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# To avoid or not to avoid? Factors influencing the discrimination of predator diet cues by a terrestrial salamander

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Many prey species use chemical cues deposited by predators to assess predation risk, and some prey are capable of discriminating among predator chemical traces based on the predator's recent diet. Here we test the influence of genetic relatedness (degree of genetic similarity between prey) and ecological relatedness (degree of refuge and trophic overlap between syntopic prey) on the evolution of predator-diet discrimination by red-backed salamanders, *Plethodon cinereus*. In experiment 1, we examined whether red-backed salamanders from Binghamton University (Binghamton, New York, U.S.A.) would differentially respond to chemical traces from garter snakes (*Thamnophis sirtalis*) fed conspecific *P. cinereus* from four populations belonging to three phylogenetic groups: Group II from Mountain Lake, Virginia, Group III from Binghamton University, New York, Group III from Hawk Mountain, Pennsylvania, and Group IV from Cherry Springs, Pennsylvania, U.S.A. The test salamanders only avoided chemical traces from garter snakes fed salamanders from the two Group III sites, although the response to the Group III-Hawk Mountain treatment was intermediate to that for the Group III-Binghamton University treatment and the Group II and IV treatments. In experiment 2, we examined whether red-backed salamanders collected from two subpopulations, syntopic or allotopic with two-lined salamanders, *Eurycea bislineata*, vary in their response to chemical cues from garter snakes foraging on two-lined salamanders. Only red-backed salamanders syntopic with *E. bislineata* avoided chemical traces from garter snakes fed *E. bislineata*. These results suggest that both genetic and ecological relatedness play a role in the evolution of predator-diet discrimination in red-backed salamanders.

Predator-prey interactions influence the ecology and evolution of most organisms, and the threat of predation may dramatically alter the behaviour of prey species (Lima & Dill 1990; Lima 1998). Organisms of nearly every trophic level have evolved defence mechanisms to reduce predation or predation risk. These defence mechanisms range from predator avoidance behaviours, which reduce the probability of encountering a predator, to antipredator behaviours, which reduce the probability of a successful predation event if a predator is encountered (Endler 1986; Brodie et al. 1991). Predator avoidance mechanisms are especially advantageous to prey species because prey may

eliminate predator encounters and reduce injuries involved with antipredator behaviour (Sih 1987; Lima & Dill 1990). One would therefore expect that the perception and identification of particularly threatening predators would be selected for in a wide variety of animal species.

Chemical cues from predators or predation events are used by some prey organisms to greatly reduce predation risk (Weldon 1990; Chivers & Smith 1998; Kats & Dill 1998). The ability of prey to discriminate among predation-related chemical cues in the environment is quite refined in many organisms, and some species use chemical information about a predator's recent diet to modify their defence response (Jacobsen & Stabell 1999; Madison et al. 1999a; Murray & Jenkins 1999; Venzon et al. 2000; Persons et al. 2001). For example, prey may increase their antipredator response to individual predators that have recently foraged on conspecific prey (Belden et al. 2000; Venzon et al. 2000; Chivers & Mirza 2001a; Persons et al. 2001; Madison et al. 2002). The differential responsiveness of prey to predators foraging on different prey species (hereafter referred to as diet discrimination) has been

examined in some detail for aquatic vertebrates, especially among fish (Mathis & Smith 1993; Brönmark & Pettersson 1994; Brown et al. 1995) and larval amphibians (Wilson & Lefcourt 1993; Laurila et al. 1997; Chivers & Mirza 2001b), but diet-related predator effects among terrestrial vertebrates have received limited attention (Madison et al. 1999a, b; Murray & Jenkins 1999).

