EVOLUTIONARY RESPONSE OF PREDATORS TO DANGEROUS PREY: PREADAPTATION AND THE EVOLUTION OF TETRODOTOXIN RESISTANCE IN GARTER SNAKES

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Abstract.—Coevolutionary interactions typically involve only a few specialized taxa. The factors that cause some taxa and not others to respond evolutionarily to selection by another species are poorly understood. Preadaptation may render some species predisposed for evolutionary response to new pressures, whereas a lack of genetic variation may limit the evolutionary potential of other taxa. We evaluate these factors in the predator-prey interaction between toxic newts (*Taricha granulosa*) and their resistant garter snake predators (*Thamnophis sirtalis*). Using a bioassay of resistance to tetrodotoxin (TTX), the primary toxin in the prey, we examined phenotypic evolution in the genus *Thamnophis*. Reconstruction of ancestral character states suggests that the entire genus *Thamnophis*, and possibly natricine snakes in general, has slightly elevated TTX resistance compared to other lineages of snakes. While this suggests that *T. sirtalis* is indeed predisposed to evolving TTX resistance, it also indicates that the potential exists in sympatric congeners not expressing elevated levels of TTX resistance. We also detected significant family level variation for TTX resistance in a species of *Thamnophis* that does not exhibit elaborated levels of the trait. This finding suggests that evolutionary response in other taxa is not limited by genetic variability. In this predator-prey system, species and population differences in resistance appear to be largely determined by variation in the selective environment rather than preadaptation or constraint.

Key words.—Coevolution, garter snake, preadaptation, predator-prey, resistance, tetrodotoxin.

The reciprocal nature of coevolutionary interactions provides an opportunity to study virtually all levels of evolutionary process from frequency-dependent changes in allele frequencies within populations (e.g., Dybdahl and Lively 1998) to cladogenesis and the sources of biodiversity (e.g., Farrell 1998; Janz and Nylin 1998; Page et al. 1998). Despite the wealth of research on coevolving systems (e.g., Thompson 1994), we know relatively little about why some taxa become caught in coevolutionary interactions while others do not.

The initial evolutionary response by one species to selection imposed by another is the first step down the coevolutionary path. For any given species that represents a selective force, many taxa potentially interact with and experience that force, yet only one or a few will respond and become caught up in the reciprocal cycle of coevolution. A recent review (Thompson 1994) emphasizes the integral link between specialization and coevolution, suggesting that the majority of interactions result in a restricted set of interacting species. What determines why some species enter into a coevolutionary interaction while others do not?

The answer to this question lies in the factors that influence adaptive evolutionary change—selection and the genetic potential to respond to selection. The strength and form of selection is determined by details of the ecological interactions among taxa. Although many taxa might interact with one species, the fitness effects of such an interaction can vary greatly. Both the consequence of the interaction and the predictability of that consequence contribute to the overall strength of selection (Brodie and Brodie 1999a). Specifics of local environment, differences in the assemblage of other

interactants, and a variety of other features can all generate locally variable selection that can lead to geographic differences in the pattern of coevolutionary partners (Thompson 1994).

Assuming that selection is equal for all potentially interacting taxa, limited genetic variation for traits at the phenotypic interface of the interaction might prevent the initial evolutionary response in some taxa (Futuyma et al. 1995). Although empirical quantitative genetic studies generally lead to the conclusion that substantial genetic variation exists for virtually all phenotypic traits (Barker and Thomas 1987; Mousseau and Roff 1987), there is a limit to this extrapolation. Strong abiotic selection sometimes fails to generate evolutionary responses in species with limited genetic variation, as in studies of heavy metal tolerance in grasses (Bradshaw 1991). Estimates of the heritability of threshold temperatures in turtles with environmental sex determination suggest that genetic variability is insufficient to evolutionarily track global warming (Janzen 1994). Phytophagous beetles of the genus *Ophraella* appear to lack genetic variation for exploitation of host plants utilized by their congeners, thus limiting their potential to shift to new hosts and constraining the macroevolutionary pattern of coevolution (Futuyma et al. 1995).

Some species may be predisposed to entering into a coevolutionary interaction even when the requisite criteria of selection and genetic potential are met by a number of taxa. The presence of traits that allow immediate utilization of a resource could provide the initial headstart that permits some species to enter an interaction and leaves others behind. Preadaptation may be especially important in determining the

participants in coevolution because of the dynamic nature of the process. Species not involved at the beginning are likely to be outpaced as traits at the phenotypic interface are quickly elaborated through reciprocal selection and response. Preadaptation appears to be important in determining participants in mutualisms among yucca and yucca moths (Pellmyr et al. 1996) and among obligate plant-ant associations (Ward 1991), wherein specialization seems to result from elaboration of existing traits rather than novel phenotypes.

