COEVOLUTION OF DEADLY TOXINS AND PREDATOR RESISTANCE: SELF-ASSESSMENT OF RESISTANCE BY GARTER SNAKES LEADS TO BEHAVIORAL REJECTION OF TOXIC NEWT PREY

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ABSTRACT: Deadly toxins and resistance to them are an evolutionary enigma. Selection for increased resistance does not occur if predators do not survive encounters with toxic prey. Similarly, deadly toxins are of no advantage to individual prey if it dies delivering the toxins. For individual selection to drive the coevolutionary arms race between resistant predators and lethal prey, the survivorship of individual predators must covary with their resistance. The extreme toxicity of the rough skinned newt *Taricha granulosa* appears to have coevolved with resistance in its predator, the common garter snake *Thamnophis sirtalis*, yet the mechanism by which individual selection can operate has been unclear in this and other lethal prey-predator systems. We show that individual snakes assess their own resistance relative to newt toxicity and reject prey too toxic to consume. Rejected newts all survived attacks and attempted ingestion by snakes that sometimes lasted over 50 min. Behavioral moderation of toxin exposure by snakes provides the association between individual resistance and fitness necessary for coevolution of lethal toxins and resistance to occur.

Key words: Predation; Predator-prey; Resistance; Selection; *Taricha*; Tetrodotoxin; *Thamnophis*; Toxin

ANTIPREDATOR defenses that kill predators, such as deadly toxins, represent a paradox for coevolutionary theory. On the one hand, the advantages of killing a potential predator or resisting the effects of a lethal toxin appear obvious. At the same time, it is difficult to understand how traits with an "all-or-none" fitness consequence for both players in an interaction can undergo coevolution in an escalating arms race that is typically assumed to occur in predator-prey interactions (Brodie and Brodie, 1999*a*; Dawkins and Krebs, 1979; Sih, 1985). If all predators die when they attack deadly toxic prey, no selection for increased resistance can take place. Because resistance does not increase, there is no longer selection for increased toxicity. How, then, can deadly toxins and resistance to them evolve?

The problem is similar to that encountered in the evolution of distastefulness and aposematism. Fisher (1930) noted that individual selection was unlikely to drive the evolution of unpalatability because dis-

tasteful individuals would die (or be seriously injured) when sampled by a predator. Subsequent research has shown that unpalatable prey often survive encounters with predators and, in many cases, escape unharmed (e.g., Fisher, 1930; Järvi et al., 1981). This realization has stimulated considerable research into how individual advantage may drive the diversity of distastefulness and warning signals (Alatalo and Mappes, 1996; Leimar et al., 1986; Servedio, 2000; Yachi and Higashi, 1998). Because unpalatability has an immediate repellent effect without a lethal consequence for predators, predators can experience selection to decrease their sensitivity to the distastefulness of a given prey. The resulting coevolutionary escalation of distastefulness and sensitivity can lead to the so-called "palatability spectrum" (Speed and Turner, 1999), whereby prey unpalatable to one predator may be acceptable to another (Brower et al., 1963).

Deadly toxins and other lethal defenses present a more difficult puzzle for the coevolution of predators and prey. Many taxa have toxins that present lethal consequences for predators that ingest them, as

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found for dendrobatid frogs (Daly et al., 1984, 1987), sea cucumbers (Mebs, 2001; Russell, 1965), and cinnabar moths (Formanowicz and Brodie, 1985). In such cases, toxins cannot be considered in the same selective context as unpalatability. More potent (i.e., deadly) toxins can be the advantage that allows individual prey to survive predation attempts. However, if all predators die in encounters with prey, they neither experience selection for increased resistance nor exert selection on increased prey defense (Brodie and Brodie, 1999*a*). When prey are deadly, the continued coevolution of toxicity and resistance requires that individual predators assess the level of toxicity of individual prey relative to their own resistance and reject those prey that possess too high a level of toxicity.

