Evolution, 56(10), 2002, pp. 2067–2082

THE EVOLUTIONARY RESPONSE OF PREDATORS TO DANGEROUS PREY: HOTSPOTS AND COLDSPOTS IN THE GEOGRAPHIC MOSAIC OF COEVOLUTION BETWEEN GARTER SNAKES AND NEWTS

EDMUND D. BRODIE, JR.,[1] B. J. RIDENHOUR,[2] AND E. D. BRODIE III[2]
[1]*Department of Biology, Utah State University, Logan, Utah, 84322-5305*
[2]*Department of Biology, Indiana University, Bloomington, Indiana, 47405-3700*

Abstract.—The ''geographic mosaic'' approach to understanding coevolution is predicated on the existence of variable selection across the landscape of an interaction between species. A range of ecological factors, from differences in resource availability to differences in community composition, can generate such a mosaic of selection among populations, and thereby differences in the strength of coevolution. The result is a mixture of hotspots, where reciprocal selection is strong, and coldspots, where reciprocal selection is weak or absent, throughout the ranges of species. Population subdivision further provides the opportunity for nonadaptive forces, including gene flow, drift, and metapopulation dynamics, to influence the coevolutionary interaction between species. Some predicted results of this geographic mosaic of coevolution include maladapted or mismatched phenotypes, maintenance of high levels of polymorphism, and prevention of stable equilibrium trait combinations.

To evaluate the potential for the geographic mosaic to influence predator-prey coevolution, we investigated the geographic pattern of genetically determined TTX resistance in the garter snake *Thamnophis sirtalis* over much of the range of its ecological interaction with toxic newts of genus *Taricha*. We assayed TTX resistance in over 2900 garter snakes representing 333 families from 40 populations throughout western North America. Our results provide dramatic evidence that geographic structure is an important component in coevolutionary interactions between predators and prey. Resistance levels vary substantially (over three orders of magnitude) among populations and over short distances. The spatial array of variation is consistent with two areas of intense evolutionary response by predators (''hotspots'') surrounded by clines of decreasing resistance. Some general predictions of the geographic mosaic process are supported, including clinal variation in phenotypes, polymorphism in some populations, and divergent outcomes of the interaction between predator and prey. Conversely, our data provide little support for one of the major predictions, mismatched values of interacting traits. Two lines of evidence suggest selection is paramount in determining population variation in resistance. First, phylogenetic information indicates that two hotspots of TTX resistance have evolved independently. Second, in the one region that TTX levels in prey have been quantified, resistance and toxicity levels match almost perfectly over a wide phenotypic and geographic range. However, these results do not preclude the role the nonadaptive forces in generating the overall geographic mosaic of TTX resistance. Much work remains to fill in the geographic pattern of variation among prey populations and, just as importantly, to explore the variation in the ecology of the interaction that occurs within populations.

Key words.—Arms-race, coevolution, geographic mosaic, predator-prey, *Taricha*, tetrodotoxin resistance, *Thamnophis*.

Received November 26, 2001. Accepted July 12, 2002.

Ecological and behavioral interactions between individual organisms generate the reciprocal selection that drives coevolution. It is this observation that has led to the realization that the process of coevolution occurs primarily at the scale of populations and is only then translated into species levels patterns (Thompson 1994, 1997, 1999a, b; Nuismer et al. 1999, 2000; Gomulkiewicz et al. 2000). Because interactions and their consequences can vary from one locality to another, the outcomes of coevolution and the adaptive processes that drive it are expected to vary geographically. If we look across a broad geographic range of species interaction, we are likely to observe a patchwork of genotypes and phenotypes that reveal the geographic structure of populations.

This scenario is the general framework of the geographic mosaic theory of coevolution (Thompson 1994, 1997, 1999b). At one level this theory is little more than an observation of the scale of microevolution, but at another it is a fundamentally novel view of the dynamic of coevolution. Geographic structure leads to population subdivision and a concomitant variety of phenomena that influence evolutionary trajectories (Wade and Goodnight 1998). Heterogeneous selection, drift, gene flow among local populations, metapopulation dynamics including extinction and recolonization, and contributions from nonadditive genetic architecture all impact evolution in structured populations. If selection varies geographically, then we expect to see a mixture of ''hotspots,'' wherein the reciprocal selection of coevolution is strong, and ''coldspots,'' wherein selection is weak, throughout the range of an interaction (Thompson 1994, 1999b). Population structure and its associated phenomena may lead to a remixing of coevolving traits between hotspots and coldspots. Consequently, predictions regarding both the dynamics and the patterns of coevolution change if we consider the scale of interaction and the intermixing of traits that results from population structure (Thompson 1994, 1999b, c). Recent models of coevolution indicate that the ramifications of geographic structure can include maintenance of genetic polymorphism, prevention of stable equilibria, and considerable local maladaptation (Nuismer et al. 1999, 2000; Gomulkiewicz et al. 2000).

Thompson (1999b) outlines some of the geographic patterns that would corroborate the geographic mosaic theory, and evidence has recently begun to accumulate along these lines. (1) The strength of coevolution differs among populations such that there are hotspots and coldspots of coevolution through a species' range. For example, crossbills (*Loxia* sp.) prey upon

the cones of lodgepole pines (*Pinus contorta*) and the two appear to coevolve in some isolated populations. However, competition from red squirrels (*Tamiasciurus hudsonicus*) largely excludes crossbills from utilizing lodgepole pine cones where the predators co-occur and, consequently, the phenotypes of both cones and crossbill bills differ in these areas (Benkman 1999; Benkman et al. 2001). (2) Traits at the coevolutionary interface show clinal or mosaic variation. Evidence for such interpopulational variation includes host-parasite (Parker 1985; Dybdahl and Lively 1996; Kraaijeveld and Godfray 1999) and plant-herbivore (Berenbaum and Zangerl 1998) interactions, wherein both mosaic and continuous clinal variation in traits at the phenotypic interface are observed. (3) Some populations maintain coevolved traits primarily through connections to other populations. This is a particularly difficult phenomenon to demonstrate because it requires knowledge of gene flow, historical population relationships, and local selection pressures. Nonetheless, such factors are implicated in the maintenance of disease resistance and virulence in plant-pathogen (Burdon and Thrall 1999) and host-parasite systems (Dybdahl and Lively 1996; Burdon and Thrall 1999) and antipredator traits of salamanders (Storfer and Sih 1998). (4) The characters at the coevolutionary interface, as well as the form of the interaction, differ among populations. The interaction between the prodoxid moth *Greya politella* and its host plant *Lithophragma parviflorum* differs geographically with availability of other host plants and potential pollinators (Thompson 1999b). The interaction can range from mutualism to antagonism depending on the local community composition. (5) Phenotypic mismatches and maladaptation occur because of the intermixing of traits among populations. This prediction results from a synthesis of other effects of geographic structure, but implies that local selection is not the only driving force in coevolution. Examples of mismatched abilities between interacting species come from several types of systems, including plant-pathogen (Kaltz et al. 1999), predator-prey (Storfer and Sih 1998) and plant-herbivore (Berenbaum and Zangerl 1998) interactions.

Studies of interactions that are replicated over broad landscapes are requisite for evaluating these predictions and the view that geographic structure is an important influence on the dynamics and patterns of coevolution. The fundamental component of the geographic mosaic theory is that differences among populations exist and influence evolutionary dynamics and that such patterns can only be detected by surveying wide geographic ranges. Moreover, general support for the hypothesis of the geographic mosaic of coevolution depends upon investigations of varied types of interaction and taxonomic groups.

To evaluate the potential importance of geographic structure in predator-prey coevolution, we examine the pattern of geographic variation in tetrodotoxin (TTX) resistance in western North American populations of the garter snake *Thamnophis sirtalis*. TTX resistance is a phenotype that has coevolved in *T. sirtalis* through interaction with toxic newts of the genus *Taricha* (Brodie and Brodie 1999a). This system is one of the only predator-prey interactions, particularly among those involving vertebrates, where evidence of coevolution is clear. The contiguous nature of the distribution of both snakes and newts in western North America renders this an especially important system in which to investigate the geographic mosaic. Most previously examined coevolutionary systems include at least one species with relatively disjunct distributions or limited gene flow (see Thompson 1999b), which is likely to lead to more exaggerated population subdivision and therefore more pronounced geographic structure.

Garter Snakes versus Newts—An Arms Race between Predators and Dangerous Prey

The phenotypic interface of the interaction between the garter snake *T. sirtalis* and newts of the genus *Taricha* revolves around the chemical defense, tetrodotoxin (TTX). Tetrodotoxin is one of the most potent neurotoxins known; molecules of the toxin bind to Na^+ channels in nerve and muscle tissue, blocking action potential propagation (Narahashi et al. 1967; Hille 1992; Lipkind and Fozzard 1994). The toxicity of *Taricha* results almost entirely from high amounts of TTX in the skin (Mosher et al. 1964), and these newts are lethal to a wide range of potential predators (Brodie 1968). All species of the genus *Taricha* possess TTX, but interspecific and interpopulational variation in toxicity is known (Hanifin et al. 1999). *Taricha granulosa* appears to be more toxic than its congeners—one adult *T. granulosa* may contain enough toxin to kill approximately 25,000 white mice (Brodie et al. 1974). Although TTX is found in a variety of animals spanning at least four phyla including arthropods, platyhelminthes, mollusks, and vertebrates (reviewed in Daly et al. 1984; Kodama et al. 1985; Daly et al. 1987), *Taricha* are the only animals that possess TTX for which a resistant predator is known (Brodie 1968; Brodie and Brodie 1990, 1991, 1999a).

