

Multiple inducible defences against multiple predators in the anuran tadpole, *Rana pirica*

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ABSTRACT

Question: What conditions are required for evolution of predator-specific inducible defences?

Hypotheses: (1) Prey organisms distinguish among predators to which they are exposed. (2) Prey individuals with a predator-specific defence must attain higher survivorship than those with a mismatched defensive phenotype.

Organisms: Prey, anuran tadpoles (*Rana pirica*); biting type predator, dragonfly larvae (*Aeshna nigroflava*); swallowing type predator, salamander larvae (*Hynobius retardatus*).

Methods: *Rana pirica* tadpoles were exposed to the predator signal in close proximity to or remote from the dragonfly larvae or the salamander larvae to determine whether the tadpoles develop predator-specific morphologies and whether they utilize predator-specific signals in the induction process. We conducted predation trials to determine whether the tadpoles with induced phenotypes were more resistant to the attack in the corresponding predator environment.

Results: *Rana pirica* tadpoles developed predator-specific morphologies in response to exposure to two different types of predator. The tadpoles discriminated between the predators – that is, different signals were required to develop the specific phenotypes in the induction process. The survival rate of tadpoles of specific phenotypes was higher than that of tadpoles of mismatched or non-induced phenotypes when exposed to predation by the corresponding predators.

Keywords: cue, induced defence, morphology, phenotypic plasticity, polymorphism.

INTRODUCTION

Inducible defences are a ubiquitous form of phenotypic plasticity that involve altering behaviour, life history or morphology in response to predation risk (reviewed by Tollrian and Harvell, 1999). Organisms adopting an inducible defence strategy must first receive a cue from a predator and then develop a defensive phenotype before the actual attack (Adler and Harvell, 1990; Tollrian and Harvell, 1999). General arguments for the evolution of phenotypic plasticity suggest the following prerequisites for the evolution of inducible defences. First, prey organisms are exposed to unpredictably infrequent attacks by predators (Moran, 1992;

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Van Tienderen, 1997). Second, a reliable cue indicates the potential for future danger (Lloyd, 1984; Lively, 1986; Moran, 1992). Finally, the defensive phenotype is beneficial if expressed in the presence of predators, but costly in their absence (Lively, 1986; Clark and Harvell, 1992).

In natural systems, prey organisms are seldom subject to a single type of predator; they usually experience numerous environments created by various types of predators (Sih *et al.*, 1998; Thompson, 1998). A prey organism that exhibits inducible defence phenotypes to various types of predators has two options: induction of a non-specific defence as a generalist or induction of specific defences as a specialist in response to the corresponding predators. For most predator–prey interactions, it remains unclear whether prey develop a non-specific inducible defence or specific defences to different predators.

General inference and empirical studies on adaptive strategies in multiple predator environments have suggested the following prerequisites for evolution of induction of predator-specific defences. First, different predators employ different hunting methods (Sih *et al.*, 1998). Second, when exposed to a predation risk by a predator, prey individuals with a predator-specific defensive phenotype must attain higher survivorship than individuals with a mismatched defensive phenotype or a general defensive phenotype. Although several researchers have revealed predator-specific plastic phenotypes in various taxonomic groups (Dodson, 1989; DeWitt, 1998; Relyea, 2001; Van Buskirk, 2001; Laurila *et al.*, 2002), few researchers have studied whether specific phenotypes provide higher survival rates in the corresponding predator environments (but see DeWitt *et al.*, 2000). Third, prey organisms face multiple types of predation hazard unsynchronously. If a specific response to a predator species has a negative survival effect in the presence of the other predator species, co-existence of both predator species results in a reduction of benefits of multiple inducible defences (Sih *et al.*, 1998; Poitrineau *et al.*, 2003). Finally, prey should be selected to distinguish among the predators to which they are exposed (Tollrian and Harvell, 1999). Prey organisms might therefore demonstrate predator-specific defences in response to unique predator cues or different concentrations of the same cue. A few studies in which multiple inducible phenotypes responding to different predators were examined suggested that prey species utilize predator-specific cues for predator discrimination (Relyea, 2001; Van Buskirk, 2001). Evidence of cue-detection ability for predator-specific responses requires experimental determination of whether prey organisms discriminate among predators by detecting different cues or by detecting different concentrations of the same cue.