Many predators behaviorally modify their exposure to prey toxin by ingesting only selected body parts or portions of prey. Meadow voles (generalist feeders) incorporate toxic *Lespedeza* and *Penstemon* into their natural diet, but cannot survive on a strict diet of either in the laboratory (Lindroth et al., 1986). Larvae of the green lacewing minimize exposure to pyrrolizidine alkaloids in the eggs of *Utetheisa* moths by sampling single eggs and rejecting clutches with high toxin loads (Eisner et al., 2000). Grasshopper mice disarm bombardier beetles by lodging the posterior of the beetle in the substrate before preying upon them (Eisner, 1966). Otters in the Sierra de Gredos of central Spain circumvent the toxins in the Eurasian common toad by consuming only the internal organs or posterior of the body, leaving the head and toxin-rich parotoid glands intact (Lizana and Mellado, 1990). Unknown predators have similarly disemboweled newts of the genus *Notophthalmus* (Shure et al., 1989), which contain some tetrodotoxin (TTX) in their skin (Yotsu-Yamashita and Mebs, 2001). However, snakes are limited in their ability to modify a predatory interaction in this manner because they are constrained to swallow prey whole (Greene, 1997). Snakes that seize toxic prey risk exposure to the full toxin load if they ingest the item. Yet snakes have commonly evolved resistance to toxins that kill other predators, such as *Heterodon* and *Xenodon* that eat *Bufo* (Duellman and Trueb, 1986), *Liophis* that eat *Atelopus, Dendrobates,* and *Phyllobates* (Myers et al., 1978), and *Thamnophis fulvus* that eat *Bolitoglossa rostrata* (Brodie and Ducey, 1991).

One striking example of resistance to a deadly toxic prey involves the garter snake *Thamnophis sirtalis* and the rough-skinned newt *Taricha granulosa.* The skin of *T. granulosa* contains extremely high and variable concentrations of tetrodotoxin (TTX; Hanifin et al., 2002), a potent neurotoxin that blocks voltage-gated sodium channels in nerve and muscle tissue, thereby inhibiting the propagation of action potentials (e.g., Lipkind and Fozzard, 1994; Narahashi et al., 1967). Tetrodotoxin is an effective defense against predators for the newt (Brodie, 1968; Brodie et al., 1974; Mosher et al., 1964; Wakely et al., 1966), for example, the toxic secretions of *T. granulosa* have allowed newts to escape unharmed after being swallowed by a bullfrog for 15 min and survive being swallowed by fish for 13–20 min (Brodie, 1968). *Taricha granulosa* have also been observed to crawl out of the mouths of *T. couchi* that were immobilized after ingesting the toxic newt; newts have survived up to 85 min of ingestion by garter snakes (Brodie and Brodie, 1990). The only predator known to be resistant to this toxin is the common garter snake *T. sirtalis* (Brodie and Brodie, 1990, 1991, 1999*a*).

Historical, geographic, and intrapopulational patterns of resistance and toxicity all indicate that garter snake resistance and newt toxicity are in the midst of a coevolutionary arms race. Within populations, resistance to TTX varies among neonate snakes and has a heritable basis (Brodie and Brodie, 1990). Among populations, the levels of both newt toxicity and garter snake resistance are variable, but roughly matched (Brodie and Brodie, 1990, 1991, 1999*a*), suggesting a geographic mosaic of coevolutionary outcomes (Thompson, 1994, 1997, 1999). Garter snake populations allopatric with newts are not resistant to TTX, while sympatric populations are

resistant (Brodie and Brodie, 1990, 1999*a*). Populations of newts from some areas (e.g., Vancouver Island, British Columbia) lack TTX (Hanifin et al., 1999), and garter snakes from these populations are nonresistant (Brodie and Brodie, 1991). Other garter snakes that co-occur with *Taricha* are susceptible to TTX (Brodie, 1968; Brodie and Brodie, 1999*a*; Motychak et al., 1999), supporting the view that resistance to TTX is an adaptation by a predator to its toxic prey. What is unclear in this scenario is how selection at the individual level can drive the evolution of resistance; if a population of predators cannot survive the ingestion of toxic prey, then all individuals that feed on the prey die, and no variance in fitness of predators exists.

To explore how individual level selection might operate on predator resistance and, consequently, on toxicity of prey, we examined interactions between toxic *T. granulosa* and their resistant garter snake predator *T. sirtalis.* We exploited population differences in resistance and toxicity to pair snakes with newts that were, on average, more toxic than the snake would naturally encounter in the wild. By using snakes with known levels of resistance, we were able to investigate whether or not snakes could assess their own ability to withstand the effects of TTX and whether such behavior would have fitness consequences for snakes and newts. If positive directional selection for resistance is operating, we predict that higher resistance should increase the probability that an individual snake can eat a toxic newt and, in doing so, exploit a locally abundant energy source. Furthermore, higher resistance should decrease costs by reducing the time a snake is impaired and at risk while intoxicated after ingesting a newt.

