12-2008

Evolutionary Genetics of Tetrodotoxin (TTX) Resistance in Snakes: Tracking a Feeding Adaptation from Populations Through Clades

Chris R. Feldman
Utah State University

EVOLUTIONARY GENETICS OF TETRODOTOXIN (TTX) RESISTANCE IN SNAKES: TRACKING A FEEDING ADAPTATION FROM POPULATIONS THROUGH CLADES

by

Chris R. Feldman

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Biology

Approved:

_____	_____
Michael E. Pfrender Major Professor	Karen E. Mock Committee Member
_____	_____
Edmund D. Brodie, Jr. Committee Member	Ethan P. White Committee Member
_____	_____
Paul G. Wolf Committee Member	Byron R. Burnham Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2008

ABSTRACT

Evolutionary Genetics of Tetrodotoxin (TTX) Resistance in Snakes:

Tracking a Feeding Adaptation from

Populations Through Clades

by

Chris R. Feldman, Doctor of Philosophy

Utah State University, 2008

Major Professor: Dr. Michael E. Pfrender
Department: Biology

Understanding the nature of adaptive evolution has been the recent focus of research detailing the genetic basis of adaptation and theoretical work describing the mechanics of adaptive evolution. Nevertheless, key questions regarding the process of adaptive evolution remain. Ultimately, a detailed description of the ecological context, evolutionary history, and genetic basis of adaptations is required to advance our understanding of adaptive evolution. To address some of the contemporary issues surrounding adaptive evolution, I examine phenotypic and genotypic changes in a snake feeding adaptation.

Adaptations can arise through fixation of novel mutations or recruitment of existing variation. Some populations of the garter snakes *Thamnophis sirtalis*, *T. couchii*, and *T. atratus* possess elevated resistance to tetrodotoxin (TTX), the lethal toxin of their

newt prey. I show that TTX resistance has evolved independently through amino acid changes at critical sites in a voltage-gated sodium channel protein ($Na_v1.4$) targeted by TTX. Thus, adaptive evolution has occurred multiple times in garter snakes via *de novo* acquisition of beneficial mutations.

Detailing the genetic basis of adaptive variation in natural populations is the first step towards understanding the tempo and mode of adaptive evolution. I evaluate the contribution of $Na_v1.4$ alleles to TTX resistance in two garter snake species from central coastal California. Allelic variation in $Na_v1.4$ explains 29% and 98% of the variation in TTX resistance in *T. atratus* and *T. sirtalis*, respectively, demonstrating that $Na_v1.4$ is a major effect locus. The simple genetic architecture of TTX resistance in garter snakes may significantly impact the dynamics of trait change and coevolution.

Patterns of convergent evolution are cited as some of the most compelling examples of the strength of natural selection in shaping organismal diversity. Yet repeated patterns may tell us as much about the constraints that restrict evolution as about the importance of natural selection. I present data on convergent molecular adaptations in parallel arms-races between diverse snakes and amphibians from across the globe. Six snake species that prey on TTX bearing amphibians have independently acquired amino acid changes in $Na_v1.4$. The derived mutations are clustered in two portions of the gene, often involving the same sites and substitutions. While a number of amino acid changes can make $Na_v1.4$ insensitive to TTX, most of these negatively impact or abolish the ion-conducting function of the protein. Thus, intramolecular pleiotropy likely prevents most replacements from becoming fixed and imposes limits on protein evolution.

(149 pages)

ACKNOWLEDGMENTS

Fieldwork is often the most challenging component of an integrated field/lab project, and so I am grateful to Butch Brodie III, Mike Edgehouse, Dan Mulcahy, Becky Williams, and Jim Parham for aid, levity, and above all, companionship while in the field. I also thank Butch Brodie Jr, Amanda Mortensen, and Ashley Wilkinson for additional field assistance and Butch Brodie III, Dave Cook, Robert Hansen, Butch Brodie Jr, Mike Pfrender, Steve Arnold and Robin Lawson for helpful field advice. For supporting fieldwork I thank Butch Brodie Jr who bankrolled trips (NGS 7531-03), Marjorie Matocq who generously lent her *Truckosaurus*, and Jeanine and Jean Matocq who opened their home and provided a welcome way station between field forays where the ice was always ready for the Picon Punch. While in the field Jan Pedotti generously allowed access to his family ranch, and Denise Cadman (City of Santa Rosa) and the Santa Lucia Preserve also permitted access to their holdings. For providing scientific collecting permits I thank California Department of Fish and Game, California Department of Parks and Recreation, and Idaho Department of Fish and Game.

I am indebted to Alan de Queiroz for loaning live specimens, and Kevin Wiseman, Tom Sinclair, Scott Boback, Don Schultz (Mfezi LLC), Peter Ducey, Bruce Christman, Shawn Kuchta, Ted Papenfuss, and Cliff Fontenot for providing live material. Butch Brodie Jr did a tremendous job "running the snakes" and he received assistance with the bioassays and with captive care from Amanda Mortensen, Jerry Scoville, Ashley Wilkinson, Jacob Pluid, and Mike Edgehouse. I thank Jens Vindum and Michelle Koo

(CAS), and Jonathan Campbell and Carl Franklin (UTA) for help with the curation of vouchers and USU IACUC for approving specimen protocols.

I am grateful to Jimmy McGuire, Dave Wake, and Carla Cicero (MVZ), Jens Vindum and Bob Drewes (CAS), Donna Dittmann and Chris Austin (LSUMNS), Jonathan Campbell and Carl Franklin (UTA), Frank Burbrink, Mo Donnelly, Raúl Manyero and Fred Kraus for generous tissue loans. For lab assistance I thank Michelle Andrews and Erin O'Leary-Jepsen (ISU MRCF), Kris Kruse (UNR NGC), and Marjorie Matocq. For helpful advice in the lab, and often good company while there, I thank Mike Pfrender, Marjorie Matocq, Amy Runck, Ali Scoville, John Chuckalovcak, and Quinn Shurtluff. I am indebted to Mike Pfrender, Marjorie Matocq, Butch Brodie Jr, Paul Wolf, Carol von Dohlen, and Rosemary Smith for accommodating me in their labs, and Mike Pfrender, Butch Brodie Jr, and Butch Brodie III for funding lab work (NSF DEB-0315172; NSF DEB-021212487; USU Center for Integrated BioSystems Research Grant).

For computational aid I thank Luobin Yang and Mike Thomas, and I appreciate statistical assistance from Leigh Latta and Mike Pfrender. I am grateful for helpful discussions on the physiology and biochemistry of sodium channels with Shana Geffeney, Caryn Evilia, Charles Hanifin, and Jim Groome. I enjoyed useful discussions on herp natural history with Butch Brodie Jr, Mike Pfrender, Butch Brodie III, Harry Greene, Neil Das, Peter Janzen, Bob Drewes, and Shi Haitao. I appreciate thoughtful conversations on this and other works with the USU Herp Group and especially Mike Pfrender, Butch Brodie Jr, Butch Brodie III, Marjorie Matocq, Becky Williams, Eric O'Neill, Dan Mulcahy, Kristin Bakkegard, Joe Mendelson III, Leigh Latta, Michelle

Cummer, Ryan O'Donnell, and Ali Scoville. My dissertation committee, Mike Pfrender, Butch Brodie Jr, Paul Wolf, Karen Mock, and Ethan White, kept things rolling and also provided insightful comments and discussions. I am indebted to Butch Brodie Jr, Alicia Pool, Jonathan Campbell, William Flaxington, Peter Janzen, Brian Kubicki (CRARC), Samuel Li, and Hidetoshi Ota for permission to use photographs.

Salary support while at USU came through teaching assistantships provided by the USU Department of Biology and I thank Mike Pfrender, Frank Messina, and Joe Mendelson III for encouraging me to work alongside them and for sharing their teaching expertise and philosophies. I would also like to acknowledge a USU School of Graduate Studies Dissertation Fellowship as well as Butch Brodie Jr and Mike Pfrender for providing finishing funds (NSF DEB-0315172) that allowed me to focus entirely on research.

Finally, I would like to thank my peers in the USU Herp Group, my two mentors, and my friends and family outside USU. My comrades in the USU Herp Group, Eric O'Neill, Becky Williams, Dan Mulcahy, Kristin Bakkegard, Leigh Latta, Michelle Cummer, Ryan O'Donnell, Ali Scoville, and Mike Edgehouse, created an atmosphere that was rigorous and challenging while at the same time warm and engaging. Working with Mike Pfrender and Butch Brodie Jr has been a privilege and a pleasure. It is Mike and Butch that foster the demanding yet genial camaraderie of Herp Group, creating an environment where scientific pursuits and young careers bloom. Through their actions it's clear that Mike and Butch put their student's interests above all others, and I can't possibly thank them for the time, the resources, and the effort they poured into my research, into my career, and into helping all the students of the Herp Group succeed. I

cherish my experiences working with them and strive to become a mentor in their mold. Lastly, Mike Thomas, Alissa Salmore, Jim Parham, Scott Scheff, Jason Izakowitz, John and Marina Durrant, my parents Robert and Kathy, my sister Joy, my parents-in-law Jeanine and Jean Matocq, my wife Marjorie Matocq and our son Rowan, all merit special attention for their unending support, the inspiration their successes provide, and the delight their friendships give.

Chris R. Feldman

CONTENTS

Page

ABSTRACT iii

ACKNOWLEDGMENTS v

LIST OF TABLES xi

LIST OF FIGURES xii

CHAPTER

1. INTRODUCTION 1

2. PARALLEL ARMS RACES AND CONVERGENT MOLECULAR EVOLUTION: GARTER SNAKES (*THAMNOPHIS*) REPEATEDLY ADAPT TO DEADLY PREY 6

Introduction 6
Materials and Methods 9

Bioassays 9
Sequence Data 10
Phylogenetic Analyses 12

Results and Discussion 14

Elevated TTX Resistance in *Thamnophis* 14
Genetic Basis of TTX Resistance in *Thamnophis* 15
Evolution of TTX Resistance in *Thamnophis* 19

3. SIMPLE GENETIC ARCHITECTURE OF A SNAKE FEEDING ADAPTATION: GENETICS OF TETRODOTOXIN RESISTANCE IN GARTER SNAKES (*THAMNOPHIS*) 25

Introduction 25
Materials and Methods 27

Bioassays 27
Sequence Data 28
Phenotype-Genotype Matching 28

Results and Discussion 30

Variation in TTX Resistance 30
Variation in $Na_v1.4$ 30
Relationship between Variation in $Na_v1.4$ and TTX Resistance 31
Simple Genetic Architecture and Coevolution 35

4. CONVERGENCE AND CONSTRAINTS IN SNAKE-AMPHIBIAN COEVOLUTION 43

Introduction 43
Materials and Methods 47

Bioassays 47
Sequence Data 47
Assessing Constraints on $Na_v1.4$ 48

Results and Discussion 50

TTX-Mediated Snake-Amphibian Coevolution 50
Genetic Basis of TTX Resistance in Snakes 53
Constraints on Convergent Evolution 57

5. SUMMARY 64

REFERENCES 68

APPENDICES 87

APPENDIX A. Specimens examined 88
APPENDIX B. Primers used to amplify and sequence $Na_v1.4$ 126
APPENDIX C. Exon/intron structure of *Thamnophis* $Na_v1.4$ 128

CURRICULUM VITAE 130

LIST OF TABLES

Table Page

2.1 Locality and sample information for *Thamnophis* and relatives assayed 22

3.1 Differences in TTX resistance between $Na_v1.4$ genotypes in central California populations of *T. atratus* and *T. sirtalis* 38

3.2 Percentage of phenotypic variance explained by $Na_v1.4$ genotype (PVE) in central California populations of *T. atratus* and *T. sirtalis*. 39

A.1 List of specimens examined for TTX bioassay (PE) and $Na_v1.4$ sequence variation (GE) 89

B.1 Oligonucleotide primers used to amplify and sequence genomic DNA and messenger RNA (cDNA) in *Thamnophis* and other squamate reptiles. 127

LIST OF FIGURES

Figure Page

2.1 Predator-prey interactions between *Thamnophis* and *Taricha* in western North America. 23

2.2 Map and alignment of adaptive $Na_v1.4$ amino acid replacements alongside phylogeny of $Na_v1.4$ alleles. 24

3.1 Geographic distribution and collection sites of *T. atratus* and *T. sirtalis* in California. 40

3.2 TTX resistance in central California populations of *T. atratus* allocated by $Na_v1.4$ genotype. 41

3.3 TTX resistance in central California populations of *T. sirtalis* allocated by $Na_v1.4$ genotype. 42

4.1 Phylogenetic distribution of TTX resistance in snakes that prey on TTX bearing amphibians and also possess adaptive variation in $Na_v1.4$. 61

4.2 Ecological and molecular convergence in TTX resistant snakes from across the globe. 62

4.3 Regression of inhibitory concentrations of TTX against single channel Na^+ conductance. 63

C.1 Map of *Thamnophis* $Na_v1.4$ intron/exon structure emphasizing pore regions 129

CHAPTER 1

INTRODUCTION

Genetic studies combined with the analysis of phenotypic variation are fundamental to our understanding of the process of adaptive evolution (Futuyma, 1998). Recently, the study of adaptive variation has benefited from theoretical developments (e.g., Orr, 2005a, 2005b; Phillips, 2005) as well as major gains in molecular genetic techniques, and growing number of studies are beginning to document the molecular basis of adaptation (e.g., Wichman et al., 1999; Rokyta et al., 2005; Gompel et al., 2005; Joron et al., 2006). Nevertheless, a number of unresolved issues regarding the generality of the mechanisms of adaptive evolution remain (Barton, 2001; Carroll, 2005; Hoekstra and Coyne, 2007; Barrett and Schluter, 2008; Pennisi, 2008). Further development of the field will come from empirical data that explicitly address the outstanding questions that surround adaptive evolution (Feder and Mitchell-Olds, 2003; Ellegren and Sheldon, 2008; Stinchcombe and Hoekstra, 2008). Progress will be made by studies that describe the genetic basis of adaptation and analyze the process of adaptive evolution in natural settings (e.g., Hoekstra et al., 2006; Storz et al., 2007). Yet genetic characterization of ecologically relevant traits in wild populations is rare, typically because the selective agents generally remain vague, the characters under selection often difficult to define, and the molecular basis of the traits thought to be under selection usually unknown. Thus, an impediment to our progress is the availability of empirical systems with well-defined ecological contexts and selection pressures, and with a clearly defined genetic basis for adaptation.

The interaction between toxic Pacific newts (*Taricha*) and the resistant predatory garter snakes (*Thamnophis*) provides a model system for the study of adaptation variation and predator-prey coevolution (Brodie and Brodie, 1999). This system is ideal because the traits that mediate the coevolution are easily decomposed (Brodie and Ridenhour, 2003), geographically variable (Brodie et al., 2002; Hanifin et al., 2008), and in the predator, at least, seemingly under control by a well-studied gene family (Geffeney et al., 2002, 2005). Furthermore, arm-race dynamics between populations of newts and garter snakes appears to have evolved multiple times independently (Brodie et al., 2005). I exploit these features of the newt-snake system to address three issues that surround the study of adaptive evolution: 1) the source of adaptations; 2) the genetic architecture of adaptations; 3) the constraints on adaptations.