As expected for a trait at the phenotypic interface of coevolution, the level of TTX resistance in *T. sirtalis* varies with the presence of toxic newts. Where newts are absent or nontoxic (as is the case on Vancouver Island, British Columbia; Brodie and Brodie 1991; Hanifin et al. 1999), *T. sirtalis* are minimally resistant to TTX. TTX resistance in western *T. sirtalis* is clearly derived; resistance in these populations is 10–1000 times that of any other member of the genus. However, phylogenetic analysis also reveals the ancestral condition in the genus *Thamnophis* and its close relatives to be 10 times more resistant to TTX than other colubrid snakes (Motychak et al. 1999), suggesting that there may be some predisposition to TTX resistance within the lineage.

The reciprocal selection driving the coevolutionary arms race between toxicity and resistance results from the behavioral interaction between predator and prey. Some snakes can assess their own resistance relative to newts they attack and reject prey too toxic to ingest (Williams et al. 2002). *Thamnophis sirtalis* that seize newts exhibit severe symptoms of TTX poisoning, and snakes that do not accurately assess their resistance to an individual prey item sometimes die after ingesting a newt. One of the first symptoms of TTX poisoning is lack of muscle control and immobility, and even snakes resistant enough to successfully swallow a newt are typically incapacitated for a period ranging from 30 min to seven hours (Williams et al. 2002). Such consequences of TTX poisoning may represent further selection for resistance, because an individual that cannot move is unable to effectively ther-

moregulate or escape predation (Brodie and Brodie 1990, 1999a). Rejected newts survive encounters with snakes, even after more than 50 minutes in the mouth of the predator.

TTX resistance is an individually and genetically variable phenotype within populations of garter snakes that has the potential to respond to such selection. Heritability estimates based on full-sibling analyses are generally high (h^2 ranges from 0.65 to 0.80; Brodie and Brodie 1990, 1999a). Environmental influences on resistance appear minimal, and experimental attempts to induce or increase resistance through repeated exposure to TTX have had no effect (Brodie and Brodie 1990; Ridenhour et al. 1999).

The ability to withstand the effects of TTX comes at the cost of reduced locomotor performance. Evidence of a trade-off between sprint speed and resistance at both the phenotypic and genetic level comes from multiple populations (Brodie and Brodie 1999b). The source of this trade-off may lie in the proximate mechanism of TTX resistance. Variation in TTX resistance at the organismal level, both among individuals and among populations, correlates closely with differences in Na^+ channel susceptibility to TTX (Geffeney et al. 2002). The trade-off between locomotor performance and resistance might stem from altered function of these Na^+ channels in terms of organismal performance. Whatever the proximate source of the trade-off, its presence may be an important factor in slowing the evolution of resistance or possibly generating the coevolutionary dynamics predicted by some models (e.g., Saloniemi 1993; Dieckmann et al. 1995; Abrams and Matsuda 1996; Gavrilets 1997).

Research on prey defense has focused mainly on the most widespread member of the genus, *Taricha granulosa*. Although it has been assumed that *T. granulosa* are toxic throughout their range, and that level of toxicity is constant within the species (Mosher et al. 1964; Twitty 1966; Brodie 1968), recent observations suggest this is not the case (Hanifin et al. 1999). The presence of TTX in *T. granulosa* was initially documented in the 1960s for specimens from the Willamette Valley of Oregon and the San Francisco Bay area, both regions with TTX-resistant *T. sirtalis* populations (Mosher et al. 1964; Wakely et al. 1966; Brodie 1968; Brodie and Brodie 1999a). Subsequent studies revealed that *T. granulosa* populations vary in the presence or absence of TTX in skin, as well as in the amount of TTX when it is present (Hanifin et al. 1999). Tetrodotoxin has been detected but not well quantified in the other two members of the genus, *T. torosa* and *T. rivularis* (Mosher et al. 1964; Brodie et al. 1974). *Taricha torosa* is common throughout coastal California and the Sierra Nevada, co-occurring with *T. granulosa* along most of the coast. *Taricha rivularis* is sympatric with both congeners in a limited range north of the San Francisco Bay area.

The emerging picture of geographic variation in newt TTX levels suggests a mosaic of selection that might generate the kinds of dynamics predicted by the geographic mosaic theory of coevolution. Where newts lack TTX, there is no selection for increased resistance. In fact, given the cost of TTX resistance in *T. sirtalis*, there may even be selection against resistance in areas where newts lack the toxin. Previous work suggests that populations of western *T. sirtalis* do vary in resistance, but no systematic survey across the geographic range of the interaction between snakes and newts yet has been conducted. In this paper, we present the results of a long-term study of the geographic patterns of variation in TTX resistance in *T. sirtalis* throughout western North America. Our study suffers from a common deficiency of research on species interactions in that we know much more about one of the interacting species than the other. Nonetheless, the accumulated evidence suggests that many features predicted by the geographic mosaic, including hotspots and coldspots of evolutionary escalation, clinal variation, and maintained polymorphism, hold for this vertebrate predator-prey system. Conversely, we do not find strong support for maladapation or trait mismatches, one of the key predictions of the geographic mosaic approach.

Materials and Methods

We collected TTX resistance data from a total of 40 populations of *Thamnophis sirtalis* throughout western North America between 1985–2001 (see Appendix 1). These localities spanned the southern half of the range of the genus *Taricha* and included populations of snakes both sympatric and allopatric with newts. Snakes from one midwestern population (Whiteside Co., Illinois) were also sampled for comparison.

Data were generally collected on neonates born in the laboratory to wild-caught females. Field collections were made between mid-May and late-June in each year, when female snakes could be recognized as pregnant by gently palping the abdomen for developing embryos. Females were returned to the laboratory at Utah State University (or University of Texas at Arlington in 1985–1988) within 10 days of collection and housed individually until parturition. Females gave birth between mid-July and early-October, depending on the population of origin. During the intervening captivity, each female was held in a 10-gallon glass aquarium with a thermal gradient (24–30°C) and a 14:10 L:D photoperiod. Adult snakes had constant access to water and were fed fish (farm raised minnows or mollies) once weekly. Within 24 hrs of parturition, neonates were measured for mass, snout-vent length (SVL), and total length, and housed individually in plastic tubs (15 cm diameter × 10.5 cm tall). Neonates were watered once daily.

Two populations deviated from the above protocol. Snakes from East Bay, Contra Costa Company, California, were wild caught young-of-the-year collected in August 1998. Based on body size and time of year, these individuals were less than four weeks old, but of unknown experience and relatedness. Snakes from the San Mateo Company California, locality belong to *T. s. tetrataenia*, the federally protected San Francisco garter snake (= *T. s. infernalis*; Rossman et al. 1996). The Forth Worth Zoo provided two families of neonates from their captive breeding program in 1985. The crosses that produced these snakes were known to involve close relatives (one litter resulted from an uncle-niece mating), and some level of inbreeding occurred in the prior generations in the zoo-bred population of *T. s. tetrataenia*. All neonates from the San Mateo Company sample were tested for resistance at 107–115 days of age following the procedure described below.

Measuring TTX Resistance

We scored resistance to TTX using a bioassay based on whole-organism performance (Brodie and Brodie 1990). The bioassay is highly repeatable and enables us to assess individual and population differences in susceptibility to TTX. At three-to-five days after birth, each individual was raced for 2 m on a 4 m × 0.1 m linear racetrack lined with indoor/outdoor carpet; the maximum interval speed in a trial was taken as the maximum sprint speed score. Trials conducted before 1990 used 1 m interval speeds hand-timed with a stopwatch. All subsequent trials were conducted on an identically designed racetrack equipped with infrared sensors to electronically time sprint speed over 0.5 m intervals (see Brodie and Brodie 1990). Both measurement methods produce equivalent results (E. D. Brodie, Jr., unpubl. data). Individuals were tested repeatedly and the mean of all scores was taken as an individual's ''baseline speed.'' Snakes tested before 1990 were measured on two successive days for a total of four repeated measures, whereas those tested after 1990 were sampled only twice on a single day. A single individual (EDB, Jr.) conducted all race trials throughout the study, including those described below, to prevent interobserver variance.

Eighteen to 21 hours after the last sprint test, neonates were given intraperitoneal injections of known doses of TTX (crystalline 3X in citric acid–sodium citrate buffer, from Sankyo Co, Tokyo, Japan) diluted in amphibian ringer solution. Thirty minutes after injection, snakes were tested again following the sprint speed protocol to obtain a measure of ''postinjection speed.'' Forty-eight hours after the first injection trial, snakes were tested again, up to four times total. Some individuals were repeatedly tested at the same dose whereas others received two or three different doses (see below). Control injections of physiological saline have no effect on snake performance (Brodie and Brodie 1990), and TTX resistance is not affected by repeated short- or long-term exposure (Brodie and Brodie 1990; Ridenhour et al. 1999). For cases in which individuals were tested more than once at the same dose of TTX, the average of repeated trials was taken as an individual's measure of post-injection speed. Resistance was scored as the percentage of an individual's baseline speed crawled after injection (100 × postinjection 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 100% of their baseline speed.

The primary goal of this study was to compare population level differences in response of snakes to TTX. Because populations differ greatly in their susceptibility to TTX, not all populations could be tested at the same doses. Consequently, we took two separate approaches to characterizing population differences in TTX resistance.

Resistance at common doses

A number of populations were tested at one of two standard doses of TTX, allowing direct comparisons of resistance at these doses. Eight or more individuals were tested from 25 populations at 0.1 μg TTX and 18 or more individuals from eight different populations were tested at 1.0 μg TTX. Comparisons of resistance within each of these two groups were performed via ANOVA. Individual mass was used as a covariate to adjust for population differences in neonate body size. Differences in resistance among population means were tested using Tukey's HSD (honestly significant difference) test at $\alpha = 0.05$. This comparison adjusts experiment-wise Type I error rate for the number of multiple comparisons (300 at 0.1 μg and 28 at 1.0 μg) and is conservative when sample sizes differ among means, as is the case for this data set (SAS Institute 1994). Eight populations could not be compared in this fashion because few or no individuals were tested at these or other common doses.