MATERIALS AND METHODS

Assaying Snake Resistance

All *T. sirtalis* used in our study were born in the laboratory to wild-caught females collected from Warrenton, Clatsop Co., Oregon, in 1996, then reared in captivity for 4 yr. During this time, snakes were housed in 38-l aquaria with access to thermal gradients and exposed to a 12:12 light/dark photoperiod. Diets consisted entirely of fish; at the time of the experiment, snakes had no previous experience with newts. Twenty individuals born to eight different females were used.

Resistance to TTX was measured for all individuals as neonates. We scored resistance using a bioassay based on whole organism locomotor performance (Brodie and Brodie, 1990, 1991, 1999*b*; Ridenhour et al., 1999). Trials were conducted at 25 C. Each snake was raced on a 2-m linear racetrack equipped with infrared sensors to electronically time sprint speed (Brodie and Brodie, 1999*b*; Ridenhour et al., 1999). The maximum speed in a trial for a 0.5-m segment of the track was taken as the sprint speed score. Individuals were tested repeatedly, and the average of two scores was taken as an individual's baseline speed. Twenty hours after the last sprint test, neonates were given intraperitoneal (IP) injections of TTX (0.5 µg). Oral doses and IP injections of TTX given to garter snakes produce qualitatively identical symptoms, and these resistance measures are directly proportional to each other (Williams et al., 2002). Thirty minutes after injection, snakes were tested again to obtain a measure of post-injection speed. Two repeated measures of post-injection speed were taken at 48-h intervals. Again, the average of repeated trials was taken as an individual's measure of post-injection speed. Resistance to TTX was scored as the percentage of an individual's baseline speed crawled after injection (100 × post-injection speed/baseline speed). Individuals that are greatly impaired by TTX crawl only a small proportion of their normal speed, while those that are unaffected by an injection of TTX crawl at 100% of their baseline speed. The bioassay is highly repeatable and enables us to assess individual differences in susceptibility to TTX (Brodie and Brodie, 1990, 1991, 1999*b*). Previous experience with TTX has no effect on resistance, and relative differences in resistance are consistent from birth to adulthood (Ridenhour et al., 1999).

Assaying Snake-newt Interactions

By pairing snakes (Warrenton, Clatsop Co., Oregon) and newts (Soap Creek, Benton Co., Oregon) from different populations, we were able to observe predators interacting with prey more toxic than they would normally encounter in nature. Sympatric snakes and newts are expected to be well matched in terms of resistance and toxicity, respectively, but variation among populations in both traits is extensive (Brodie and Brodie, 1999a). We calculated, based on the average body size (70 g) and resistance of snakes from Warrenton, that ingesting 0.65 mg of TTX would reduce a snake's crawl speed to 50% (see Williams et al., 2002 for calculation). Adult female newts from Soap Creek contain an average of 4.3 mg (range 1.7–7.0 mg) of TTX in their skin (Hanifin et al., 2002), which is approximately five times as much as newts from Warrenton (C. T. Hanifin, unpublished data). Thus, an average snake from Warrenton could not ingest an average newt from Soap Creek without suffering extreme impairment (including death) from TTX. This greater than 4-fold variation in toxicity is much larger than the 1.3-fold variation in newt mass of this experiment; in fact, newt toxicity is largely independent of newt mass (Hanifin et al., 2002). Though within-population snake resistance varies greatly as well, even the most resistant snakes from Warrenton would not be able to ingest the most toxic newts from Soap Creek without severe performance consequences.

Because we were primarily interested in how snakes of varying TTX resistance respond to toxic prey, we focused on the interaction between predator and prey after initial contact. Interactions were observed in the home chambers of the snakes. Newts were introduced to the chamber by placing them into the water bowl in the same manner that meals of fish were introduced in the past. In accordance with the standard feeding procedure over the previous 4 yr, snakes were not prevented from viewing adjacent cages where other snakes had been offered newts. Nineteen of the 20 snakes offered newts seized prey; the one snake that did not attempt to eat was excluded from further analysis.