Because some prey species discriminate among predators foraging on different prey, it is important to study the factors that favour this discrimination in a prey species to better understand the ecology of predator–prey interactions. Chivers & Mirza (2001b) hypothesized that the evolution of this diet-based discrimination is a direct result of varying predation intensity, and that the ability of prey to discriminate between chemical cues from predators should increase when predation intensity is relatively uncertain. Thus, if predation pressure is consistently high (e.g. a specialist predator), then differential responsiveness to diet cues is less valuable to the target prey, because essentially all members of the predator species feed on the focal prey species at all times. The prey should therefore respond to cues from all individuals of this predator species because they represent a continuous threat. If predation pressure is relatively uncertain (e.g. a generalist predator) or fluctuates temporally, the target prey may benefit by only avoiding those predators currently foraging on conspecifics, or heterospecifics in the same prey guild, to prevent unnecessarily compromising foraging or mating (Lima & Dill 1990; Lima 1998).

How species assign risk to predators foraging on different types of prey has not been fully determined experimentally. There are a number of factors that could contribute to diet discrimination of predator chemical cues, and we suggest that these factors fall into two broad categories: genetic and ecological relatedness. Genetic relatedness is defined as the degree of genetic similarity between species who by virtue of their similarity share chemical defences, such as the common alarm trigger among ostariophysian fish (Smith 1992; Brown et al. 2000). Applying the genetic relatedness concept, one would predict, for example, that a target prey species would avoid chemical cues from predators feeding on a congeneric prey because of shared prey chemistry and defence mechanisms, despite possible allopatry between the target and congeneric prey. We define ecological relatedness as the degree of refuge (microhabitat) and trophic overlap between syntopic prey species that are likely to share the same predators. By this definition, three prey species that share similar retreats and are members of the same prey guild would have a high degree of ecological relatedness, even though they may not be closely related phylogenetically.

## The Prey and the Predator

One well-studied, chemically mediated, predator–prey system ideal for examining predator-diet discrimination is the interaction between red-backed salamanders, *Plethodon cinereus*, and garter snakes, *Thamnophis sirtalis*. The natural histories of these species suggest a long evolutionary

history of predator–prey interactions. Red-backed salamanders are one of the most abundant vertebrates inhabiting the forests of eastern North America where they prey on a variety of small invertebrates associated with the leaf litter (Burton & Likens 1975; Jaeger 1980a, b). Eastern garter snakes are sympatric with *P. cinereus* throughout much of their range (Conant & Collins 1991) and are a common predator in forests where they forage on invertebrate and vertebrate prey including amphibian species (Hamilton 1951; Carpenter 1952; Kephart & Arnold 1982; Rossman et al. 1996; Bronikowski & Arnold 1999). Red-backed salamanders and garter snakes are syntopic in many areas where each will take refuge beneath logs or rocks on the forest floor (Jaeger 1980b; personal observation). Garter snakes prey on red-backed salamanders (Hamilton 1951), show fidelity to foraging sites (Gillingham et al. 1990), and are active concomitantly with red-backed salamanders on warm evenings (Jaeger 1980b; Reichenbach & Dalrymple 1986). Therefore, identifying and responding to areas with garter snake chemical cues may yield fitness benefits to *P. cinereus*.

Red-backed salamanders respond to garter snake chemical cues in laboratory and field trials (Sullivan et al. 2002, in press), but their response varies with the time of day and the recent diet of the predator (Madison et al. 1999a, b; Murray & Jenkins 1999; Maerz et al. 2001). During daytime trials, red-backed salamanders show a pronounced avoidance of garter snake chemical traces regardless of snake diet. During late-night trials, however, red-backed salamanders avoid traces from garter snakes fed *P. cinereus*, but not traces from *T. sirtalis* fed goldfish or earthworms (Madison et al. 1999b, 2002). Garter snakes are primarily, but not exclusively, diurnal foragers in the northeastern United States (when *P. cinereus* is active), and presumably they pose less threat to *P. cinereus* at night, unless they have recently fed on *P. cinereus*. These results suggest that red-backed salamanders can assess predation risk through chemical cues and can respond to an increased risk of predation with avoidance.