Estimation of population dose-response curves

To obtain population-level dose-response curves, we tested snakes from each population at a series of doses ranging from 0.001 μg to 100 μg TTX. A total of 2960 snakes from 334 families representing 41 populations were examined (the breakdown of samples for each population is reported in Appendix 1). For any given population, 3–10 different doses were used (mean dose/population = 4.5).

Because TTX resistance is related to body size within and among some populations of snakes (Brodie and Brodie 1990, 1999b), we controlled for mass differences among populations using a population-level mass-adjusted measure of dose. Tetrodotoxin doses were converted to mass-adjusted mouse units (MAMUs; Brodie and Brodie 1990, 1999a) 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 (Brown and Mosher 1963; one ''mouse unit'' = 0.01429 μg TTX). One MAMU is therefore one mouse unit of TTX per gram of snake.

The TTX-dose response curve was estimated separately for each population using curvilinear regression with the general transform $y' = \ln[1/y - 1]$. In addition to fitting the data well (see Appendix 1), this transform provides a convenient means for estimating the ''50% dose,'' or the population average dose of TTX that reduces performance to 50% of baseline speed. At $y = 0.5$ (i.e., 50%), $y' = 0$ and the 50% dose is estimable as $\hat{x} = -\alpha/\beta$ (where α is the intercept and β the slope from the curvilinear regression). Because $\hat{x}$ takes the form of a ratio, the standard error for the estimated 50% dose is calculated using the standard methods for the variance of a ratio (Lynch and Walsh 1998, p. 818). Confidence intervals of 95% were calculated as ± (1.96 × SE). Due to limitations of the transformation, a score of resistance = 0 (resulting in division by zero) was revised to be a resistance = 0.01. In some cases, resistance scores were > 1 (i.e., post-injection speed was slightly faster than baseline speed), resulting in improper transformation ($\ln[1/y - 1]$) is undefined if $y > 1$ or $y \leq 0$); resistance scores ≥1 were coded as resistance = 0.99. Distribution and leverage analysis indicated that a transformation of the x variable (MAMU of TTX) was needed to fix positive skew; data on administered doses were therefore transformed using $x' = \ln(x + 1)$. Because the estimated 50% dose and its confidence interval were calculated using x', the estimated 50% dose and CI were converted back to standard MAMU scores prior to further pre-

sentation (graphical and textual). All statistical analyses were performed using SAS version 8.2.

Families from one population (Willow Creek, Sonoma Co., CA) were too variable to estimate the 50% dose in the above fashion. However, 54 individuals from five families of snakes from this population were tested at different doses that yielded family mean resistance measures of 50% ± 5% (overall mean = 49.5%). For this population, the 50% dose was estimated as the mean of these five doses (and the confidence interval base on the associated standard error).

Geographic patterns of resistance

The 50% doses estimated for each population were used to visualize the geographic distribution of resistance. An isocline map of western North America populations was generated using inverse distance-weighted interpolation based on observed resistance values and the latitude and longitude coordinants for each population. Because of the nonlinear scale of resistance, values of resistance ≥ 100 MAMU were entered as 100 MAMU in this analysis. The function's power was set at 2, and the neighborhood at 300 mi. Other neighborhood sizes (the radius of influence of a locality) produce similar results. Analyses were performed in ArcView GIS 3.2 with Spatial Analyst 2.0.

To determine if geographic variation in TTX resistance levels was consistent with radiating clines of decreasing resistance from hotspots, we compared resistance levels to distance from observed regions of highest resistance. Because results suggested two regions of extreme resistance (see below), separate analyses for each hotspot were conducted. Centers of hotspots were defined as the local populations with the highest resistance levels: Tenmile for the northern hotspot and San Mateo for the southern hotspot. Linear regression was used to determine if observed resistance levels were a simple function of distance from hotspots. For each hotspot, all populations within a given radius were included. We report results from analyses using 300-km radii, although distances up to 650 km (the distance between northern and southern hotspots) provide similar results.

RESULTS

Resistance at Common Doses

The mass adjusted resistance of populations tested at both common doses differed significantly (Fig. 1; 1.0 μg TTX: $F_{7, 1087} = 105.46$, $P < 0.0001$; 0.1 μg TTX: $F_{24,960} = 30.82$, $P < 0.0001$). At the 0.1 μg dose, the number of populations compared and overlapping significance groups generated a complicated picture of general resistance levels (Fig. 1A). Generally speaking, one group of relatively high resistance populations (Harvey Hall Lake, Furnace Flats, Bear Ridge, Hoquiam, Willits, San Simeon, Inland Lake, Parsnip Lakes) was statistically distinguishable from a group of low resistance populations (Anderson Lake, Vandenburg, McCumber, Orick, Mahogany, Lofton Lake, Peony Spring), but populations in neither group were distinct from most of the intermediate populations. Other pairs of populations differed significantly, but this general grouping of low, intermediate, and high resistance populations captures the basic pattern of differences (Fig. 1A). At the higher common dose, 1.0 μg, the eight populations fell into three distinct significance groups (Fig. 1B): Tenmile > Dupont and Benton > Wildboy, Chinook, McGribble, Gilroy, and Warrenton. The two sets of populations tested at different TTX doses could not be compared directly. However, populations compared at 0.1 μg TTX were considered less resistant than those tested at 1.0 μg TTX because the doses differed by an order of magnitude.

Population dose response curves

The curvilinear regression model described the relationship between resistance and dose well for all populations analyzed ($0.41 < R^2 < 0.88$; $P < 0.0001$ for all cases; Appendix 1). Details of regression equations and dose response curves for each population are reported in Appendices 1 and 2.

To simplify comparisons of population level resistance, 50% MAMU doses were estimated from the regression equations. The 50% doses for each population spanned a range over three orders of magnitude. The least resistant population (Peony Spring) was reduced to 50% crawl speed at just over 1 MAMU (comparable to the lethal dose for a mouse), whereas the most resistant population (San Mateo) tolerated over 1000 MAMU before a similar depression of performance. Although all populations exhibited variation in TTX resistance, one population was noteworthy in its degree of variation. Differences among families within the Willow Creek population were greater than those observed between many populations. Fifty percent doses estimated separately for each of five families at Willow Creek ranged over two orders of magnitude (Fig. 2).

We assigned eight levels of TTX resistance based on the distribution of the 50% doses and their 95% confidence intervals as well as the statistical analysis from the common dose comparisons above (Fig. 3). The top five resistance levels had virtually nonoverlapping confidence intervals and correspond directly to significant differences detected in comparisons at common doses where possible: Level VIII = 560–1279 MAMUs; Level VII = 52–53 MAMUs; Level VI = 27–35; MAMUs, Level V = 15–22 MAMU, and Level IV = 10–11 MAMUs.

The majority of populations fell within the bottom three levels of resistance. The 50% doses of these groups were more continuously distributed and all fell below 10 MAMUs: Level III = 5.6–7.1 MAMUs; Level II = 4–5 MAMU; Level I = 1.1–3.8 MAMUs. The delineations between these groups were less obvious than for their more resistant counterparts, but were nonetheless supported by the comparison of resistance at 0.1 μg TTX. Inconsistencies between levels assigned based on dose response curves and significantly different groups in the common dose comparison were limited to shifts between Levels I ↔ II (Bear Lake, Orick, Latah) or II ↔ III (Scott Lake, Inland Lake) and involved populations at level boundaries.

The geographic distribution (Fig. 4) of resistance levels reveals two areas of relatively high resistance around the San Francisco Bay area of California (Level VIII) and from the central Coast Range of Oregon to the Puget Sound of Washington (Level VII, VI). Somewhat lower levels of resistance are detected in the regions surrounding these two areas. The