We assessed a snake's exposure time to the toxin of newts as the time between a snake's initial strike and subsequent release (i.e., no longer in physical contact with the snake) or swallowing (i.e., no longer protruding from the mouth of the snake) of the newt. This measure of exposure time is supported by previous work that indicates toxin from the skin of the newt may be primarily absorbed by the soft tissues of the upper gastrointestinal track (Williams et al., 2002). Although one might expect that exposure to TTX continues after consumption of a newt, Williams et al. (2002) and Brodie and Tumbarello (1978) observed consumption of newts and poison dart frogs by garter snakes, respectively, and hypothesized that most exposure to the toxin occurs as toxic prey are swallowed; the rate of exposure is reduced after prey reach the stomach where the toxin may be at least partially neutralized or degraded. Our score is likely to be a conservative estimate of exposure time, particularly for those snakes that ingested newts.

The righting reflex of snakes was used as an assay of TTX intoxication. The toxin blocks sodium channels and results in inhibition of action potentials in nerve and muscle, thus impairing muscle function (e.g., Lipkind and Fozzard, 1994; Narahashi et al., 1967) and is known to inhibit righting reflex in snakes (Brodie, 1968). After snakes had either swallowed or released a newt, the recovery time (from the initial attack) was determined by testing the righting reflex of the snake at 5-min intervals. The snake was considered recovered when it either could not be turned over on its dorsum or immediately righted itself. Testing the righting reflex of snakes was less disturbing and exhausting to the animal than repeatedly measuring crawl speed and, thus, gave a more precise estimate of recovery time. Newt mass was recorded before introduction of newts into the chambers, and snake mass was measured at the completion of the trial; for snakes that consumed newts, mass was es-

TABLE 1.—Comparison of predation attempts during which newts were consumed versus rejected. Exposure time, recovery time, and resistance were fourth root transformed to better approximate a normal distribution. Results of *t*-tests on snake mass, exposure time, and newt mass for all snakes were not different than those for surviving snakes.

	Surviving snakes				
	Newt consumed (n = 4)		Newt rejected (n = 13)		
	Range	Mean (SE)	Range	Mean (SE)	*t*
Snake mass (g)	54.5–77.2	69.9 (5.2)	38.3–80.9	61.4 (3.4)	1.364
Exposure time (min)	51–76	65.3 (8.0)	1–50	11.4 (4.0)	7.618***
Recovery time (min)	76–161	136.3 (20.2)	23–197	74.2 (14.1)	2.874*
Newt mass (g)	13.6–18.0	15.0 (1.0)	9.5–15.6	13.3 (0.5)	1.153
Percent resistance	70.4–88.2	81.0 (4.3)	21.1–100.0	53.7 (7.2)	3.196**
	All snakes				
	Newt consumed (n = 6)		Newt rejected (n = 13)		
Percent resistance	35.6–88.2	68.9 (9.2)	21.1–100.0	53.7 (7.2)	1.234

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

timated by subtracting the mass of the newt from the total measured mass.

Statistical Analyses

Path analysis was employed to investigate the relationship between various factors that potentially influence the exposure time and recovery time of snakes that attack toxic newts. The path model was based on the hypothesis that snake resistance and mass might influence both the exposure time and recovery time directly and that exposure time might also directly influence recovery time. Newt mass was also included as a potential direct physical effect on exposure and recovery time (newt mass and toxicity are largely uncorrelated; Hanifin et al., 2002). Individual measures of toxicity for the newts used in our study were unavailable because toxicity assays are destructive (Hanifin et al., 1999; Wakely et al., 1966). We also compared snakes that rejected newts to those that consumed newts for differences in the above variables with *t*-tests. Resistance, exposure time, and recovery time were fourth root transformed for all analyses to approximate better a normal distribution.