To understand adaptive predator-specific inducible defences, we examined morphologies of anuran tadpoles (*Rana pirica*) exposed to two different types of predator: larval salamander (*Hynobius retardatus*) and larval dragonfly (*Aeshna nigroflava*). Anuran tadpoles represent an excellent model system for studying inducible morphological defence. Tadpoles of more than 20 species, in five genera and four families, distributed in Europe and North America are reported to exhibit morphological change induced by various predators, including fish, salamander and aquatic insects such as the larval dragonfly (e.g. Van Buskirk, 2002). These studies have shown common features of inducible morphology of tadpoles; that is, the tadpoles increase their tail fin depth and reduce their body size in the presence of various predators. Tadpoles with induced phenotypes survive better when attacked by predators (McCollum and Van Buskirk, 1996; Van Buskirk and McCollum, 1999), although the exact mechanisms underlying their increased survival are not well understood (Van Buskirk and McCollum, 2000a,b).

We previously found that tadpoles of *Rana pirica* exhibit a unique induced morphological defence in the presence of larval salamanders, *Hynobius retardatus* (Kishida and

Nishimura, 2004). The salamander-induced phenotype exhibits not only a higher tail fin but also a bulgy body (Fig. 1c). The bulgy body prevents the tadpole from being swallowed by the gape-limited predator larval *H. retardatus*. Furthermore, the induction of the bulgy morph differs from that of the existing predator-induced morphologies in other anuran tadpoles. While the predator-induced morphological change in other anuran tadpoles can be triggered by remote chemical cues from predators or injured conspecifics (McCollum and Leimberger, 1997; Laurila *et al.*, 2001; Relyea 2001; Van Buskirk, 2002; Van Buskirk and Arioli, 2002; LaFiandra and Babbitt, 2004), induction of the defensive bulgy morph of *R. pirica* tadpoles in response to the presence of larval *H. retardatus* was not triggered by only a remote cue. Close proximity to the predator was required for the induction (Kishida and Nishimura, 2004).

The salamander-induced bulgy morph is thought to have been selected under the condition of an intimate predator–prey interaction with larval *H. retardatus* (Kishida and Nishimura, 2004). *Rana pirica* tadpoles, however, are exposed to predation risk by not only *H. retardatus* larvae but also other predatory aquatic insects. One of the major insect predators is the larval dragonfly, *Aeshna nigroflava*. In natural habitats, predator composition temporally and spatially varies across environments. In general, while larval *H. retardatus* are the dominant predator in small ponds from the early developmental season of *R. pirica* tadpoles, *A. nigroflava* are dominant in relatively large ponds (approximately more than 10 m²) from the middle developmental season of the tadpoles (unpublished data). In contrast to the prey capturing method of larval *H. retardatus*, which is a swallowing type, *A. nigroflava* larvae bite into prey with a protrusive labium and chew it.

In summary, the tadpoles, which exhibit an inducible morphological defence (the bulgy morph) against one type of predator (the larval salamander) (Kishida and Nishimura, 2004), also experience a threat of different type of predator. And the two types of predation threat tend to be separately experienced temporally and spatially. These circumstances seem to be



Fig. 1. Tadpoles with basic, high-tail and bulgy morphs. (a) Basic morph in the absence of predators. (b) High-tail morph induced by the dragonfly. (c) Bulgy morph induced by the salamander.

satisfied as part of the prerequisites for evolution and maintenance of multiple specific inducible defences.

However, if the tadpoles adopt indiscriminate cues to deal with the predation threats and the bulgy body also functions as armour to protect the tadpoles from attack by dragonfly larvae, *R. pirica* tadpoles may show a bulgy morph in response to the presence of dragonfly larvae as a general strategy. Alternatively, if the tadpoles discriminate cues from the two types of predators and an alternative inducible phenotype other than the bulgy one is a better option in the presence of dragonfly larvae, the prerequisites of evolution and maintenance of multiple specific inducible defences are completed.