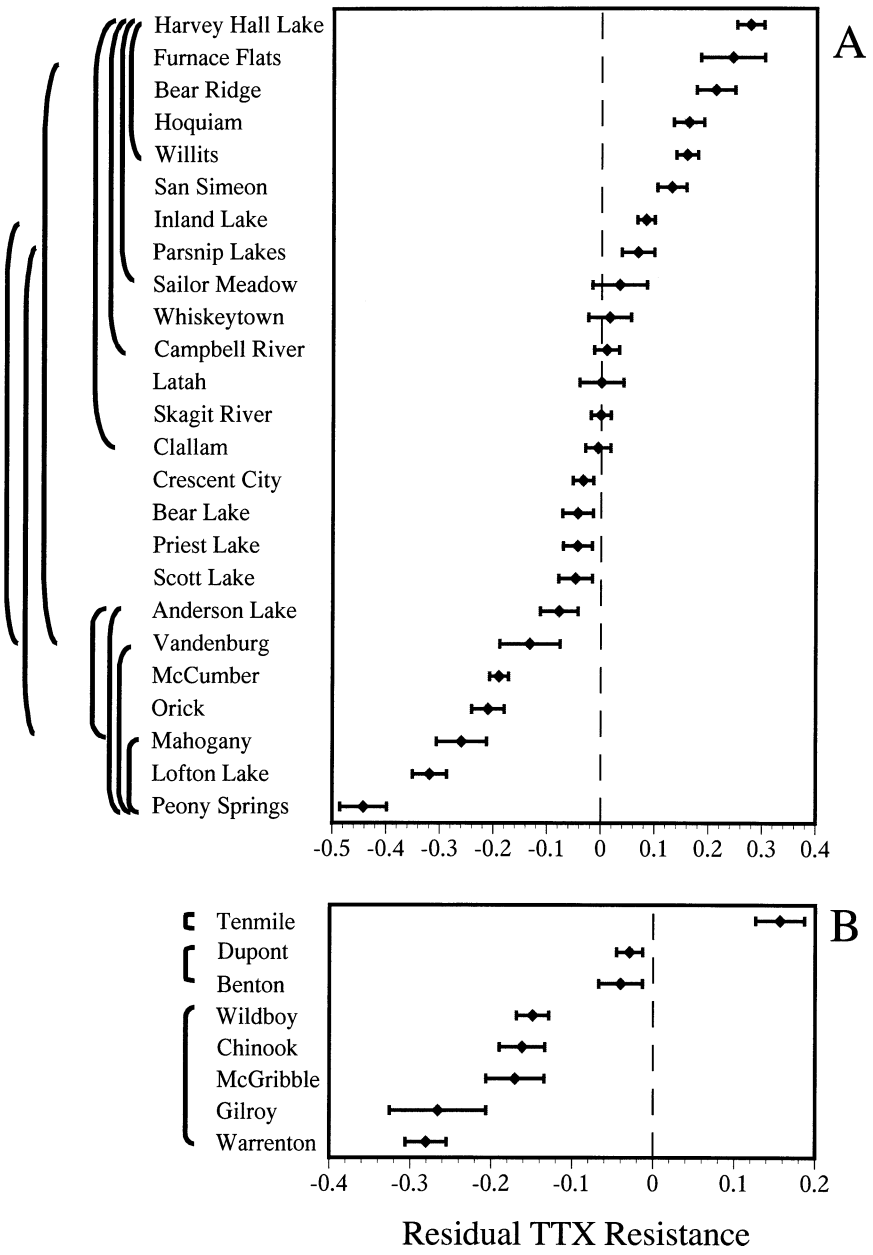


FIG. 1. Population differences in TTX resistance at common doses. Residual (mass-adjusted) resistance is shown for (A) 25 populations sampled at 0.1 μg TTX and (B) eight populations sampled at 1.0 μg TTX. Groups of populations that are not statistically distinguishable are connected by overlapping lines.

lowest levels (I–III) of resistance are widely distributed across western North America. The Whiteside Co., Illinois population, which occurs well outside the range of *Taricha*, exhibited the lowest level (I) of resistance. These general patterns were confirmed by the visualization of isoclines of TTX resistance (Fig. 4), although absolute levels of resistance estimated were often higher than those observed due to the heavy influence of extreme levels of resistance and uneven geographic sampling.

Population level resistance declined monotonically with distance from each hotspot center. Regressions of 50% dose on distance from the hotspot explained 58% (for the northern hotspot: $F_{1,8} = 10.89$, $R^2 = 0.577$, $P = 0.011$) and 82% (for the southern hotspot: $F_{1,7} = 31.4$, $R^2 = 0.818$, $P = 0.0008$) of the variance among populations within 300 km of each center. Including populations at greater distances reduced R^2 values, but gave similar significant results up to a radius of 650 km, which is the distance between hotspots.

DISCUSSION

Geographic Patterns of Phenotypic Diversity

Considerable variation in TTX resistance is evident among populations of western *Thamnophis sirtalis*. Our measure of population level resistance, the 50% dose, spans three orders of magnitude, from 1.1–1279 MAMU (Fig. 3). Few pheno-

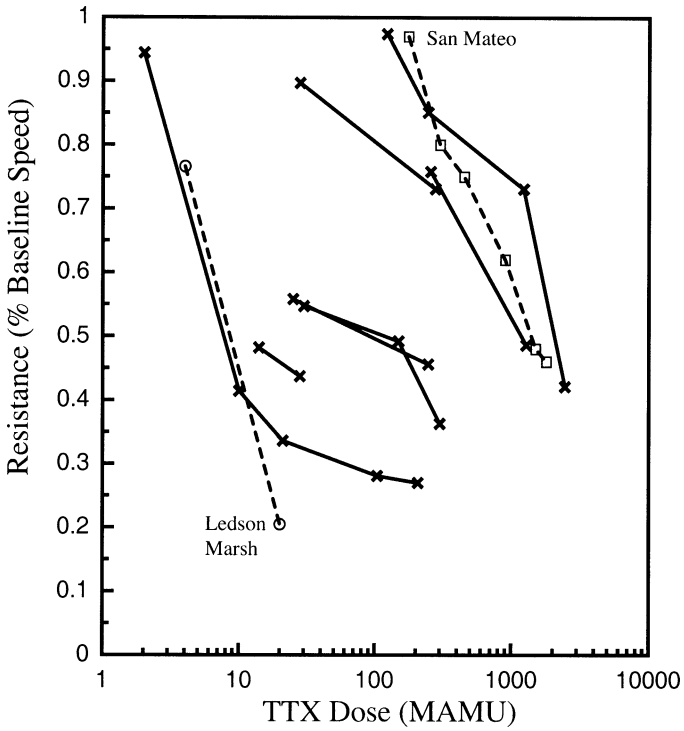


FIG. 2. TTX resistance in Willow Creek, CA population. Dose-response plots (solid lines) are shown for each of seven families of snakes tested from Willow Creek, Sonoma Co. CA. Points indicate means at each dose for a given family; curves are not fit because of insufficient data within individual families. Dose-response plots for two adjacent populations are shown as dashed lines for comparison (Ledson Marsh, CA, open circles; San Mateo, open squares). Note that range of variation among families within Willow Creek overlaps the mean levels in both adjacent populations even though this range spans four levels of resistance as denoted in Figures 3 and 4.

typic characters, particularly those with little or no plasticity, exhibit this degree of variation within a single species. More importantly, the geographic distribution of this variation is consistent with a mosaic of hotspots and coldspots (cf., Thompson 1994, 1999b) of evolutionary response by snakes to prey toxin.

Population variation in resistance is arrayed in a set of radiating clines emanating from two regions of high resistance (Fig. 4). The area of highest resistance (Level VIII) is found near San Francisco Bay in California. Resistance levels drop off to the north and south of this hotspot, but are similarly high in the closest Sierra Nevada population (Omo). The second area of high resistance is found in northern Oregon and southern Washington. The highest resistance here is found on the central coast of Oregon (Level VII at Tenmile), with slightly decreased levels to the east in the Willamette Valley (Level VI at Benton). To the north and south of these populations, resistance drops two to fivefold to Level V. A second Level VI population (Dupont) is present at the southern end of Puget Sound. Surrounding these two ''hotspots'' of resistance are populations with resistance between Levels I–III. These populations include localities outside the range of newts, localities well within the range of newts, and localities lying between the California and Oregon hotspots.

Although distance from a hotspot explains much of the variation in TTX resistance among populations, the geographic scale over which resistance levels change is uneven (Fig. 4). Dramatic phenotypic differences can be found over very small geographic distances. Resistance drops two orders of magnitude over 73 km in the Sierra Nevada (from 560 to 6.4 MAMU from Omo to Sailor Meadow). North of San Francisco Bay a similar reduction occurs over only 38 km (from 784 to 10 MAMU from Willow Creek to Ledson Marsh). At the same time, moderate levels of resistances (Level VI) are present along either side of the Columbia River in southern Washington and northern Oregon. Low resistance is spread throughout the range of localities allopatric with newts.

Phylogenetic and historical biogeographic data indicate independent evolutionary origins of the two hotspots (Geffeney et al. 2002). California populations of *T. sirtalis* likely arose from a southern radiation, whereas populations of *T. sirtalis* along the northwest coast and intermountain west represent two distinct lineages with a probable northern origin (Janzen et al. 2002). Comparative analyses have shown that the ancestral state of resistance for western *T. sirtalis* and indeed the entire genus *Thamnophis* is comparable to Level I (approximately 1–3 MAMU; (Motychak et al. 1999). Thus the high levels of resistance in California and Oregon populations appear to represent separate evolutionary events, further underscoring the lability of TTX resistance in space and time (phylogenetic comparisons will be treated rigorously elsewhere; Ridenhour, Janzen, Brodie and Brodie, unpubl. data).

Does a Selection Mosaic Exist?

The labels ''hotspot'' and ''coldspot'' imply differences in the strength of reciprocal selection that cannot be gleaned from a geographical distribution of phenotypes in a single species alone. In fact, the geographic mosaic approach predicts that the level of exaggeration of phenotypic traits may not correspond directly to the strength of reciprocal selection on those traits because of the effects of population subdivision (Thompson 1994, 1999b; Gomulkiewicz et al. 2000; Nuismer et al. 2000). However, in the case of TTX resistance in garter snakes, we have a growing, but as yet incomplete, picture of spatial variation in selection on resistance (i.e., newt toxicity) that supports the hotspot/coldspot perspective. We adopt the term hotspot to refer to areas of exaggerated resistance because it is unlikely that such extreme levels of TTX resistance could be produced in the absence of strong reciprocal selection (Brodie and Brodie 1990, 1999a, b).