RESULTS

Nineteen of 20 snakes seized newts, but only four snakes successfully consumed the prey (Table 1). All the newts rejected after attack survived. Snakes that con-

sumed a newt and survived were, on average, more resistant, endured longer exposure times, and had longer recovery times than snakes that seized and rejected a newt. Mean snake mass and mean newt mass did not differ for these two groups of snakes. One extremely large snake (mass = 110.5 g) of low resistance (35.6%) that ate a newt in 33 min and one snake (mass = 66.5 g) of moderate resistance (54.0%) that took 198 min to swallow a newt never recovered. The latter snake appeared paralyzed with the newt inside its mouth for at least 2 h. This moderate resistance snake died 6 h after first seizing a newt; the low resistance snake could not right itself or react in any manner to provocation 24 h after first biting the newt and was euthanized at this time. In order to compare and evaluate differences in snakes that consumed a newt and survived and those that did not consume a newt and survived, the two snakes that died were not included in the path analysis or *t*-tests. To evaluate the hypothesis that snakes of lower resistance simply do not eat newts and are, thus, under negative selection for resistance, a separate *t*-test was conducted (Table 1). The results revealed that, of all snakes, mean resistance did not differ between those that ate newts and those that did not (Table 1).

All snakes exhibited obvious symptoms of TTX poisoning. Even snakes with ex-
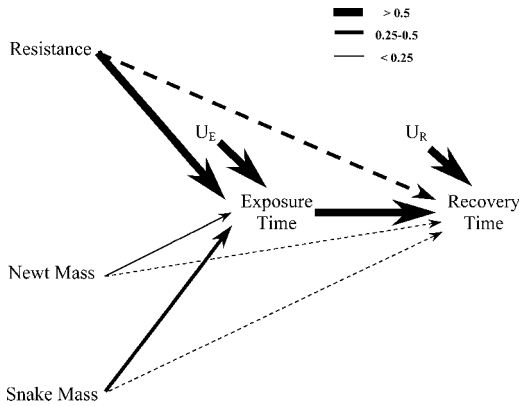
FIG. 1.—Path diagram of causal factors influencing recovery time of garter snakes that attack newts. Recovery time is only directly influenced by exposure time during a predation attempt. Exposure time to TTX is directly influenced by resistance and body mass of individual snakes. Effects of unmeasured variables are shown for exposure time (U_E) and recovery time (U_R). Solid arrows denote positive effects and dashed arrows denote negative effects. Arrow weight indicates strength of effect (path coefficients).

posure times of 1–3 min could not hold their heads steady. After releasing or swallowing the newt, all snakes began gaping their mouths and rubbing their open mouths on the substrate. In extreme cases, snakes writhed around with open mouths, sometimes biting themselves as close as 5 cm behind their head. Some snakes could not elevate their head for extended periods of time and lay flaccid on the substrate even when disturbed to test righting reflex. Although snakes were considered recovered by their righting reflex, some continued to exhibit occasional unsteadiness and

head wobbles for several minutes after "recovery."

Path analysis (Fig. 1; Table 2) revealed that resistance and mass of snakes directly influenced exposure time to TTX. Exposure time, in turn, had a large positive and direct effect on recovery time. Although not significant in this analysis, higher resistance led to shorter recovery times after controlling for differences in exposure time. Newt mass was not an important causal factor for either exposure time or recovery time. Unmeasured variables contributed substantially to variation in both exposure time and recovery time.

DISCUSSION

The primary factor influencing the length of exposure to newt toxin is a snake's own resistance (Fig. 1), indicating that snakes can somehow assess their own susceptibility to TTX and reject prey that are too toxic to handle. The significance of this behavioral moderation of toxin exposure is that it provides a mechanism whereby predators can interact with potentially deadly prey without necessarily suffering the lethal consequences of the toxin. Only snakes of high resistance consumed a newt and survived (Table 1). As a result, quantitative differences in resistance to TTX among individuals can have fitness consequences for snakes. Similarly, individual differences in toxicity among newts, therefore, may correspond to differences in predation risk. Thus, self-assessment of TTX resistance by snakes represents the behavioral mechanism by

TABLE 2.—Path coefficients and effect tests (df = 1) from partial regressions on standardized variables. Exposure time, recovery time, and resistance were fourth root transformed to better approximate a normal distribution.

	Exposure time		Recovery time	
	β =	F =	β =	F =
Snake mass	0.490	7.24 $P = 0.018$	−0.102	0.160 NS
Newt mass	0.197	1.19 NS	−0.057	0.073 NS
Resistance	0.675	13.55 $P = 0.003$	−0.256	0.748 NS
Exposure time	—	—	0.884	8.014 $P = 0.015$

which reciprocal selection between *T. sirtalis* and *T. granulosa* can occur.

