Flexible architecture of inducible morphological plasticity

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Summary

1. Predator-induced morphological defences are produced in response to an emergent predator regime. In natural systems, prey organisms usually experience temporal shifting of the composition of the predator assemblage and of the intensity of predation risk from each predator species. Although, a repetitive morphological change in response to a sequential shift of the predator regime such as alteration of the predator species or diminution of the predation risk may be adaptive, such flexible inducible morphological defences are not ubiquitous.

2. We experimentally addressed whether a flexible inducible morphological defence is accomplished in response to serial changes in the predation regime, using a model prey species which adopt different defensive morphological phenotypes in response to different predator species. *Rana pirica* (Matsui) tadpoles increased body depth and tail depth against the predatory larval salamander *Hynobius retardatus* (Dunn); on the other hand, they only increased tail depth against the predatory larval dragonfly *Aeshna nigroflava* (Martin).

3. *Rana pirica* tadpoles with the predator-specific phenotypes were subjected to removal or exchange of the predator species. After removal of the predator species, tadpoles with each predator-specific phenotype changed their phenotype to the nondefensive basic one, suggesting that both predator-specific phenotypes are costly to maintain. After an exchange of the predator species, tadpoles with each predator-specific phenotype reciprocally, flexibly shifted their phenotype to the now more suitable predator-specific one only by modifying their body part. The partial modification can effectively reduce time and energy expenditures involved in repetitive morphological changes, and therefore suggest that the costs of the flexible morphological changes are reduced.

Key-words: bulgy, inducible defence, morphogenesis, phenotypic plasticity, reversibility.

Introduction

Organisms improve their fitness by changing their ontogenic trajectory to produce different phenotypes in response to environmental changes (Pigliucci 2001; DeWitt & Scheiner 2004). This adaptive design, called developmental plasticity, is important with respect to both the life history of the individual organism and interspecies interactions within an ecosystem (Tollrian & Harvell 1999; Pigliucci 2001; Werner & Peacor 2003; DeWitt & Scheiner 2004).

Predation is a biological environmental factor that is stochastic at both micro- and mesoscales, and it is one of the important selection factors influencing the design of a prey organism (Kerfoot & Sih 1987; Tollrian & Harvell 1999; Barbosa & Castellanos 2005). A predator-induced morphological defence is one type of adaptive developmental plasticity, produced in response to an emergent predation risk, which has been observed in a variety of taxa (reviewed by Tollrian & Harvell 1999). A number of past studies have examined how prey morphologically responds to a predator species (Bronmark & Pettersson 1994; Tollrian 1995; Trussell 2000; Van Buskirk 2002; Relyea 2003; Kishida, Mizuta & Nishimura 2006), and several studies have compared different morphological phenotypes of prey organisms induced by predators with different predatory tactics (DeWitt, Robinson & Wilson 2000; Relyea 2001; Van Buskirk 2001). These studies have shown diversity and/ or predator-specificity of the inducible morphologies, and have identified the functional significance of the

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induced phenotype (DeWitt *et al.* 2000; Kishida & Nishimura 2004; Teplitsky *et al.* 2005; Wilson, Kraft & van Damme 2005). However, most of them focus on the induction of morphological defences after exposure of prey organisms to an environment with a constant predation risk by a single predator species (see Teplitsky, Plenet & Joly 2004). In natural systems, however, prey organisms experience more complex predation regimes. That is, the composition of predator species and their interaction intensity can vary over time, and therefore the prey organisms can experience temporal shifting of the predation regime rather than a constant predation risk.

Sequential encounters with different types of predator or a temporal reduction of the predation risk are important possible scenarios that reflect the phenological schedules of prey and predators in natural environments. Suppose that prey have already been induced to exhibit a defensive phenotype in response to a certain type of predator. We have a simple expectation regarding the subsequent phenotype of the prey organism after a sequential environmental change: if the original predator leaves and another type of predator comes, the prey discard their present defence and immediately exhibit a new adaptive defence appropriate to the new predation regime; or, if the predation risk is relaxed and the present defence is costly to maintain, the prey disinvest in their defence and revert to the nondefensive phenotype. Thus, a flexible change of alternative defence morphologies according to the alteration of predator types or a change of the degree of predation risk could be one adaptive design.