In this paper, we explore the determinants of adaptive evolutionary response of garter snake predators to toxic prey. Scant evidence exists supporting the view that predators and prey are generally caught in coevolutionary interactions (Brodie and Brodie 1999a). Current views predict that selection imposed by prey on their predators is rarely expected to balance the much stronger selection of predators on prey (Abrams 1986; Vermeij 1994; Abrams and Matsuda 1996). Selection is expected to be more equitable when prey are dangerous and can harm or kill predators (Vermeij 1987; Brodie and Brodie 1999a), but this point still does not explain why some predators respond to selection while others do not.

From a microevolutionary perspective, one of the best documented predator-prey systems includes the newt *Taricha granulosa* and its predator, the garter snake *Thamnophis sirtalis* in the Pacific Northwest of North America (Brodie and Brodie 1990, 1991, 1999a). Over much of its range, *Taricha granulosa* possesses the potent neurotoxin tetrodotoxin (TTX) as a highly effective defense against potential predators (Mosher et al. 1964; Wakely et al. 1966; Brodie 1968; Brodie et al. 1974; Johnson and Brodie 1975; Daly et al. 1987). The only known predator of this toxic newt is the garter snake *Thamnophis sirtalis*. This species is highly resistant to TTX in some regions of its sympatry with *Taricha* (Brodie and Brodie 1990). Within populations, resistance to TTX varies among individual snakes and has a heritable basis (Brodie and Brodie 1990). Resistance is not affected by either short-term or long-term exposure to TTX (Brodie and Brodie 1990; Ridenhour et al. 1999), thus maternal effects are unlikely to explain familial or population differences. Geographic variation in resistance among populations of *Thamnophis sirtalis* corresponds to variation in TTX toxicity of sympatric newts (Brodie and Brodie 1991, 1999a; Hanifin et al. 1999), suggesting that a geographic mosaic of predator resistance has evolved in response to newt toxicity.

This system provides a unique opportunity to evaluate the factors that influence the initiation of evolutionary response by predator to prey and thereby gain insight into what determines the participants in coevolutionary escalation. *Thamnophis sirtalis* is the only known member of its genus that has evolved high levels of resistance to the toxins of *Taricha*, despite the existence of up to six congeners (*Thamnophis atratus, T. couchii, T. elegans, T. gigas, T. hammondii,* and *T. ordinoides*) in sympatry with *Taricha*. We posit that an unelaborated but genetically variable form of TTX resistance would have been essential in the ancestor of *Thamnophis sirtalis* for some populations to evolve extreme resistance to newt toxins. We tested this prediction using a whole organism locomotor performance assay to measure TTX resistance in snakes. This method allowed the use of small amounts of TTX and was sensitive enough to measure even low levels

of resistance. By sampling a number of *Thamnophis* species and other snake genera, we reconstructed the ancestral state of TTX resistance in *Thamnophis sirtalis* and in the genus in general. We also examined family level variation in TTX resistance in a *Thamnophis* species (*T. elegans*) that does not have elevated levels of resistance, to determine whether other taxa in the genus have the genetic potential to respond to selection by dangerous prey.

METHODS

Protocols for husbandry and testing were identical for all populations and species sampled. Pregnant females collected from the field were brought into the laboratory, housed individually, and fed live fish weekly. To minimize maternal and environmental sources of variation all animals were housed in a single temperature controlled room. After parturition, neonates were measured and weighed. Snakes were then housed individually and watered daily, and were not fed until testing was completed. All subjects were tested between 3–10 days of age and trials were conducted at 25–26°C.

Resistance to TTX was tested by means of a whole organism performance bioassay developed by Brodie and Brodie (1990). Each individual snake was raced on a 2 m linear racetrack, lined with artificial turf and equipped with infrared sensors at 0.5 m intervals to electronically time sprint speed. The maximum 0.5 m speed in a given trial was taken as the sprint speed score. Individuals were tested two times and the mean was taken as an individual's "Baseline Speed." Twenty-four hours after the last sprint test, neonates were given 0.1 ml intraperitoneal injections of TTX. Experimental doses of TTX were created by serial dilution of purified TTX in citrate buffer (Sankyo) with physiological saline to achieve a range of TTX concentrations. Thirty minutes after injection, snakes were tested again to obtain a measure of "Post-injection Speed." Repeated measures of postinjection speed were taken at 48-h intervals. "Resistance" was scored as the percentage of an individual's baseline speed crawled after injection (Post-injection Speed/Baseline Speed). Individuals that are greatly impaired by TTX crawl only a small proportion of their normal speed, whereas those that are unaffected by an injection of TTX crawl 100% of their baseline speed. Control injections of physiological saline have no effect on snake performance (Brodie and Brodie 1990), and TTX resistance is not affected by repeated exposure (Brodie and Brodie 1990; Ridenhour et al. 1999). The bioassay is highly repeatable and enables us to assess individual differences in susceptibility to TTX.