Populations can adapt to novel environments in two ways: selection on new mutations or selection on pre-existing variation (Orr and Betancourt, 2001). The dynamics of natural selection can differ dramatically when adaptations result from either novel mutations or changes in the frequency of standing genetic variation, influencing the rate and fate of adaptive evolution (Orr and Betancourt, 2001; Hermisson and Pennings, 2005; Barrett and Schluter, 2008). New mutations start at extremely low frequencies and are prone to loss through genetic drift (Nei et al., 1975; Allendorf, 1986). However, natural selection will promote highly advantageous alleles more quickly than alleles with smaller effects (Falconer and Mackay, 1996). Thus large effect alleles will be more likely to escape drift, and so we expect that adaptive evolution through new mutations involves alleles with major fitness advantages (Orr and Betancourt, 2001; Hermisson and Pennings, 2005; Barrett and Schluter, 2008). Regardless, the fixation of new adaptive

variation will occur slowly because the initial frequencies of new mutations are close to zero (Barrett and Schluter, 2008). However, if a beneficial allele is present in standing variation, then adaptive evolution can proceed rapidly, not only because beneficial alleles are immediately available but also because they start at higher frequencies than new mutations (Barrett and Schluter, 2008). This also means that small effect alleles have a higher chance of contributing to adaptations because they have already escaped drift and can more easily rise to fixation (Orr and Betancourt, 2001; Hermisson and Pennings, 2005; Barrett and Schluter, 2008). Thus, assessing the relative contribution of new versus old variation is important in understanding how adaptive evolution proceeds in natural settings (Barrett and Schluter, 2008). To this end, I examine whether parallel feeding adaptations in three species of *Thamnophis* are the result of independent mutational events or the recruitment of the same standing variation (Chapter 2).

The underlying genetic architecture of adaptations affects not only the tempo and mode of phenotypic evolution, but also patterns of coevolution. Coevolutionary cycles are predicted to be more stable and dynamic under a multilocus model because adaptive alleles in the predator rarely change in harmony, allowing the prey to stay "ahead" in the arms-race (Sasaki, 2000; Agrawal and Lively, 2002, 2003; Kopp and Gavrilets, 2006; Nuismer et al., 2007). Conversely, when the traits that mediate the coevolution have a simple genetic basis, selection can more easily fix all the adaptive alleles, leading to the evolution of extreme phenotypes in which the prey "loses" the arm-race (Sasaki, 2000; Kopp and Gavrilets, 2006; Nuismer et al., 2007). Thus, detailing the genetic architecture of adaptations is essential in understanding coevolutionary dynamics (Bohannan and Lenski, 2000; Thompson, 2005; Wade, 2007; e.g., Yoshida et al., 2007). I assess the

contribution of genetic variation to toxin resistance in two garter snake species, and how this bears on the tempo and stability of newt-snake coevolution (Chapter 3).

A long-standing debate in evolutionary biology is the relative role of evolutionary constraints in restricting the course and pattern of adaptive evolution (Gould and Lewontin, 1979; Maynard Smith et al., 1985; Wake, 1991; Arnold, 1992; Futuyma, 1998; Pigliucci and Kaplan, 2000; Brakefield and Roskam, 2006; Brakefield, 2007). Can natural selection always fit organisms optimally to their environments, or do physical, historical, and genetic factors often bias the amount and pattern of variation upon which natural selection can act? Experimental manipulations have shown that heritable variation is rarely lacking, and given the right demographic conditions and enough time, natural selection can push populations over perceived hurdles (Travisano et al., 1995; Teotónio and Rose, 2000; Beldade et al., 2002; Frankino et al., 2005, 2007). There are ample reasons, however, to assume that biochemical and structural limitations at lower levels of organization have cascading effects on how organisms can become suited to their environments (Maynard Smith et al., 1985; Wake, 1991; Futuyma, 1998; Pigliucci and Kaplan, 2000; DePristo et al., 2005; e.g., Miller et al., 2006; Weinreich et al., 2006). Furthermore, historical contingencies and bias in how variation is generated may also limit selective responses (Gould 1989a; Arnold, 1992; Futuyma, 1998; Brakefield and Roskam, 2006; Brakefield, 2007). Taking a broad phylogenetic view of amphibian-snake coevolution, I assess the role of constraints by estimating bias in the molecular convergence evident across snake lineages (Chapter 4). I then relate the narrow genetic response of snakes back to possible biophysical tradeoffs that likely serves as an evolutionary constraint.

The coevolution between resistant snakes and their toxic amphibian prey is well poised for addressing some of the most fundamental and fascinating issues surrounding ecology and evolution (Brodie and Brodie, 1999). In collaboration with my mentors (see Acknowledgments), I look forward to making continual strides in our understanding of the natural history, ecology, and evolution of this chemically mediated system. It is my hope that the reader finds this research as compelling as I have—from the questions being posed, to the data collected, to the organisms themselves.

CHAPTER 2

PARALLEL ARMS RACES AND CONVERGENT MOLECULAR EVOLUTION: GARTER SNAKES (THAMNOPHIS) REPEATEDLY ADAPT TO DEADLY PREY

INTRODUCTION

Since the early days of the modern synthesis, genetic studies combined with the analysis of phenotypic variation have been fundamental to our understanding of the process of adaptive evolution (Futuyma, 1998). In recent years the study of adaptive variation has benefited from major gains in molecular genetic techniques and a growing number of studies documenting the molecular basis of adaptation (e.g., Nachman et al., 2003; Abzhanov et al., 2004; Alberston et al., 2005; Rokyta et al., 2005; Storz et al., 2007). The result of these studies is a rapid increase in our understanding of the process of, and constraints to adaptive evolution. Among the unresolved issues are a number of important questions regarding the generality of the mechanisms underlying the process of adaptive evolution. For example, is adaptive evolution typically the result of novel mutations, or changes in the frequency of standing genetic variation in natural populations (Orr and Betancourt, 2001; Hermisson and Pennings, 2005; Barrett and Schluter, 2008)? Do gene flow among populations and hybridization among species facilitate or retard adaptive evolution, and under what circumstances (Arnold, 1997; Barton, 2001; Seehausen, 2004)? What are the relative contributions of changes in gene regulation and gene function (Carroll, 2005; Hoekstra and Coyne, 2007; Wray, 2007; Pennisi, 2008)? While the theoretical framework describing the process of adaptive evolution is rapidly advancing (Orr, 2005a, 2005b; Phillips, 2005), further development

is dependent on empirical data to explicitly address these questions. Major advances will be achieved through studies that describe the genetic basis of adaptation and analyze the process of adaptive evolution in structured populations. One ideal setting is empirical systems with a repeated pattern of convergent evolution in phylogenetically independent species with geographically structured populations experiencing common selective pressures.

I exploit such a system in the coevolutionary relationship between garter snake predators (*Thamnophis*) and their toxic newt prey (*Taricha*). Newts of the genus *Taricha* possess the neurotoxin tetrodotoxin (TTX) (Mosher et al., 1964; Wakely et al., 1966; Brodie et al., 1974; Yotsu et al., 1990), which acts as a powerful chemical defense against vertebrate predators (Brodie, 1968; Brodie et al., 1974). Tetrodotoxin binds to voltage-gated sodium channels in nerves and muscles, blocking the movement of sodium ions (Na^+) across the cell membrane and halting the propagation of action potentials that control nerve impulses (Kao and Levinson, 1986; Hille, 2001). By paralyzing nerves and excitable muscle cells, TTX causes immobilization, respiratory failure, and often, death (Brodie, 1968; How et al., 2003; Isbister and Kiernan, 2005). Despite the fact that TTX is one of the most potent neurotoxins known (Medinsky and Klaassen, 1996), *T. sirtalis* in a number of populations are able to prey on toxic *T. granulosa* (Brodie and Brodie, 1990, 1991). The levels of TTX resistance in garter snakes and concentrations of TTX in newts often covary over much of western North America, suggesting the two species have entered coevolutionary arms-races characterized by adaptation and counter-adaptation (Brodie et al., 2002; Hanifin et al., 2008). While the means of TTX production in newts remains unknown (Hanifin et al., 2002, 2003; Tsuruda et al., 2002;

Cardall et al., 2004; Lehman et al., 2004), the physiological and genetic mechanisms at least partially responsible for elevated TTX resistance in *T. sirtalis* have been recently uncovered (Geffeney et al., 2002, 2005). A few amino acid changes in the skeletal muscle sodium channel ($Na_v1.4$) alter the molecular environment of the channel pore and dramatically alter TTX binding affinity to this protein (Geffeney et al., 2005).

A parallel ecological interaction between garter snake and newt was recently described in the Sierra Nevada of California: *T. couchii* prey on both *T. torosa* (Brodie et al., 2005) and *T. sierrae* (Wiseman and Pool, 2007) and are resistant to TTX at levels seen in sympatric *T. torosa* (Brodie et al., 2005). Here I document a third arms race involving an additional garter snake and newt interaction: *T. atratus* sympatric with TTX bearing *Taricha* are resistant to TTX. The repeated occurrence of TTX resistance in multiple *Thamnophis* species provides a context to examine alternative hypotheses for convergent adaptations. Are convergent adaptations among multiple *Thamnophis* species the result of (i) independent evolution via new mutation, (ii) the recruitment of standing variation, or (iii) the transfer of beneficial alleles via gene flow? Resistance to TTX appears in both closely and distantly related garter snake taxa, given the significant difference in coalescence times for nuclear and mitochondrial genes (Moore, 1995; Palumbi et al., 2001) we cannot *a priori* rule out incomplete lineage sorting. Furthermore, there is evidence of infrequent hybridization between garter snake species, even across diverse *Thamnophis* clades (Rossman et al., 1996; Shine et al., 2004). For alleles with a substantial fitness advantage, even low levels of introgression may be sufficient to allow the transfer of adaptive variation among species (Arnold, 1997; Barton, 2001). To reconstruct the evolutionary sequence of elevated TTX resistance in

Thamnophis, I first examine genetic underpinnings of resistance across garter snakes, and then use gene trees to distinguish the signature of (i) independent molecular evolution from that of (ii) incomplete lineage sorting or horizontal transfer.

MATERIALS AND METHODS

Bioassays

I collected TTX resistance data from 22 *T. atratus*, 84 *T. couchii* and 85 *T. sirtalis* from California (Fig. 2.1; Table 2.1; Appendix A). To provide a phylogenetic perspective on the evolution of elevated TTX resistance, I also collected resistance data from 240 garter snakes from 8 species representing the major *Thamnophis* clades (de Queiroz et al., 2002) and from 34 snakes from 4 outgroup taxa representing pertinent New World natricine lineages (Alfaro and Arnold, 2001) (Table 2.1). Some TTX resistance data came from Motychak et al. (1999) and Brodie et al. (2005).

I measured TTX resistance using a bioassay of whole organism performance (Brodie and Brodie, 1990; Brodie et al., 2002). I first established an individual's "baseline speed" by racing it down a 4m racetrack equipped with infrared sensors. I averaged the speed of two time trials to obtain an individual's baseline crawl speed. Following a day of rest, I gave each snake an intra-peritoneal injection of a known, mass-adjusted dose of TTX (Sigma). Thirty minutes post-injection I raced snakes on the track to determine "post-injection speed." I repeated this process, resting snakes for a day then increasing the dose of TTX (0.5 μg, 1 μg, 2 μg, 5 μg and 10 μg) and running snakes, up to five total sequential TTX tests per snake. I scored "resistance" as the reduction of an individual's baseline sprint speed following an injection of TTX (post-injection

speed/baseline speed). I calculated a population (or species) dose response curve from individual responses to the serial TTX injections using a simple linear regression (Ridenhour et al., 2004). From this regression model I estimated the "50% dose," defined as the amount of TTX required to reduce the average snake to 50% of its baseline speed. This measure is analogous to a 50% inhibition concentration (IC_{50}). Because TTX resistance is related to body size (Brodie and Brodie, 1990; Ridenhour et al., 2004), I transformed doses into mass-adjusted mouse units (MAMU), the amount of TTX (mg) required to kill a 20g mouse in 10 minutes (see Ridenhour et al., 2004). This correction allows direct comparison of TTX resistance between individuals, populations, or species. Further details of the bioassay and information on captive care of snakes can be found elsewhere (Brodie et al., 2002; Ridenhour et al., 2004).

Sequence Data

I collected sequence data from $Na_v1.4$ from a subset of garter snakes and outgroup taxa assayed for TTX resistance (Table 2.1). The single α-subunit of Na_v loci forms a membrane-spanning channel that allows selective permeation of Na^+ ions (Hille, 2001). This subunit consists of four domains (DI-DIV) each containing six transmembrane helices (S1-S6) with the polypeptide chains linking S5 and S6 creating the outer pore of the channel (Fig. 2.2) (Hille, 2001). The four pore forming segments (P-loops) fold back into the membrane to create the outer pore, modeled as a cone at the base of which lies a narrow selectivity filter (Lipkind and Fozzard, 2000; Hille, 2001) that preferentially allows Na^+ ions to pass through the channel (the DEKA motif; Terlau et al., 1991; Heinemann et al., 1992). The funnel shape of the outer pore is thought to form from four

α-helix-turn-β-strand structures (one from each S5-S6 linker), with the last residue of each turn facing the pore to create the selectivity filter (Lipkind and Fozzard, 2000). These same structures that line the outer pore and permit selectivity and permeability of Na^+ through the channel bind strongly to TTX. TTX apparently fits into the vestibule through a combination of hydrogen and ionic bonds, steric attraction, and cation-π interaction (Terlau et al., 1991; Kontis and Goldin, 1993; Penzotti et al., 1998; Yamagishi et al., 2001; Choudhary et al., 2003; Tikhonov and Zhorov, 2005; Scheib et al., 2006; Santarelli et al., 2007), essentially docking in the outer pore and blocking Na^+ movement (Hille, 2001). Thus, I focused my investigation on amino acid variation in the four P-loops of $Na_v1.4$, paying attention to the α-helix-turn-β-strand structures. I obtained the entire coding sequence (CDS) of $Na_v1.4$ from seven garter snakes (four species) to check for post-transcriptal modification (Liu et al., 2004; Song et al., 2004).

I isolated and purified genomic DNA from muscle or liver tissue with the DNeasy Tissue Kit (Qiagen, Inc.). I amplified (Saiki et al., 1988) the four regions of $Na_v1.4$ between the S5 and S6 transmembrane segments that form the P-loop using primers I designed specifically for snake $Na_v1.4$ (Appendix B). My amplicons included a linked intron in DI and portions of two introns in DIII (Appendix C). I cleaned amplified products using the ExcelaPure PCR Purification Kit (Edge Biosystems) and used purified template in cycle-sequencing reactions with Big Dye 3.1 (Applied Biosystems, Inc.). Following an isopropanol/ethanol precipitation, I ran cycle-sequenced products on an ABI 3130 automated sequencer (Applied Biosystems, Inc.). I sequenced all samples in both directions.

I isolated and purified mRNA from fresh skeletal muscle with the RNeasy Mini Plus Kit (Qiagen, Inc.). I reverse transcribed total mRNA to cDNA with the iScript Select cDNA Synthesis Kit (BioRad) and oligo(dT) primer. I then amplified a series of overlapping pieces of $Na_v1.4$ to construct a complete contig of the locus using primers I designed specifically for snake $Na_v1.4$ (Appendix A). I cleaned and sequenced amplified products as above. I edited sequences by eye in Sequencher 4.2 (Gene Codes Corp.), aligned sequences with Clustal W 1.83 (Thompson et al., 1994), and translated coding regions into amino acid sequences using MacClade 4.08 (Maddison and Maddison, 2005). I deposited all sequences in GenBank.

Phylogenetic Analyses

I used maximum parsimony (MP; Farris, 1983), maximum likelihood (ML; Felsenstein, 1981), and Bayesian (BI; Larget and Simon, 1999) methods on the combined P-loop and flanking intron sequences to infer phylogenies of $Na_v1.4$ alleles. I excluded 1.3Kb of a highly variable region of intron 19 (flanking DIII P-loop) where I could not confidently establish positional homology. The final alignment for phylogenetic analyses included over 2.9Kb of $Na_v1.4$ sequence, 1.0Kb from the four coding regions containing the P-loops, and over 1.9Kb from three flanking introns. Because indels often contain phylogenetic signal (Rokas and Holland, 2000; Kawakita et al., 2003) I coded indels in the introns as an additional character (deletion 0, insertion 1). I polarized the dataset with the natricine *Virginia striatula* (Alfaro and Arnold, 2001).