Although flexible morphological shifts are desirable from an ecological viewpoint, their evolution is constrained by, for example, maintenance and production costs of the mechanistic architecture required for the flexible morphological change, and limitations on the benefit conferred by the flexible change (DeWitt, Sih & Wilson 1998). Evolution of the desired flexible phenotypic plasticity would occur only when the benefit conferred outweighed the constraints (DeWitt & Scheiner 2004). Identifying whether flexible inducible morphological defences are accomplished in response to serial changes in the predation regime is crucial for understanding the evolution of multiple developmental plasticity and therefore adaptive phenotypic design. In this paper, we address these problems using a model prey–predator system in which the prey species displays different morphological phenotypes against different types of predator species and in which the composition of the predator assemblage and the intensity of the predation risk from each predator species can vary over time and space.

The *Rana pirica* (Matsui) tadpole is a prey organism that has evolved multiple predator-induced defensive morphologies. That is, *R. pirica* tadpoles develop distinctive predator-specific morphological responses in the presence of predators with different prey capturing methods (Kishida & Nishimura 2005). *R. pirica* tadpoles exhibit a 'high-tail' morphological phenotype when

they are responding to predation risk by the larval dragonfly *Aeshna nigroflava* (Martin), which bites its prey; however, they develop not only the high-tail morphology but also a bulgy body, the 'bulgy' phenotype, in the presence of larval salamanders *Hynobius retardatus* (Dunn), which swallows its prey whole (see fig. 1 in Kishida & Nishimura 2005). The tadpoles with these specific morphologies have higher survival rates compared with those with mismatched or noninduced phenotypes when exposed to predation by the corresponding predators. That is, when exposed to predation risk by the dragonfly, more of the dragonfly induced 'high-tail' tadpoles survive than 'bulgy' or noninduced 'basic' tadpoles; on the other hand, more of the salamander-induced 'bulgy' tadpoles survive than 'high-tail' or 'basic' tadpoles when exposed to predation risk by the salamander (Kishida & Nishimura 2005).

During the developmental period of *R. pirica* tadpoles, they frequently seem to experience in their natural habitat a sequential shift in the predator environment, such as successive encounters with the two types of predators or a reduction in the predation risk from one or both of the predators (Fig. 1). Thus, this model system provides an opportunity to investigate flexible developmental plasticity in response to sequential changes in the predator environment. We hypothesize that *R. pirica* tadpoles will shift their predator-specific phenotype
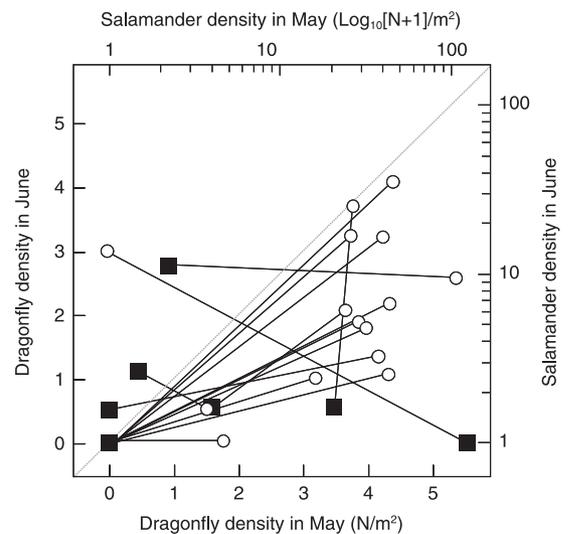
Fig. 1. Densities of a late instar larval dragonfly, *Aeshna nigroflava* (filled square), and a larval salamander, *Hynobius retardatus* (open circle), in samples taken in natural ponds on Oshima Peninsula, Hokkaido, Japan. Samples were collected from May to June in 2003 from 15 ponds that contained *Rana pirica* tadpoles. The grey dotted line is the isoline (equal predator densities in May and June). Paired marks connected by a solid black line represent predator densities in the same pond. The region above and to the left of the isoline represents increasing predator density, and that below and to the right represents decreasing predator density. A solid line crossing the isoline indicates an alteration of the main predator species between May and June in the pond. Density was calculated from net samples collected from more than 10% of the total area of each pond.

in response to sequential alteration of the predator species in accordance with the adaptive significance of the predator-induced morphs. In addition, if *R. pirica* tadpoles, having adopted a defensive phenotype, revert to a nondefensive phenotype in response to a reduction in the predation risk from predators, we can deduce that the defensive morphologies are costly to maintain.

