Evolutionary Response of Predators to Dangerous Prey: Reduction of Toxicity of Newts and Resistance of Garter Snakes in Island Populations

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EVOLUTIONARY RESPONSE OF PREDATORS TO DANGEROUS PREY: REDUCTION OF TOXICITY OF NEWTS AND RESISTANCE OF GARTER SNAKES IN ISLAND POPULATIONS

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The primary weakness of the arms race analogy of coevolution between predator and prey is the paucity of direct evidence of predator evolution in response to adaptations of prey (Abrams, 1986). Recently, we presented microevolutionary evidence of predators' potential ability to respond to antipredator mechanisms of prey, and argued that selection for such response exists in natural populations of the garter snake *Thamnophis sirtalis* that eat the toxic newt *Taricha granulosa* (Brodie and Brodie, 1990).

Coevolution between predator and prey lineages should be observable among isolated populations of the two species. Interpopulational variation in either prey defense or predator exploitation should be mirrored in the complementary ability of sympatric opponent populations. If increased adaptation of one species drives the evolution of the other, then predator and prey should show measurable responses to each other in their relative abilities regardless of the absolute level of either defense or exploitation (Brodie and Brodie, 1990).

Preliminary observations suggested that *Thamnophis sirtalis* from Vancouver Island, British Columbia, were much less resistant to tetrodotoxin (TTX) than previously studied populations of conspecifics that feed on *Taricha granulosa* (Brodie and Brodie, 1990). Vancouver Island *Thamnophis sirtalis* are known to feed frequently on *Taricha granulosa* (Macartney and Gregory, 1981; P. Gregory, pers. comm.), so their lack of resistance to the major toxin of the newt (TTX) would

TABLE 1. Geographic variation of toxicity of skin of *Taricha granulosa* to white mice. Sample sizes are in parentheses; NL = Not Lethal.

Dose cc of skin	Mean time to death (min)	
	Willamette Valley*	Vancouver Island
0.01	—	69.2 (6)**
0.008	—	NL (2)
0.004	2.0 (1)	NL (2)
0.002	2.0 (1)	—
0.0015	2.0 (1)	—
0.001	2.0 (2)	—
0.0005	4.5 (12)	—
0.0004	5.5 (12)	—
0.0002	10.3 (26)	—
0.0001	10.1 (31)	—
0.00007	6.5 (3)	—
0.00005	13.4 (42)	—

* Brodie (1968) and Brodie et al. (1974).
** 2 additional mice recovered fully.

be incongruous with a coevolutionary explanation for the toxicity and resistance of these species (Brodie and Brodie, 1990). To elucidate this relationship, we investigated the toxicity of *Taricha granulosa* and the resistance to TTX of *Thamnophis sirtalis* from Vancouver Island.

METHODS

Newt toxicity was assessed with intraperitoneal injections of skin extracts into white mice (17.0–20.7 g, $\bar{x}$ = 18.9 g) using techniques outlined by Brodie (1968) and Brodie et al. (1974). Skin extract was prepared from the back skin of five adult *Taricha granulosa* from an island (Read Island) adjacent to Vancouver Island, British Columbia (=Vancouver Island).

Resistance of *Taricha granulosa* to TTX was measured by intraperitoneal injection of purified TTX in citrate buffer (Sankyo) diluted with physiological saline to produce the reported dosages. Subjects were observed for 2 hr after injection for any signs of poisoning. Resistance to TTX was examined in newts from Vancouver Island (4.1–6.6 g, $\bar{x}$ = 5.5 g) and from a population of established toxicity (Brodie, 1968; Brodie et al., 1974) from the Willamette Valley, Benton Co., Oregon (=Willamette Valley; 7.5–14.9 g, $\bar{x}$ = 11.8 g). Six newts from each population were injected, two with each of three dosages (0.00286, 0.0286, and 0.286 mg of TTX).

Twenty-five neonate *Thamnophis sirtalis* from four laboratory born litters from Vancouver Island, British Columbia (=Vancouver Island) were tested for resistance to TTX using the locomotor performance bioassay developed by Brodie and Brodie (1990). This bioassay provides an estimate of an individual's resistance to TTX in terms of the proportion of its normal sprint speed it is capable of crawling 30 min after an intraperitoneal injection of TTX. Various concentrations of TTX were used. Individuals were tested at up to three concentrations; only the first injection at any given concentration was used to calculate resistance. Multiple injections of TTX do not affect the resistance bioassay, and control injections of saline do not reduce crawl speed (Brodie and Brodie, 1990).