I conducted MP reconstructions in PAUP* (Swofford, 2002) with the branch-and-bound algorithm. I weighted characters equally and treated multiple state positions as

polymorphic. To assess nodal support, I used the bootstrap resampling method (Felsenstein, 1985) employing 1000 pseudoreplicates of heuristic searches using TBR branch swapping and 100 random sequence additions in PAUP*.

I executed ML analyses in PAUP* under the HKY+I+Γ model (Hasegawa et al., 1985; Gu et al., 1995; Yang, 1994), the best fit substitution model determined in Modeltest 3.7 (Posada and Crandall, 1998). I performed ML searches with the heuristic search algorithm using TBR branch swapping with 10 random sequence additions (with 10 Γ rate categories). I estimated nodal support with 100 bootstrap pseudoreplicates using TBR branch swapping and 10 random sequence additions.

I performed mixed-model BI analyses in MrBayes 3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). I partitioned nucleotides and indels into two data partitions and conducted searches under the HKY+I+Γ and parsimony approximation model (Tuffley and Steel, 1997). I ran BI analyses for 10 million generations using the default temperature (0.2) with four Markov chains per generation, sampling trees every 1000 generations. I assessed nodal support (i.e., posterior probability) by the frequency of recovered clades sampled after the stable equilibrium (Huelsenbeck and Ronquist, 2001).

Finally, I assessed the congruence between $Na_v1.4$ gene trees and expectations of allelic relationships under a model of a single origin of beneficial alleles with repeated recruitment or horizontal transfer of these alleles. I constrained the MP and ML searches in PAUP* to retain only those trees with a monophyletic TTX resistant clade. I then compared the constrained and unconstrained MP and ML estimates of $Na_v1.4$ phylogeny in PAUP* using a two-tailed Wilcoxon signed-ranks test (Templeton, 1983) and a one-

tailed multiple-comparisons likelihood ratio test (Shimodaira and Hasegawa, 1999) with 1000 RELL bootstrap pseudoreplicates.

Results and Discussion

Elevated TTX Resistance in Thamnophis

Thamnophis atratus from a portion of the central California coast (Molino Creek, Santa Cruz Co., and Pilar Point Harbor, San Mateo Co.; Fig. 2.1) display high levels of resistance to TTX (Table 2.1). I estimated the amount of TTX required to slow the average *T. atratus* from the central coast to 50% of its normal crawl speed to be >100 MAMU. In fact, these *T. atratus* are among the most TTX resistant snakes ever recorded, exceeded only by a few populations of *T. sirtalis* (Brodie et al., 2002). Newts would have to be exceptionally toxic to impair these *T. atratus*. Since TTX levels vary extensively within and among populations of *Taricha* (Hanifin et al., 1999, 2008), we might expect a complex pattern of match and mismatch (hotspots and coldspots) between newt toxicity and *T. atratus* TTX resistance across the landscape, consistent with the geographic mosaic model of coevolution (Thompson, 1994, 2005).

Similarly, multiple populations of *T. couchii* in the southern Sierra Nevada possess elevated TTX resistance, with an estimated mean 50% dosage of 86.5 MAMU. The population of *T. sirtalis* from Willow Creek also exhibits extreme TTX resistance, with a mean 50% dosage >100 MAMU, on par with some of the most TTX resistant snake populations known (Brodie et al., 2002). Finally, my broad taxonomic survey confirms the notion that elevated TTX resistance within *Thamnophis* is a derived trait (Motychak et al., 1999); other species of *Thamnophis* (including an eastern population of

T. sirtalis) and four natricine species display low levels of TTX resistance (<1-2 MAMU).

Genetic Basis of TTX Resistance in Thamnophis

I examined the genetic underpinnings of TTX resistance by characterizing molecular changes in the skeletal muscle sodium channel gene $Na_v1.4$. This locus produces a channel forming protein essential in muscle function that TTX selectively blocks (Hille, 2001). A great deal of literature on the architecture of Na_v loci suggests that TTX fits into the outer pore of the channel (see Lipkind and Fozzard, 2000; Hille, 2001), and replacements at certain residues in the pore dramatically alter TTX binding affinity (Noda et al., 1989; Terlau et al., 1991; Kontis and Goldin, 1993; Pérez-García et al., 1996; Chen et al., 1997; Penzotti et al., 1998; Yamagishi et al., 2001; Choudhary et al., 2003) whereas substitutions elsewhere in the protein appear to have little effect (Hille, 2001).

The entire α-subunit of $Na_v1.4$ in garter snakes encodes 1875 residues (5658bp) and shows high structural and amino acid homology with mammalian $Na_v1.4$. Additionally, $Na_v1.4$ intron/exon boundaries appear identical between garter snakes and mammals (*Homo* and *Rattus*) (Appendix B). I found no difference between the genomic and coding sequences (cDNA) of $Na_v1.4$, suggesting splice variation or RNA editing does not play a role in modulating TTX resistance in garter snakes. I detected additional variation in $Na_v1.4$ outside the P-loops, ranging from two replacements between *T. sirtalis* alleles to as many as seventeen between *T. couchii* and *T. sirtalis*. However, most

substitutions occur in the linkers that connect transmembrane segments that have no contact with TTX (Hille, 2001).

Pore loop amino acid sequences of $Na_v1.4$ are nearly invariant across garter snakes and relatives and almost identical to mammalian sequences, suggesting the locus is under strong stabilizing selection because of its critical functional role. Eastern *T. sirtalis* share an I764L in DII and the *T. ordinoides* possesses a K1537R in DIV (positions follow $Na_v1.4$ CDS from *T. sirtalis* AY851746). These substitutions involve nearly equivalent amino acid changes at the extracellular start of the P-loop connecting to the S5 segment. Amino acid replacements in the pore forming structures (pore α-helix, selectivity filter, β-strand; Lipkind and Fozzard, 2000) that interact with TTX are only found in TTX resistant snakes (Fig. 2.2). In contrast to previous work linking TTX resistance to functional changes in the DIV P-loop of $Na_v1.4$ (Geffeney et al., 2005), both *T. atratus* and *T. couchii* show changes in the P-loop of DIII.

Southern Sierra Nevada *T. couchii* have a single M1276T substitution in β-strand of DIII. This site plays a large role in the binding of TTX to the outer pore (Terlau et al., 1991; Pérez-García et al., 1996; Chen et al., 1997; Penzotti et al., 1998; Choudhary et al., 2003; Tikhonov and Zhorov, 2005). Site directed mutagenesis at this position in rat $Na_v1.2$ (brain) and $Na_v1.4$ shows that the amount of TTX required to block current (IC_{50}) generally increases by orders of magnitude when M is replaced (Terlau et al., 1991; Pérez-García et al., 1996; Chen et al., 1997; Penzotti et al., 1998; Choudhary et al., 2003). TTX binding affinity is still dramatically altered (down to 2000-fold decrease) when M is replaced by another neutral or even negatively charged amino acid (Terlau et al., 1991; Penzotti et al., 1998; Choudhary et al., 2003). Thus, the phenotypic effect of the M1276T

change in *T. couchii* is probably drastic even though it does not involve a charge change. Because M is hydrophobic and larger than hydrophilic T, I suspect that the M1276T replacement alters the shape of the pore and hence the docking orientation of TTX. An identical M→T replacement occurs in both $Na_v1.4a$ and $Na_v1.4b$ of TTX bearing pufferfish (Venkatesh et al., 2005) (teleost fish possess functional duplicates of most Na_v genes; Novak et al., 2006) supporting the view that this substitution in *T. couchii* $Na_v1.4$ has a considerable affect on TTX affinity.

Thamnophis atratus possess three amino acid changes in the P-loops of $Na_v1.4$: two in DIII (D1277E and A1281P) and one in DIV (D1568N). The D1277E replacement seems an unlikely candidate for TTX resistance because D and E possess the same charge, are nearly equal in size, and muations at this position generally lead to only minor changes in TTX binding affinity (Terlau et al., 1991; Pérez-García et al., 1996; Choudhary et al., 2003). However, a substitution also involving similar amino acids at a position thought to have little reactivity with TTX still produced a measurable reduction in TTX binding to $Na_v1.4$ in *T. sirtalis* (Geffeney et al., 2005). The I1561V substitution common to the three TTX resistant populations of *T. sirtalis* previously surveyed involves a nearly equivalent replacement, yet this substitution reduced the sensitivity of $Na_v1.4$ to TTX by 50% (Geffeney et al., 2005). It is also interesting to note that the pufferfish *Tetraodon nigroviridis* displays substitutions at the corresponding position in both $Na_v1.4$ copies, one of which is the same D→E (Venkatesh et al., 2005). The A1281P change occurs C-terminal to the β-strand and may be too superficial to influence TTX ligation (Li et al., 2000). However, other residues at the extracellular mouth of the pore have been shown to interact with TTX despite their distance from the selectivity

filter (Kontis and Goldin, 1993; Yamagishi et al., 2001; Carbonneau et al., 2002). Moreover, P always modifies polypeptide secondary structure with a considerable bend because of its ring shape. I cannot be certain this structural change will impact the conformation of the outer pore, but it is intriguing that the identical substitution is observed in $Na_v1.4a$ from tetrodotoxic pufferfish of the genus *Takifugu* (Yotsu-Yamashita et al., 2000; Venkatesh et al., 2005). The third P-loop change in *T. atratus* is a D1568N in the β-strand of DIV. This substitution occurs at a site known to play a major role in TTX ligation (Terlau et al., 1991; Pérez-García et al., 1996; Chen et al., 1997; Penzotti et al., 1998; Choudhary et al., 2003; Tikhonov and Zhorov, 2005). Changing D→N at this position in rat $Na_v1.4$ (D1532) yields a 30–40-fold increase in TTX resistance (Penzotti et al., 1998; Choudhary et al., 2003). The large effect of this substitution probably occurs because the hydrogen bond normally formed between TTX and D1568 (Chen et al., 1997; Choudhary et al., 2003; Scheib et al., 2006) is neutralized by uncharged N. Interestingly, the D1568N substitution occurs independently in Willow Creek *T. sirtalis*. Here it is part of a TTX insensitive $Na_v1.4$ allele that contains three other replacements, including I1561V (Geffeney et al., 2005).

One final consideration regarding the $Na_v1.4$ genotype of *T. atratus* is that some of the P-loop substitutions may be compensatory changes (DePristo et al., 2005). Specifically, the two DIII replacements might be required to maintain the function of the outer pore following the drastic D1568N replacement in DIV. Likewise, perhaps only the I1561V and identical D1568N substitutions in Willow Creek *T. sirtalis* actually alter TTX binding affinity and one or two of the other changes in DIV are compensatory.

Evolution of TTX Resistance in Thamnophis

Natural selection is often considered the main force behind convergent evolution by exposing independent lineages to similar selective environments (Futuyma, 1998), yet the genetic basis of convergence may result from a number of factors (Maynard Smith et al., 1985; Wake, 1991; True and Haag, 2001; Budd, 2006). Lineages may independently acquire beneficial mutations at the loci under selection (Zhang and Kumar, 1997; Wood et al., 2005; Arendt and Reznick, 2008; e.g., Stewart et al., 1987; Wichman et al., 1999; Mundy et al., 2004; Woods et al., 2006). Common evolutionary responses and parallel molecular changes may be due to genetic constraints in closely related lineages with similar genetic architecture that bias the response to selection (Schluter, 1996; Schluter et al., 2004; Brakefield, 2007). Alternatively, beneficial alleles may have a single origin and become repeatedly fixed by selection in common environments (Orr and Betancourt, 2001; Hermisson and Pennings, 2005; Barrett and Schluter, 2008; e.g., Colosimo et al., 2005; Hartley et al., 2006). Similarly, adaptive variation may be introduced to populations or species through introgression (Arnold, 1997; Barton, 2001; e.g., Cahan and Keller, 2003; Rieseberg et al., 2003; Grant et al., 2004; Herder et al., 2006).

To trace the origins of elevated TTX resistance in this system as it relates to $Na_v1.4$ evolution, I established the evolutionary relationships of $Na_v1.4$ alleles in *Thamnophis*. If elevated TTX resistance in *Thamnophis* has evolved through (i) independent changes in $Na_v1.4$, then a $Na_v1.4$ gene tree should roughly match the accepted garter snake phylogeny (de Queiroz et al., 2002). On the other hand, if elevated TTX resistance in *Thamnophis* has occurred through (ii) the recruitment of preexisting adaptive variation or horizontal transfer of beneficial alleles, then the three TTX resistant

taxa will form a clade in a $Na_v1.4$ phylogeny, contrary to the *Thamnophis* phylogeny. The three phylogenetic methods (MP, ML, BI) largely agree, and there are only a few areas of disagreement (only BI tree shown), though nodal support is generally weak. Overall, phylogenetic relationships of *Thamnophis* $Na_v1.4$ alleles (Fig. 2.2) are surprisingly concordant with independent estimates of garter snake relationships based on mitochondrial loci (de Queiroz et al., 2002). Assaying TTX resistance across garter snakes and relatives and mapping resistance data onto the $Na_v1.4$ phylogeny indicates that elevated TTX resistance is a derived trait, whereas the ancestral condition for garter snakes and relatives is low TTX resistance (Motychak et al., 1999). Elevated TTX resistance appears to have originated independently at least two times within *Thamnophis* (Fig. 2.2): once in western *T. sirtalis* which are part of a basal group, and possibly once in the common ancestor of *T. atratus* and *T. couchii* which form a well-nested clade. However, if other populations of *T. atratus* or *T. couchii* display ancestral levels of TTX resistance then the reconstruction of this character would be more complex and separate origins of TTX resistance in *T. atratus* and *T. couchii* might well be more parsimonious. Furthermore, the functional mutations in the $Na_v1.4$ P-loops of *T. atratus* and *T. couchii* are not shared but appear uniquely derived. Finally, the hypothesis of a single origin of adaptive $Na_v1.4$ variation linking TTX resistant garter snakes through either incomplete lineage sorting or gene flow was rejected by statistical tests of hypothesis compatibility (MP, Wilcoxon signed-ranks test: L difference = 26, $z = -5.099$, $p < 0.0001$; ML, SH test: -lnL difference = 88.8277, $p < 0.0001$). Thus, the topology of the $Na_v1.4$ gene tree and the nature of the changes in the P-loops allow us to reject the hypothesis (ii) that elevated TTX resistance occurred through the either the recruitment of standing genetic variation

in $Na_v1.4$ or introgression of $Na_v1.4$ alleles. Instead, the data are consistent with the hypothesis (i) elevated TTX resistance has evolved three times independently within *Thamnophis* through convergent changes in $Na_v1.4$.

We now have three parallel ecological arms races between garter snakes and newts. In each case we have a deadly prey species with the same quantifiable defense (TTX), a predator known to consume the prey in the wild, a measurable capacity in the predator to overcome the prey defense (TTX resistance), and phylogenetic information to show that resistance to the prey defense is derived. Thus, the ecological and evolutionary context of elevated TTX resistance in *Thamnophis* displays all the hallmarks of an adaptation (Gould and Vrba, 1982; Rose and Lauder, 1996). I have shown that the predatory adaptation has evolved independently through changes at the same gene. Studying convergent, phylogenetically independent adaptations is a powerful approach to develop generalities about the process of adaptation. These empirical data are useful for understanding the processes generating adaptive variation in parallel ecological settings and the molecular genetic basis of coevolutionary interactions. Future work will examine the roles of standing genetic variation and gene flow among populations within species. As genomic tools become available to non-model systems (McGaugh et al., 2007) and coalescent approaches advance (Hey and Nielsen, 2004; Beerli, 2006; Knowles and Carstens, 2007), it will be possible to examine how selection and gene flow promote the transfer and fixation of beneficial alleles (e.g., Rosenblum et al., 2007) across the coevolutionary landscape.