Because resistance is related to body size within and among some populations (Brodie and Brodie 1990, 1999b), we controlled for mass differences among populations using a population level mass adjusted measure of resistance. Tetrodotoxin doses were converted to mass-adjusted mouse units (MAMUs) by dividing a given dose by the mean neonate mass for each population, then dividing by the amount of tetrodotoxin sufficient to kill one gram of mouse in 10 min.

Reconstruction of the Ancestral State of Tetrodotoxin Resistance

We tested the level of tetrodotoxin resistance for each of 761 neonates from 70 snake litters (Table 1, Fig. 1). One

TABLE 1.   Mean TTX resistance for *Thamnophis* species, *Nerodia* and *Coluber*.

Species	Locality	Litters/individuals	MAMUs TTX required for 50% baseline speed	Relative resistance
T. sirtalis	Benton (Benton Co., OR)	28/233	25.6	High
	Illinois (Whiteside Co.)	1/31	1.7	Low
	Maine (York Co.)	1/29	1.8	Low
T. elegans	Bear Lake (Bear Lake Co., ID)	7/85	1.1	Low
	Laurendell (Sonoma Co., CA)	5/38	1.0	Low
	Willow Creek (Sonoma Co., CA)	9/85	1.2	Low
T. couchii	(Curry Co., OR)	1/8	1.4	Low
T. cyrtopsis	(Santa Cruz Co., AZ)	2/34	2.1	Low
T. ordinoides	(Benton Co., OR)	10/127	1.8	Low
T. proximus	(Denton Co., TX)	2/36	1.4	Low
N. taxispilota	(Aiken Co., SC)	3/51	1.5	Low
C. constrictor	(Mendocino Co., CA)	1/4	0.2	Very Low

population of *Thamnophis sirtalis* known to prey upon toxic newts (Benton Co., OR; Brodie and Brodie 1990, 1991, 1999b) was compared to two populations of *T. sirtalis* allopatric with respect to newts, as well as to seven populations representing five *Thamnophis* species known not to prey upon newts. This latter group included two species that are nowhere sympatric with *Taricha* (*Thamnophis cyrtopsis* and *T. proximus*) and three species that are sympatric with *Taricha*

in at least part of their ranges (*Thamnophis couchii, T. elegans*, and *T. ordinoides*). One population each of *Coluber constrictor* and *Nerodia taxispilota* also were tested for purposes of outgroup comparison. For additional outgroup comparison we used TTX resistance data (minimal lethal dose trials) from Brodie (1968) for six genera of snakes from three families: *Charina, Coluber, Contia, Crotalus, Masticophis*, and *Pituophis*.
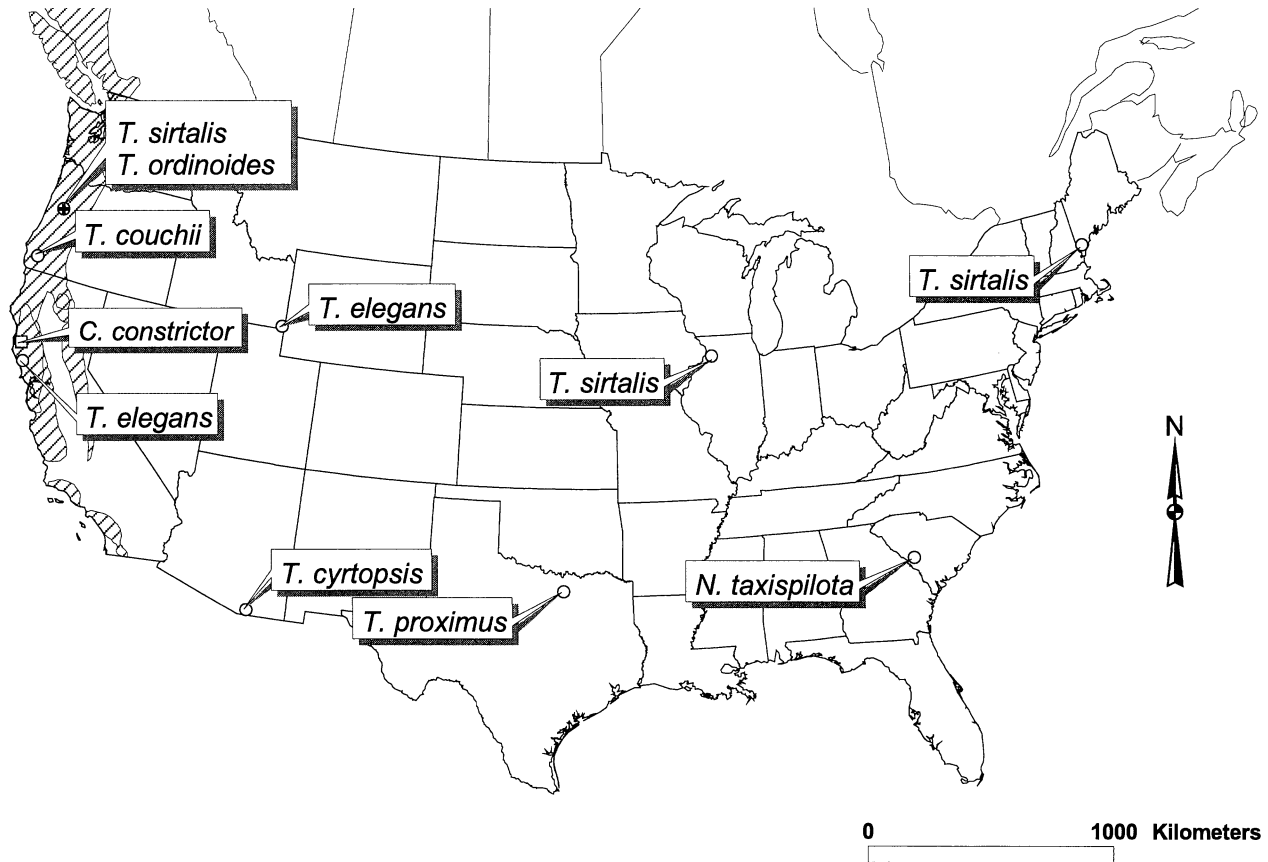