Previously published data (Brodie and Brodie, 1990) on two populations of *Thamnophis sirtalis* were compared to the results of this study; one population is sympatric with toxic *Taricha granulosa* and resistant to TTX (Willamette Valley, Benton Co., Oregon), the other is allopatric with *Taricha granulosa* and nonresistant (Bear Lake Co., Idaho). For comparison with published data, dosages were reported in mass-adjusted mouse units to account for size differences among populations (one mass-adjusted mouse unit = the amount of TTX needed to kill one gram of mouse in 10 min times the mean mass of each snake population; based on the amount of TTX needed to kill a 20-g mouse in 10 min). Only one common dosage was used in all three populations (0.00015 mg of TTX). At this dosage, a Jonckherre Test for Ordered Alternatives (Siegel and Castellan, 1988) was used to test the a priori hypothesis that TTX resistance in *Thamnophis sirtalis* increases with sympatry and toxicity of *Taricha granulosa*. Paired comparisons also were made with a posteriori multiple comparisons tests (Siegel and Castellan, 1988 p. 213) to determine which of the three populations contributed to the order effect.

RESULTS AND DISCUSSION

Newt Toxicity

Skin extract from Vancouver Island newts was at least 1,000 times less toxic than that of Willamette

TABLE 2. Geographic variation in mean resistance of *Thamnophis sirtalis* measured as % baseline speed to intraperitoneal injections of tetrodotoxin (TTX).

Dose mg TTX	% Baseline speed ± SE (*N*)		
	Sympatric/toxic*	Vancouver Island	Allopatric*
0.015	11 ± 3.7 (14)	—	—
0.0015	37 ± 7.0 (15)	—	9 ± 6.6 (2)
0.001	—	12 ± 3.0 (5)	—
0.00075	59 ± 7.7 (7)	—	13 ± 1.0 (2)
0.0005	—	16 ± 1.6 (5)	—
0.0002	—	34 ± 2.4 (5)	—
0.00015	97 ± 3.9 (3)	63 ± 15.9 (20)	18 ± 2.5 (3)
0.0001	—	77 ± 10.0 (4)	—
0.000075	—	—	64 ± 5.5 (3)
0.000015	—	—	94 ± 11.7 (3)

* Brodie and Brodie (1990).

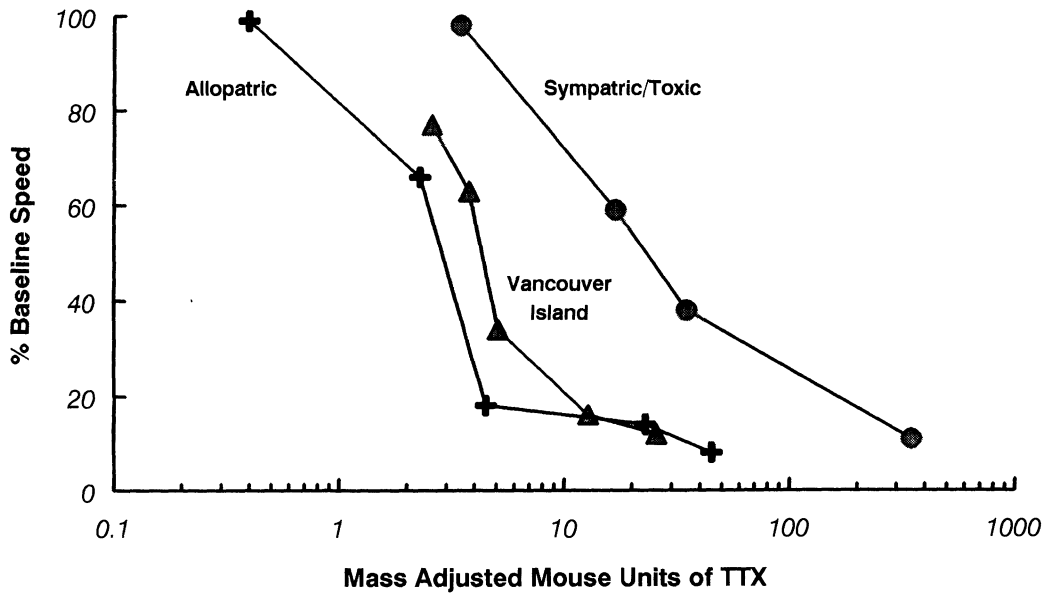

FIG. 1. Mean resistance (in % baseline speed) as a function of mass-adjusted mouse units of TTX (shown on a $\log_{10}$ scale) for each of the three snake populations. (Data on sympatric/toxic and allopatric populations from Brodie and Brodie, 1990.)

Valley newts (Table 1). Moreover, the qualitative responses of mice to injections of Vancouver Island newt skin differed markedly from those to either Oregon newt skin or pure TTX. The classic symptoms of TTX poisoning include loss of righting reflex and muscular coordination, clonic convulsions, regurgitation, flaccid paralysis, a fall in blood pressure, and a continued heartbeat after cessation of breathing (Brodie, 1968). Subjects injected with skin extract from Vancouver Island newts showed no clonic convulsions or loss of coordination, and no heartbeat after breathing had stopped. At fatal doses of the skin extract, time to death was much longer than ever observed in mice injected with either TTX or skin extract from newts known to have TTX (Table 1). These results suggest that Vancouver Island *Taricha granulosa* have little or no TTX in their skin and are generally far less toxic than conspecifics from Oregon and California (Brodie, 1968; Brodie et al., 1974; Wakely et al., 1966). The toxicity of Vancouver Island newts may result from a high molecular weight toxin mentioned by Brandon and Huheey (1981).