TABLE 2.1. Locality and sample information for *Thamnophis* and relatives assayed. Mean TTX resistance, and samples sizes for TTX bioassay, individuals genotyped for the four $Na_v1.4$ P-loops, and samples sequenced for entire CDS of $Na_v1.4$.

Taxon	Locality	*n* Bioassay (adult/offspring)[a]	*n* $Na_v1.4$ P-loop/CDS	$\bar{x}$ 50% MAMU[b]
T. atratus	Molino Creek, Santa Cruz Co., CA & Pilar Point Harbor, San Mateo Co., CA	22 (9/13)	9/1	>100
T. couchii	Cold Springs Creek, Tulare Co., CA & Wishon, Tulare Co., CA	84 (17/67)[c]	8/1	86.5
T. cyrtopsis	Ladder Ranch, Sierra Co., NM & Patagonia Lake, Santa Cruz Co., AZ	38 (4/34)[d]	1/-	1
T. elegans	San Simeon Creek Ranch, San Luis Obispo Co., CA & Prewitt Creek, Monterey Co., CA	24 (24/-)	9/1	1
T. errans	Mil Diez, Durango, MEX	1 (1/-)	1/-	<1
T. nigronuchalis	Mil Diez, Durango, MEX	1 (1/-)	1/-	1
T. ordinoides	Adair, Benton Co., OR	127 (-/127)[e]	1/-	2
T. proximus	Krugerville, Denton Co., TX & Splendora, Montgomery Co., TX	37 (1/36)[d]	1/-	2
T. radix	Corrumpa Creek, Union Co., NM	2 (2/-)	1/-	1
T. sirtalis	Willow Creek, Sonoma Co., CA	85 (26/59)	10/4	>100
T. sirtalis VA	Mountain Lake Biological Station, Giles Co., VA	10 (10/-)	4/-	5
Nerodia sipedon	Auburn, Lee Co., AL	4 (4/-)	1/-	<1
Storeria occipitomaculata	Cincinnatus, Cortland Co., NY	20 (8/12)	1/-	<1
Tropidoclonion lineatum	Luray, Russell Co., KS	5 (5/-)	1/-	<1
Virginia striatula	North Houston, Harris Co., TX	5 (5/-)	1/-	2

[a]total number of snakes assayed for TTX resistance including wild caught adults and their offspring born in the lab.
[b]mass-adjusted measure of the amount of TTX required to slow a snake to 50% of its normal crawl speed (see methods).
[c]from Brodie et al. (2005) but supplemented with additional samples.
[d]from Motychak et al. (1999) but supplemented with additional samples.
[e]from Motychak et al, (1999).

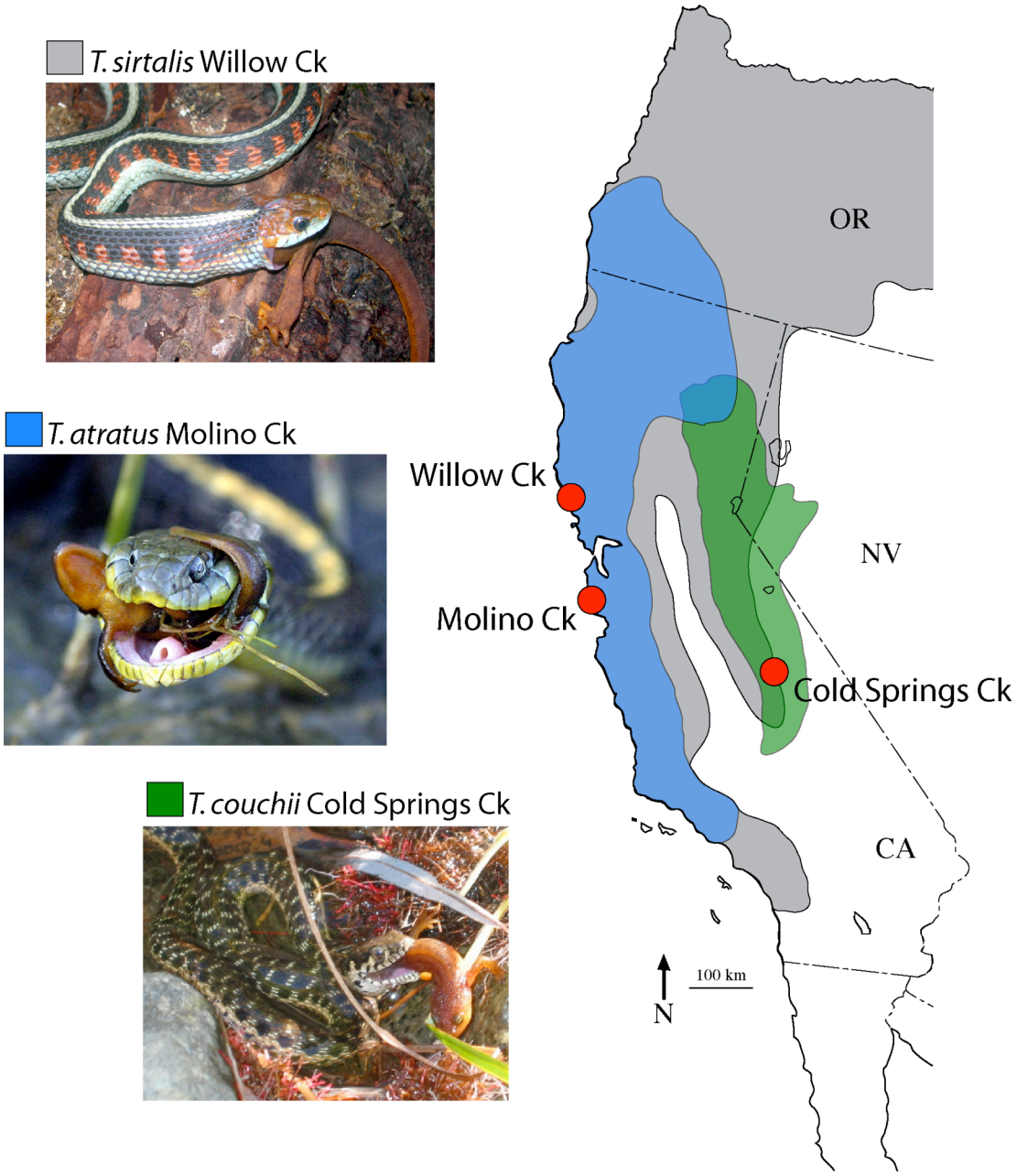

FIGURE 2.1. Predator-prey interactions between *Thamnophis* and *Taricha* in western North America. Coevolution between *T. sirtalis* and Pacific newts (*Taricha*) which possess the potent neurotoxin tetrodotoxin (TTX) has been the focus of ongoing research for decades (e.g., Brodie, 1968; Brodie et al., 2002), but it was recently discovered that similar interactions between *T. couchii* and their newt prey occur in the southern Sierra Nevada Mountains of California (Brodie et al., 2005). Herein a third parallel arms race is described, involving *T. atratus* and sympatric newts along a portion of the central California coast. Geographic distributions of *T. sirtalis* (grey), *T. atratus* (blue), and *T. couchii* (green) in California and Oregon (Stebbins, 2003) highlighting the three focal populations (red) where garter snakes take newts and are also highly resistant to TTX.

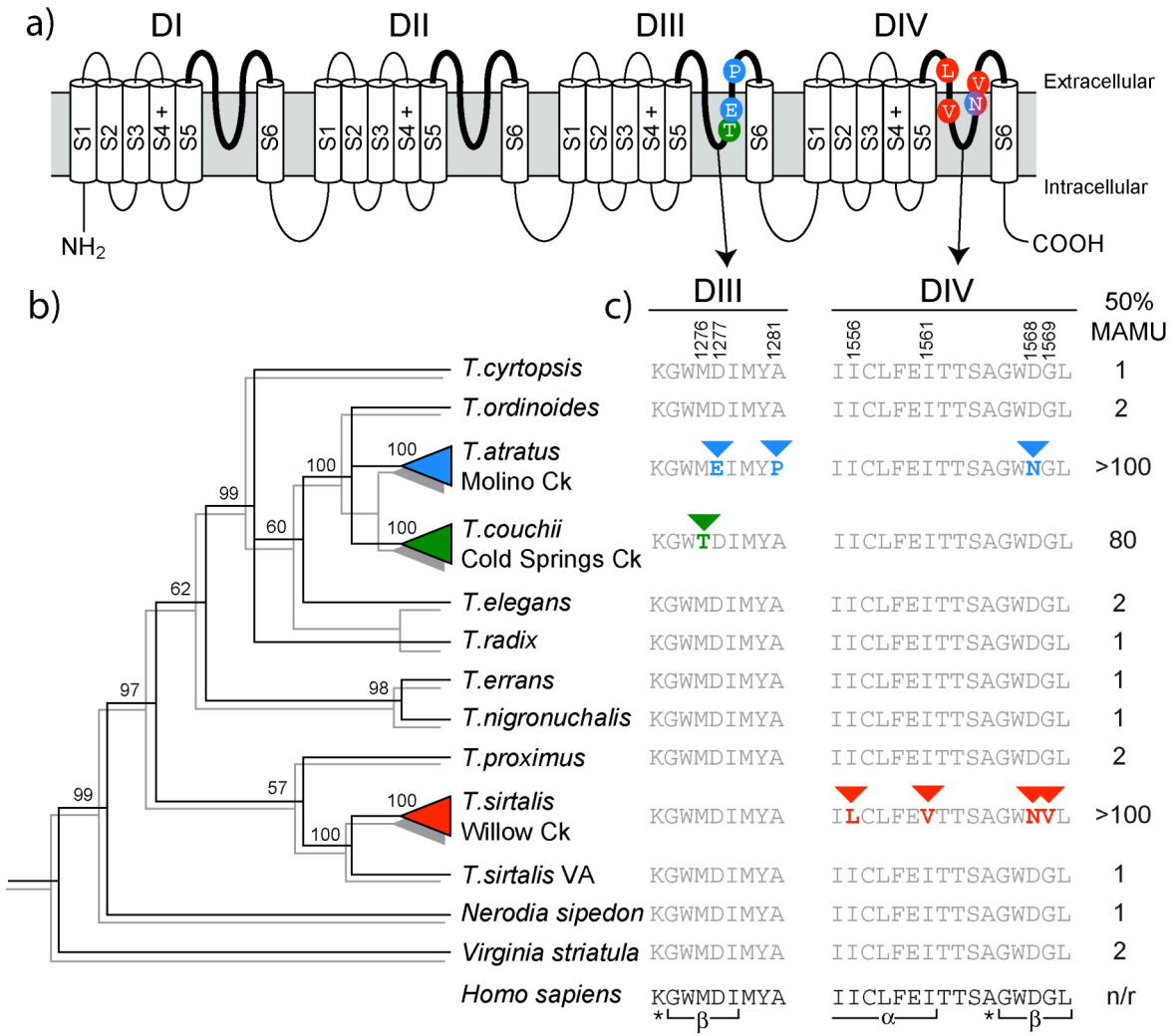

FIGURE 2.2. Map and alignment of adaptive $Na_v1.4$ amino acid replacements alongside phylogeny of $Na_v1.4$ alleles. *(Continued)*

FIGURE 2.2. *(Continued)* Replacements in the pore forming loops (P-loops) of the skeletal muscle sodium channel ($Na_v1.4$) that interact with TTX occur only in the TTX resistant garter snakes assayed, appear fixed in the three resistant populations, and occur at sites important in TTX ligation. (a) Structure of the α-subunit of $Na_v1.4$ showing the four domains (DI-DIV), their six transmembrane segments (S1-S6), and the linkers that connect segments (Hille, 2001). The four polypeptide chains that link S5 to S6 (bold) form the outer pore of the channel by folding back into the membrane to create a funnel at the base of which lies a narrow selectivity filter (Lipkind and Fozzard, 1994, 2000; Hille, 2001) that preferentially conducts Na^+ ions. A number of residues that form the external mouth, lining, and selectivity filter of the pore bind strongly to TTX (Noda et al., 1989; Terlau et al., 1991; Kontis and Goldin, 1993; Pérez-García et al., 1996; Chen et al., 1997; Penzotti et al., 1998; Yamagishi et al., 2001; Choudhary et al., 2003), which occludes the pore and halts Na^+ movement (Hille, 2001). Approximate location of amino acid substitutions in DIII and DIV P-loops (color coded to species) discussed in text. (b) Phylogeny of $Na_v1.4$ alleles from *Thamnophis* and relatives based on the coding regions of all four P-loops (1.0kb) and linked introns (1.9kb). The gene tree (black) closely resembles independent estimates of garter snake phylogeny based on mtDNA (grey; de Queiroz et al., 2002) and shows that elevated TTX resistance has evolved multiple times in garter snakes and does not involve the recruitment of preexisting adaptive alleles (e.g., Colosimo et al., 2005) or introgression of adaptive variation (e.g., Rieseberg et al., 2003). Topology and nodal support values estimated via mixed-model Bayesian tree searches (Huelsenbeck and Ronquist, 2001); some outgroups pruned for simplicity; colors on branches correspond to colors on map (Fig. 2.1) except for resistant *T.sirtalis* (red). (c) Amino acid sequences of the DIII and DIV P-loops alongside measures of whole animal resistance to TTX. Though all four P-loops were examined, only the DIII and DIV regions hold variation in garter snakes, but here amino acid substitutions (arrows and replacements color coded to species) occur at critical residues that change the structure and electrostatic environment of the pore and alter TTX binding. Human sequence given for comparison (M81758), but positions follow $Na_v1.4$ CDS from *T. sirtalis* AY851746; structures of the pore labeled below human sequence (✶, selectivity filter; α, α-helix; β, β-strand).

CHAPTER 3

SIMPLE GENETIC ARCHITECTURE OF A SNAKE FEEDING ADAPTATION: GENETICS OF TETRODOTOXIN RESISTANCE IN GARTER SNAKES (*THAMNOPHIS*)

INTRODUCTION

A central goal of evolutionary biology is to understand the genetics surrounding adaptive variation (Futuyma, 1998; Feder, 2007; Ellegren and Sheldon, 2008). The major promise of the modern synthesis is that theoretical and empirical work on variation at the population level firmly links microevolution to the broad scale macroevolutionary patterns apparent in nature (Futuyma, 1998; Mayr and Provine, 1998). We can certainly measure the origins, evolution, and persistence of consequential mutations in controlled laboratory settings, and place these data into a well-developed framework of adaptive evolution (Orr, 2005a, 2005b; Phillips, 2005; e.g., Wichman et al., 1999; Rokyta et al., 2005; Miller et al., 2006; Weinreich et al., 2006). Yet our ability to describe the effects of like genetic changes on the rate and direction of phenotypic evolution in real communities is only now emerging (Feder and Mitchell-Olds, 2003; Ellegren and Sheldon, 2008; Stinchcombe and Hoekstra, 2008; e.g., Gompel et al., 2005; Hoekstra et al., 2006; Joron et al., 2006; Storz et al., 2007). A significant impediment to progress is the availability of empirical systems with well-defined ecological contexts and selection pressures, and with a clearly defined genetic basis for adaptation.