FIG. 1.   Geographic distribution of snake species and populations sampled. Populations expressing high-level TTX resistance are represented by a plus symbol; populations of medium-level resistance are represented by circles; populations of low-level resistance are represented by squares. Hatching represents the distributions of *Taricha* from Stebbins (1985).
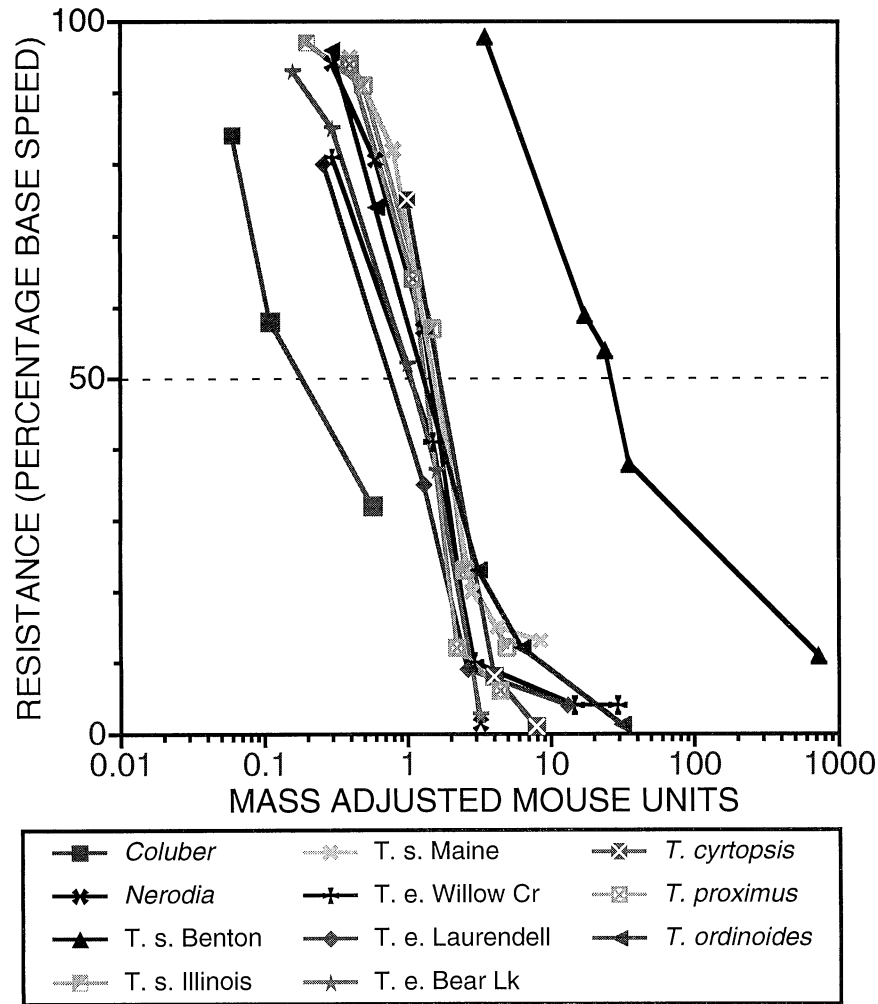
Fig. 2. Means TTX resistance as a function of Mass Adjusted Mouse Units of TTX (log scale). Data plotted are from neonates of five species of *Thamnophis*, including two populations of *T. sirtalis* allopatric to newts and one population of TTX "resistant" *T. sirtalis* from Benton County, Oregon. One populations each of *Coluber constrictor* and *Nerodia taxispilota* are included for comparison.