None of the *Taricha granulosa* from either population injected with any dosage of TTX exhibited any sign of poisoning. The dosages tested correspond to 10, 100, and 1,000 mouse units of TTX. The resistance of Vancouver Island newts to TTX suggests that TTX toxicity is an ancestral trait of this population. Additional evidence for this conclusion comes from the occurrence of TTX in many salamandrid genera closely related to *Taricha* (Wakely et al., 1966; Brodie et al., 1974; Wake and Özeti, 1969) and in all congeners of *Taricha granulosa* (Mosher et al., 1964; Wakely et al., 1966; Brodie et al., 1974). The Vancouver Island newts then have lost most or all TTX toxicity.

Several nonexclusive phenomena might explain the evolutionary reduction of TTX toxicity in this population. If the founding *Taricha granulosa* encountered no resistant predators, then selection may have reduced their toxicity because of some cost associated with TTX synthesis. If TTX production is not a costly adaptation, then the lack of selection favoring toxicity (in the absence of a resistant predator) would render TTX production a neutral character and would allow drift to take place, possibly resulting in reduced toxicity. Founder effects may also have contributed to the observed patterns if the initial colonizers of Vancouver Island were less toxic, on average, than the parent population.

A nonevolutionary explanation is also possible. In some taxa, TTX is produced by symbiotic bacteria (e.g., Noguchi et al., 1986, 1987; Yotsu et al., 1987; Thuesen and Kogure, 1989). If this is the case for *Taricha granulosa,* and the appropriate symbiont does not occur on Vancouver Island, then the reduction of TTX toxicity may be a purely environmental effect. However, amphibians are currently thought to synthesize their own TTX specifically as a defensive compound (Daly et al., 1987), so this explanation seems unlikely.

Snake Resistance

TTX resistance of Vancouver Island *Thamnophis sirtalis* is intermediate between that of conspecifics from populations sympatric with toxic newts (=sympatric/toxic; Willamette Valley, Benton Co., Oregon) and populations allopatric to newts (=allopatric; Bear Lake Co., Idaho) (Table 2). Resistance increased significantly across the three populations, progressing from the allopatric to the sympatric/toxic population ($J^* = 3.77$, $P < 0.0001$). Allopatric *Thamnophis sirtalis* were significantly less resistant than Vancouver Island *Thamnophis sirtalis* ($z = 10.75$, $P < 0.0001$), which were significantly less resistant than sympatric/toxic *Thamnophis sirtalis* ($z = 10.34$, $P < 0.0001$).

At all concentrations of TTX injected, Vancouver Island snakes (which are sympatric with less toxic newts) were less resistant than those from the sympatric/toxic population (Table 2). The resistance curve of the Vancouver Island population is closer to that of the nonresistant, allopatric population than that of the resistant, sympatric/toxic population (Fig. 1).

Depending on the ancestral condition of the Vancouver Island population of *Thamnophis sirtalis,* they have either lost or not evolved TTX resistance. If the *Thamnophis sirtalis* that colonized Vancouver Island came from a resistant population, then either selection to eliminate a costly adaptation (resistance) or drift in the absence of selective advantage (no toxic prey) could explain the lack of resistance. Alternatively, the founders may have come from a nonresistant population (or have been less resistant members of a resistant population) and, because of the low toxicity of newts there, have not experienced selection to increase resistance to the level of Willamette Valley conspecifics.

Coevolution of Newt Toxicity and Snake Resistance

The above hypotheses assume that both TTX toxicity and resistance are heritable traits, and that sufficient variation exists in populations to allow evolutionary processes to occur. We have demonstrated these qualities for TTX resistance in *Thamnophis sirtalis,* but the extent and basis of intrapopulation variation in TTX toxicity of *Taricha granulosa* is still unknown (Wakely et al., 1966; Brodie et al., 1974).

Regardless of the specific explanations for the reduced toxicity of newts and nonresistance of snakes on Vancouver Island, these populations represent support for the coevolution of prey defense and predator exploitation. Coupled with the results of Brodie and Brodie (1990), we now have evidence of a crude correlation between antipredator ability of a prey and exploitative ability of a predator between sympatric populations of the two species: In the Willamette Valley, where *Taricha granulosa* is highly toxic, *Thamnophis sirtalis* is resistant to TTX, while on Vancouver Island, where *Taricha granulosa* have little or no TTX, *Thamnophis sirtalis* are barely more resistant than conspecifics occurring outside the range of *Taricha granulosa.* This apparent matching of defense and exploitative ability among populations is a predicted outcome of an evolutionary arms race between predator and prey (Brodie and Brodie, 1990).

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