The interaction between toxic newts (*Taricha*) and several resistant predatory garter snakes (*Thamnophis*) provides a model system for the study of adaptation variation

and predator-prey coevolution (e.g., Brodie and Brodie, 1999; Brodie et al., 2002). This system is ideal because the traits that mediate the coevolution are easily decomposed, geographically variable, and at least partly controlled by a well-studied gene family. Newts of the genus *Taricha* possess the neurotoxin tetrodotoxin (TTX) (Mosher et al., 1964; Wakely et al., 1966; Brodie et al., 1974; Yotsu et al., 1990), which acts as a powerful chemical defense against vertebrate predators (Brodie, 1968; Brodie et al., 1974). Tetrodotoxin binds selectively to the outer pore of voltage-gated sodium channels in nerves and muscles (Lipkind and Fozzard, 2000; Hille, 2001), blocking the movement of sodium ions (Na^+) across the cell membrane and halting the propagation of action potentials (Kao and Levinson, 1986; Hille, 2001). By arresting nerve impulses in muscle and nervous tissue, TTX causes immobilization, respiratory failure, and often, death (Brodie, 1968; How et al., 2003; Isbister and Kiernan, 2005). In spite the fact that TTX is one of the most powerful neurotoxins known (Medinsky and Klaassen, 1996), three species of *Thamnophis* have independently evolved high tolerance of TTX (Chapter 2) and prey on sympatric newts (Brodie and Brodie, 1990; Brodie et al., 2005; Chapter 2). The physiological and genetic mechanisms at least partially responsible for elevated TTX resistance involve slight alterations in the outer pore (P-loop) of the skeletal muscle sodium channel ($Na_v1.4$) that dramatically reduce the affinity of TTX to this protein (Geffeney et al., 2002, 2005; Chapter 2).

Certain P-loop replacements in Na_v genes alter TTX ligation to sodium channels (Noda et al., 1989; Terlau et al., 1991; Kontis and Goldin 1993; Pérez-García et al., 1996; Chen et al., 1997; Penzotti et al., 1998; Yamagishi et al., 2001; Choudhary et al., 2003) and these changes have visible physiological effects at the organismal level (Geffeney et

al., 2005; Venkatesh et al., 2005; Maruta et al., 2008). Thus, Na_v loci can be considered genes of major effect, yet we still lack a good grasp on the contribution of individual alleles to TTX resistance. Here, I take advantage of the striking variability in TTX resistance seen in three *T. atratus* and *T. sirtalis* populations along the coast of central California (Fig. 3.1). I examine the relationship between allelic variation in $Na_v1.4$ and TTX resistance in these populations, and how this relationship bears on our understanding of adaptive variation in garter snakes and the coevolutionary dynamics between newts and snakes.

Materials and Methods

Bioassays

I collected TTX resistance data from 83 *T. atratus* and 47 *T. sirtalis* from three populations along the central coast of California: Molino Creek, Santa Cruz Co.; Gilroy, Santa Clara Co.; Santa Lucia Preserve, Monterey Co. (Fig. 3.1; Appendix A). The data from Molino Creek *T. atratus* are from Chapter 2.

I measured TTX resistance using the same bioassay of whole organism performance detailed in Chapter 2 (see also Brodie and Brodie, 1990; Brodie et al., 2002; Ridenhour et al., 2004). However, some of the *T. sirtalis* were so resistant to TTX that they were essentially unaffected by standard injections. I administered additional TTX doses to a subset of these snakes and found they could still run above 50% of their normal ability at over 200, 500, and 1000 MAMUs. I thus assigned a minimum 50% estimate of 100 MAMUs to all unaffected *T. sirtalis* (based on the highest common dose given), recognizing that the actual measures of TTX resistance must be much higher.

Sequence Data

I collected DNA sequence data from $Na_v1.4$ from each garter snake assayed for TTX resistance. I followed the same laboratory and data editing procedures outlined in Chapter 2. And as in Chapter 2, I also focused on variation in portions of the four domains (DI-DIV) that code for the outer pore (P-loops) because TTX interacts with residues of the outer pore (Lipkind and Fozzard, 2000; Hille, 2001) and changes at some of these sites in $Na_v1.4$ are thought to contribute TTX resistance in snakes (Geffeney et al., 2005; Chapter 2, 4) and pufferfish (Yotsu-Yamashita et al., 2000; Venkatesh et al., 2005; Maruta et al., 2008).

I scored a $Na_v1.4$ sequence as a unique allele if it possessed amino acid replacements in or nearby the pore forming structures of the P-loops (pore α-helix, selectivity filter, β-strand; Lipkind and Fozzard, 2000) that interact with TTX. This notation for alleles includes the gene name followed by a subscript of one letter amino acid abbreviations given in the order those derived allelic substitutions occur in the locus. This nomenclature reflects the molecular differences between the ancestral garter snake gene sequence ($Na_v1.4_{anc}$) and derived $Na_v1.4$ variants, rather than putative phenotypic effects or dominance attributes of alleles. I deposited all sequences in GenBank.

Phenotype-Genotype Matching

I assigned each phenotype to its respective genotype then tested for overall differences in TTX resistance between the genotypes with a single factor analysis of variance (ANOVA) using restricted maximum likelihood in SAS v9.1 (SAS Institute). I then used *post hoc* tests to assess the significance of pairwise differences between

genotypes. I also determined the statistical association between genotype (allelic variation in $Na_v1.4$) and phenotype (variation in TTX resistance) by estimating the variance components using the same single factor ANOVA. The percentage of variance explained (PVE) by the genotype is simply the proportion of the total variance attributed to the genotype.

I evaluated allelic dominance in $Na_v1.4$ using the dominance coefficient (h), a simple metric for assessing the degree and direction of dominance influencing a trait. In the most straightforward case, a diallelic system with three genotypes, h can be calculated by setting the mean trait value of the genotype with the most visible effect to one (AA = 1), scaling the mean of the heterozygous class to this measure and solving for the proportion of the homozygous value expressed in heterozygotes (Aa = $1 - h$). If $h = 0.5$ then the alleles have a purely additive effect and there is no dominance, but when $h \neq 0.5$ ($0 \leq h \leq 1$) then some form of dominance is apparent, with extreme cases of overdominance and underdominance reflected by $h < 0$ and $h > 1$, respectively. Ideally, h can be calculated over a suit of loci affecting a trait and include allele frequency information to account for the effects of selection (Falconer and Mackay, 1996; Deng, 1998). Because I pooled data from separate populations and included some parent offspring pairs, these allele frequencies cannot be considered representative of the actual population values, so I did not consider the effects of selection on $Na_v1.4$ dominance. Additionally, I only assessed dominance in *T. sirtalis* because I did not find any fully heterozygous *T. atratus*.

RESULTS AND DISCUSSION

Variation in TTX Resistance

Central coastal California populations of *T. atratus* and *T. sirtalis* (Fig. 3.1) possess dramatic phenotypic variation in TTX resistance. In fact, TTX variation within populations of *T. atratus* and *T. sirtalis* is equivalent to the range of resistance seen across the entire clade of *Thamnophis*, with snakes possessing natively low levels (Motychak et al., 1999; Chapter 2) to those on par with the most TTX resistant snakes ever recorded (Brodie et al., 2002; Chapter 2). For example, the amount of TTX required to slow Santa Lucia Preserve *T. atratus* to 50% of their normal crawl speed ranged from 2–100 MAMUs. Likewise, 50% TTX dosages in *T. sirtalis* from this same locality ranged from 6–>100 MAMUs.

Variation in $Na_v1.4$

I found four $Na_v1.4$ alleles in central coastal populations of *T. atratus* ($Na_v1.4_{anc}$, $Na_v1.4_{EPN}$, $Na_v1.4_{EP}$, $Na_v1.4_{N}$). The first, $Na_v1.4_{anc}$, is the same TTX sensitive $Na_v1.4$ allele found across the garter snake phylogeny (Chapter 2). The three derived *T. atratus* alleles can be distinguished from $Na_v1.4_{anc}$ as follows: $Na_v1.4_{EPN}$ contains a D1277E in the DIII β-strand, an A1281P C-terminal to the DIII β-strand, and a D1568N in the DIV β-strand; $Na_v1.4_{EP}$ holds the two DIII changes, D1277E and A1281P; $Na_v1.4_{N}$ possesses only the DIV replacement, D1568N. The four alleles are distributed among only four genotypes in these *T. atratus* populations (Fig. 3.2).

I found only two alleles in the sympatric populations of *T. sirtalis* ($Na_v1.4_{anc}$, $Na_v1.4_{LVNV}$). As with *T. atratus*, some *T. sirtalis* possess the same allele that appears to

represent the ancestral garter snake $Na_v1.4$ sequence (Chapter 2). The derived *T. sirtalis* allele differs from this putative ancestor through four mutations in the P-loop of DIV; $Na_v1.4_{LVNV}$ is characterized by an I1555L and I1561 in the α-helix and a D1568N and G1569V in the β-strand. As expected, the two *T. sirtalis* $Na_v1.4$ alleles occur in three genotypes (Fig. 3.3).

Most of the $Na_v1.4$ alleles have been identified and detailed previously (Geffeney et al., 2005; Chapter 2), and the two *T. sirtalis* alleles have been functionally expressed (Geffeney et al., 2005). Briefly, the replacements in the P-loops of the derived alleles are known to reduce the binding affinity of TTX to the outer pore of $Na_v1.4$ by changing pore structure and environment (Geffeney et al., 2005; Chapter 2). Besides the fact that substitutions in the derived $Na_v1.4$ alleles occur at sites known to play a major role in TTX ligation, nearly every one of these sites also shows coincident replacements in distantly related snakes that also consume prey with TTX (Chapter 4) and in TTX bearing pufferfish (Yotsu-Yamashita et al., 2000; Venkatesh et al., 2005; Maruta et al., 2008).

Relationship between Variation in $Na_v1.4$ and TTX Resistance

Partitioning *T. atratus* phenotypes into their respective genotypes reveals obvious differences between the groups (Table 3.1; Fig. 3.2). Snakes homozygous for the ancestral $Na_v1.4$ allele exhibit the least amount of TTX resistance ($\bar{x}$ = 7.41, SD = 3.99) while the next lowest group is composed of snakes heterozygous for $Na_v1.4_N$ and $Na_v1.4_{anc}$ ($\bar{x}$ = 16.47, SD 18.08). These two genotypes display considerably less TTX resistance (t-value = -5.06, $P < 0.0001$; t-value = -3.77, $P = 0.0003$) than snakes with the $Na_v1.4_{EPN}$ allele ($\bar{x}$ = 47.84, SD 39.80). Thus, *T. atratus* genotypes with $Na_v1.4$ alleles

with a greater number of derived P-loop substitutions exhibit higher TTX resistance than those possessing alleles with fewer or no P-loop replacements. Whether the effects of the individual alleles is additive or ordinal remains to be determined pending additional phenotypic data from the full array of expected genotypes. Nevertheless, the association between $Na_v1.4$ polymorphism and TTX resistance is significant (F-value = 10.100, $P < 0.0001$) and the proportion of the phenotype explained (PVE) by the genotype is substantial (29%) in *T. atratus* (Table 3.2).

Matching *T. sirtalis* phenotypes to their respective genotypes also reveals dramatic differences between the genotypes (Table 3.1; Fig. 3.3). Snakes homozygous for $Na_v1.4_{anc}$ show moderate levels of TTX resistance ($\bar{x}$ = 18.97, SD = 8.58) while those heterozygous for $Na_v1.4_{LVNV}$ and $Na_v1.4_{anc}$ or homozygous for $Na_v1.4_{LVNV}$ display extremely high resistance ($\bar{x}$ = 100.00, SD = 0; $\bar{x}$ = 100.00, SD = 0). Snakes with these later two genotypes are significantly less affected by TTX than those homozygous for $Na_v1.4_{anc}$ (t-value = -45.54, $P < 0.0001$; t-value = -46.31, $P < 0.0001$), but indistinguishable from one another (t-value = 0, $P = 1$). Thus, the derived $Na_v1.4_{LVNV}$ allele appears to exhibits complete dominance ($h = 0$). Our estimate of dominance is admittedly rough because I set all the 50% MAMU scores of highly resistant *T. sirtalis* to the same value (lowest common dose), forcing heterozygotes and dominant homozygotes to appear identical (see Materials and Methods). At the molecular genetic level, it seems reasonable to assume that heterozygotes are less resistant to TTX because half of the skeletal muscle sodium channels in these snakes should have been translated from the $Na_v1.4_{anc}$ allele. Yet at the organismal level, *T. sirtalis* heterozygous or homozygous for $Na_v1.4_{LVNV}$ appear so immune to TTX that these genotypes may be functionally

equivalent. Globally, the relationship between variation in $Na_v1.4$ and TTX resistance in these *T. sirtalis* populations is strong (F-value = 1330.820, $P < 0.0001$), with a remarkable PVE (98%) by the genotype (Table 3.2). However, our procedure of setting all highly resistant *T. sirtalis* to the same 50% dosage created a very homogenous dataset and I consider this PVE an overestimate.

Here, $Na_v1.4$ is clearly a large effect locus, explaining 29% to 98% of TTX resistance in *T. atratus* and *T. sirtalis*, respectively. Nevertheless, allelic variation in $Na_v1.4$ cannot solely account for whole animal resistance in garter snakes. First, the proportion of the phenotype explained by the genotype in *T. atratus* suggests the contribution of additional loci or molecular mechanisms. Second, $Na_v1.4$ expression is confined to skeletal muscle tissue (Trimmer et al., 1989, 1990; Goldin, 2001), yet the central nervous system and some peripheral nerves are sensitive to TTX (Goldin, 2002; Geffeney and Rubin, 2006). It seems evident that these tissues must also be defended against TTX to produce a fully resistant phenotype. I propose two, non-exclusive hypotheses to explain TTX resistance at the organismal level in garter snakes: 1) changes in the spatial patterns of Na_v expression; 2) parallel changes in P-loops across the Na_v gene family.

In mammals, nine different Na_v genes are functionally expressed in specific tissues with excitable cells (Plummer and Meisler, 1999; Goldin, 2001; Hille, 2001). A third of these ($Na_v1.5$, $Na_v1.8$, $Na_v1.9$) are natively resistant to TTX (Goldin, 2002; Geffeney and Rubin, 2006). Furthermore, regional expression profiles change during organismal ontogeny, with some Na_v loci dominating certain tissues during early development then quickly yielding to other gene family members as the organism

matures (Beckh et al., 1989; Trimmer et al., 1990; Goldin, 2001). Thus, modification of the Na_v regulatory apparatus could produce resistant tissues through suppression of TTX sensitive sodium channels and expression of resistant channels. However, sodium channels appear highly specialized to their respective tissues (Goldin, 2001; Hille, 2001), so drastic alterations to the spatial expression of Na_v genes might disrupt proper tissue function (Geffeney and Rubin, 2006).

Alternatively, or perhaps in conjunction with minor changes in sodium channel expression, other TTX sensitive Na_v loci may have encountered adaptive mutations similar to those in derived $Na_v1.4$ alleles. Because even single mutations can dramatically alter TTX binding affinity (e.g., Noda et al., 1989; Terlau et al., 1991; Geffeney et al., 2005; Tikhonov and Zhorov, 2005; Venkatesh et al., 2005), only slight modification of central and peripheral nerve Na_v genes may be required. Additionally, most Na_v paralogs are physically close to their nearest relatives (Plummer and Meisler, 1999; Goldin, 2002; Novak et al., 2006), and in mammals, seven of the nine genes are regionally clustered within two chromosomes (Plummer and Meisler, 1999; Goldin, 2002). Thus, coincident P-loop changes in separate sodium channel genes might immediately become linked. If recombination rates are low, selection could easily fix such linkage groups in populations before recombination breaks apart adaptive allelic combinations.

I favor this second hypothesis of convergent gene family evolution to explain whole animal TTX resistance in garter snakes and suggest that both *T. atratus* and *T. sirtalis* demonstrate the extremes of this scenario. In the case of *T. sirtalis*, derived Na_v variants have become (or remain) linked, forming a coadapted gene complex that

produces an extreme phenotype. In *T. atratus*, on the other hand, recombination has probably disassociated the linkages between TTX resistant alleles at separate Na_v loci. Some *T. atratus* will still inherit this suit of adaptive variation and possess the "Na_v supergene" that produces acute resistance. Others will possess varying degrees of mismatch between resistant Na_v genes and express corresponding levels of TTX resistance. Identification and characterization of the P-loops in all TTX sensitive sodium channels in *Thamnophis* should eventually provide a complete picture of the molecular mechanisms responsible for elevated TTX resistance.