Population levels of resistance were estimated as the amount of TTX (in MAMUs) necessary to reduce the baseline speed of the average snake to 50%. These values were estimated by linear interpolation from the mean resistance curves in each population. Given the natural breaks between orders of magnitude, TTX resistance was graded as three discrete character states (low resistance, medium resistance, and high resistance) and traced (MacClade 3.04, Maddison and Maddison 1992) onto a phylogeny of the genus *Thamnophis*. The phylogeny used was previously published and represents a consensus tree based on mtDNA and allozymes, (adapted from de Queiroz and Lawson 1994) with outgroup structure based on Forstner et al. (1995).

Intrapopulational Variation in TTX Resistance

Within population variation in TTX resistance was examined in a single population of *Thamnophis elegans* from Bear Lake County, Idaho. The resistance level of this population was representative of the genus (Fig. 2). This population of *T. elegans* is allopatric to newts and therefore has not experienced selection for increased resistance.

Seven pregnant females were collected from the wild in July 1997 and held in the laboratory until parturition. Tetrodotoxin resistance was assayed as described above using neonates from each litter ($n = 85$). Three different doses of TTX (0.00001 mg, 0.00003 mg, and 0.00005 mg) were used to characterize variation in resistance to TTX. Among-family variation was examined with analysis of variance including family and dose as main effects, and the dose X family interaction (SAS version 6, SAS Institute 1989). Within-family variation was inspected graphically at a single dose (0.00003 mg).

RESULTS

Reconstruction of the Ancestral State of TTX Resistance

All species and populations of *Thamnophis* tested demonstrated some level of tetrodotoxin resistance as compared to *Coluber* (Table 1; Fig. 1). The population of *Thamnophis sirtalis* from Benton County, Oregon, had a comparatively high mean resistance, requiring 25.6 MAMUs to reduce Baseline Speed to 50% of normal (Fig. 2). In comparison, two
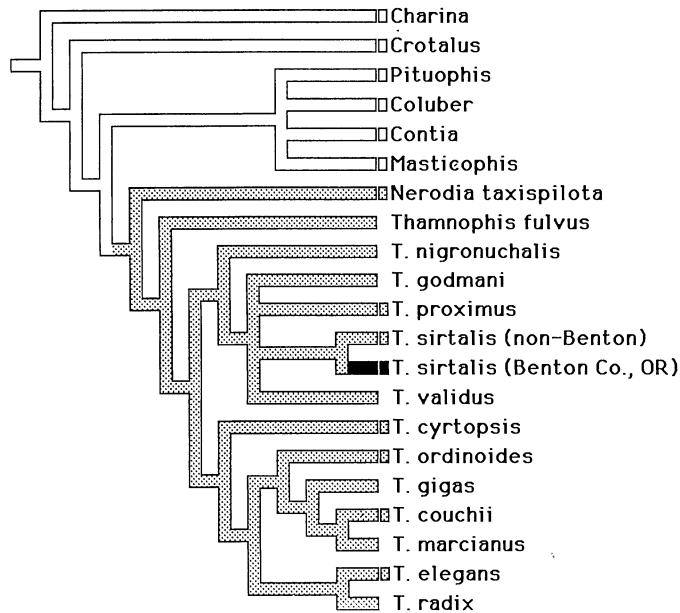
FIG. 3. Phylogenetic reconstruction of the evolution of TTX resistance in snakes. *Thamnophis* phylogeny adapted from de Queiroz and Lawson (1994) with outgroup structure from Forstner et al. (1995). Populations of *T. sirtalis* are separated on the basis of resistance character differences. Boxes mark taxa for which we report data. Branches supporting high-level resistance are black, branches supporting low-level resistance are stippled, and branches supporting very low resistance are unshaded.
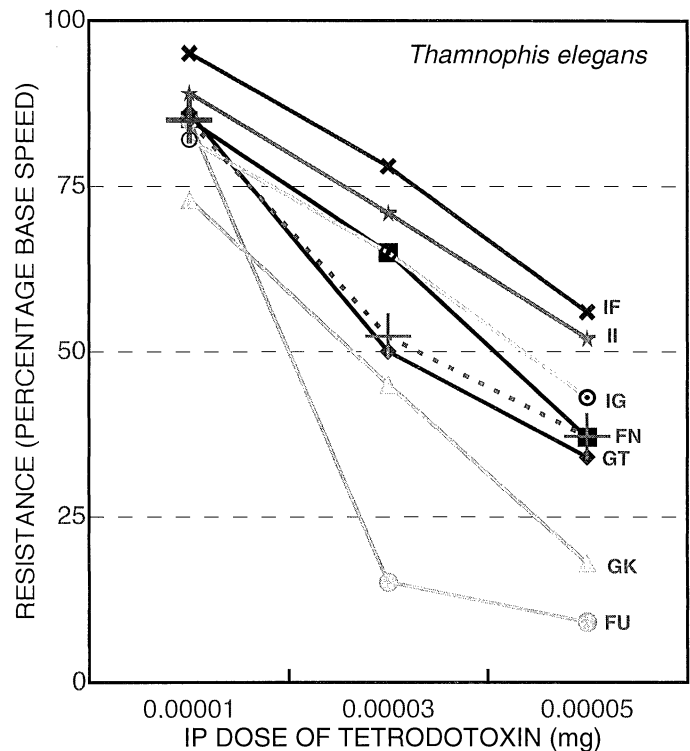