Experimentally, we address the following questions: (1) Do *R. pirica* tadpoles reciprocally modify their predator-specific morphologies in response to reciprocal changes of the predator species (larval salamander or larval dragonfly)? (2) Are both predator-specific morphologies reversible (back to the basic morph) after removal of the predation risk?

Materials and methods

DESCRIPTION OF THE NATURAL PHENOLOGICAL PROCESS

Generally, at the population level, *R. pirica* and *H. retardatus* spawn in small ponds formed by melting snow in early spring in Hokkaido, Japan (Sato 1989). For some time after the eggs hatch, the densities of the two species of amphibian larvae are frequently high, and the predator–prey interaction between them must thus be very intensive for a certain period (Fig. 1). In some ponds, larvae of *A. nigroflava* have been hibernating, and they subsequently gradually become activated as water temperature rises from mid-spring. After a while, the predator–prey interaction tends to weaken as the number of larval *H. retardatus* declines (June in Fig. 1) via predation by the aquatic insect or cannibalism.

MATERIALS AND EXPERIMENTAL METHODS

Five egg masses of *R. pirica* were collected from a pond in which both predator species are common. Three egg clusters of *H. retardatus* and 20 larvae of *A. nigroflava* were collected from other ponds. Collected eggs and larvae of each species were placed separately in 12-L stock aquaria.

The experimental units were 4·5-L (28·5 × 16·5 × 9·5 cm deep) aquaria filled with 2 L of aged tap water. Each aquarium contained a saucer-shaped colander (15 and 8 cm in diameter at the top and bottom, respectively). On 25 June 2003, we randomly assigned 40 similarly sized, 2-day-old *R. pirica* hatchlings (total mass = 24·03 ± 4·61 mg, mean ± SD, $n = 30$) to each of the aquaria and placed them outside of the colander.

On 29 June 2003, the experiments, each consisting of two treatment periods (an inducing period and a regime-switching period), were started. As morphological responses can be affected by developmental stage (i.e. a larval morphogenesis event is destroyed by metamorphosis) or overall size (Kishida & Nishimura 2004), the experiments must be conducted before the focal *R. pirica* tadpoles have reached the developmental stage at which morphological changes being induced by the predation

threat would be affected by their subsequent metamorphosis. As a preliminary experiment showed that *R. pirica* tadpoles require 1 week to develop their functional predator-specific morphologies in such experimental settings, we planned the experiments with two treatment periods, each lasting 7 days, and ended each experiment at a developmental stage early enough to avoid an effect of metamorphosis.

We conducted seven separate experiments, which were divided into four categories as follows: (1) predator-unaltered experiments, in which the predator species remained the same throughout the two experimental periods; (2) predator-exchange experiments, in which the predator species was changed (from salamander to dragonfly or from dragonfly to salamander) at the start of the second period; (3) predator-removal experiments, in which the predator was removed at the beginning of the second period; and (4) control treatment, in which no predator was assigned during either treatment period. Each experiment was replicated six times. In each of the predator experiments, the first period was used for the induction of a predator-specific morph. The morphological responses of the tadpoles during the second period of the predator-exchange and predator-removal experiments were the focus of our attention. The predator-unaltered experiments and the control experiment were references against which the morphological responses observed during our previous study could be checked (Kishida & Nishimura 2005).

The procedures used to test the predator-specific induced morphologies in response to the salamander and dragonfly predators were established by Kishida & Nishimura (2005). In that study, we found that induction of the 'high-tail' morph requires only a remote cue from dragonfly larvae, whereas induction of the 'bulgy' morph requires a cue from salamander larvae in close proximity to the tadpoles (Kishida & Nishimura 2005). Therefore, to create a dragonfly predation regime (dragonfly treatment), one dragonfly larva was introduced into the colander, which segregated it from the tadpoles being studied, so that actual predation was prevented. To create a salamander predation regime (salamander treatment), however, three salamander larvae were introduced into the aquarium outside of the colander, that is, into the same space as the tadpoles being studied, to satisfy the induction condition of the bulgy morph, even though this treatment entails actual predation. These induction procedures were not adopted to simulate natural situations but for the sake of obtaining clear expression of the distinctive morphology in response to each predator with minimal loss of the studied tadpoles by predation. Even though morphological induction caused by the larval dragonfly is underestimated compared with that caused by the larval salamander, these different procedures are valid because it was not our purpose to compare the type of induction or the rate of induction between the two types of predators.