Simple Genetic Architecture and Coevolution

Most phenotypic traits are thought to be governed by a complex interplay between many genes (Falconer and Mackay, 1996). However, it has become increasingly clear that complex phenotypic traits are often controlled by a small number of loci of major effect (e.g., Doebley and Stec, 1991; Cohn and Tickle, 1999; Abzhanov et al., 2004; Alberston et al., 2005; Steiner et al., 2007; Sutter et al., 2007). While our view of the genetic architecture of adaptations is rapidly changing, both the infinite alleles and quantitative trait locus (QTL) viewpoints are certainly valid (Orr and Coyne, 1992). Each genetic model also makes specific predictions about the tempo and mode of evolution. Adaptation can occur rapidly and through large steps when the genetic architecture of a trait under selection is relatively simple (e.g., Hawthorne and Via, 2001; Peichel et al., 2001; ffrench-Constant et al., 2004; Albertson and Kocher, 2006). Fixation of standing variation or a few new mutations of large effect can rapidly drive phenotypic evolution (Barrett and Schluter, 2008; e.g., Colosimo et al., 2005; Hartley et al., 2006).

Yet phenotypic evolution will proceed more slowly and through incremental steps if adaptive traits are controlled by numbers of loci (Falconer and Mackay, 1996). Polygenetic adaptations will require many mutations, most of which are expected to be recessive and take longer to spread to fixation than dominant alleles (Falconer and Mackay, 1996). Furthermore, some loci might be constrained by epistatic interactions or pleiotropy, others might experience different mutation or recombination rates, back-mutating or picking up disadvantageous recombinants or mutations while beneficial alleles fix at other loci. So unless multiple loci change in concert, adaptive evolution should proceed gradually.

The genetic architecture of adaptations is certainly important in understanding the dynamics of coevolution as well (Bohannan and Lenski, 2000; Thompson, 2005; Wade, 2007; e.g., Yoshida et al., 2007). Cycles of arms-race evolution wherein predator (exploiter) phenotypes continually "chase" prey (victim) phenotypes are expected regardless of whether the genetic architecture underlying the traits is simple or complex (Sasaki, 2000; Agrawal and Lively, 2002, 2003; Kopp and Gavrilets, 2006; Nuismer et al., 2007). However, when the traits that mediate the coevolution have a simple genetic basis, reciprocal selection can more easily fix all the adaptive alleles, leading to the evolution of extreme phenotypes in which the prey "loses" the arm-race (Sasaki, 2000; Kopp and Gavrilets, 2006; Nuismer et al., 2007). Conversely, coevolutionary cycles are predicted to be more stable and dynamic under a multilocus model because adaptive alleles in the predator rarely change in harmony, allowing the prey to stay "ahead" in the arms-race (Kopp and Gavrilets, 2006; Nuismer et al., 2007). Thus, detailing the genetic architecture of the traits that mediate the coevolution is vital in understanding not only

the rate and direction of phenotypic evolution, but also in considering the outcome of coevolution.

The molecular basis of adaptive TTX resistance in *Thamnophis* appears relatively simple, probably involving a few replacements in a handful of genes. Simple genetic architecture underlying the traits that mediate the coevolution should allow for a rapid back-and-forth evolutionary arms race, but also potential "escape" from the arms race by the predator (Sasaki, 2000; Kopp and Gavrilets, 2006; Nuismer et al., 2007). Though we only have a handle on the genetics underlying the predatory counter adaptation, both of these predictions appear to have been met. Coevolution between *T. sirtalis* and *T. granulosa* in the Pacific Northwest seems to have initiated only recently, probably during the Late Pleistocene (Janzen et al., 2002; Ridenhour et al., 2007). A range-wide survey of trait matching between *T. sirtalis* and *Taricha* showed that one-third of western *T. sirtalis* populations possess such acute TTX resistance that they are essentially "winning" the arms-race (Hanifin et al., 2008). Characterization of additional of Na_v loci in *Thamnophis* and continued phenotype-genotype matching at the population level will help determine whether the evolution of a Na_v supergene in garter snakes frequently creates an insurmountable counter measure to which newts cannot respond, or whether recombination and gene flow consistently decouple adaptive alleles and keep newts competitive in this arms race.

TABLE 3.1. Differences in TTX resistance between $Na_v1.4$ genotypes in central California populations of *T. atratus* and *T. sirtalis*. Differences assessed with *post hoc* t-tests of the least squares means from a single factor ANOVA.

Taxon Genotypes compared	t-value	*P*-value
T. atratus		
$Na_v1.4_{anc}$ $Na_v1.4_{anc}$ vs $Na_v1.4_{N}$ $Na_v1.4_{anc}$	-1.01	0.3147
$Na_v1.4_{anc}$ $Na_v1.4_{anc}$ vs $Na_v1.4_{EPN}$ $Na_v1.4_{EP}$	-1.10	0.2748
$Na_v1.4_{anc}$ $Na_v1.4_{anc}$ vs $Na_v1.4_{EPN}$ $Na_v1.4_{EPN}$	-5.06	< 0.0001
$Na_v1.4_{N}$ $Na_v1.4_{anc}$ vs $Na_v1.4_{EPN}$ $Na_v1.4_{EP}$	-0.79	0.4306
$Na_v1.4_{N}$ $Na_v1.4_{anc}$ vs $Na_v1.4_{EPN}$ $Na_v1.4_{EPN}$	-3.77	0.0003
$Na_v1.4_{EPN}$ $Na_v1.4_{EP}$ vs $Na_v1.4_{EPN}$ $Na_v1.4_{EPN}$	-0.22	0.8254
T. sirtalis		
$Na_v1.4_{anc}$ $Na_v1.4_{anc}$ vs $Na_v1.4_{LVNV}$ $Na_v1.4_{anc}$	-45.54	< 0.0001
$Na_v1.4_{anc}$ $Na_v1.4_{anc}$ vs $Na_v1.4_{LVNV}$ $Na_v1.4_{LVNV}$	-46.31	< 0.0001
$Na_v1.4_{LVNV}$ $Na_v1.4_{anc}$ vs $Na_v1.4_{LVNV}$ $Na_v1.4_{LVNV}$	0	1

TABLE 3.2. Percentage of phenotypic variance explained by $Na_v1.4$ genotype (PVE) in central California populations of *T. atratus* and *T. sirtalis*. Variance components estimated with a single factor ANOVA.

Taxon	Genotype variance (sum of squares)	Total variance (sum of squares)	PVE (%)	F-value	*P*-value
T. atratus	339.730	1174.233	28.932	10.100	< 0.0001
T. sirtalis	2187.000	2213.768	98.791	1330.820	< 0.0001

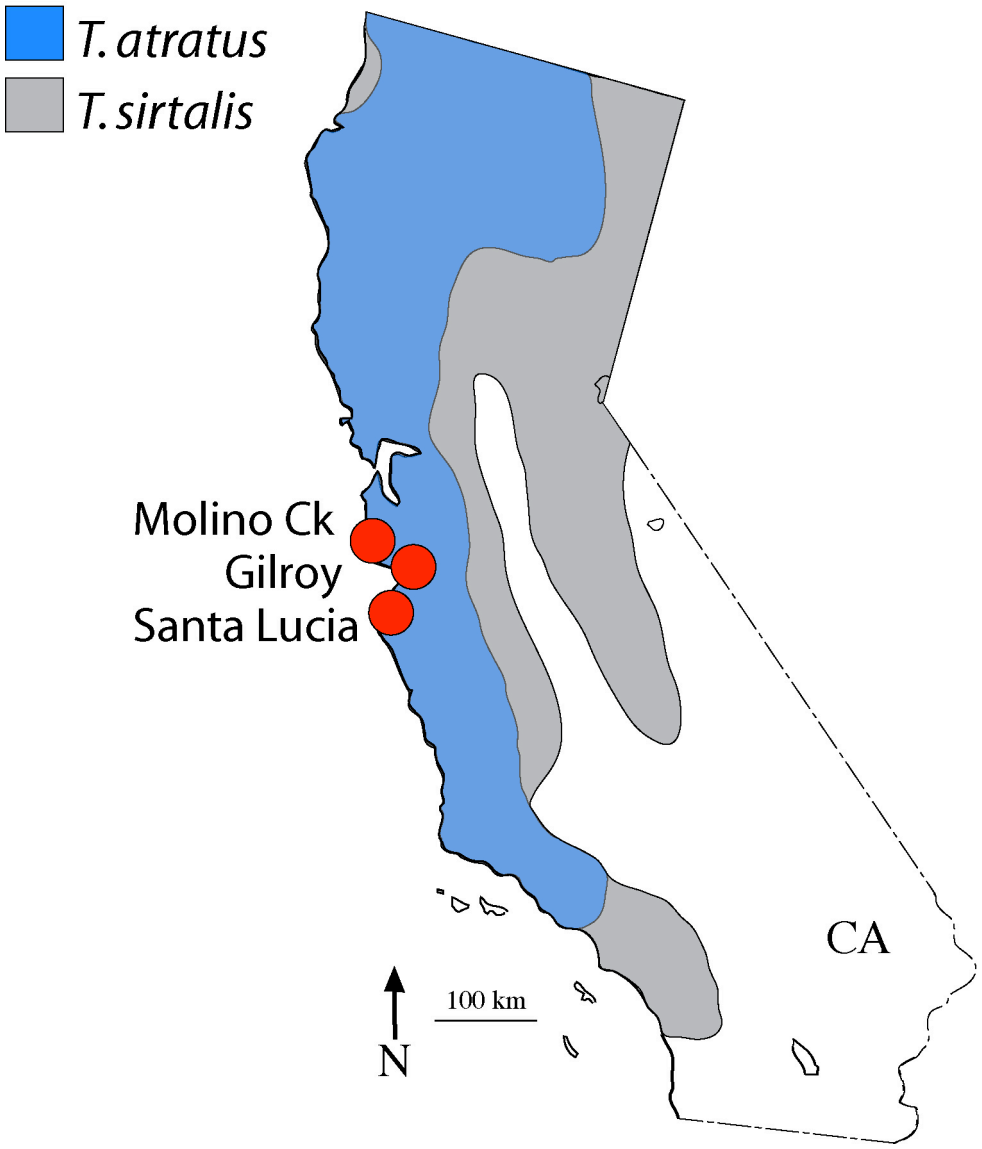

FIGURE 3.1. Geographic distribution and collection sites of *T. atratus* and *T. sirtalis* in California. Range of *T. atratus* (blue) and *T. sirtalis* (grey) follow Stebbins (2003); three focal populations (red) are: Molino Creek, Santa Cruz Co.; Gilroy, Santa Clara Co.; Santa Lucia Preserve, Monterey Co.

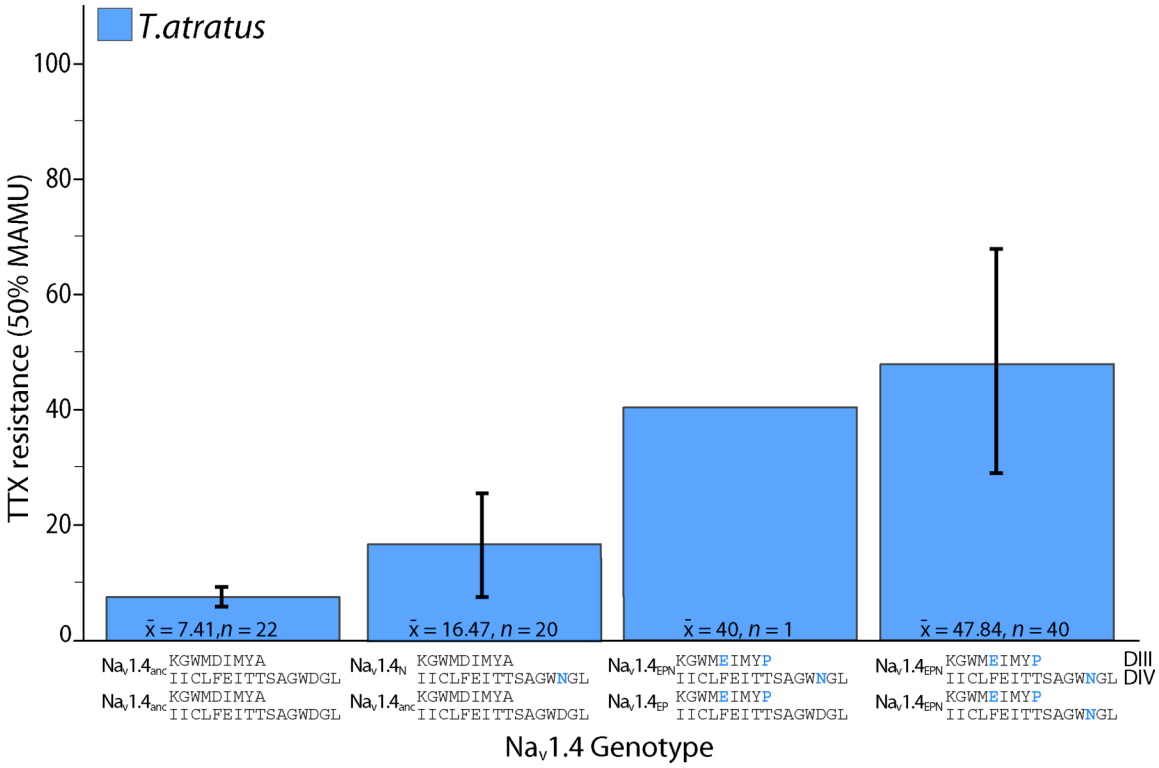

FIGURE 3.2. TTX resistance in central California populations of *T. atratus* allocated by $Na_v1.4$ genotype. Allele names and actual DIII and DIV P-loop sequences highlighting the critical residues that characterize those alleles are provided for each genotype, along with mean TTX resistance, standard deviation (error bars), and sample size.