Coldspots refer to regions in which little or no reciprocal selection occurs, so the most obvious coldspots exist where only one of a pair of interacting species occurs. Such coldspots due to allopatry are potentially important to the dynamics of the geographic mosaic because gene flow in and out of these populations can influence phenotype values just as it does among sympatric populations. In our system, coldspots due to allopatry occur outside the range of *Taricha*; where newts do not occur there can be no selection for increased resistance to TTX. Throughout the sampled range of populations allopatric with newts, snakes exhibit the lowest level of resistance, equivalent to the ancestral level found in

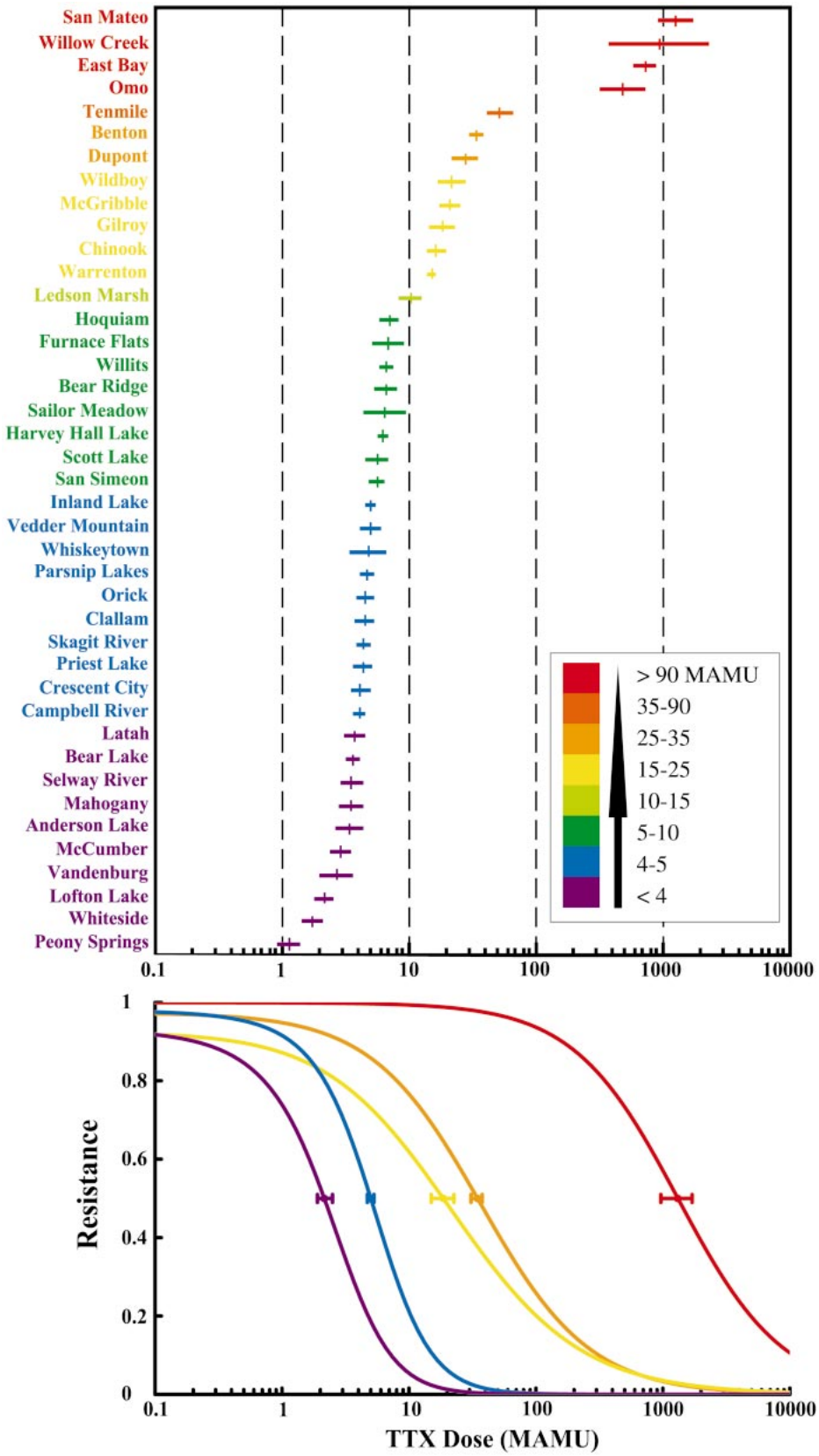


FIG. 3. 50% doses of 41 populations. (Top) The dose of TTX required to reduce crawl speed to 50% is shown with 95% confidence interval. Dose is plotted on a log scale of mass-adjusted mouse units (MAMU). 50% doses are estimated from separate regressions of resistance on TTX dose for each population. Colors indicate eight assigned levels of TTX resistance (see text). (Bottom) Representative dose-response curves and estimated 50% doses (with 95% CI) are shown for five representative populations (from left to right: Lofton Lake, Inland Lake, Gilroy, Benton, San Mateo). Analogous regressions for all populations are shown in Appendix 2.

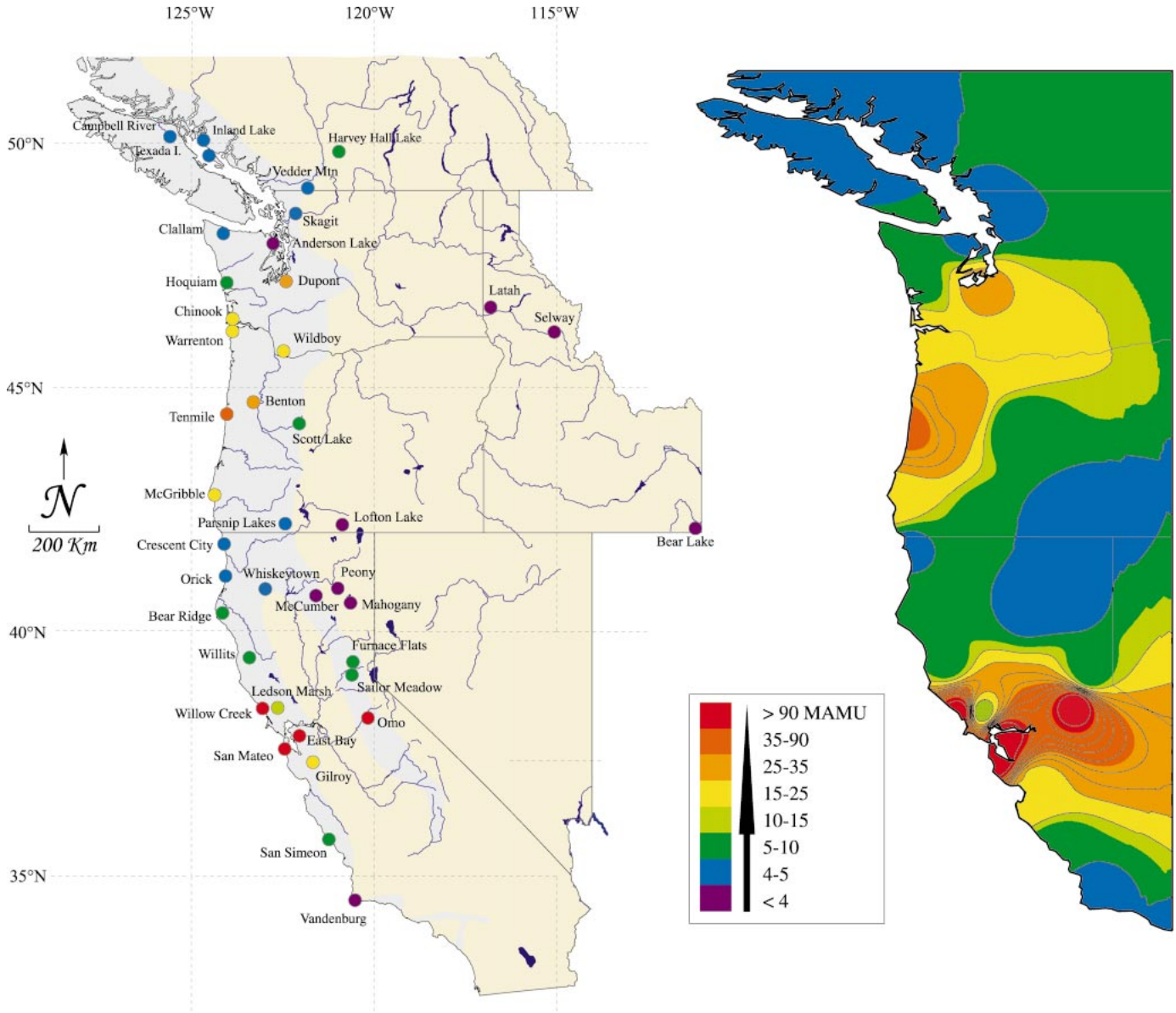


FIG. 4. Geographic distribution of TTX resistance in western North America. (Left) Forty populations of *Thamnophis sirtalis* sampled throughout western North America are shown (Whiteside, IL, not shown). Colors indicate resistance level as denoted in legend and based on 50% dose in Figure 3. The range of the genus *Taricha* is shown in gray, but extends beyond the area shown in the map to the north along the coast of British Columbia. (Right) Isoclines of TTX resistance in western North America as estimated from inverse distance weighted interpolation of the data shown in the graphic on the left. Two geographic areas of extreme TTX resistance (''hotspots'') are apparent with radiating clines of decreasing resistance values emanating from them.

other species of *Thamnophis* (Motychak et al. 1999). The one exception to this rule is the population from Harvey Hall Lake in southern British Columbia. This is a locality near the eastern edge of known *Taricha* range in the North Cascades of British Columbia (Nussbaum et al. 1983). Amphibian distributions in this part of North America are not well studied, so this population may, in fact, be sympatric with newts.