At the start of the experiment, the snout–vent length of the salamander larvae was 16·21 ± 1·23 mm ($n = 10$),

and the total length of the dragonfly larvae was 38·45 ± 7·94 mm (*n* = 10) (mean ± SD). The experiment was conducted at a water temperature of 16–18 °C. The tadpoles were fed rabbit chow *ad libitum* daily, and the water of all aquaria was changed every second day throughout the experiment. We replaced the individual predators with others from the holding tanks daily. While in the holding tanks, the predators were fed sufficient numbers of small *R. pirica* tadpoles to satiate them. However, as actual predation was unavoidable in the salamander treatments, in order to keep the densities of tadpoles in the aquaria uniform during the experimental periods, we counted the surviving tadpoles in all aquaria every 2 days and equalized the numbers of tadpoles in all aquaria by random deletion until all had the same number of tadpoles as the aquarium with the fewest tadpoles. The number of preyed-upon tadpoles in the salamander aquaria in the first and second periods was 7·17 ± 1·2 (*n* = 18) and 3·92 ± 1·24 (*n* = 12), respectively.

At the end of each treatment period, eight tadpoles were randomly selected from the survivors in each aquarium and killed in 10% ethanol. Photographs of dorsal and lateral views were taken in a small glass chamber, the photographic images were projected on to a computer monitor, and the following three traits were digitized: body length (from the tip of the snout to the tail joint), maximum body depth, and maximum tail depth. Our previous study (Kishida & Nishimura 2005) showed that body depth and tail depth relative to body length are adequate indicators for identifying the predator-specific morphologies: the shape of the dragonfly-induced tadpole is characterized by a higher tail depth, whereas the shape of the salamander-induced tadpole is characterized by not only a higher tail but also a bulgy body (i.e. increased body depth) compared with the noninduced basic tadpoles. Therefore, we used the size-adjusted body depth and tail depth [i.e. each data point was adjusted relative to that at body length mean (11·72 mm), which was calculated from all individuals, after regression against body length] of tadpoles as indicators of the predator-specific morphs.