In this study, we addressed the following issues experimentally. (1) Do the tadpoles exhibit different inducible morphologies in response to the different predators with different attack modes? (2) In the induction process, are different cues required to develop the specific phenotypes? If the tadpoles exhibit specific morphologies, (3) do the tadpoles with specific morphologies show adaptive differences in resistance to attack by the different predators?

MATERIALS AND METHODS

Collection and maintenance

Eggs of *R. pirica* and *H. retardatus* and larvae of *A. nigroflava* were collected from ponds in Hokkaido, Japan from late March to late June in 2003, and placed in 12-litre aquaria. *Rana pirica* tadpoles were fed rabbit chow *ad libitum*. The larval *H. retardatus* and *A. nigroflava* were fed small-sized *R. pirica* tadpoles *ad libitum*. Water in all aquaria was changed every third day. The experiments were conducted in a laboratory at 14–18°C, using a natural day/night (14 h/10 h) regime.

Induction experiment in the presence of different types of predator

We designed an experiment to determine (1) whether *R. pirica* tadpoles show specific morphological responses and (2) whether they utilize different cues – remote or close cues – in response to predators that employ distinct methods of prey capture. Focal tadpoles were reared in the segregated or contiguous presence of salamander or dragonfly predators (2 × 2 factorial design) to determine the effects of remote and close cues in producing the presumptive anti-predator phenotypes.

The experiment was started on 20 April 2003. The experimental units were 18-litre aquaria (37 × 25 cm in surface area, and 19 cm in height) each containing 2 litres of sand and filled with 10 litres of aged tap water. Each aquarium contained one partially submerged saucer-shaped colander (15 and 8 cm in diameter at the top and bottom, respectively). One hundred similarly sized 14-day-old tadpoles (mean ± standard deviation: body length = 8.41 ± 1.10 mm, $n = 50$) were randomly chosen from the holding tank, and were placed outside the colander in each aquarium. A predator was placed inside or outside the colander in the experimental aquarium, according to one of five treatments (see below). When a predator was placed in the colander, it was segregated from the focal tadpoles in the aquarium.

The experiment consisted of five treatments: two predator types (salamander and dragonfly) crossed with two levels of cue types (remote and close), plus a treatment with no predators. That is: (1) one salamander larva inside the colander (remote salamander

treatment), (2) one dragonfly larva inside the colander (remote dragonfly treatment), (3) one salamander larva outside the colander (close salamander treatment), (4) one dragonfly larva outside the colander (close dragonfly treatment), and (5) without a predator either inside or outside the colander (control treatment). Each treatment was repeated in seven different aquaria. At the start of the experiment, snout–vent length of the salamander larvae and total length of the dragonfly larvae were 17.78 ± 1.07 mm ($n = 20$) and 33.21 ± 5.26 mm ($n = 15$), respectively.

We defined close cues as tactile or close chemical cues, and remote cues as chemical cues. In the remote-predator aquarium, the tadpoles received remote cues that diffused from a predator or conspecifics that were attacked inside the colander. In the close-predator aquarium, the tadpoles received not only remote cues but also close cues from a predator outside the colander.

To minimize unexpected predation of focal tadpoles in the close-predator treatments, predator individuals were replaced daily with others that were kept in the holding tanks, in which predators were fed sufficient *R. pirica* tadpoles. In the replacement, predators were randomly chosen from each holding tank. However, since actual predation was unavoidable in the close-predator treatments, we also allowed the segregated predators to prey on *R. pirica* tadpoles in the remote-predator treatments, by assigning five victim tadpoles to all colanders, in order to equalize the feeding status of the predators between the ‘close’ and ‘remote’ treatments. The surviving tadpoles (not focal animals) in the colanders were removed daily and replaced by five new ones. To make the densities of tadpoles in the aquaria uniform during the experimental period, we counted the surviving tadpoles (focal animals) in all aquaria every third day, and equalized the numbers of tadpoles in all aquaria in accordance with that in the aquarium with the minimum number (by randomly reducing focal animals in other aquaria).