Whether ecological or genetic relatedness has contributed to the evolution of predator-diet discrimination by red-backed salamanders is unknown, although the available evidence can be interpreted to support both hypotheses. Red-backed salamanders from sites syntopic with two-lined salamanders, *Eurycea bislineata*, will avoid garter snakes fed *E. bislineata* in late-night laboratory trials (A. M. Sullivan, D. M. Madison & J. R. Rohr, unpublished data). Two-lined salamanders are confamilial (Plethodontidae), so homology of the chemical ‘marker’ that labels snakes may be responsible for this behavioural response. On the other hand, red-backed salamanders from a site lacking syntopic two-lined salamanders (Binghamton University, New York, U.S.A.: BU) failed to avoid garter snakes fed *E. bislineata* in late-night trials, suggesting that ecological relatedness, or ‘experience’ with the prey species in the predator’s diet is necessary for the discrimination (Sullivan et al., in press).

In the following experiments, we examine how genetic relatedness and ecological relatedness may have shaped the evolution of predator-diet discrimination by red-backed salamanders. Previous studies show that red-backed

salamanders avoid cues from snake predators (Cupp 1994; McDarby et al. 1999; Madison et al. 1999a, b; Murray & Jenkins 1999), and discriminate between garter snakes fed different prey (Madison et al. 1999b). Here, we (1) examine the role of genetic relatedness in predator-diet discrimination by subjecting *P. cinereus* from BU to chemical cues from snakes fed conspecific prey that genetically differ from the BU population, and (2) examine the role of ecological relatedness by assessing *P. cinereus* responses to snakes fed salamander species that are either allotopic or syntopic to test salamanders.

## METHODS

### Collection and Maintenance of Study Animals

We collected 224 adult red-backed salamanders in June 2002 from the Binghamton University Nature Preserve, BU (Broome County, New York) for experiment 1 and 168 adults in October 2002 from the Waterman Conservation Education Center, WC (Tioga County, New York) for experiment 2. We housed salamanders individually in 15-cm-diameter petri dishes lined with moistened paper towels and maintained them at 18°C and a 15:9 h light:dark photoperiod in a climate-controlled chamber. We tested all salamanders once within one week of capture and then released them at their collection site. We captured 10 adult garter snakes from BU as predator stimulus donors and housed them individually in 38-litre glass aquaria and provided them with a heating block and crumpled paper towels for cover. We provided water ad libitum, and food biweekly (diet varied according to experiment).

### Collection of Predator Chemical Cues

A distilled water rinse of *T. sirtalis* was used as the predator stimulus. Before collecting the rinse, we fed the source *T. sirtalis* three adult female and three adult male salamanders of the appropriate treatment group, and immediately transferred it to a 4-litre beaker that had been cleaned with Alcojet detergent and rinsed in 2% nitric acid and distilled water. After transfer, we covered the beaker with cheesecloth and placed the beaker on a heating block so that one-half of the beaker bottom was warmed. After 72 h, we carefully removed the snake and rinsed the beaker with 200 ml of distilled water. We filtered the rinse through a 0.45- $\mu$ m filter to remove solid materials, and then divided the filtrate into four 50-ml aliquots. Samples were rapidly frozen in liquid nitrogen and stored in a freezer for later use. Previous research found no differences in the anti-predator responses of salamanders to frozen and fresh snake cues, which suggests that rapid freezing does not chemically alter the sample (McDarby et al. 1999; Madison et al. 1999a; Sullivan et al. 2002). The entire collection process was repeated to provide enough snake stimuli for both experiments. In total, 48 *P. cinereus* (12 from each group) were collected and fed to snakes to provide the chemical cues for experiment 1. For experiment 2, we

collected 12 *P. cinereus* and 12 *E. bislineata* to provide the chemical stimuli.