ANALYTICAL DATA AND DATA CORRECTION

Statistical analyses were conducted in two steps. As a preliminary step, we examined whether the predator-specific inducible morphologies had been accomplished at the beginning of the second experimental period, and whether the predator-specific morphological responses were maintained in the second experimental period in the predator-unaltered experiments. At the second step, we tested our main hypotheses concerning the flexible shifting and reversibility of the predator-specific morphologies. In these analyses, the aquarium means of the size-adjusted variables were obtained from the eight sampled individuals for all of the treatments, with the exception of the salamander treatments, at the end of each experimental period (see
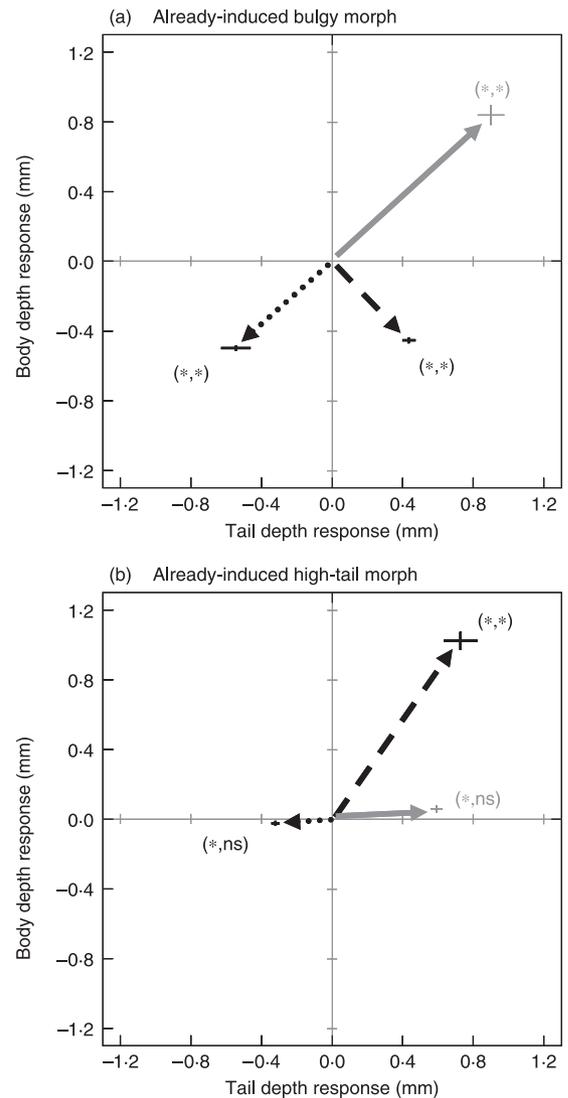
Fig. 2. Morphological responses of tadpoles subjected to each of the three treatments in the second period, that is, differences in size-adjusted body depth (vertical axis) and tail depth (horizontal axis) between the end of the first and the end of the second period. (a) Salamander treatment in the first period, in which the bulgy morph had already been induced; (b) dragonfly treatment in the first period, in which the high-tail morph had already been induced. Predator-exchange experiment (dashed arrow); predator-removal experiment (dotted arrow). Predator-unaltered experiments are represented as solid grey arrows for reference. Error bars denote one standard error (*n* = 6). Statistical significance is shown by the ordered pair next to each arrow. The first member of the pair refers to the difference in tail depth, and the second member to the difference in body depth. *Statistically significant after the sequential Bonferroni correction; ns, not significant.

below). In particular, in the analysis of the morphological responses in the second period, the response variables were the differences of the means of the variables between the end of the second and the beginning of the second treatment periods in each aquarium. The morphological responses are realized as the located vectors of the response variables in an orthogonal coordinate system (Fig. 2).

In the salamander treatment, to exclude the possibility that selective predation of individuals having less inducible morphologies biases aquarium means of the morphological responses calculated from the sampled survivors, leading to overestimation of the response, we corrected the sample data before analysis. For this correction, we deleted some individuals having more inducible morphologies from the sample in each aquarium. Deletion from the sample was determined by the following method.

We supposed an imaginary sample with some number of individuals in addition to the actual sampled number (here, eight individuals), in which the surplus individuals had been deleted by salamander predation before the actual sampling event. We estimated the number of individuals deleted in the imaginary sample by the following equation; $n_i/(40 - n_i) = x_i/8$, where 40 is the number of tadpoles at the start of the experiment, n_i is the number of individuals removed by predation in aquarium i during the experimental period, 8 is the actual sample number, and x_i is the number of tadpoles deleted from the imaginary samples.

As we had supposed that x_i individuals with less inducible morphologies had been deleted from the imaginary sample by predation, the same number of individuals having more inducible morphologies should be deleted from the actual sample to correct for the bias. The actual individuals sampled were sorted in ascending order by body depth and numbered. We next generated x_i unique random numbers from a triangular distribution with its mode toward the high end of the distribution. We then eliminated from the data the sampled individuals that had been assigned those random numbers.

Even though we could not know the morphometry of the tadpoles that had actually been removed by salamander predation (so the sample correction method does not precisely reflect the realistic details), the random removal of tadpoles having a larger body depth is an appropriate correction for possibly selective predation causing the mean value of the traits to be too large. After the correction, we calculated the aquarium means of the salamander treatments for use in the statistical analysis.

Results

INDUCTION OF SPECIFIC DEFENSIVE MORPHS

At the end of the first period, the tadpoles subjected to the salamander treatments exhibited higher bodies and tails than those subjected to the control treatment, and the tadpoles subjected to the dragonfly treatments exhibited only higher tails compared with the control tadpoles (Table 1a). Furthermore, in each of the predator-unaltered experiments, the predator-specific responses were enhanced during the second period. The body and tail depths at the end of the second period were higher than those at the end of the first period in the salamander-unaltered experiment (Table 1b, Fig. 2a). On the other hand, in the dragonfly unaltered experiment, we detected a statistically significant increase of tail depth during the second period (Table 1b, Fig. 2b). These morphogenetic changes coincided with the defensive morphologies induced in response to these predators as reported in our previous study (Kishida & Nishimura 2005), and we confirmed that morphogenesis continued throughout both periods of the experiment.