The risks associated with predation attempts on *T. granulosa* are not limited to death from TTX exposure. Snakes that seized newts were physically impaired for 23–197 min in our experiment. Those individuals that ingested newts were impaired for an average of 62 min longer than those snakes that rejected newts. In the field, we have encountered *T. sirtalis* similarly incapacitated after feeding on *T. granulosa.* Individuals in this condition are more at risk than those that have ingested non-toxic prey of similar size because the extreme physical impairment resulting from intoxication renders them even more susceptible to predation and further inhibits their ability to thermoregulate effectively (Brodie and Brodie, 1990, 1999*a*). Although exposure time was the largest causal factor of variation in recovery time, Brodie et al. (2002) found that resistance did significantly reduce recovery time. Our results agree with their conclusion when differences in exposure time were considered. Selection associated with the length of recovery time can drive the evolution of resistance even if the predation sequence does not end in ingestion.

Additionally, the hypothesis that snakes of lower resistance simply do not eat newts was rejected. Behavioral rejection of newts does not result in negative selection pressure on resistance. This is intuitive when one realizes that the extreme variation in newt toxicity and snake resistance introduce the possibility that even snakes of low resistance may encounter and consume newts of low toxicity where resistance and toxicity are roughly matched, as appears to be the case in many populations (Brodie and Brodie, 1990, 1991, 1999*a*). Thus, selection favoring higher resistance would occur in populations of *T. sirtalis* sympatric with toxic newts at all mean levels of resistance.

The overall balance of costs and benefits of feeding on toxic newts is difficult to evaluate. Clearly, there are risks associated with attacking and ingesting newts, including a genetic tradeoff between resistance and locomotor performance that renders the most resistant snakes less adept at escape (Brodie and Brodie, 1999*b*). On the other hand, TTX resistance allows *T. sirtalis* to exploit an abundant prey item that other predators cannot. A meal the size of an average adult newt could last a garter snake for as long as 8 d (Peterson et al., 1998), during which time the snake may have less exposure to predators and forgo some energy costs of foraging. The short-term risk of immobility following predation of a newt may be offset by a reduction in day-to-day vulnerability because individuals may not need to forage for a week or more.

The mechanism by which snakes judge their own resistance is unclear and may, in fact, be passive. The relationship between exposure time and resistance would exist if snakes reject prey after they begin to experience some threshold level of intoxication. As TTX is initially absorbed by the membranes of the mouth and upper gastrointestinal tract (Williams et al., 2002), snakes with relatively low resistance would become incapacitated sooner than those with higher resistance. However, it is important to note that, of the snakes that rejected newts, nearly all did so within 12 min of seizing prey. If rejection is based on a threshold level of intoxication, this level is reached quite quickly.

The behavioral termination of predation by snakes as a function of their own resistance also has critical importance for the evolution of toxicity. All newts rejected by snakes survived, including one that had been in a snake's mouth for nearly 1 h. A mix of successful and unsuccessful predation is a necessary requisite for the evolution of antipredator mechanisms (Sih, 1985; Vermeij, 1982, 1985). Unfortunately, we were unable to quantify the individual toxicity of newts in this study, so we cannot be certain that the probability of rejection is related to individual differences in toxicity. However, the effect of any toxin is a function of both the quantity of toxin and the resistance of the predator. We, therefore, expect the behavioral decision to reject a newt to be determined by an interaction of toxicity and resistance.

LITERATURE CITED

ALATALO, R. V., AND J. MAPPES. 1996. Tracking the evolution of warning signals. Nature 382:708–709.

BRODIE, E. D., JR. 1968. Investigations on the skin toxin of the adult roughskinned newt, *Taricha granulosa.* Copeia 1968:307–313.

BRODIE, E. D. III, AND E. D. BRODIE, JR. 1990. Tetrodotoxin resistance in garter snakes: an evolutionary response of predators to dangerous prey. Evolution 44:651–659.

———. 1991. Evolutionary response of predators to dangerous prey: reduction of toxicity of newts and resistance of garter snakes in island populations. Evolution 45:221–224.

———. 1999a. Predator-prey arms races. Bioscience 49:557–568.

———. 1999b. The cost of exploiting poisonous prey: tradeofffs in a predator–prey arms race. Evolution 53:626–631.