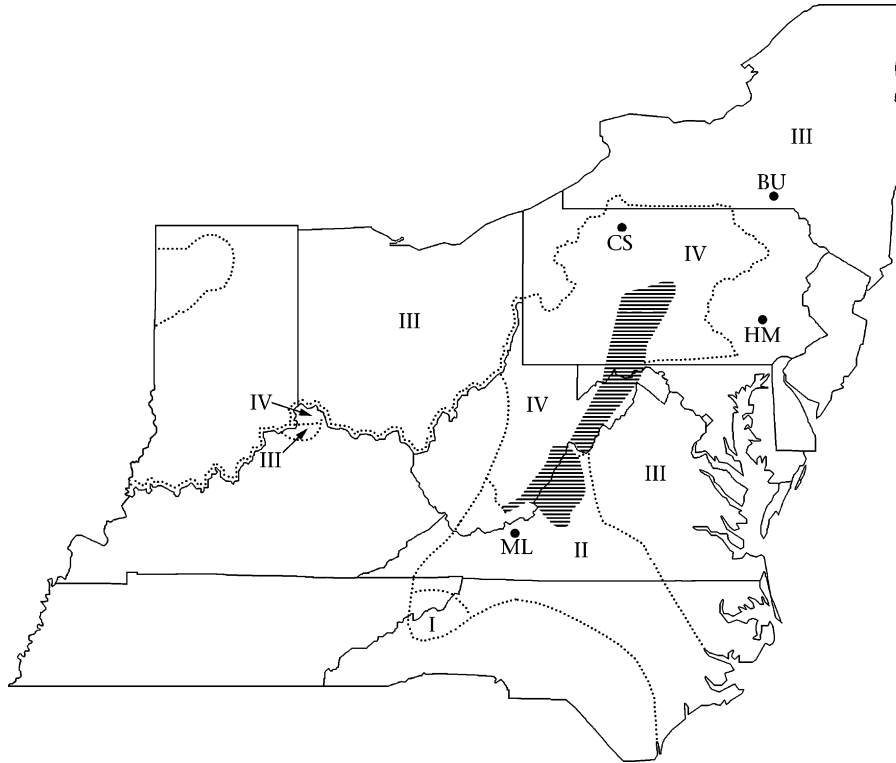
### Laboratory Bioassay

We used previously established behavioural bioassay procedures to test for salamander responses to predator cues (Madison et al. 1999a, b; Sullivan et al. 2002, 2003). In brief, we placed two filter paper semicircles on opposite sides of 15-cm-diameter  $\times$  1.5-cm-high petri dishes while maintaining a 3-mm gap between each semicircle. We added 1.5 ml of treatment solution (predator diet treatment) or control water to each semicircle using a 10-ml tuberculin syringe, and randomly distributed each treatment dish on an 8  $\times$  7 grid on the floor of our experimental room. Treatments were randomly stratified so that an equivalent number of replicates of each treatment were repeated each night. We transferred each salamander from its home dish to the assigned experimental dish with a clean cotton swab, and placed a 15-mm collar of grey paper around the dish to visually isolate the salamander within each test dish during the set-up period. After salamanders were distributed, we turned off the lights and recorded the trial in complete darkness with a video camera sensitive to infrared light. Conducting trials in total darkness forced salamanders to select substrates using only nonvisual cues and eliminated potential visual biases. We began recording trials between 2355 and 2405 hours each night.

An observer, blind to the chemical treatments, calculated the total amount of time that each salamander spent on the control (water) and stimulus (predator-diet treatment) sides of each experimental dish during each 15-min (900-s) trial. If a salamander straddled the 3-mm gap, the side with more than half of the individual was considered the occupied side. We chose this criterion, instead of the location of the sensory organs, because red-backed salamanders sometimes 'freeze' when they detect an alarm substance (Brodie et al. 1974; Madison et al. 1999a), and only the sensory receptors (less than half of the body) may be over the alarm substrate. Using location of the sensory receptors in this scenario would indicate 'a preference' for the alarm substance, whereas using the location of more than half of the body would not, and thus, provides a more accurate measure of an individual's response to the alarm substance.