Toxic newts are the obvious agent of selection for increased resistance, but this does not mean that selection is uniform throughout the range of *T. sirtalis* and *Taricha*. Variation in TTX levels among populations of *Taricha* is known, but not yet well described. Moreover, two or three species of sympatric *Taricha* are present in parts of coastal California, each of which may represent different kinds of selection on snake resistance. What is known about interpopulational differences in newt toxicity correlates closely to the pattern of resistance observed in this study. Toxic newts were first described from the San Francisco Bay Area and the Willamette Valley—coincidently the two regions at the hearts of the presumptive hotspots of resistance evolution. Early work on these populations of newts did not provide quantitative estimates of TTX levels (Brown and Mosher 1963; Mosher et al. 1964; Brodie 1968), therefore it is impossible to compare

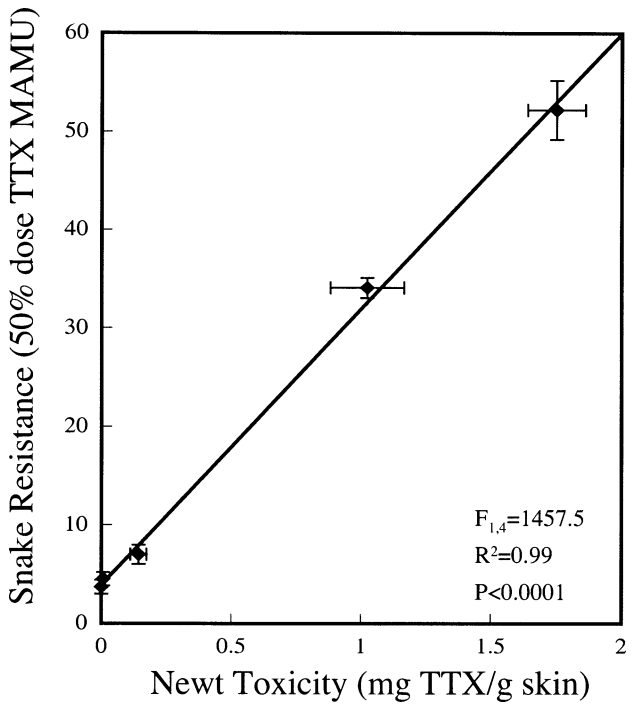


FIG. 5. TTX resistance relative to sympatric newt toxicity. TTX resistance (50% dose) for five populations of snakes is plotted against newt toxicity (mg TTX/g skin; Hanifin et al. 1999) from the same localities. Resistance is tightly predicted by toxicity of newts as shown by regression line (model: 50% dose = 3.8 + 28.03[mg TTX/g skin of newts]).

differences in snake resistance to differences in newt toxicity for these regions. Subsequent work on interpopulational variation in TTX levels has focused on northern populations of *Taricha granulosa* (Hanifin et al. 1999). The highest toxin levels have been observed at Tenmile and Benton, the center of the northern hotspot of resistance. TTX is absent from Texada Island newts in British Columbia, where resistance is only Level II. TTX is present, but at very low levels in two populations from the Olympic Peninsula of Washington where snake resistance is low (Levels II and III). Based on these five localities for which TTX levels of newts and resistance of *T. sirtalis* are known, predator resistance is tightly correlated with prey defense (Fig. 5). These results do not provide support for the hypothesis of maladaptation resulting from the geographic mosaic (Thompson 1999b; Nuismer et al. 2000). However, toxin levels of *Taricha* throughout the rest of the range are not yet known and could substantially alter the apparent pattern of coadaptation with *T. sirtalis* throughout the range of sympatry.

Selection on snake resistance does not result from mere sympatry with toxic prey; it is the predator-prey interaction between newts and snakes that results in selection. Although we have not conducted systematic diet studies in any of the populations described in this paper, some information about geographic variation in *T. sirtalis* diet is available. Anecdotal observations indicate that feeding on newts is not restricted to populations with high resistance. Snakes were found with newts in their stomachs during field collection at four of the localities studied, including populations from Level VII (Tenmile, OR), Level VI (Benton Co, OR), Level III (Hoquiam, WA), and Level II (Parsnip Lakes, OR). Previous research on diet breadth indicated that snakes from San Mateo (Level VIII; S. Barry, pers. comm.) and Vancouver Island, British Columbia (Level II; Macartney and Gregory 1981) forage on *Taricha*. Juvenile snakes from Orick, California (Level II) readily eat pieces of *Taricha* in captivity (Arnold 1978). These observations demonstrate that the ecological interaction, and therefore potential coevolution, between newts and snakes is not limited to populations in which resistance has evolved to extreme levels.

It is also critical to realize that other aspects of the behavioral ecology of the snake-newt interaction can alter the apparent selection represented by predation on toxic newts. For example, selection for increased resistance does not require successful ingestion of a toxic newt. Individual *T. sirtalis* have some ability to judge their own resistance and reject newts too toxic to handle. However, snakes that seize and then release a toxic newt ingest enough toxin that they may be immobilized for several hours (Williams et al. 2002). Immobility and loss of coordination can lead to further selective costs in terms of increased risk of predation or thermal stress. Conversely, trade-offs with resistance, such as locomotor performance (Brodie and Brodie 1999b), may ameliorate selection on resistance if toxin levels in prey are low. Behavioral prey preferences may also influence the interplay of selective forces. Chemosensory responses of neonate snakes vary among species, populations, and families, including responses of *T. sirtalis* to newt odor from three western populations (Arnold 1992). More in-depth investigation of the fitness costs and benefits of the garter snake-newt interaction is required to fully evaluate the geographic patterns of selection on resistance and toxicity.

Evidence for Remixing of Traits

The critical corollary of geographically variable selection is that gene flow will lead to a remixing of traits among populations (Nuismer et al. 1999, 2000; Thompson 1999b; Gomulkiewicz et al. 2000). This process in turn generates a variety of phenomena that do not result from coevolution in unstructured populations. The maintenance of genetic variation, stability of polymorphisms, and existence of phenotypic mismatches in antagonistic interactions among species are but some of the results of geographically structured coevolution in which selection is spatially variable and gene flow occurs among populations. The precise predictions depend on the spatial scale and arrangement of selection and gene flow, but relatively simple models suggest that the general phenomena might be quite common (Nuismer et al. 1999, 2000; Gomulkiewicz et al. 2000). Unfortunately, direct data on the scale of population subdivision and migration in *Thamnophis sirtalis* are unavailable. We can, however, examine patterns of variation within and among populations that should result if remixing occurs.

One of the strongest predictions is that some populations should be highly variable if gene flow occurs between populations with different genetic states. All populations of snakes studied show substantial variation in TTX resistance, so this is a difficult prediction to evaluate across the land-

scape. Nonetheless, one population in particular shows evidence of extreme variation that is most likely maintained through remixing of traits (Fig. 2). Willow Creek, (Sonoma Co., CA) is a Level VIII population, but families from this locality vary so greatly that many could not even be tested at the same doses of TTX, and the 50% dose for the population could not be calculated with the regression approach. Family mean 50% doses (based on doses that yield 45–55% crawl speeds) ranged from less than 10 to over 1000 MAMU (Fig. 2). The low end of this range is comparable to the 50% dose for the geographically closest population studied, Ledson Marsh (10.4 MAMU, 38 km to the east). The high end of this range is comparable to the nearby Bay Area populations to the south (East Bay, 945 MAMU, and San Mateo, 1279 MAMU). This pattern of intrapopulational variation would be expected if migration from regions with different levels of resistance maintained polymorphism, but direct investigations of gene flow will be required to test this hypothesis.

Another piece of suggestive evidence is the high level of resistance around San Francisco Bay and in the Omo population in El Dorado Co., California. Genetic studies in other reptiles (Feldman 2000) and amphibians (Wake and Yanev 1986; Moritz et al. 1992) suggest a migration corridor in the Central Valley (a ''trans-valley leak''; Rodriguez-Robles et al. 2001) through which gene flow occurs between the central Sierra Nevada and coastal California. If similar patterns of gene flow occur in *T. sirtalis*, they could be at least partially responsible for the extreme resistance (Level VIII) observed at Omo. Selection cannot be ruled out as an explanation of the Omo resistance level because we do not know the toxicity of sympatric newts. However, this population is conspicuously over two orders of magnitude more resistant than populations less than 100 km to the north.

The continuous quantitative variation among populations of *T. sirtalis* presents a unique opportunity to explore the factors that generate the intermediate levels of TTX resistance. The general pattern of clinal variation centered on hotspots of high resistance (Levels VII and VIII; Fig. 4) ranging through intermediate levels, down to the ancestral level of resistance (Level I) is consistent with several scenarios. This pattern is predicted if selection varies geographically from strong to nonexistent and gene flow along such clines maintains intermediate levels of resistance. This pattern is consistent not only with the monotonically decreasing clines from either of the major hotspots to populations outside of newt range, but also with the reduction in resistance (to Level II) in the Northern Californian region between the two hotspots. Conversely, a similar clinal pattern exists in the populations north of the Oregon hotspot for which levels of toxicity and resistance at each locality are almost perfectly matched (Fig. 5). Such phenotype matching implies, but does not demonstrate, reciprocal selection between newt toxicity and snake resistance at each locality. Matched intermediate levels of resistance and toxicity could result from similar scales of gene flow in newts and snakes, with no reciprocal selection occurring in the intermediate populations. Of course, a single explanation for clines, gene flow, or variable selection need not apply to all cases, and both factors may influence a single case. The elucidation of these processes awaits data on the geographic pattern of newt toxicity, as well as studies of the scale of gene flow in both species.

Conclusions

The pattern of variation in TTX resistance of garter snakes provides dramatic evidence that geographic structure is an important component in coevolutionary interactions between predators and prey. Resistance levels vary substantially among populations and over short distances. The spatial array of variation is consistent with two areas of intense evolutionary response by predators (hotspots) surrounded by clines of decreasing resistance. The factors that maintain this geographic variation are not yet clear and may vary across the landscape of the interaction; both gene flow among populations and tight reciprocal selection between garter snakes and newts are implicated in different areas.

Although these results generally corroborate the geographic mosaic view of coevolution, they fall far short of constituting a test. Some general predictions of the process are supported, including clinal variation in phenotypes, polymorphisms in some populations, and divergent outcomes of the interaction between predator and prey. Conversely, one of the major predictions, maladaptation or mismatching of interacting traits, is not strongly supported by the available data. Two lines of evidence suggest that selection is extremely important in generating the geographic pattern observed. First, phylogenetic information (Janzen et al. 2002) indicates that the Oregon and California hotspots of TTX resistance have evolved independently. Second, in the one region that TTX levels in prey have been quantified, resistance and toxicity levels match almost perfectly over a wide phenotypic and geographic range. It is important to realize that this transect represents only one of many regions of clinal variation and as the pattern of TTX toxicity is revealed other regions may show different patterns. Also, the prediction of maladaptation is dependent on details of spatial scale and relative strengths of gene flow and selection that have not been quantified in this system. As is true for many coevolutionary systems, our knowledge of one species in the interaction dwarfs the other. Much work remains to fill in the geographic picture of variation among prey populations and, just as importantly, to explore the variation in the ecology of the interaction that occurs within populations.