BRODIE, E. D., JR., AND P. K. DUCEY. 1991. Antipredator skin secretions of some tropical salamanders (*Bolitoglossa*) are toxic to snake predators. Biotropica 23:58–62.

BRODIE, E. D., JR., AND M. S. TUMBARELLO. 1978. The antipredator functions of *Dendrobates auratus* (Amphibia, Anura, Dendrobatidae) skin secretion in regard to a snake predator (*Thamnophis*). Journal of Herpetology 12:264–265

BRODIE, E. D., JR., J. L. HENSEL, JR., AND J. A. JOHNSON. 1974. Toxicity of the urodele amphibians *Taricha, Notophthalmus, Cynops* and *Paramesotriton* (Salamandridae). Copeia 1974:506–511.

BRODIE, E. D. III, E. D. BRODIE, JR., AND J. E. MOTYCHAK. 2002. Recovery of garter snakes (*Thamnophis sirtalis*) from the effects of tetrodotoxin. Journal of Herpetology 36:95–98.

BROWER, L. P., J. V. Z. BROWER, AND C. T. COLLINS. 1963. Experimental studies of mimicry. 7. Relative palatability and mullerian mimicry among neotropical butterflies of the subfamily Heliconiiae. Zoologica: New York Zoological Society 48:65–84.

DALY, J. W., R. J. HIGHET, AND C. W. MYERS. 1984. Occurence of skin alkaloids in non-dendrobatid frogs from Brazil (Bufonidae), Australia (Myobatrachidae), and Madagascar (Mantellinae). Toxicon 22:905–919.

DALY, J. W., C. W. MYERS, AND N. WHITTAKER. 1987. Further classification of skin alkaloids from neotropical poison frogs (Dendrobatidae), with a general survey of toxic, noxious substances in the Amphibia. Toxicon 25:1021–1095.

DAWKINS, R., AND J. R. KREBS. 1979. Arms races between and within species. Proceedings of the Royal Society of London B 205:489–511.

DUELLMAN, W. E., AND L. TRUEB. 1986. Biology of the Amphibians. McGraw–Hill Book Co., New York, New York, U.S.A.

EISNER, T. E. 1966. Beetle's spray discourages predators. Natural History 75:42–47.

EISNER, T., M. EISNER, C. ROSSINI, V. K. IYENGAR, B. L. ROACH, E. BENEDIKT, AND J. MEINWALD. 2000. Chemical defense against predation in an insect egg. Proceedings of the National Academy of Sciences 97:1634–1639.

FISHER, R. A. 1930. The Genetical Theory of Natural Selection. Oxford University Press, Oxford, U.K.

FORMANOWICZ, D. R., JR., AND E. D. BRODIE, JR. 1985. Unpalatability and toxicity of an introduced species (cinnabar moth larvae) to native predators. American Midland Naturalist 133:401–403.

GREENE, H. W. 1997. Snakes: the Evolution of Mystery in Nature. University of California Press, Berkeley, California, U.S.A.

HANIFIN, C. T., M. YOTSU-YAMASHITA, T. YASUMOTO, E. D. BRODIE III, AND E. D. BRODIE, JR. 1999. Toxicity of dangerous prey: variation of tetrodotoxin levels within and among populations of the newt *Taricha granulosa.* Journal of Chemical Ecology 25:2161–2175.

HANIFIN, C. T., E. D. BRODIE III, AND E. D. BRODIE, JR. 2002. Tetrodotoxin levels of the rough-skin newt, *Taricha granulosa,* increase in long-term captivity. Toxicon 40:1149–1153.

JÄRVI, T., B. SILLÉN-TULLBERG, AND C. WIKLUND. 1981. The cost of being aposematic: an experimental study of predation on larvae of *Papilio machaon* by the great tit *Parus major.* Oikos 36:267–272.

LEIMAR, O., M. ENQUIST, AND B. SILLÉN-TULLBERG. 1986. Evolutionary stability of aposematic coloration and prey unprofitability: a theoretical analysis. American Naturalist 128:469–490.

LINDROTH, R. L., G. O. BATZLI, AND S. I. AVILDSEN. 1986. *Lespedeza* phenolics and *Penstemon* alkaloids: effects on digestion efficiencies and growth of voles. Journal of Chemical Ecology 12:713–728.

LIPKIND, G. M., AND H. A. FOZZARD. 1994. A structural model of the tetrodotoxin and saxitoxin binding site of the Na+ channel. Biophysics Journal 66: 1–13.