## EXPERIMENT 1: GENETIC RELATEDNESS AND DIET DISCRIMINATION

In this experiment, we examined whether individual *P. cinereus* were capable of discriminating and differentially responding to chemical cues from garter snakes fed red-backed salamanders from different phylogenetic lineages. The phylogenetic lineages for *P. cinereus* were established by Hass (1985), who compared protein-coding loci from red-backed salamanders in 52 populations throughout the southern portion of its range. The analysis of protein variation to study genetic relationships in salamanders is well documented in the literature (e.g. Highton 1995, 1999a, b; Nishikawa et al. 2001), and Hass' (1985) data



**Figure 1.** Ranges of the four groups (I–IV) of *P. cinereus* as determined by comparing protein-coding loci among populations (Hass 1985). Distributions are indicated by dotted lines. Horizontal lines indicate the portion of the range from which *P. cinereus* is largely excluded. The four collection sites for experiment 1 were: Binghamton University Nature Preserve (BU), Cherry Springs State Park (CS), Hawk Mountain State Park (HM), and Mountain Lake Biological Station (ML).

indicate that *P. cinereus* includes four distinct phylogenetic groups (see Fig. 1). We examined the response of red-backed salamanders from BU (Hass' Group III) to garter snakes from BU that were fed *P. cinereus* from three of the four lineages to examine whether intraspecific genetic variation among the prey influenced red-backed salamander antipredator responses. The four treatments were *T. sirtalis* fed: (1) Group II *P. cinereus* collected 655 km away at Mountain Lake Biological Station (ML), (2) Group III *P. cinereus* from BU, (3) Group III *P. cinereus* collected 161 km away at Hawk Mountain State Park (HM), and (4) Group IV *P. cinereus* collected 161 km away at Cherry Springs Park (CS). If close genetic relatedness of the prey is required to elicit avoidance, then we suggest that prey *P. cinereus* from Groups II and IV would be the least likely to elicit avoidance, and Group III *P. cinereus* from BU would be the most likely to elicit avoidance.

We used a two-tailed Wilcoxon signed-ranks tests to determine whether the amount of time that test salamanders spent on the substrates labelled with predator cues differed significantly from the reference value of 450 s, which we would expect if salamanders spent an equivalent amount of time on control and predator substrates (Sheskin 1997). We used a Kruskal–Wallis one-way analysis of variance by ranks to determine whether the amount of time on predator substrates differed between treatments with post hoc multiple comparisons when the main effect was significant (Siegel & Castellan 1988; Sheskin 1997). We adjusted the experiment-wise error rate using sequential

Bonferroni tests using the Dunn–Šidák method because it is more powerful than a blanket Dunn–Šidák probability for all comparisons as described in Sokal & Rohlf (1995).

## Results

The amount of time that red-backed salamanders spent on predator substrates did not differ significantly from 450 s when the predator was maintained on Group II (Wilcoxon signed-ranks test:  $T = 774.00$ ,  $N = 56$ ,  $P = 0.845$ ) or Group IV salamanders ( $T = 708.00$ ,  $N = 56$ ,  $P = 0.463$ ; Table 1), suggesting that salamanders neither avoided nor were attracted to these diet treatments.

**Table 1.** Median time that *P. cinereus* from Binghamton University Nature Preserve spent on substrates inoculated with chemical cues from garter snakes fed *P. cinereus* from different phylogenetic groups

Predator-diet treatment	Time		$T$	$P$
	on snake substrate (s)	Interquartile range		
Group II (ML)	459.50	49.50–800.50	774.00	0.845
Group III (BU)	105.50	4.50–286.50	171.00	<0.001
Group III (HM)	229.00	6.50–523.50	426.00	0.002
Group IV (CS)	447.50	98.00–878.00	708.00	0.463

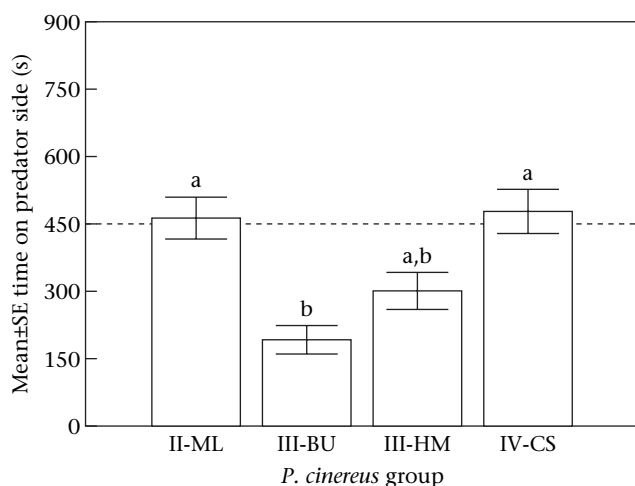
Wilcoxon signed-ranks tests:  $N = 56$  in all cases. Collection sites are: ML = Mountain Lake Biological Station, Virginia; BU = Binghamton University Nature Preserve, New York; HM = Hawk Mountain State Park, Pennsylvania; CS = Cherry Springs State Park, Pennsylvania.