FIG. 4. Variation in family mean TTX resistance at three doses of TTX. Seven litters of *Thamnophis elegans* from Bear Lake County, Idaho, are shown. Each curve denotes a single family and the population mean is represented by the dashed line.

populations of *Thamnophis sirtalis* occurring well outside the range of *Taricha* (Whiteside Co., Illinois, and York Co., Maine; Fig. 1) were reduced to 50% Baseline Speed by only 1.7 and 1.8 MAMUs, respectively. Each population of the five other species of *Thamnophis* tested fell within the same range (50% Baseline Speed at 1.0–2.1 MAMUs).

A population of *Nerodia taxispilota* from South Carolina had the same mean resistance as the majority of the *Thamnophis* group (50% Baseline Speed at 1.5 MAMUs). In contrast, a population of *Coluber constrictor* from California had a mean TTX resistance much lower than that of the *Thamnophis* group (50% Baseline Speed at 0.2 MAMUs). Previously reported TTX resistance data (minimum lethal dose, Brodie 1968) for other outgroup taxa (*Charina, Coluber, Contia, Crotalus, Masticophis,* and *Pituophis*) indicate the same low levels of TTX resistance (high susceptibility) as we measured in *Coluber* using the performance bioassay.

Graphical inspection of the mean TTX resistance levels for all populations revealed that resistance in *Thamnophis sirtalis* from Benton County, Oregon, differed from all other *Thamnophis* tested by an order of magnitude (Fig. 2). All other *Thamnophis* and *Nerodia* grouped closely at a level intermediate between *Thamnophis sirtalis* from Benton County, and the low resistance levels of the outgroup taxa (represented by *Coluber* in Fig. 2). Tracing TTX resistance on the phylogeny of *Thamnophis* (Fig. 3) supported low-level TTX resistance (50% ability at 1.0–2.1 MAMUs) as the ancestral condition in the genus *Thamnophis*. Low-level resistance in a single species of *Nerodia* also suggests this may be the primitive condition for all natricine snakes.

Intrapopulational Variation in TTX Resistance

Tetrodotoxin resistance was variable within the population of *Thamnophis* that has not evolved high resistance to TTX. Significant among family variation in TTX resistance was found in *Thamnophis elegans* from Bear Lake County, Idaho (df = 6; $F = 17.88$; $P < 0.0001$; Figs. 4, 5). Performance was also affected by dose (df = 2; $F = 341.75$; $P < 0.0001$). The significant interaction between family and dose (df = 12; $F = 7.51$; $P = 0.0001$) indicated that families differed with respect to their resistance to different doses of TTX. At the 0.00003 mg dose of TTX, family means ranged from 78% to 15% of baseline speed. In addition to differences among families, extensive variation in individual resistance was seen within families (Fig. 5).