Table 1. Results of preliminary analyses to check induction of the predator-specific morphologies
(a) Values of the size-adjusted morphological variables for each treatment at the end of the first experimental period and the results of *t*-tests comparing the values of the variables in the predator treatments with those in the control treatment. All aquarium means in the predator treatments were used in the analyses

Treatment	Tail depth			Body depth		
	Mean ± SE (*n*)	t_{22}	*P*	Mean ± SE (*n*)	t_{22}	*P*
Salamander	7·87 ± 0·076 (18)†	10·05	< 0·001	6·54 ± 0·051 (18)†	9·91	< 0·001
Dragonfly	7·40 ± 0·066 (18)	7·45	< 0·001	5·70 ± 0·031 (18)	1·43	0·166
Control	6·50 ± 0·065 (6)	–	–	5·61 ± 0·037 (6)	–	–

(b) Values of the size-adjusted morphological variables in the control and the predator-unaltered treatments at the end of the second period and the results of *t*-tests comparing the values of the variables in the predator-unaltered experiments at the end of the second period with those at the end of the first period. In these analyses, means from the same aquarium were paired between the two periods (i.e. *n* = 6 pairs of means)

Treatment	Tail depth			Body depth		
	Mean ± SE (*n*)	t_5	*P*	Mean ± SE (*n*)	t_5	*P*
Salamander	8·79 ± 0·081 (6)†	5·96	0·002	7·46 ± 0·115 (6)†	7·20	< 0·001
Dragonfly	7·90 ± 0·120 (6)	8·84	< 0·001	5·79 ± 0·020 (6)	1·30	0·250
Control	6·47 ± 0·066 (6)	−0·56	0·602	5·64 ± 0·084 (6)	0·39	0·711

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†Represents the corrected mean (see Materials and methods).

Table 2. Results of MANOVA testing of the effects of the already induced phenotypes (i.e. bulgy or high-tail morph) and the alterations of the predator environment (i.e. predator exchange or removal in the second period) on the response variables

Source	Wilks' λ	$F_{2,19}$	P
Phenotype	0·040	228·389	< 0·0001
Environmental change	0·184	42·115	< 0·0001
Phenotype × environmental change	0·125	66·335	< 0·0001

FLEXIBLE SHIFTING AND REVERSIBILITY OF THE PREDATOR-SPECIFIC MORPHOLOGIES

To determine the effect of the already induced phenotypes (i.e. the bulgy morph induced by the salamander treatment and the high-tail morph induced by the dragonfly treatment in the first period) and alteration of the type of predator environment (i.e. predator exchange or removal in the second period) on the morphological response, we conducted a two-way MANOVA on the response variables of the tadpoles subjected to the predator-exchange experiments and on those of the tadpoles subjected to the predator-removal experiments. The MANOVA tested the effects of the type of the already induced phenotype (high-tail or bulgy morph), the effects of the type of change in the predator environment (predator exchange or removal), and their interaction term as predictors. The result of the MANOVA indicated that the interaction term was highly significant, implying that the morphological responses depended on the combination of the already induced phenotype and the change in the predator environment in the second period (Table 2).

After the test of overall difference, two decomposed MANOVAS were conducted, comparing the two predator-exchange experiments (i.e. the dragonfly–salamander and the salamander–dragonfly experiments) and the two predator-removal experiments (the dragonfly–no predator and the salamander–no predator experiments).

The morphological responses were significantly different between the two predator-exchange experiments (Wilks' λ = 0·019, $F_{2,9}$ = 232·81, P < 0·0001) and between the two predator-removal experiments (Wilks' λ = 0·063, $F_{2,9}$ = 67·193, P < 0·0001) (Fig. 2). These results indicated that the tadpoles with the already induced morph caused by the presence of salamanders and those with the already induced morph caused by the dragonfly exhibited different morphological changes in response to the environmental changes.