After 14 days, the induction experiment was terminated. During the experiment, the number of tadpoles consumed in the close-salamander and the close-dragonfly aquaria was 20.22 ± 8.21 ($n = 7$) and 18.24 ± 5.43 ($n = 7$) respectively. Ten tadpoles were randomly selected from the survivors in each aquarium and killed using 10% ethanol. Photographs of the dorsal and lateral views were taken in a small glass chamber. The photographic images were projected onto a computer monitor and the following four traits were digitized: body length (from the tip of the snout to the tail joint), maximum body depth, maximum body width and maximum tail depth (total length was not measured because the tail tip of many tadpoles was clipped by predator attacks in the close-predator treatments).

Predation experiment using tadpoles with induced phenotypes

Understanding the adaptive significance of induced phenotypes requires investigation of the survival value of induced individuals. We conducted a series of predation trials, in which an individual predator was allowed a binary choice between a pair of the three distinct morph tadpoles, to determine whether the tadpoles with induced phenotypes were more resistant to attack in the corresponding predator environment.

Predation trials were conducted using paired combinations of two tadpoles, each selected from one of the three distinct phenotypes (basic, high-tail and bulgy) identified in the induction experiment (see Results), in the presence of each predator (salamander and dragonfly). Distinct phenotypes were created using the same protocols as those of the induction experiment (i.e. basic, high-tail and bulgy phenotypes were induced in the

‘no-predator’, ‘remote-dragonfly’ and ‘close-salamander’ treatments, respectively). We selected intact tadpoles that had a body size of approximately 11 mm (mean body length = 10.77 ± 0.50 mm, $n = 60$) for each phenotype.

An aquarium (28.5×16.5 cm in surface area and 9.5 cm high) that contained 2 litres of water and 2 g willow moss (*Fontinalis antipyretica*) for physical structure was used as a trial tank. We arrayed 10 tanks at a time on a rack 1.2 m high, with a distance of 0.6 m between tanks. We randomly assigned a pair of tadpoles of distinct phenotypes and placed one small cage ($10 \times 7 \times 4$ cm) containing one predator in each tank. The predators used in the experiment had not been fed for at least 2 days. We used the maximum size class in natural ponds: the snout–vent length of larval *H. retardatus* was 25.65 ± 1.69 mm ($n = 20$) and the total length of larval *A. nigroflava* was 43.29 ± 5.73 mm ($n = 20$).

After the tadpoles had been acclimated to the setting of the tanks for 20 min, the predators were released from the cages, and then the trials started. All trials were confirmed by two observers who could distinguish the phenotypes. The observers checked around the aquaria at a very slow pace so as not to disturb the animals. If predation had occurred (i.e. a tadpole had been killed or was in captivity without any possibility of escape), the survivor phenotype was confirmed by both observers. If an observer failed to identify the first victim (i.e. both tadpoles had been preyed on), the trial was excluded from the data. Each trial set consisted of 25 replicates.

RESULTS

Induction experiment in the presence of different types of predator

We focused on three morphological traits: body width, body depth and tail depth. The data for these morphological traits were corrected for variation in size measurement (i.e. the data were the residuals of each trait after regression against a size measurement). Although a composite variable of various morphological traits (e.g. the first principal component of a principal component analysis) was used as an overall size criterion in many previous studies of inducible morphology in anuran tadpoles (e.g. Van Buskirk, 2002), we used body length as the size measurement because body length is the most conservative morphometric measurement for any morphological changes in tadpoles (i.e. body length was not different among the treatments; $F_{4,34} = 1.01$, $P = 0.41$) and the residuals of each trait showed no curvilinear trends. We defined the morphology of the tadpoles subjected to the control treatment as a basic morph, and defined any induced responses of morphological traits of the tadpoles subjected to the predator treatments as the difference from the basic morph. In the following statistical analyses, the aquarium mean of the size-adjusted morphological variables was used.