Acknowledgments

Specimens were collected with permission to EDB III by the California Department of Fish and Game and the California Department of Parks and Recreation; to EDB, Jr., by the Washington Department of Fish and Wildlife, the Idaho Department of Fish and Game, and the British Columbia Wildlife Branch; and to BJR by the California Department of Fish and Game. We are indebted to the following individuals for providing additional specimens or assisting with field collections: A. Agrawal, S. Barry, J. Beatty, S. Brodie, D. Foley, R. Hoyer, F. Janzen, M. Jennings, R. Monello, J. Motychak, A. Snedden, C. Steele, and R. Storm. We were also helped, over the last 15 years, by a multitude of undergraduate and graduate students without whom we could not have done this study. C. Garrard helped develop the isocline

map of resistance and S. Brodie provided the cover artwork. The presentation of this paper was improved by comments from A. Agrawal, T. Gardner, C. Hanifin, L. Lehman, J. Motychak, S. Welter, B. Williams, and K. Young. This research was approved by the Utah State University Institutional Animal Care Committee (IACUC protocol no. 712), the Bloomington Institutional Animal Care and Use Committee (BIACUC protocols 97–119, 98–116, 99–080, 99–067, 00–071, 00–123, 00–020, 01–071), the University of Texas at Arlington Animal Care Committee, and the University of Kentucky Research Subject Office. The research was supported by National Science Foundation grants DEB9796291 and DEB9903829 to EDB III and DEB9521429 and DEB9904070 to EDB, Jr.

Literature Cited

Abrams, P. A., and H. Matsuda. 1996. Fitness minimization and dynamic instability as a consequence of predator-prey coevolution. Evol. Ecol. 10:167–186.

Arnold, S. J. 1978. Some effects of early experience on feeding responses in the common garter snake, *Thamnophis sirtalis*. Anim. Behav. 26:455–462.

———. 1992. Behavioural variation in natural populations. VI. Prey responses by two species of garter snakes in three regions of sympatry. Anim. Behav. 44:705–719.

Benkman, C. W. 1999. The selection mosaic and diversifying coevolution between crossbills and lodgepole pine. Am. Nat. 153: S75–S91.

Benkman, C. W., W. C. Holman, and J. W. Smith. 2001. The influence of a competitor on the geographic mosaic of coevolution between crossbills and lodgepole pine. Evolution 55:282–294.

Berenbaum, M. R., and A. R. Zangerl. 1998. Chemical phenotype matching between a plant and its insect herbivore. Proc. of the Natl. Acad. Sci., USA 95:13743–13748.

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: tradeoffs in a predator-prey arms race. Evolution 53:626–631.

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

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.

Brown, M. S., and H. S. Mosher. 1963. Tarichatoxin:isolation and purification. Science 140:295–296.

Burdon, J. J., and P. H. Thrall. 1999. Spatial and temporal patterns in coevolving plant and pathogen associations. Am. Nat. 153: S15–S33.

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.

Dieckmann, U., P. Marrow, and R. Law. 1995. Evolutionary cycling in predator-prey interactions: population dynamics and the Red Queen. J. Theor. Biol. 176:91–102.

Dybdahl, M. F., and C. M. Lively. 1996. The geography of coevolution: comparative population structures for a snail and its trematode parasite. Evolution 50:2264–2275.

Feldman, C. R. 2000. Comparative phylogeography of three Californian reptiles: *Contia tenuis*, *Diadophis punctatus*, *Elgaria multicarinata*. M.Sc. thesis, San Francisco State University, San Francisco, CA.

Gavrilets, S. 1997. Coevolutionary chase in exploiter-victim systems with polygenic characters. J. Theor. Biol. 186:527–534.

Geffeney, S., P. Ruben, E. D. Brodie, Jr., and E. D. Brodie, III. 2002. Mechanisms of adaptation in a predator-prey arms race: TTX-resistant sodium channels. Science 297:1336–1339.

Gomulkiewicz, R., J. N. Thompson, R. D. Holt, S. L. Nuismer, and M. E. Hochberg. 2000. Hot spots, cold spots, and the geographic mosaic theory of coevolution. Am. Nat. 156:156–174.

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*. J. Chem. Ecol. 25:2161–2175.

Hille, B. 1992. Ionic Channels of Excitable Membranes. Sinauer, Sunderland, MA.

Janzen, F. J., J. G. Krenz, T. S. Haselkorn, E. D. Brodie, Jr., and E. D. Brodie, III. 2002. Molecular phylogeography of common garter snakes (*Thamnophis sirtalis*) in Western North America: implications for regional historical forces. Mol. Ecol. 11: 1739–1751.

Kaltz, O., S. Gandon, Y. Michalakis, and J. A. Shykoff. 1999. Local maladaptation in the anther-smut fungus *Microbotryum violaceum* to its host plant *Silene latifolia*: evidence from a cross inoculation experiment. Evolution 53:395–407.

Kodama, M., T. Ogata, and S. Sato. 1985. External secretion of tetrodotoxin from puffer fishes stimulated by electric shock. Mar. Biol. 87:199–202.

Kraaijeveld, A. R., and H. C. J. Godfray. 1999. Geographic patterns in the evolution of resistance and virulence in *Drosphila* and its parasitoids. Am. Nat. 153:S61–S73.

Lipkind, G. M., and H. A. Fozzard. 1994. A structural model of the tetrodotoxin and saxitoxin binding site of the Na+ channel. Biophys. J. 66:1–13.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.

Macartney, J. M., and P. T. Gregory. 1981. Differential susceptibility of sympatric garter snake species to amphibian secretions. Am. Midl. Nat. 106:271–281.

Moritz, C., C. J. Schneider, and D. B. Wake. 1992. Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. Syst. Biol. 41:273–291.

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.

Narahashi, T., J. W. Moore, and R. N. Poston. 1967. Tetrodotoxin derivatives: chemical structure and blockage of nerve membrane conductance. Science 156:976–978.

Nuismer, S. L., J. N. Thompson, and R. Gomulkiewicz. 1999. Gene flow and geographically structured coevolution. Proc. R. Soc. Lond. B 266:605–609.

———. 2000. Coevolutionary clines across selection mosaics. Evolution 54:1102–1115.

Nussbaum, R. A., E. D. Brodie, Jr., and R. M. Storm. 1983. Amphibians and reptiles of the Pacific Northwest. Univ. Press of Idaho, Moscow, ID.

Parker, M. 1985. Local population differentiation for compatability in an annual legume and its host-specific fungal pathogen. Evolution 43:540–547.

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.

Rodriguez-Robles, J. A., G. R. Stewart, and T. J. Papenfuss. 2001. Mitochondrial DNA-based phylogeography of North American rubber boas, *Charina bottae* (Serpentes: Boidae). Mol. Phylog. Evol. 18:227–237.

Rossman, D. A., N. B. Ford, and R. A. Seigel. 1996. The garter snakes. University of Oklahoma Press, Norman, OK.

Saloniemi, I. 1993. A coevolutionary predator-prey model with quantitative characters. Am. Nat. 141:880–896.
SAS Institute. 1994. JMP User's Guide, Vers. 3. SAS Institute, Inc, Cary, NC.
Storfer, A., and A. Sih. 1998. Gene flow and ineffective antipredator behavior in a stream-breeding salamander. Evolution 52: 558–565.
Thompson, J. N. 1994. The coevolutionary process. The University of Chicago Press, Chicago, IL.
———. 1997. Evaluating the dynamics of coevolution among geographically structured populations. Ecology 78:1619–1623.
———. 1999a. Coevolution and escalation: are ongoing coevolutionary meanderings important? Am. Nat. 153:S92–S93.
———. 1999b. Specific hypotheses on the geographic mosaic of coevolution. Am. Nat. 153:S1–S13.
———. 1999c. The evolution of species interactions. Science 284: 2116–2118.
Twitty, V. C. 1966. Of scientists and salamanders. W. H. Freeman, San Francisco, CA.
Wade, M. J., and C. J. Goodnight. 1998. The theories of Fisher and Wright in the context of metapopulations: when nature does many small experiments. Evolution 52:1537–1553.
Wake, D. B., and K. P. Yanev. 1986. Gegoraphic variation in allozymes in a ''ring species,'' the plethodontid salamander *Ensatina eschscholtzii* of western North America. Evolution 40: 702–715.
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. Evolution of predator resistance to deadly toxin: Self-assessment of resistance leads to behavioral rejection of toxic prey. Herpetology: *In Press*.