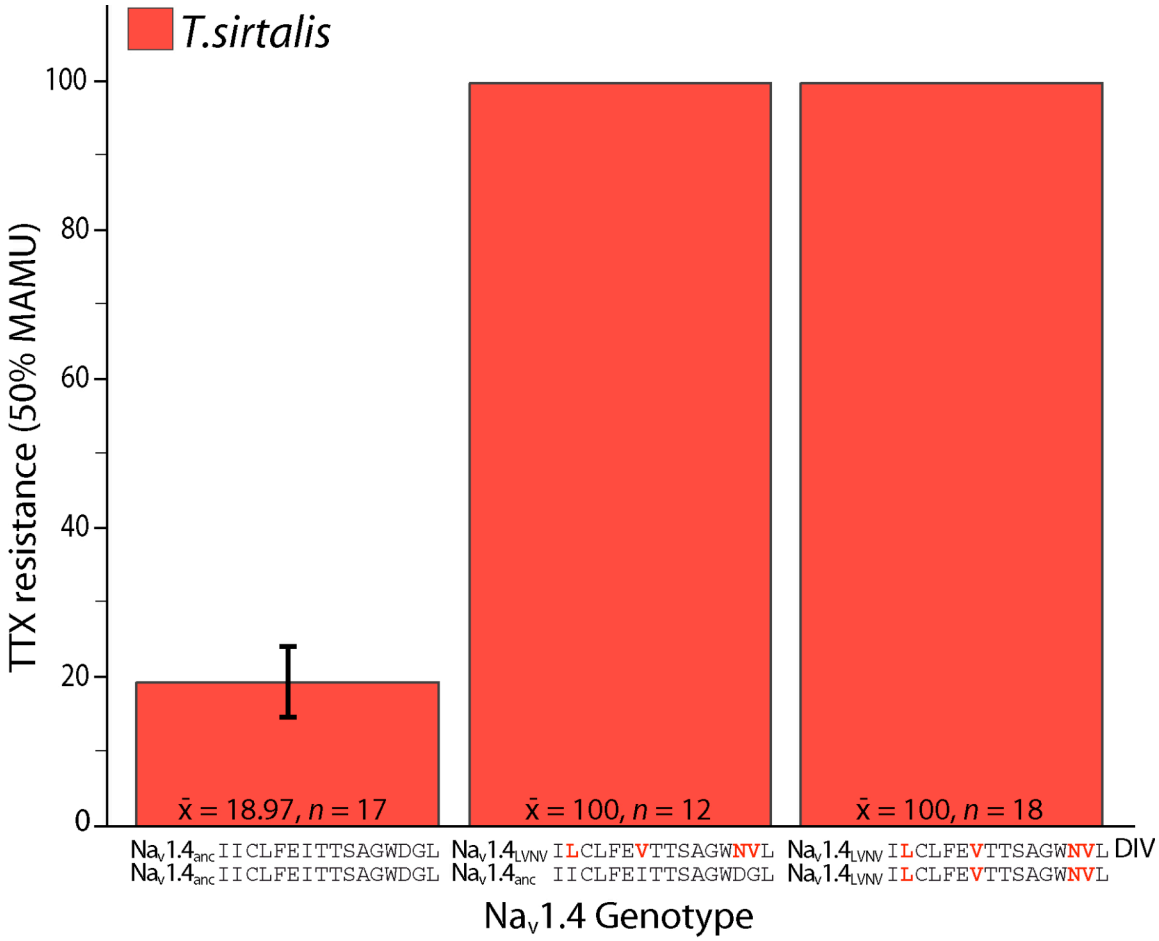

FIGURE 3.3. TTX resistance in central California populations of *T. sirtalis* allocated by $Na_v1.4$ genotype. Allele names and actual DIII and DIV P-loop sequences highlighting the critical residues that characterize those alleles are provided for each genotype, along with mean TTX resistance, standard deviation (error bars), and sample size.

CHAPTER 4

CONVERGENCE AND CONSTRAINTS IN SNAKE-AMPHIBIAN COEVOLUTION

INTRODUCTION

Darwin (1859) considered evolutionary convergence to be a highly unlikely outcome of the process of natural selection, yet the repeated evolution of similar phenotypes in response to similar ecological pressures provides some of the most compelling evidence of adaptive evolution. Striking examples such as replicated adaptations in microbial populations (Wichman et al., 1999; Cooper et al., 2003; Woods et al., 2006), the rapid appearance of identical insecticide resistance across diverse taxa (ffrench-Constant, 1994; ffrench-Constant et al., 2004), the repeated evolution of specific ecomorphologies in lizard (Losos et al., 1998, 2003) and fish (Meyer et al., 1990; Meyer, 1993) communities, and even redundancy in plant and animal guilds through time (Cowen, 2004) all suggest that phenotypic convergence is a pervasive evolutionary trend. What is less clear, however, is the role evolutionary constraints play in producing phenotypic similarity (Maynard Smith et al., 1985; Wake, 1991; Brakefield and Roskam, 2006; Brakefield, 2007).

Evolutionary constraints, that is, physical, structural, or genetic (including development) factors that restrict or bias the amount and pattern of variation upon which natural selection can act (Futuyma, 1998) have certainly influenced the tempo and mode of evolution (Maynard Smith et al., 1985; Wake, 1991; Brakefield and Roskam, 2006; Brakefield, 2007). Ideally, a quantitative genetics approach would be instrumental to the study of constraints by helping define the adaptive landscape of traits, detailing the

variation accessible to natural selection, and thus predicting the genetic and phenotypic response (Arnold, 1992). Yet there are few empirical examples of an evolutionary constraint at the molecular level because tracing the origin and fitness consequence of each failed or successful mutation through time is rarely feasible (Miller et al., 2006; Weinreich et al., 2006). An effective alternative for assessing the role of constraints is to look for bias in the outcome of replicated events against a background of possible solutions. Aside from microbial systems experiencing artificial selection, natural systems wherein the same adaptation has arisen independently multiple times should provide a useful vehicle for the study of evolutionary constraints. What is additionally required is a detailed knowledge of the ecology, genetics, and phylogeny of the adaptation to understand whether evolution has been restricted or unencumbered.

I investigated the genetic basis of convergent adaptations in snake-amphibian coevolution. Snakes are the chief vertebrate predators of amphibians (Greene, 1997; Toledo et al., 2007), which rely heavily on noxious or poisonous secretions for defense (Brodie, 1983; Duellman and Treub, 1986). Because snakes do not masticate and lack limbs, they cannot mechanically separate the toxic portions of amphibians, but must consume prey whole and thus become fully exposed to amphibian toxins (Williams et al., 2003). Nevertheless, a number of snake and amphibian taxa have entered coevolutionary arms-races characterized by adaptation and counter-adaptation (Greene, 1997). Here, I focus on the coevolution between snakes that prey on frogs and salamanders that possess tetrodotoxin (TTX) because the neuro-physiological effects of TTX are understood (Hille, 2001), the interaction between one predator-prey pair is well characterized (Brodie et al., 2002; Hanifin et al., 2008), and knowledge of the biochemical basis of TTX

resistance is emerging (Terlau et al., 1991; Kontis and Goldin, 1993; Penzotti et al., 1998; Yamagishi et al., 2001; Choudhary et al., 2003; Tikhonov and Zhorov, 2005; Scheib et al., 2006; Santarelli et al., 2007). Tetrodotoxin binds to the pore of voltage-gated sodium channels (Na_v) in nerves and muscles, blocking the movement of sodium ions (Na^+) into these cells and halting action potentials that control nerve impulses (Kao and Levinson, 1986; Hille, 2001). By paralyzing nerves and excitable muscle cells, TTX causes immobilization, respiratory failure, and often, death (Brodie, 1968; How et al., 2003; Isbister and Kiernan, 2005). However, in some populations of the garter snake *Thamnophis sirtalis* that prey on the TTX bearing newt *Taricha granulosa*, muscle sodium channels ($Na_v1.4$) are resistant to TTX (Geffeney et al., 2002). Examination of $Na_v1.4$ in resistant *T. sirtalis* and pufferfish that possess TTX shows that amino acid substitutions at the outer pore changes the binding affinity of TTX to this sodium channel (Geffeney et al., 2005; Venkatesh et al., 2005; Maruta et al., 2008).

To understand the role of evolutionary constraints in snake-amphibian coevolution, I examined the genetic underpinnings of TTX resistance across a number of unique snake lineages. Specifically, I determined whether repeated phenotypic evolution results from convergent genetic changes more frequently than would be expected by chance, and the possible sources predisposing snakes to repeated genetic responses. If adaptive evolution is unencumbered by biophysical or genetic constraints then we might expect the repeated evolution of similar phenotypes to occur through dissimilar genetic pathways because of the unique evolutionary history and genetic makeup of independent species, the stochastic nature of mutation, redundancy in developmental systems, and the over-riding power of natural selection (Gould, 1989a; True and Haag, 2001; Budd, 2006).

However, if adaptive evolution is often channeled along genetic lines of least resistance (Schluter, 1996; Schluter et al., 2004; Brakefield and Roskam, 2006; Brakefield, 2007), constrained by the biophysical properties of interacting molecules (DePristo et al., 2005; Miller et al., 2006; Weinreich et al., 2006), or limited by developmental and structural constraints (Maynard Smith et al., 1985; Gould, 1989b; Wake, 1991), then we might expect the independent evolution of phenotypic similarity to occur commonly through the same genetic steps. A relatively narrow genetic response of independent lineages to the same selective pressure could be taken as the genetic signature of an evolutionary constraint. Here, the chemically mediated coevolution involves prey species that share a common deadly toxin, so their respective predators might have responded by evolving the same means of circumventing the toxin, or they might have evolved different mechanisms of exploitation. Because TTX binds so selectively to the pore of nerve and muscle sodium channels, however, there may be only a few mechanistically similar adaptations that allow the predator species to overcome the toxin. Given an identical selective agent (TTX), possible biophysical constraints in circumventing that agent, similarities in the genetic architecture of the traits under selection due to common ancestry, and the often oligogenic nature of adaptations, I predict that convergent phenotypic evolution in this case has been tightly constrained to occur through the same genetic pathway. To test this prediction I examine the genetic underpinnings of TTX-mediated coevolution between snakes and amphibians from across the globe.

MATERIALS AND METHODS

Bioassays

To provide a phylogenetic perspective on the evolution of elevated TTX resistance in snakes, and aid in the interpretation of $Na_v1.4$ sequence data, I collected TTX resistance data from a diverse sample of colubroids (Appendix A). I collected data from 12 snake species ($n = 36$) representing most of the major colubroid lineages (Lawson et al., 2005; Wiens et al., 2008) and from one outgroup taxon ($n = 5$). I augmented these data with results from Brodie (1968), Motychak et al. (1999), Brodie et al. (2005), and Chapter 2 to provide the most complete picture of TTX resistance in squamate reptiles to date (Fig. 4.1). I measured TTX resistance using the same bioassay of whole organism performance detailed in Chapter 2 (see also Brodie and Brodie, 1990; Brodie et al., 2002; Ridenhour et al., 2004).

Sequence Data

To determine whether snake lineages have independently acquired TTX resistance through similar genetic modifications, I examined DNA sequence variation in portions of the four domains (DI-DIV) that code for the outer pore (P-loops) of $Na_v1.4$. I sequenced snakes known to prey on TTX laden amphibians, their sister groups, and additional taxa to provide a robust phylogenetic perspective (Fig. 4.1). I focused on the P-loops because TTX interacts with residues of the outer pore (Lipkind and Fozzard, 2000; Hille, 2001) and changes in some of these sites in $Na_v1.4$ are at least partly responsible for TTX resistance in *T. sirtalis* (Geffeney et al., 2005) and TTX bearing

pufferfish (Venkatesh et al., 2005). I followed the same laboratory and data editing procedures outlined in Chapter 2. I deposited all sequences in GenBank.

Assessing Constraints on $Na_v1.4$

Sodium channels are highly specialized proteins, and the amino acids that form the outer pore and selectivity filter interact in complex ways to create the optimal environment for the selective permeation of Na^+ ions (Hille, 2001). However, the same P-loop residues that permit selectivity and permeability of Na^+, also interact strongly with TTX through a combination of hydrogen and ionic bonds, steric attraction, and cation-π interaction (Terlau et al., 1991; Kontis and Goldin, 1993; Penzotti et al., 1998; Yamagishi et al., 2001; Choudhary et al., 2003; Tikhonov and Zhorov, 2005; Scheib et al., 2006; Santarelli et al., 2007). As such, changes that reduce the affinity of TTX to the outer pore will likely negatively impact the molecular sieving of the sodium channel. If antagonistic pleiotropy exists within $Na_v1.4$, then we should find convergence at the molecular level because there is probably a limited pool of replacements that can reduce TTX ligation and preserve channel function. Thus, to understand whether constraints have influenced the evolution of TTX resistance in snakes, I sought to: 1) quantify any tradeoff between TTX resistance and sodium channel function; 2) assess bias in the pattern of observed mutations.

I evaluated the potential tradeoff between TTX resistance and sodium channel performance by pulling data from studies that measured the effects of individual replacements on both TTX block and Na^+ permeability. I excluded reports that provided only ratios from which we could not calculate actual values of either TTX resistance or

Na^+ conductance. This left three studies of site directed mutagenesis (Terlau et al., 1991; Backx et al., 1992; Yamagishi et al., 2001) where the data on TTX sensitivity are inhibition concentrations of TTX (nM) required to reduce peak Na^+ current by 50% (IC_{50}) in the wild type and single mutant channels, and the data on Na^+ permeability are measures of single channel conductance (pS). I log-transformed the TTX data, and adjusted the Na^+ conductance data of each study by setting wild type measures to 1 and scaling the conductance values of each mutant to its respective wild type. I then performed a simple regression on all the data ($n = 31$) in StatView 5.0.1. (SAS Institute Inc.).

Ionic selectivity is also determined by the molecular architecture of the outer pore and is an essential function the protein. Unfortunately, few studies provided data on both TTX sensitivity and Na^+ selectivity in a manner that I could use to estimate functional tradeoffs. However, Terlau et al. (1991) modified the DIII selectivity filter (K), the single most important residue determining selectivity of the sodium channel; a change at this site completely abolishes Na^+ selectivity (Heinemann et al., 1992; Favre et al., 1996; Chen et al., 1997; Sun et al., 1997; Tsushima et al., 1997). Thus, I removed this mutant and again conducted a simple regression on the data ($n = 30$) in an attempt to add another variable in our estimate of functional tradeoffs. Nevertheless, future efforts are needed to examine Na^+ selectivity, permeability, and TTX resistance to provide a more complete picture of the contrasting demands on Na_v loci and the limited range of mutational options open to selection.

Second, I determined whether the pattern of mutations we observed in snakes is clustered or follows a random distribution. If the observed mutations are not distributed

randomly among P-loop sites, but are instead clustered, then we have evidence that the genetic response of snakes has been narrowed. I tallied the number of times a site was hit by a mutation for two sets of potentially available sites: 1) all sites of the P-loops ($n = 96$) (Lipkind and Fozzard, 2000); 2) only sites experimentally verified to reduce TTX sensitivity two-fold versus wild type ($n = 33$) (Terlau et al., 1991; Backx et al., 1992; Kontis and Goldin, 1993; Pérez-García et al., 1996; Chen et al., 1997; Penzotti et al., 1998; Yamagishi et al., 2001; Carbonneau et al., 2002; Choudhary et al., 2003), but also including sites with parallel substitutions between snakes and pufferfish still unstudied ($n = 2$) (Yotsu-Yamashita et al., 2001; Venkatesh et al., 2005). I then used a simple binomial test to compare the distribution of our data against the null expectation of a Poisson distribution. I calculated the mean and variance of the samples and then the coefficient of dispersion (CD = variance/mean). In a Poisson distribution the mean and variance are roughly equal (CD = 1), while in a clumped distribution the variance should be much greater than the mean (CD > 1) (Krebs, 1999). Because the CD is approximately χ^2 distributed (Krebs, 1999) I calculated χ^2 scores for our CD measures to obtain *P*-values against the random expectation (CD = 1). Here $\chi^2 = I(n - 1)$ where I is the CD and $n - 1$ is the degrees of freedom (DF), in this case the number of sites minus 1 (Krebs, 1999).

Results and Discussion

TTX-Mediated Snake-Amphibian Coevolution

The neurotoxin TTX is ecologically and taxonomically widespread in marine environments, yet in terrestrial ecosystems the poison occurs only in amphibians (Daly,

2004). Here, TTX is found across diverse amphibian lineages, from ambystomatid salamanders (Yotsu et al., 1990) to dendrobatid and brachycephalid frogs (Daly et al., 1994; Pires et al., 2005). As one of the most lethal, naturally occurring toxins known (Medinsky and Klaassen, 1996), TTX is a powerful chemical weapon in the amphibian defensive arsenal. Nevertheless, a single group of predators has repeatedly overcome this chemical defense; snakes are the only group of vertebrates known to consume TTX laden prey (Brodie et al., 2002). Here I identify the major cases of TTX-mediated coevolution between snakes and amphibians, and suggest these interactions are widespread but have received little attention.