Following the tests of overall morphological changes, we tested the expectation of flexible adaptive morphological shifting in the predator-exchange and predator-removal experiments (Table 3). In the predator-exchange experiment from salamander to dragonfly, body depth decreased and tail depth increased (Fig. 2a and Table 3). In the predator-exchange treatment from dragonfly to salamander, body depth and tail depth significantly increased (Fig. 2b and Table 3). In the salamander-removal experiment, the already induced deep body and high tail were decreased (Fig. 2a and Table 3). In the dragonfly removal experiment, the already induced high tail was decreased, and body depth was unchanged (Fig. 2b and Table 3).

Discussion

R. pirica tadpoles repetitively changed their morphology in response to environmental change. They reciprocally changed their predator-specific phenotype when the predator species changed, and they reverted to the basic phenotype when the predation risk was removed. Thus, they revised their ontogenetic trajectory without rigid canalization after having already once completed this major developmental event.

Theoretical studies suggest that flexible phenotypic plasticity should be favoured when temporal variation of an environmental factor is fine-grained and when the benefit of the plasticity outweighs its cost (Moran 1992; Padilla & Adolph 1996; Van Tienderen 1997). The benefits of phenotypic plasticity derive from adaptive

Table 3. Results of *t*-tests on the response variables in the second period in the predator-exchange and the predator-removal experiments. In all these tests, the null hypothesis was that *R. pirica* tadpoles with already induced predator-specific phenotypes exhibit no morphological changes in response to a change in the predator environment. To set the type I error rate (α = 0·05) of each comparison under the null hypothesis, we used the sequential Bonferroni procedure to adjust the comparison-wise significance levels for a total of eight specific comparisons (Sokal & Rohlf 1995). According to our prior knowledge of the functional or adaptive significance of the primary morphological traits observed in each environmental regime (no predator, salamander predator, and dragonfly predator) (Kishida & Nishimura 2005), each test was one-tailed except for that comparing the body depth between the two periods in the dragonfly–no predator treatment

Experiment		Tail depth		Body depth	
First period	Second period	t_5	P	t_5	P
Salamander	Dragonfly	−5·49	0·002*	−12·08	< 0·001*
Dragonfly	Salamander†	3·83	0·006*	10·37	< 0·001*
Salamander	No	−3·22	0·012*	−7·95	< 0·001*
Dragonfly	No	−6·45	< 0·001*	2·85	0·474

*Statistically significant after sequential Bonferroni adjustment for α = 0·05 across eight tests.
†The corrected means were used in the analysis (see Materials and methods).

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matching between phenotypes and environments, and the concept relies on the important assumption that phenotypic trade-offs exist (i.e. a single phenotype does not confer the most fitness in all environments.) (Via & Lande 1985; Gomulkiewicz & Kirkpatrick 1992; Moran 1992; Van Tienderen 1997). Our previous study (Kishida & Nishimura 2005) showed adaptive significance of the predator-specific phenotypes in *R. pirica* tadpoles in the corresponding predator environments. That is, when exposed to predation risk by the dragonfly, more of the dragonfly induced 'high-tail' tadpoles survive than 'bulgy' or noninduced 'basic' tadpoles; on the other hand, more of the salamander-induced 'bulgy' tadpoles survive than 'high-tail' or 'basic' tadpoles when exposed to predation risk by the salamander. These evidences suggest that the reciprocal shifts between the predator-specific phenotypes in *R. pirica* tadpoles are adaptive matching of phenotype with the predator environment. On the other hand, it remains unknown whether the reversible response of the predator-specific phenotypes to removal of the predation risk represents adaptive matching, because the adaptive significance of the nondefensive basic phenotype over the predator-specific phenotype in the absence of predation risk has not been established. In general, defensive traits are considered to be costly to produce and/or maintain (Lively 1986; Pettersson & Bronmark 1997; Trussell 2000). The fact that reversibility of the predator-specific phenotypes in *R. pirica* tadpoles exists suggests that these morphologies are costly to maintain.