However, the amount of time that red-backed salamanders spent on predator substrates was significantly less than 450 s when the predator was fed Group III salamanders from BU (Wilcoxon signed-ranks test:  $T = 171.00$ ,  $N = 56$ ,  $P < 0.001$ ) and HM ( $T = 426.00$ ,  $N = 56$ ,  $P = 0.002$ ; Table 1).

The amount of time that red-backed salamanders spent on the stimulus side differed significantly between predator-diet treatments (Kruskal–Wallis ANOVA:  $H_{3,224} = 21.12$ ,  $P < 0.001$ ). Paired comparisons indicated that salamanders spent significantly less time on substrates labelled with predators fed Group III prey from BU versus Group II ( $P = 0.001$ ) and Group IV ( $P < 0.001$ ) (Fig. 2). Response scores from salamanders exposed to snakes fed Group III prey from HM represented an intermediate value, not differing significantly from Group III-BU ( $P = 0.954$ ), Group II ( $P = 0.138$ ) or Group IV ( $P = 0.084$ ) (Fig. 2).

## EXPERIMENT 2: HABITAT OVERLAP AND PREDATOR-DIET DISCRIMINATION

The ecological relatedness model of diet discrimination of predator cues by prey implies that learning and/or experience are necessary for prey species to modify their defensive responses based on fluctuations in predation risk. Our goal in this study was to determine whether red-backed salamanders collected from two subpopulations over a small geographical scale vary in their responses to garter snakes foraging on two-lined salamanders, which either coexist with *P. cinereus* (syntopic) or do not coexist with *P. cinereus* (allotopic) in the local environment. If familiarity of other prey species is important in eliciting



**Figure 2.** Time that *P. cinereus* from Binghamton University Nature Preserve spent on substrates inoculated with chemical cues from snakes fed *P. cinereus* belonging to Group II from Mountain Lake Biological Station, Virginia, Group III from Binghamton University Nature Preserve, New York, Group III from Hawk Mountain State Park, Pennsylvania, and Group IV from Cherry Springs State Park, Pennsylvania. Different letters denote significant differences between groups.

avoidance in *P. cinereus*, then red-backed salamanders that are allotopic to two-lined salamanders will not avoid garter snakes fed two-lined salamanders, but those syntopic to two-lined salamanders will avoid this stimulus. We collected red-backed salamanders from two subpopulations at the Waterman Conservation Education Center (WC). Long-term mark–recapture data suggest that red-backed salamanders are distributed throughout the woodland area, whereas two-lined salamanders tend to be located within 100 m of the stream margin at this site (J. C. Maerz, personal communication). We collected test red-backed salamanders from a lowland area within 50 m of a stream that were syntopic with two-lined salamanders, and from an upland forested region 500 m from the same stream that were allotopic to two-lined salamanders. Members of each subpopulation were exposed to chemical test stimuli from garter snakes that had been fed either red-backed salamanders (TS<sub>PC</sub>) or two-lined salamanders (TS<sub>EB</sub>) collected from WC.

We used a two-tailed Wilcoxon signed-ranks test to determine whether the amount of time that test salamanders from each subpopulation spent on the substrates labelled with predator cues differed significantly from the hypothesized null value of 450 s (Sheskin 1997).