We did not test the hypothesis about overall difference of these morphological traits among the treatments, because we already knew that some specific morphological traits change in response to some factors incorporated in the experiment (Kishida and Nishimura, 2004). First, we conducted a two-way analysis of variance (ANOVA) to test for the effects of predator type (dragonfly vs salamander) and cue type (close vs remote) on each of the morphological traits (body width, body depth and tail depth). We found strong interaction effects between the factors of predator and cue type for all morphological traits (Table 1). Second, we conducted *ad hoc* multiple comparisons (Scheffé’s tests with the experiment-wise error, $P = 0.05$) of trait means among the possible pairs of basic morph tadpoles

Table 1. Two-way ANOVA tables of the effects of predator, cue type and their interaction on size-adjusted morphological traits: (a) body width, (b) body depth, (c) tail depth

	SS	d.f.	<i>F</i>	<i>P</i>
(a) Body width				
Predator	1.67	1	23.52	<0.001
Cue type	3.52	1	49.63	<0.001
Predator × Cue type	4.64	1	65.52	<0.001
Error	1.70	24		
(b) Body depth				
Predator	2.25	1	24.05	<0.001
Cue type	3.88	1	41.53	<0.001
Predator × Cue type	4.33	1	46.37	<0.001
Error	2.24	24		
(c) Tail depth				
Predator	0.51	1	11.46	<0.001
Cue type	0.92	1	20.84	<0.001
Predator × Cue type	2.29	1	51.70	<0.001
Error	4.79	24		

subjected to the control treatment and the tadpoles subjected to each experimental treatment.

For body width and depth, only the close-salamander treatment indicated greater mean values than the control treatment (Fig. 2a,b). The close-salamander and both dragonfly treatments had significantly greater mean values for tail depth than the no-predator (control) treatment. That is: (1) tadpoles subjected to the salamander-close treatment had a bulgy body and higher tail fin, (2) tadpoles exposed to dragonfly had only a higher tail fin, and (3) tadpoles that were remotely exposed to a salamander did not exhibit any plastic morphological response.

Since approximately 20% of tadpoles were preyed on by salamander or dragonfly larvae in the close-predator treatments, we suggest the alternative hypothesis that the morphology of the close-predator treatments differs from that of the remote-predator treatments due to selective predation. The selective predation bias hypothesis and our original hypothesis that the morphological change is induced by predation risk are not mutually exclusive. Anyway, the predation bias hypothesis is not our concern here. There did not appear to be any particular predation bias and we can disregard the hypothesis in the close-dragonfly treatment, because the means of the morphology traits exhibited no differences between the close- and remote-dragonfly treatments.

The unique morphologies of the tadpoles subjected to the close-salamander treatment require that we consider the predation bias hypothesis, in which less bulgy and/or less high-tail morphs were preyed upon by the predator. The predation bias exaggerates the induction effect of the close-salamander treatment. In the close-salamander treatment, we removed 30% of the morphometric data of individual tadpoles, beginning with the