LIZANA, M., AND V. P. MELLADO. 1990. Depradacion por la nutria (*Lutra lutra*) del sapo de la Sierra Gredos (*Bufo bufo gredosicola*). Doñana, Acta Vertebrata 17:109–112.

MEBS, D. 2001. Toxicity in animals. Trends in evolution? Toxicon 39:87–96.

MOSHER, H. S., F. A. FUHRMAN, H. D. BUCHWALD, AND H. G. FISCHER. 1964. Tarichatoxin-tetrodotoxin: a potent neurotoxin. Science 144:1100–1110.

MOTYCHAK, J. E., E. D. BRODIE, JR., AND E. D. BRODIE III. 1999. Evolutionary response of predators to dangerous prey: preadaptation and the evolution of tetrodotoxin resistance in garter snakes. Evolution 53:1528–1535.

MYERS, C. W., J. W. DALY, AND B. MALKIN. 1978. A dangerously toxic new frog (*Phyllobates*) used by Emberá Indians of western Columbia, with discussion of blowgun fabrication and dart poisoning. Bulletin of the American Museum of Natural History 161:307–365.

NARAHASHI, T., J. W. MOORE, AND R. N. POSTON. 1967. Tetrodotoxin derivatives: chemical structure and blockage of nerve membrane conductance. Science 156:976–978.

PETERSON, C. C., B. M. WALTON, AND A. F. BENNETT. 1998. Intrapopulation variation in ecological energetics of the garter snake *Thamnophis sirtalis,* with analysis of the precision of doubly labeled water measurements. Physiological Zoology 71:333–349.

RIDENHOUR, B. J., E. D. BRODIE, JR., AND E. D. BRODIE III. 1999. Effects of repeated injection of tetrodotoxin on growth and resistance to tetrodotoxin in the garter snake *Thamnophis sirtalis.* Copeia 1999:531–535.

RUSSELL, F. E. 1965. Comparative pharmacology of some animal toxins. Federal Proceedings 26:1206–1224.

SERVEDIO, M. R. 2000. The effects of predator learning, forgetting, and recognition errors on the evolution of warning coloration. Evolution 54:751–763.

SHURE, D. J., L. A. WILSON, AND C. HOCHWENDER. 1989. Predation on aposematic efts of *Notophthalmus viridescens.* Journal of Herpetology 23:437–439.

SIH, A. 1985. Evolution, predator avoidance, and unsuccessful predation. American Naturalist 125:153–157.

SPEED, M. P., AND J. R. G. TURNER. 1999. Learning and memory in mimicry: II. Do we understand the mimicry spectrum? Biological Journal of the Linnean Society 67:281–312.

THOMPSON, J. N. 1994. The Coevolutionary Process. The University of Chicago Press, Chicago, Illinois, U.S.A.

———. 1997. Evaluating the dynamics of coevolution among geographically structured populations. Ecology 78:1619–1623.

———. 1999. Coevolution and escalation: are ongoing coevolutionary meanderings important. American Naturalist 153:S92–S93.

VERMEIJ, G. J. 1982. Unsuccessful predation and evolution. American Naturalist 120:701–720.

———. 1985. Adaptations, effects, and fortuitous survival: comment on a paper by A. Sih. American Naturalist 125:470–472.

WAKELY, J. F., G. J. FUHRMAN, F. A. FUHRMAN, H. G. FISCHER, AND H. S. MOSHER. 1966. The occurrence of tetrodotoxin (tarichatoxin) in Amphibia and the distribution of the toxin in the organs of newts (*Taricha*). Toxicon 3:195–203.

WILLIAMS, B. L., E. D. BRODIE, JR., AND E. D. BRODIE III. 2002. Comparisons between toxic effects of tetrodotoxin administered orally and by intraperitoneal injection to the garter snake *Thamnophis sirtalis.* Journal of Herpetology 36:112–115.

YACHI, S., AND M. HIGASHI. 1998. The evolution of warning signals. Nature 394:882–884.

YOTSU-YAMASHITA, M., AND D. MEBS. 2001. The levels of tetrodotoxin and its analogue 6-*epi*tetrodotoxin in the red-spotted newt, *Notophthalmus viridescens.* Toxicon 39:1261–1263.