Corresponding Editor: J. Wiens

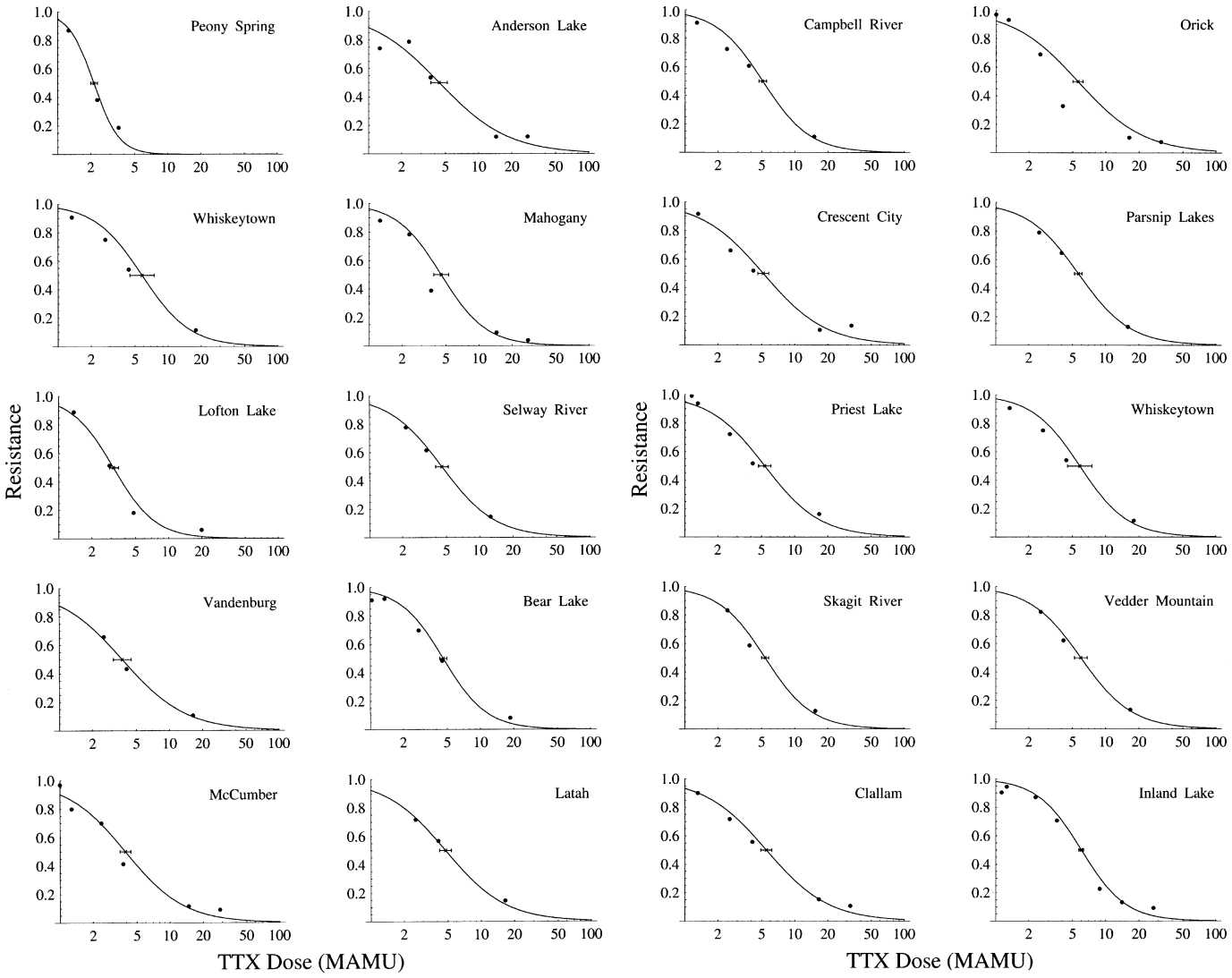
APPENDIX 1

Summary table of the geographic sampling of 2960 individual *Thamnophis sirtalis* from 41 populations, along with results of population specific dose-response regressions. The estimated 50% doses and upper and lower 95% confidence interval (CI) bounds are estimated as described in Methods. Statistics (P, R^2) refer to overall model fit; α is the intercept and β is the slope of the curvilinear regression.

Locality	County	No. of families	No. of individuals	Year(s)	Mean mass	Estimated 50% dose	Lower 95% CI	Upper 95% CI	$P <$	R^2	α	β
Anderson Lake	Jefferson Co., WA	4	23	96, 97	2.62	3.4	2.7	4.2	0.0001	0.684	2.06	1.39
Bear Lake	Bear Lake Co., ID	22	200	97, 98	1.99	3.6	3.3	4.0	0.0001	0.507	3.41	2.23
Bear Ridge	Humboldt Co., CA	3	23	98	1.87	6.6	5.6	7.9	0.0001	0.418	4.32	2.12
Benton	Benton Co., OR	32	291	85, 86, 98, 99, 00	2.78	34.1	30.8	37.7	0.0001	0.657	3.61	1.01
Campbell River	Vancouver Island, BC	5	53	98	2.50	4.1	3.7	4.5	0.0001	0.799	3.32	2.04
Chinook	Pacific Co., WA	9	59	96	3.09	16.6	14.4	19.1	0.0001	0.585	4.63	1.62
Clallam	Clallam Co., WA	8	52	96, 97	2.25	4.5	3.9	5.2	0.0001	0.685	2.63	1.54
Crescent City	Del Norte Co., CA	11	78	98	2.19	4.2	3.6	4.8	0.0001	0.615	2.53	1.54
Dupont	Pierce Co., WA	9	63	97	2.93	27.9	22.5	34.5	0.0001	0.524	3.98	1.18
East Bay	Contra Costa Co., CA		3	98	2.38	945.1	390.6	2,284.7	0.0001	0.719	4.22	0.62
Furnace Flats	Nevada Co., CA	2	8	99	1.78	7.0	5.4	8.9	0.0001	0.723	3.89	1.88
Gilroy	Santa Cruz Co., CA	11	116	98	2.24	18.4	15.0	22.6	0.0001	0.422	2.50	0.84
Harvey Hall Lake	BC	9	53	97	2.25	6.3	5.9	6.8	0.0001	0.703	5.87	2.95
Hoquiam	Grays Harbor Co., WA	7	47	96, 98	2.90	7.0	6.1	8.0	0.0001	0.479	3.35	1.61
Inland Lake	BC	7	110	97	2.69	5.0	4.7	5.3	0.0001	0.797	3.89	2.17
Latah	Latah Co., ID	4	19	98	2.25	3.8	3.2	4.4	0.0001	0.777	2.50	1.60
Ledson Marsh	Sonoma Co., CA	4	20	01	1.66	10.4	8.8	12.3	0.0001	0.753	3.89	1.60
Lofton Lake	Lake Co., OR	7	67	96, 97	1.84	2.2	1.9	2.5	0.0001	0.612	2.63	2.29
Mahogany	Lassen Co., CA	5	36	96	2.61	3.5	2.9	4.3	0.0001	0.678	3.27	2.16
McCumber	Shasta Co., CA	12	92	96	2.54	2.9	2.5	3.4	0.0001	0.575	2.23	1.63
McGribble	Curry Co., OR	7	79	87, 98	2.38	21.1	17.8	25.0	0.0001	0.418	3.29	1.06
Omo	El Dorado Co., CA	1	4	98	2.07	560.0	382.6	819.3	0.0001	0.665	10.18	1.61
Orick	Humboldt Co., CA	23	253	96, 97, 98, 99	2.29	4.6	4.0	5.2	0.0001	0.572	2.51	1.46
Parsnip Lakes	Jackson Co., OR	8	31	98	2.36	4.7	4.2	5.1	0.0001	0.809	3.24	1.87
Peony Spring	Lassen Co., CA	4	27	96	2.71	1.1	1.0	1.3	0.0001	0.555	2.91	3.83
Priest Lake	Texada Island, BC	4	39	97, 98	2.22	4.3	3.7	5.1	0.0001	0.653	2.88	1.72
Sailor Meadow	Placer Co., CA	2	12	99	1.85	6.4	4.5	9.1	0.0001	0.492	2.39	1.19
San Mateo	San Mateo Co., CA	2	51	86	4.72	1,279.0	963.5	1,697.7	0.0001	0.737	7.54	1.05
San Simeon	San Luis Obispo Co., CA	5	49	98	2.12	5.6	5.0	6.3	0.0001	0.686	3.72	1.96
Scott Lake	Lane Co., OR	2	34	98	3.33	5.6	4.7	6.7	0.0001	0.612	2.58	1.36
Selway River	Idaho Co., ID	1	8	98	3.04	3.6	3.0	4.2	0.0001	0.856	2.73	1.80
Skagit River	Skagit Co., WA	12	82	98	2.42	4.4	4.0	4.8	0.0001	0.661	3.45	2.05
Tenmile	Lane Co., OR	41	470	87, 88, 97	2.46	52.2	42.7	63.7	0.0001	0.655	3.76	0.95
Vandenburg	Santa Barbara Co., CA	1	9	98	2.27	2.7	2.1	3.5	0.0001	0.756	1.99	1.51
Vedder Mountain	BC	1	7	97	2.23	5.0	4.2	5.8	0.0001	0.885	3.35	1.88
Warrenton	Clatsop Co., OR	21	171	96, 97, 99	2.73	15.2	14.4	16.1	0.0001	0.412	4.83	1.73
Whiskeytown	Shasta Co., CA	3	19	98	2.06	4.8	3.5	6.5	0.0001	0.480	3.54	2.01
Whiteside	Whiteside Co., IL	1	31	97	1.42	1.8	1.5	2.0	0.0001	0.693	3.75	3.70
Wildboy	Skaminia Co., WA	5	46	98	2.40	21.7	17.2	27.1	0.0001	0.424	3.27	1.05
Willits	Mendocino Co., CA	12	71	98	2.03	6.7	6.1	7.3	0.0001	0.751	3.48	1.71
Willow Creek	Sonoma Co., CA	7	54	96, 98	2.79	*729.65	608.7	850.7				

APPENDIX 2

For each population, the dose-response curve and 50% Dose ($\bar{x}$, ± 95% confidence interval) as estimated from curvilinear regression are shown. On each plot, the mean resistance for a population at each measured dose is shown as a solid circle. Populations are presented in order of increasing resistance, based on the 50% Dose as reported in Figure 2 and Appendix 1. Regressions were estimated as described in the Methods, based on individual resistance data. The regression for the Tenmile population was conducted on family means because the large number of observations at a single dose (n = 470) generated a severely kurtotic distribution that violated the assumptions of regression analysis. Not shown is the curve for Whiteside Co., IL. The population from Willow Creek, Sonoma Co., CA, was too variable to characterize by regression (see text and Fig. 5). (Appendix 2 continued on p. 2082.)



APPENDIX 2. CONTINUED