## Results

The median amount of time that red-backed salamanders spent on substrates inoculated with TS<sub>PC</sub> was significantly less than 450 s for individuals from subpopulations syntopic (Wilcoxon signed-ranks test:  $T = 192.50$ ,  $N = 42$ ,  $P = 0.001$ ) and allotopic ( $T = 282.00$ ,  $N = 42$ ,  $P = 0.034$ ) to two-lined salamanders (Table 2). However, the median amount of time that red-backed salamanders spent on substrates inoculated with TS<sub>EB</sub> was significantly less than the 450-s reference time only for individuals from subpopulations syntopic ( $T = 293.00$ ,  $N = 42$ ,  $P = 0.047$ ), not allotopic ( $T = 403.00$ ,  $N = 42$ ,  $P = 0.544$ ), to two-lined salamanders (Table 2).

## DISCUSSION

Red-backed salamanders are able to distinguish among and differentially respond to predatory garter snakes based on diet-related chemical cues. Although such discrimination of predator chemical cues based on an individual predator's recent diet has been documented in other vertebrate and invertebrate prey species (Gelowitz et al. 1993; Wilson & Lefcourt 1993; Madison et al. 1999b; Chivers & Mirza 2001b; Persons et al. 2001), our study is unique in that we examined how genetic and ecological relatedness of prey influence antipredator responses. The evidence presented here suggests that red-backed salamanders are acutely aware of their chemical environment and both genetic and ecological relatedness between test salamanders and snake prey play a role in *P. cinereus* predator assessment.

Such fine-scale discrimination of predator cues by red-backed salamanders may be used to minimize the costs associated with antipredator behaviour, especially when

**Table 2.** Median time that *P. cinereus* from subpopulations syntopic or allotopic to *E. bislineata* spent on substrates inoculated with test stimuli from garter snakes fed either *P. cinereus* (TS<sub>Pc</sub>) or *E. bislineata* (TS<sub>Eb</sub>)

Subpopulation	Treatment	Time on snake substrate (s)	Interquartile range	<i>T</i>	<i>P</i>
Syntopic	TS <sub>Pc</sub>	98.50	0.00–303.00	192.50	0.001
	TS <sub>Eb</sub>	128.50	15.00–741.00	293.00	0.047
Allotopic	TS <sub>Pc</sub>	117.50	0.00–495.00	282.00	0.034
	TS <sub>Eb</sub>	324.00	57.00–826.00	403.00	0.544

Wilcoxon signed-ranks tests: *N* = 42 in all cases.

considering the physiological requirements of *P. cinereus* (Feder 1983). Red-backed salamander activity generally is restricted to damp environmental conditions (Jaeger 1978, 1980a), so foraging and reproductive activity may be suppressed by climatic factors, especially during the summer. Furthermore, red-backed salamanders respond to predator chemical cues in natural habitats by delaying emergence from their refuges at night (Sullivan et al. 2002, in press) when their invertebrate prey tend to be most active in the leaf litter (Jaeger 1978; Holomuzki 1980). In addition, the threat of snake predation declines after sunset, especially late at night, when garter snakes tend to be relatively inactive (Reichenbach & Dalrymple 1986; Madison et al. 1999a), so red-backed salamanders may gain fitness benefits by responding only to the most imminent of predation threats during an important activity period.

## Genetic Relatedness

The genetic relatedness hypothesis, which states that a target species can recognize homologous predation-related chemical cues from closely related heterospecifics, is most often associated with studies of chemical alarm cues (e.g. the nitrogen oxide alarm cue of ostariophysan fish, Brown et al. 2000). Our study validates the application of this hypothesis to studies of predator-diet discrimination, since our focal species differentially avoided chemical cues from predators fed prey from the same phylogenetic lineage (Hass 1985). Salamander avoidance of cues from garter snakes fed red-backed salamanders from the same lineage (Group III), but not from a different lineage (Group IV) from an equivalent distance (161 km), suggests that genetic variation between the different groups influenced the recognition of predator-diet cues. The intermediate response of Group III salamanders from BU to cues from garter snakes fed Group III salamanders from HM may arise from two scenarios. The chemical cues from snakes fed Group III salamanders from HM may not be identical to snakes fed Group III salamanders from BU, but may be similar enough to have triggered an avoidance response in our test salamanders from BU. Conversely, associated cues from snakes fed Group III salamanders from HM may allow test salamanders to distinguish between predators fed salamanders of the same lineage but from different populations. This pattern of graded antipredator responses is similar to the response of fish to

alarm cues from different confamilial fish species (e.g. Mirza & Chivers 2001; Mirza et al. 2001). An experiment using red-backed salamander prey collected from contact zones between groups may yield valuable data regarding the evolution of predator-diet discrimination and the nature of the chemical cues that label individual garter snakes as an increased threat.