Evolution of phenotypic plasticity is constrained by the costs of the phenotypic plasticity itself and by various limits on the benefits of plasticity (Moran 1992; Padilla & Adolph 1996; Van Tienderen 1997). The huge time and energy expenditures involved in the destruction of an existing phenotype and the construction of a new phenotype should limit the benefits of flexible phenotypic plasticity (DeWitt, Sih & Wilson 1998). Thus, if it is to be accomplished, flexible shifting is expected to occur only among phenotypes constructed similarly. Increased tail depth is common to both predator-specific phenotypes studied here. The morphological differences between the two predator-specific phenotypes are observed in epithelial tissues of the entire body in *R. pirica* tadpoles (see fig. 1 in Kishida & Nishimura 2005). Therefore, the reciprocal shifts between the predator-specific phenotypes are equivalent to changing the epithelial tissues of the entire body. In fact, in the predator-exchange experiments, the flexible morphological changes seem to have been achieved by partial modifications. That is, in the salamander to dragonfly exchange treatment, destruction, but not reconstruction, occurred only in the body tissues, and the response in the tail tissues was maintained during the second period (Fig. 2a). In the dragonfly to salamander exchange treatment, the tadpoles newly developed a bulgy body without the destruction of the existing morph being necessary (Fig. 2b). These partial modifications might effectively reduce the time and energy expenditures involved in the repetitive morphological changes.

It is noteworthy that, with regard to the reversibility of the bulgy phenotype, a previous study (Kishida & Nishimura 2004) demonstrated that the bulgy tadpoles reduce their bulgy body by actually shrinking the hypertrophied epithelial tissue in response to the removal of the predation risk from larval salamanders. We observed an apparently similar shrinking process in the phenotypic shift from the bulgy phenotype to the high-tail phenotype in the second period of the salamander–dragonfly experiment. Given that *R. pirica* tadpoles can withdraw some resources by shrinking their bulgy body and then reallocate them to other developmental requirements during their subsequent life history, resource reallocation may also reduce the cost of adaptive shifting among the specific phenotypes.

A modern molecular genetic approach is needed for understanding the proximate mechanisms of developmental plasticity (Pigliucci 2001; DeWitt & Scheiner 2004). Mori *et al*. (2005) identified a difference in the transcription of a set of genes expressed in body epithelial tissue between the typical and bulgy tadpoles by cDNA subtraction. In the bulgy tadpoles, genes concerned with fibrinolysis were down-regulated and genes having a critical role in intracellular assembly were up-regulated. The down-regulation of the fibrinolysis-related genes results in the retention of blood vessels by the bulgy morph, facilitating the circulation of blood, and the up-regulation of the intracellular assembly genes modifies the intracellular structure of the epithelial tissue of the morph (Mori *et al*. 2005). The application of this molecular method to the high-tail morph and comparison of the genetic cascades leading to the development of both morphological phenotypes could significantly contribute to our understanding of the genetic architecture and evolution of flexible phenotypic plasticity.

The adaptive significance of, and the mechanism maintaining, the shifting of the specific phenotypes can be understood in terms of the temporal variability of the predator regime, which can be generated by an abiotic environmental change, and the varying phenologies of the organisms in the pond community. In lentic pond habitats, community composition is strongly affected by local environmental variation associated with pond desiccation (Urban 2004). In particular, in temporary ponds, the community composition is highly variable, depending on recent weather changes (Jefferies 1994; Schneider & Frost 1996; Skelly 1996). *R. pirica*, *H. retardatus* and *A. nigroflava* larvae commonly live in small lentic ponds that expand and merge with neighbouring ponds or dry up in response to stochastic weather changes such as heavy rainfall or drought (Sato 1989). In such ponds, abiotic environmental changes thus may be important determinants of temporal variability in the pond community and therefore of the temporal shifting in the predator composition for *R. pirica* tadpoles.

An additional mechanism affecting shifts of predation risk may be the phenology of the pond community. *R. pirica* and *H. retardatus* spawn vast numbers of eggs in small ponds created by melting snow in early spring.

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For some time after the eggs hatch, the density of larval *H. retardatus* is frequently high (May in Fig. 1), and thus the predator–prey interaction between these two species of amphibian larvae must be very intensive for a certain period. After a while, the predator–prey interaction tends to weaken as the number of larval *H. retardatus* declines (June in Fig. 1) via predation by aquatic insects or cannibalism. These mechanisms might impose on *R. pirica* tadpoles various changes in the predation regime (e.g. relaxation or intensification of the predation risk, or a predator exchange), and thus might shape and maintain the flexible morphological shifting capacity in *R. pirica* tadpoles.

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