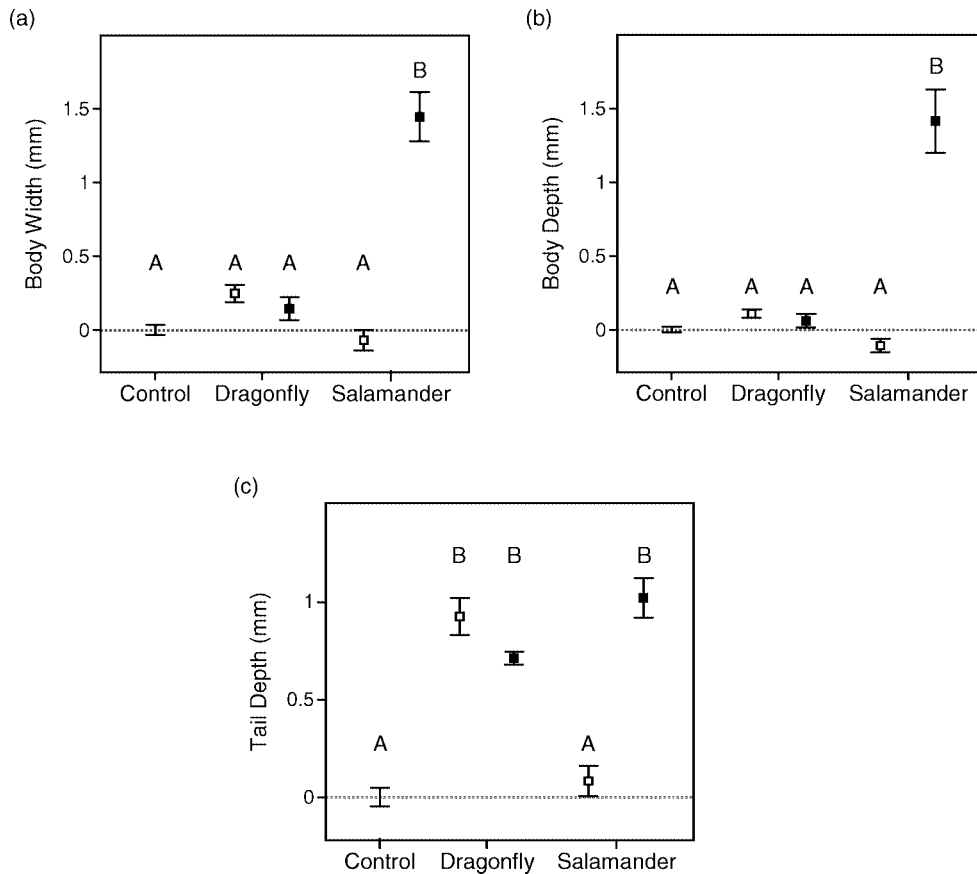


Fig. 2. Induced responses of morphological traits of tadpoles subjected to five treatments (i.e. differences in size-adjusted mean values from the no-predator treatment): (a) body width; (b) body depth; (c) tail depth. Open squares are remote-treatments, and solid squares are close-treatments. Error bars denote one standard error ($n = 7$). Results of each overall two-way ANOVA are shown in Table 1. Homogeneous treatment groups are indicated by horizontal ordered letters (Scheffé-adjusted).

largest one in each aquarium, to adjust the possible exaggeration in the original analysis given that we accept the predation bias hypothesis, and evaluated the morphological differences between the close- and remote-salamander treatments. We found that the tadpoles in the close-salamander treatment exhibited a significantly bulgier body morph and a higher tail than the tadpoles in the remote treatment (results of t -tests: body width, $t_{12} = 5.73$, $P < 0.001$; body depth, $t_{12} = 4.75$, $P < 0.001$; tail depth, $t_{12} = 3.80$, $P = 0.003$).

In summary, *R. pirica* tadpoles developed predator-specific morphologies: a salamander-specific 'bulgy morph' (bulgy body and higher tail fin) and a dragonfly-specific 'high-tail morph' (only higher tail fin) (Fig. 1). Furthermore, we demonstrated that *R. pirica* tadpoles have different signal detection systems for induction of predator-specific morphologies; that is, while the induction of the bulgy morph requires close proximity of the salamander, the high-tail morph can be remotely cued by the larval dragonfly.

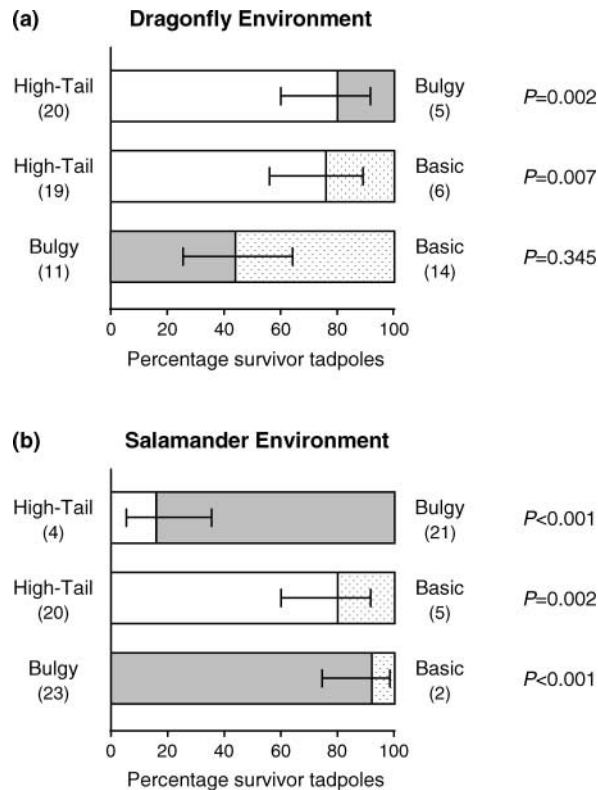


Fig. 3. Percentage of survivors in the predation trials in the presence of two predator species: (a) dragonfly environment, (b) salamander environment. Number in parentheses represents the actual number of survivors in the 25 trials. P -values indicate the results of the binomial test. Error bars denote 95% confidence limits for proportions ($n = 25$).