The source of the chemical cue that labels the garter snake is uncertain, but it is unlikely to be an alarm cue assimilated into predator tissues or secreted in waste products, as observed in some species (e.g. fish: Mathis & Smith 1993; Brown et al. 1995; sea anemones: Howe & Harris 1978). Red-backed salamanders do avoid damage-release cues from con- and heterospecifics (Sullivan et al. 2003), but a study by Madison et al. (2002) showed that the labelling of snakes by salamander prey does not require ingestion of the prey, or damage to salamander tissues. For example, red-backed salamanders do not avoid cues from distressed conspecifics or unfed garter snakes when they are exposed to these cues independently. However, red-backed salamanders do avoid a combination of these two cues. An interesting future study would be to examine the avoidance of salamanders to macerated tissue extracts from the different red-backed salamander groups.

We hypothesize that the substance ‘labelling’ some garter snakes as an increased risk may be a social pheromone produced by *P. cinereus*. Plethodontid salamanders possess well-developed integumentary glands for chemical cue production (Houck & Verrell 1993; Jaeger & Gabor 1993; Simons et al. 1994; Houck & Sever 1995), and use such cues to mediate reproduction (Houck 1986; Houck et al. 1998), homing (Madison 1969, 1972), individual recognition (Madison 1975; Dawley 1984), and territorial defence (Mathis 1990; Mathis et al. 1998). Recent studies show that in some cases, there is enough intraspecific geographical variation in the sex pheromones of red-cheeked salamanders, *P. jordani* (Rollman et al. 2000), and red-sided garter snakes, *T. s. parietalis* (LeMaster & Mason 2003), to reduce the likelihood of reproduction between conspecifics from different populations. Low vagility among red-backed salamanders may contribute to the variation in chemical make-up among the different populations. Kleeberger & Werner (1982) showed that the daily movements of radioactively tagged *P. cinereus* averaged 0.43 m, with home ranges of 13–24 m<sup>2</sup>, suggesting that genetic divergence and the subsequent variation of social cues used by this species may be plausible over the geographical distances considered in this study.

## Ecological Relatedness

The avoidance by individual red-backed salamanders of cues from snakes fed two-lined salamanders only when the red-backed salamanders were syntopic with two-lined salamanders in the local population suggests the importance of ecological familiarity (experience) in responding to predator-diet cues. Because of our experimental design, the results of experiment 2 do not allow us to unequivocally state that the pattern of diet discrimination by the different red-backed salamander subpopulations was the result of individual experience with two-lined salamanders. An experiment that maintains red-backed salamanders naïve to two-lined salamanders in captive syntopy may more completely address this aspect. The present results do suggest that individual red-backed salamanders are acutely aware of their surroundings and can assess predation risk based on their familiarity with other members of the woodland community. Ecological familiarity benefits prey species that recognize and respond to alarm and distress cues from ecologically related individuals in order to minimize the threat of predation (Pakarinen 1992; Chivers et al. 1997; Smith 1999).

In conclusion, this study suggests that genetic and ecological relatedness play a role in the detection and differential avoidance of chemical cues from predators foraging on assorted prey. Our results suggest that these mechanisms are not necessarily mutually exclusive, and an environmental component to cue variation should not be ruled out. For example, the different diets for salamanders from different populations may affect cue structure. In our study, red-backed salamanders appeared to be sensitive to their chemical surroundings, and varied their antipredator response to predation-related chemical cues using information gleaned from the predator's recent prey. Future studies should look further at genetic and ecological relatedness as possible contributors to the evolution of other predator-prey systems.

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