DISCUSSION

Our results indicate that a low level of TTX resistance was present in the ancestor of the predator, *Thamnophis sirtalis*, prior to its evolutionary response to the toxic prey *Taricha granulosa*. The high levels of this exploitative ability observed in some populations of *Thamnophis sirtalis* (Brodie and Brodie 1999a) are clearly derived with respect to their congeners. However, neither interspecific patterns of genetic variation nor preadaptation for TTX resistance adequately explain why *Thamnophis sirtalis* is the only species that has undergone adaptive escalation of TTX resistance. We evaluate the evidence for these points below, and suggest that geographic and interspecific variation in selection may ex-
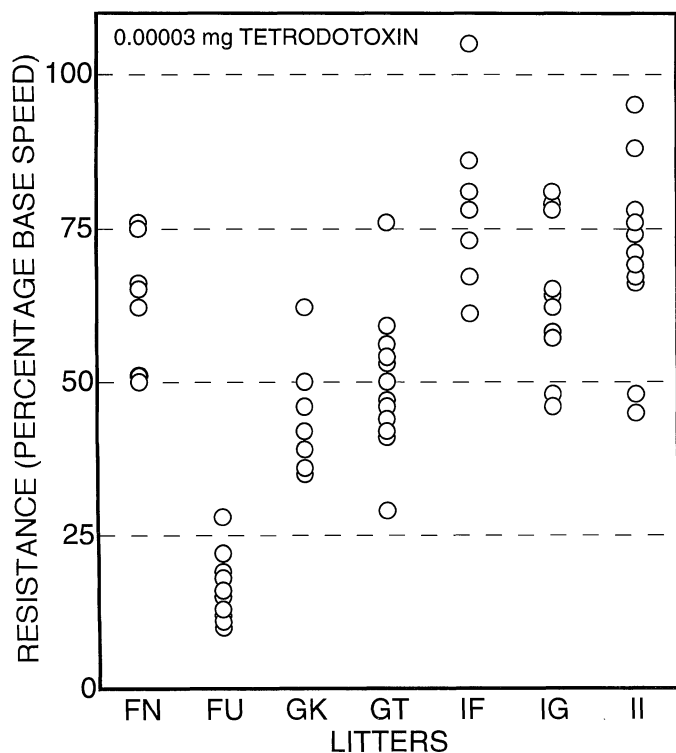
FIG. 5. Variation within and among families at a single dose of TTX (0.00003 mg TTX). Resistance of individual neonates from each of seven litters of *Thamnophis elegans* from Bear Lake County, Idaho, is shown.

plain the observed patterns of evolutionary response to poisonous prey.

Preadaptation for TTX Resistance

Low-level tetrodotoxin resistance, characterized by a 50% reduction of locomotor ability at 1.0–2.1 MAMUs of TTX, is known in most major branches of the genus *Thamnophis* and at least one species of *Nerodia*. Our phylogenetic reconstruction indicates that TTX resistance is a derived condition in the genus *Thamnophis* and possibly the entire subfamily Natricinae (Fig. 3); our minimal sampling outside the genus *Thamnophis* precludes great confidence in the reconstruction of the ancestral character state of natricines. The level of TTX resistance in *Thamnophis* is at least five times that of other colubrid snakes (Table 1, Fig. 2). Populations of *T. sirtalis* allopatric with respect to newts (and presumably not adapted to newts in their recent evolutionary history) demonstrate the same low levels of TTX resistance as do their congeners. Assuming that all populations we refer to *T. sirtalis* represent a monophyletic lineage, these allopatric populations are the best representatives of the unelaborated state of resistance ancestral to the species. While *T. sirtalis* appears to be preadapted to TTX resistance, this is not a feature that distinguishes it from other garter snake species sympatric with toxic newts.

Preadaptation may be a requisite for, rather than a determinant of, adaptive response to dangerous prey or other biotic selective forces. The existence of the toxin resistance char-

acter is necessary before a population can experience and respond to selection by that toxin. If no individuals are able to ingest toxic prey and survive, there is no variance in fitness and thus no opportunity for selection (sensu Crow 1958; Arnold and Wade 1984). Assuming that TTX was present in ancestral newts, some degree of TTX resistance would have been necessary for snakes to initially exploit newts as prey and thereby experience selection from the toxin. Although we do not know the ancestral level of TTX toxicity in *Taricha granulosa* when the coevolutionary interaction between snakes and newts began, TTX is widespread in newts of the family Salamandridae and in all North American species of newts (Mosher et al. 1964; Yotsu et al.1990), suggesting that the common ancestor of *Taricha* did possess TTX.