Predation experiment using tadpoles with induced phenotypes

The results of binomial tests revealed that *R. pirica* tadpoles of specific phenotypes are adaptive against the corresponding predators: (1) in the dragonfly environment, the survival rate of tadpoles with the high-tail phenotype was higher than the survival rate of tadpoles with the basic phenotype and the bulgy phenotype (Fig. 3a); and (2) in the salamander environment, the survival rate of tadpoles with the bulgy phenotype was higher than that of tadpoles with mismatched phenotypes, the high-tail phenotype or the basic phenotype (Fig. 3b). One especially noteworthy result is that the tadpoles with the dragonfly-induced morphology had a higher survival rate than the tadpoles with the non-induced basic morphology in the presence of predation risk by both larval *A. nigroflava* and *H. retardatus*. However, the salamander-induced tadpoles had a higher survival rate than the tadpoles with basic morph only in the *H. retardatus* environment.

DISCUSSION

Predator-specific morphologies

Predator-specific inducible defences should be favoured by natural selection in the presence of alternative predators with different attack modes. Determining the adaptive significance of specific phenotypes is important for understanding the evolution of multiple inducible defences against multiple predators. We demonstrated that *R. pirica* tadpoles are induced to develop the predator-specific morphologies in response to exposure to two different types of predator – larval salamanders, *H. retardatus* (swallowing-type predator), and larval dragonflies, *A. nigroflava* (biting-type predator) – and the survival rate of *R. pirica* tadpoles of specific phenotypes is higher than that of tadpoles of mismatched or non-induced phenotypes when exposed to predation by the corresponding predators.

General defence, high-tail phenotype

A predator-induced high-tail morph is a common feature of morphology induced in larvae of many amphibian species in response to various predators (e.g. Van Buskirk and Schmidt, 2000; Van Buskirk, 2002), and anuran tadpoles of such a predator-induced high-tail phenotype have a higher survival rate than those of a non-induced phenotype in the presence of predators (McCollum and Van Buskirk, 1996; Van Buskirk and McCollum, 1999). *Rana pirica* tadpoles also developed a high tail fin in the presence of larval dragonfly, *A. nigroflava*, and the tadpoles with the high-tail phenotype attained higher survival rate in the dragonfly environment.

A noteworthy result in the predation experiment is that the survival rate of the tadpoles with the dragonfly-induced high-tail phenotype was higher than that of the tadpoles with the basic phenotype when exposed to predation by the mismatched predator, larval salamander *H. retardatus* (Fig. 3b). That is, the dragonfly-specific high-tail phenotype is effective against not only the inducing corresponding predator (larval dragonfly, *A. nigroflava*) but also the mismatched predator (larval salamander, *H. retardatus*). Such a general adaptation of high-tail phenotypes has been observed in allied species. For example, in the grey tree frog (*Hyla versicolor*), the rate of survival of the higher-tail tadpoles induced by the larval dragonfly (*Anax longies*) was higher than that of non-induced phenotypes not only when the dragonfly was present but also when the other predator species (larval salamander, *Ambystoma tigrinum*) was present (Van Buskirk and McCollum, 2000a).

Induction of the high-tail phenotype in *R. pirica* tadpoles could be also considered to be a common response with an adaptive function to various potential predators that live in the lentic habitats of these anuran species. Determining the mechanistic function of the general adaptation is important for our further understanding of the evolution and maintenance of the induction of the high-tail phenotype. Although a higher tail fin has been suggested to enhance swimming performance (e.g. McCollum and Van Buskirk, 1996; Lardner, 1998), this hypothesis has not been verified (Van Buskirk and McCollum, 2000b). Further investigations are needed to elucidate the function of the high-tail morph.

