	0.017	0.9829
Predation threat	6.114E-5	1, 48	2.543E-4	0.9873
Morphotype	0.043	1, 48	17.957	0.0001
Day of experiment x Morphotype	0.005	2, 48	1.993	0.1475
Predation threat x Morphotype	3.973E-5	1, 48	0.017	0.8983
Tail depth				
Day of experiment	2.353E-5	2, 48	0.006	0.9944
Predation threat	0.111	1, 48	26.647	<0.0001
Morphotype	3.890E-4	1, 48	0.094	0.7610
Day of experiment x Morphotype	0.002	2, 48	0.530	0.5919
Predation threat x Morphotype	0.082	1, 48	19.686	<0.0001
Head width				
Day of experiment	2.299E-6	2, 48	0.001	0.9995
Predation threat	0.011	1, 48	2.572	0.1153
Morphotype	0.184	1, 48	48.727	<0.0001
Day of experiment x Morphotype	0.029	2, 48	6.859	0.0024
Predation threat x Morphotype	0.001	1, 48	0.244	0.6235
Mouth width				
Day of experiment	1.500E-5	2, 48	0.003	0.9973
Predation threat	0.010	1, 48	1.814	0.1843
Morphotype	0.432	1, 48	77.351	<0.0001
Day of experiment x Morphotype	0.064	2, 48	11.547	<0.0001
Predation threat x Morphotype	9.982E-5	1, 48	0.018	0.8942