Data from other types of coevolutionary interaction also suggest that preadaptation is necessary but not sufficient for coevolution to ensue. Pseudomyrmecine ants that are involved in obligate ant-plant associations ("plant-ants") indicate at least twelve independent origins of the habit. All are characterized by several ancestral character states that include inhabitation of holes in plants left by stem boring insects and the tending of scale insects for food (Ward 1991). Presumably, these features generate the predisposition to utilize plant parts (such as thorns and other cavities) as domatia, whereas the tending of scale insects that live on plants (often in crevices or cavities) provides the trophic resource that pulls the ants into close relationship with plants. Yucca moths that are the obligate pollinators of yucca flowers belong to a single family (Prodoxidae), of which not all members have evolved this mutualistic habit (Pellmyr et al. 1996). Of the four traits that are critical for the evolution of obligate mutualism, three appear to be present in the ancestor of yucca moths before the colonization of yuccas as hosts (local host specificity, oviposition on flowers, and limited seed destruction). Only one trait, pollination-causing behavior, appears to be novel in yucca moths alone.

It is not clear why the entire genus *Thamnophis* (or possibly the subfamily Natricinae) should exhibit a low-level resistance to TTX. Unlike the preadaptations described above for plant-ants and yucca moths, resistance to TTX provides no obvious fitness benefits to organisms that do not interact with TTX toxic prey. Garter snakes eat a diversity of prey types; some species specialize on slugs and worms, others on fish, others on amphibians (Rossman et al.1996). The only possible prey type of *Thamnophis* and *Nerodia* other than *Taricha* that is expected to have even slight amounts of TTX is the eastern newt *Notophthalmus viridescens*. The most likely explanation for the broad taxonomic distribution of TTX resistance is some as yet unidentified aspect of the physiology of natricine snakes that confers resistance to TTX. The mechanism of TTX resistance in snakes is not yet known, but may be related to Na+ channel structure or function (Brodie and Brodie 1999b).

Genetic Variation in TTX Resistance

Genetic variation for appropriate characters is clearly a requisite for evolutionary response to any source of selection, and biotic selective agents are no exception. At one extreme, a trait cannot be genetically variable unless it exists, but this

is essentially the same issue as preadaptation—there can be no selection on or response to selection by a trait that is not expressed (e.g., Futuyma et al. 1995). For phenotypes that do exist, the short and long term direction of evolution may be biased by the multivariate dimensions of greatest genetic variation (Schluter 1996). Conversely, a lack of appropriate variation could prevent some populations or species from following a path of adaptive escalation (e.g., Futuyma et al. 1995).

Substantial genetic variation for TTX resistance has been detected in several populations of *Thamnophis sirtalis* that exhibit high levels of TTX resistance (Brodie and Brodie 1990, 1999b). In one population of *T. sirtalis* allopatric with respect to newts that exhibits the ancestral low level of TTX resistance, substantial family level variation also has been observed (Brodie and Brodie 1999b). We show here that similar variation is present in another member of the genus, *T. elegans*, that exhibits low-level resistance. This conclusion is based on differences among full-sib families of neonate snakes, and therefore might be attributable to common family environments or nonadditive genetic variation. However, direct exposure to TTX in short and long term experiments does not induce or alter individual levels of resistance (Brodie and Brodie 1990; Ridenhour et al. 1999), thus differences in environmental (including maternal) effects are not likely to influence the family differences. We have not sampled genetic variation in other *Thamnophis* species, but the presence of family differences in both *T. elegans* and populations of *T. sirtalis* allopatric to newts, suggests that genetic variability exists in the other species sampled. Indeed, all species and populations of *Thamnophis* examined exhibit individual differences in TTX resistance among neonate snakes. Given the lack of influence of exposure and high repeatability of TTX resistance (Brodie and Brodie 1990), these individual differences likely reflect at least some genetic variation.

The absence of genetic variation is not expected to be a common constraining force in evolution (Barker and Thomas 1987) and does not appear to explain the low level of TTX resistance in some western *Thamnophis*. Seven species of garter snakes, including *T. sirtalis* and *T. elegans*, are sympatric with toxic newts (*Taricha*), and each is known to prey upon amphibians (Nussbaum et al. 1983). Despite phenotypic and genetic variation in these taxa, only *T. sirtalis* has evolved resistance to newt toxins.

The geographic mosaic view of coevolutionary process suggests that variation in the outcome or evolutionary consequence of species interactions is due, in part, to selective differences among localities (Thompson 1999a,b). An extension of this argument leads to the conclusion that sympatric, related taxa may differ with respect to coevolutionary interactions for the same reasons, even though they occur syntopically. Clearly, many factors can contribute to the strength and mode of selection experienced by any given population or species, but for predators attacking prey, the most obvious and most likely are features of the diet including availability of alternative prey, specificity of diet, and foraging habits and behaviors. Genetically based differences in prey preferences exist among populations and species of *Thamnophis* (Arnold 1981, 1992), and could have been responsible for the initial divergence among species with respect to newt

feeding. In any case, predisposition and genetic constraint do not appear to be important determinants of the taxonomic distribution of TTX resistance, leaving an adaptive explanation as the next possibility to explore.

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