Salamander-specific defence, bulgy phenotype

The result of induction of the salamander-induced bulgy morph, which is a unique response in allied anuran species, is in line with the results of a previous study (Kishida and Nishimura, 2004). Kishida and Nishimura (2004) showed that the bulgy body functions to prevent the tadpoles from being swallowed by the gape-limited predator, larval *H. retardatus*. In

the present study, we demonstrated that the bulgy phenotype was ineffective against the biting-type predator larval dragonfly, *A. nigroflava*. These results imply that the bulgy phenotype is the specific defensive phenotype against gape-limited predators.

Among the various potential predators in the habitat of *R. pirica* tadpoles, *H. retardatus* is the only predator that adopts a swallowing-type gape-limited predation (unpublished data). There is evidence of a reciprocal phenotypic plasticity, in which *R. pirica* tadpoles develop the functional defensive bulgy morph specialized to the *H. retardatus* larvae, and larval *H. retardatus* develop a carnivorous broad-headed morph in a high number of *R. pirica* tadpoles, which is advantageous for consumption of larger prey (Michimae and Wakahara, 2002). Accordingly, the bulgy morph might have been selected as the result of an arms race with larval *H. retardatus*.

Predator-specific signal detection

Prey organisms that adopt predator-specific phenotypic plasticity need to distinguish the types of predators to which they are exposed, either by predator-specific unique cues or by different concentrations of the same cue (Tollrian and Harvell, 1999). The type of cue utilized has been suggested in several studies in which significant variation of plastic phenotypes among different predator species was detected (Relyea, 2001; Van Buskirk, 2001). We demonstrated that the cues necessary for induction of specific phenotypes in *R. pirica* tadpoles differ depending on the predator-specific morphology.

Signals associated with a predation risk must have been selected according to the combination of their reliability, the time lag between occurrence of the cue and the actual risk, and the time required to respond to an impending crisis. Reliability and the time lag between the cue and the attack are related not only to the sensory ability and escape ability of the prey, but also to the hiding strategy and attacking strategy of the predator. Signal adoption is closely related to those characteristics that depend on the life histories of the predator and prey.

It is generally agreed that the predator-induced morphological change in many anuran tadpoles can be triggered by remote chemical cues from predators or injured conspecifics (McCollum and Leimberger, 1997; Laurila *et al.*, 2001; Relyea, 2001; Van Buskirk, 2002; Van Buskirk and Arioli, 2002; LaFiandra and Babbitt, 2004). Also, *R. pirica* tadpoles exhibited the high-tail morph in the presence of remote cues of the larval dragonfly *A. nigroflava*. On the other hand, a close cue of larval *H. retardatus* must be a necessary condition for the induction of the bulgy morph [this finding is in line with the results of our previous study (Kishida and Nishimura, 2004)].

Dragonfly larvae of the genera *Aeshna* and *Anax* are the common predators of anuran species in lentic habitats worldwide. They spawn in summer, and their larvae overwinter. In the next spring, as water temperature rises, the growing larvae feed actively and the size of the larvae is sufficient for them to prey on tadpoles (i.e. their killing power is high). Co-existing dragonfly larvae are a consistently serious predator for the tadpoles. In general, a remote chemical cue is a good signal for an aquatic organism to detect the presence of an obligate predator, since chemical substances that can be dispersed over relatively long distances and persist for long periods may allow organisms to deliberately change their phenotype (Havel, 1987; Adler and Harvell, 1990; Tollrian and Harvell, 1999).

Rana pirica and *H. retardatus* spawn a greater number of eggs in small ponds transiently formed by melting snow in early spring. Generally, *R. pirica* tadpoles hatch earlier than

H. retardatus larvae, and thus the hatched tadpoles live alongside salamander eggs or early-stage small salamander larvae (Sato, 1990). For a certain period in the early stage of development, *R. pirica* tadpoles chronically experience close proximity with small *H. retardatus* larvae that are not substantial predators. The salamanders around the tadpoles become dangerous predators as they grow. In the phenological process with unsynchronized size development, utilization of a 'close' signal detection system might have been evolutionarily favoured rather than utilization of a remote signal detection system.

Rana pirica tadpoles may have acquired the facultative development of the bulgy morph and the close-signal detection system, in addition to the high-tail morph and the remote signal detection system, against the gape-limited predator salamander larvae, *H. retardatus*, based on the intimate relationship between them.

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