In western North America, coevolutionary interactions are well established between Pacific newts (*Taricha*) and three garter snake species (*Thamnophis*): *T. sirtalis* take *T. granulosa* (Brodie, 1968) and show varying levels of physiological resistance to TTX (Brodie et al., 2002); *T. couchii* prey on *T. torosa* (Brodie et al., 2005) and *T. sierrae* (Wiseman and Pool, 2007) and are resistant to TTX at levels seen in sympatric *T. torosa* (Brodie et al., 2005); *T. atratus* consume *T. granulosa* (Greene and Feldman, 2008) and probably *T. torosa* (Fox, 1951) and possess high levels of TTX resistance (Chapter 2, 3). The landscape mosaic of match and mismatch between prey (TTX) and predator (TTX resistance) traits provides a foundation for the study of the ecological and evolutionary dynamics of newt-garter snake coevolution (Brodie and Brodie, 1999; Brodie et al., 2002; Hanifin et al., 2008). Yet parallel interactions between other sets of ecologically and phylogenetically separate snake and amphibian pairs may provide novel insights in our understanding of coevolution.

The East Asian natricine, *Rhabdophis tigrinus*, preys heavily on frogs (Mori and Moriguchi, 1988; Mori et al., 1992; Hirai, 2004) and includes the treefrog *Polypedates leucomystax* in its diet (Zhao et al., 1998). Recently, members of the rhacophorid tree frog genus *Polypedates* (cf *P. leucomystax*; P. Janzen pers. comm.) were found to possess low to high levels of TTX (30–920 Mouse Units (MU); Tanu et al., 2001). Another natricine, the Japanese *Amphiesma pryeri*, takes the newt *Cynops ensicauda* (Mori and Moriguchi, 1988; Goris and Maeda, 2004), which contains moderate to acute levels of TTX (60–7000 MU; Mosher et al., 1964; Wakely et al., 1966; Yotsu et al., 1990; MU estimates from Daly, 2004). The Eurasian genus *Natrix*, sister group to North American natricines (Alfaro and Arnold, 2001; Lawson et al., 2005), contains a few amphibian specialists known to feed on sympatric *Triturus* newts (Arnold and Ovenden, 2002). For example, some Italian populations of *N. natrix* prey heavily on *T. alpestris* and *T. carnifex* (Luiselli et al., 1997; Filippi and Luiselli, 2002), and *N. megalocephala* of the Caucuses is a predator of *T. vittatus* (Szczerbak, 2003). Levels of TTX recorded in newts of the genus *Triturus* range from negligible to moderate amounts (<1–190 MU; Mosher et al., 1964; Wakely et al., 1966; Yotsu et al., 1990; MU estimates from Daly, 2004). The Central and South American xenodontine, *Liophis epinephalus*, is a frog specialist known to take a number of highly dangers prey (Toledo et al., 2007) including several species of *Atelopus* toads (Myers et al., 1978; Greene, 1997) that possess TTX and similar alkaloids (10-100 MU; Kim et al., 1975; Daly et al., 1994; MU estimates from Daly, 2004). Several East Asian pitvipers also consume TTX bearing prey. In Japan, *Gloydius blomhoffii* takes *Cynops pyrrhogaster* and *Protobothrops flavoviridis* preys on

C. ensicauda (Mori and Moriguchi, 1988; Goris and Maeda, 2004), while Taiwanese *P. stejnegeri* is known to eat *Polypedates* (Creer et al., 2002).

Mapping TTX resistance data and diet records onto the colubroid phylogeny indicates that high sensitivity to TTX is the ancestral condition, and that TTX resistance has originated repeatedly in snakes (Fig. 4.1). Elevated TTX resistance has evolved independently at least five times within natricines and once in xenodontines. If we consider all the known prey records, then elevated TTX resistance has probably evolved twice in pitvipers and possibly once among members of *Natrix* (not mapped because I could not acquire live or genetic material). The parallel arms-races between potentially lethal prey and resistant predators has evolved independently across the globe in diverse amphibians and snakes. Unfortunately, TTX detection is difficult (Daly, 2004), and the cryptic nature of snakes leads to only rare observations of feeding in the wild (Greene, 1997). Thus, I suspect numerous coevolutionary interactions between TTX bearing amphibians and their ophidian predators await discovery.

Genetic Basis of TTX Resistance in Snakes

I examined the genetic underpinnings of TTX resistance in snakes by characterizing molecular changes in the skeletal muscle sodium channel gene $Na_v1.4$. This locus produces a channel forming protein essential in muscle function that TTX selectively blocks (Hille, 2001). A great deal of literature on the architecture of Na_v loci suggests that TTX fits into the outer pore of the channel (see Lipkind and Fozzard, 2000; Hille, 2001), and replacements at certain residues in the pore dramatically alter TTX binding affinity (Noda et al., 1989; Terlau et al., 1991; Kontis and Goldin, 1993; Pérez-

García et al., 1996; Chen et al., 1997; Penzotti et al., 1998; Yamagishi et al., 2001; Choudhary et al., 2003).

Consistent with the critical functional role of $Na_v1.4$, the amino acids of the pore forming structures (pore α-helix, selectivity filter, β-strand; Lipkind and Fozzard, 2000) are highly conserved across colubroid snakes and nearly identical to mammalian sequences (though I detected appreciable variation N-terminal to the DII and DIII α-helices that may warrant further investigation). Excluding TTX resistant taxa, I found only eight mutations in the P-loops of squamates: three reversals or plesiopmorphies shared with mammals (*Enhydris* DI; *Causus* DII; Colubroidea DIV); three autapomorphies (*Charina* DI, DIV; *Elapsoidea* DII); and two variable sites in DII that may reflect phylogeny or unsorted polymorphism. None of these sites are thought to be important in TTX ligation, though it is interesting to note that non-colubroid reptiles share an E1560Q (positions follow $Na_v1.4$ CDS from *T. sirtalis* AY851746) and also display heightened sensitivity to TTX compared to colubroids (Fig. 4.1).

I found twelve derived mutations in the six species that consume TTX bearing prey, greater variation than that seen across all squamate reptiles (Fig. 4.2). Of the twelve replacements, eight occur in amino acids of the β-strand, whose side chains face the pore thus directly interact with TTX (Lipkind and Fozzard, 1994, 2000). Additionally, seven out of the twelve substitutions are confined to the same three sites, two of which involve substitutions to the same amino acid. A detailed discussion of the P-loop changes in *Thamnophis* can be found elsewhere (Geffeney et al., 2005; Chapter 2), here I briefly describe novel discoveries in the Old World natricines *A. pryeri*, *R. tigrinus*, and the New World xenodontine *L. epinephalus*.

Japanese *A. pryeri* share a single D1277E substitution in the β-strand of DIII. The replacement involves biochemically similar amino acids, and mutations at this position generally lead to only minor changes in TTX binding affinity (Terlau et al., 1991; Pérez-García et al., 1996; Choudhary et al., 2003). However, it is worth pointing out that a substitution also involving similar amino acids at a position thought to have little reactivity with TTX still produced a dramatic reduction in TTX binding affinity; the I1561V substitution seen in some *T. sirtalis* reduced the sensitivity of $Na_v1.4$ to TTX by 50% (Geffeney et al., 2005). Furthermore, an identical D→E substitution occurs in highly resistant *T. atratus* (Chapter 2) and in $Na_v1.4b$ of the TTX bearing pufferfish, *Tetraodon* (Venkatesh et al., 2005), again suggesting this replacement provides some level of TTX resistance.

The *Rhabdophis tigrinus* from Korea holds an I1555M change in DIV α-helix. The substitution involves relatively similar amino acids, but the loss of a rigid alphatic side chain and gain of a larger functional group may slightly alter the orientation or conformation of the α-helix. While this position is also quite superficial, a number of putatively extracellular sites clearly interact with TTX (Kontis and Goldin, 1993; Yamagishi et al., 2001; Carbonneau et al., 2002; Choudhary et al., 2003; Geffeney et al., 2005) and I1555 is considered a TTX sensing residue (Tikhonov and Zhorov, 2005). Perhaps most telling is the fact that an identical replacement is seen in $Na_v1.4b$ of the pufferfish *Tetraodon* and *Takifugu* (Venkatesh et al., 2005).

The Neotropical *L. epinephalus* displays two flanking mutations in the β-strand of DIV. The first, a D1568S, occurs at a site known to play a major role in TTX ligation (Terlau et al., 1991; Pérez-García et al., 1996; Chen et al., 1997; Penzotti et al., 1998;

Choudhary et al., 2003; Tikhonov and Zhorov, 2005; Scheib et al., 2006). A hydrogen bond is thought to form between TTX and D1568 (Chen et al., 1997; Yotsu-Yamashita et al., 1999; Choudhary et al., 2003; Tikhonov and Zhorov, 2005; Scheib et al., 2006), so changes that neutralize the charged D1568 should dramatically reduce TTX affinity to the outer pore. Substitutions at this position in rat $Na_v1.2$ and $Na_v1.4$ require 30 to 500 times the amount of TTX to block Na^+ current (Terlau et al., 1991; Pérez-García et al., 1996; Chen et al., 1997; Penzotti et al., 1998; Choudhary et al., 2003). Interestingly, *L. epinephalus* shares a change at D1568 with the two highly resistant garter snakes, *T. atratus* and *T. sirtalis*, though to a different amino acid (Geffeney et al., 2005; Chapter 2). The second P-loop change seen in *L. epinephalus* is a G1569D substitution. Similar replacements occur in TTX resistant *T. sirtalis* (Geffeney et al., 2005) and $Na_v1.4a$ of pufferfish (Venkatesh et al., 2005; Maruta et al., 2008). Functional expression in rat $Na_v1.2$ of one of the naturally occurring pufferfish replacements required a 2 to 3-fold increase in TTX to block Na^+ current (Maruta et al., 2008). Given current understanding of the docking orientation of TTX (Choudhary et al., 2003; Tikhonov and Zhorov, 2005; Scheib et al., 2006), drastic changes at G1569 should alter the positioning or fit of TTX into the outer pore and may further weaken the affinity of TTX to the neighboring N1568 residue (Maruta et al., 2008). In *L. epinephalus*, the G1569D substitution involves dramatically different amino acids and likely changes the conformation of the external mouth of the outer pore and possibly pore volume because D is large, charged and hydrophobic, whereas G is tiny, uncharged and hydrophilic.

Finally, it is interesting to note that I did not detect adaptive variation in *Natrix*. However, some species of *Natrix* show extensive geographic variation in diet (Arnold

and Ovenden, 2002; Luiselli et al., 2005) and predation on newts seems to be a restricted local phenomenon (Luiselli et al., 2005). Furthermore, most newts of the genus *Triturus* possess only low to only trace amounts of TTX (Mosher et al., 1964; Wakely et al., 1966; Yotsu et al., 1990; Daly, 2004). Thus it is uncertain whether my samples of *N. maura* and *N. natrix* represent newt feeding populations, and to what degree physiological resistance is required to consume European newts.

Constraints on Convergent Evolution

At first glance natural selection may appear to play the dominant role driving phenotypic convergence across independent lineages. However, such convergence might also bespeak biases inherent in the evolution of the traits under selection. There may be few available responses to selection because of potential biophysical or biochemical constraints on the traits under selection (Wake, 1991; DePristo et al., 2005; Miller et al., 2006; Weinreich et al., 2006) and commonalities in the genetic architecture underlying the phenotype (i.e., genetic channeling; Schluter, 1996), all of which might produce similarities in the adaptive landscape and bias trait evolution (Arnold, 1992; Arnold et al., 2001). Thus, both natural selection and evolutionary constraints are likely involved in convergent evolution (Wake, 1991; Schluter et al., 2004; Brakefield, 2007).

Despite the intuitive relevance of constraints, their role in evolution remains contentious because few have convincingly demonstrated a constraint working with or against natural selection to bias character evolution (Pigliucci and Kaplan, 2000; Brakefield and Roskam, 2006; Brakefield, 2007; e.g., Miller et al., 2006; Weinreich et al., 2006). One roadblock is that addressing the role of constraints ideally requires

knowledge of the genetic and epigenetic causes of traits and how those traits relate to fitness, as well as information on the potential sources of constraints to adequately describe trait space and fitness landscapes (Arnold, 1992; Pigliucci and Kaplan, 2000; Brakefield and Roskam, 2006; Miller et al., 2006; Brakefield, 2007). In snake-amphibian coevolution, the ecological players are long-lived and often cryptic taxa, so collecting population level fitness data to describe the adaptive landscape of TTX resistance may not be feasible. Nonetheless, TTX resistance is clearly adaptive (Motychak et al., 1999; Brodie et al., 2002; Chapter 2) and the trait appears to have a simple genetic basis (Geffeney et al., 2005; Chapter 3), so the evolution of TTX resistance may offer a tractable system for the study of constraints. Furthermore, a great deal of research on the molecular biology and neuro-physiology of the sodium channel provides us with a good picture of the adaptive topology of Na_v loci despite the absence of quantitative genetic data.

The molecular architecture of the outer pore of $Na_v1.4$ is strikingly conserved across snakes and mammals, suggesting intense stabilizing selection to preserve this design. Indeed, amino acid substitutions in the P-loops that alter TTX binding affinity to sodium channels also tend to either reduce Na^+ permeability ($r^2 = 0.417$, $P < 0.0001$), Na^+ selectivity, or both ($r^2 = 0.497$, $P < 0.0001$), due to the neutralization of negative charges that line the outer pore and changes in pore volume or aperture (Noda et al., 1989; Terlau et al., 1991; Backx et al., 1992; Chiamvimonvat et al., 1996; Chen et al., 1997; Sun et al., 1997; Yamagishi et al., 2001). The tradeoff between TTX sensitivity and sodium channel performance is readily apparent at the molecular level (Fig. 4.3), suggesting strong antagonistic pleiotropy within $Na_v1.4$. Such intramolecular pleiotropy should limit

selection to only those mutational options that both preserve pore structure (muscle function) and also defend against TTX.

If there are severe biophysical constraints on $Na_v1.4$, then the fitness landscape of this locus may have just a few peaks and natural selection will repeatedly move independent species up those paths. Thus, the repeated occurrence of adaptive mutations at the same few beneficial sites in $Na_v1.4$ would provide the genetic signature of constrained evolution. A noticeable departure from the null expectation of a Poisson distribution of mutations (random events) is observed whether I liberally considered all P-loop sites available for substitution (CD = 3.5626, $\chi^2 = 338.4483$, $P < 0.0001$) or conservatively just those sites verified to reduce TTX (CD = 1.5973, $\chi^2 = 54.3077$, $P = 0.015$). Instead, P-loop substitutions are underdispersed, and in TTX resistant taxa the majority of replacements (seven out of twelve) are confined to the same three sites in DIII and DIV, two of which involve substitutions to the same amino acid. The striking coincidence of replacements between resistant snakes and pufferfish (Yotsu-Yamashita et al., 2000; Venkatesh et al., 2005; Soong and Venkatesh, 2006) further strengthens the notion that the genetic response of snakes (and perhaps all TTX resistant vertebrates) has been restricted.

Phenotypic convergence may well be the hallmark of adaptive evolution, yet the primacy of natural selection should not be accepted without scrutiny (Gould and Lewontin, 1979; Pigliucci and Kaplan, 2000; Brakefield, 2007). Understanding biases in the generation and accessibility of variation are critical to resolving how evolutionary constraints work with or against natural selection to produce repeated patterns. Underlying similarities in codon bias, genetic variance (G_{max}), genome structure, and

other similarities due to common descent may bias the amount and pattern of variation available to natural selection (Arnold, 1992; Schluter, 1996; Schluter et al., 2004). The ability of natural selection to act on that variation may be further restricted through biophysical, biochemical, developmental, or pleiotropic constraints, predisposing some outcomes over others (Maynard Smith et al., 1985; Wake, 1991; Miller et al., 2006; Weinreich et al., 2006). In the snake-amphibian coevolution examined here, convergent molecular evolution appears to underlie the phenotypic convergence. Similar selective pressures, simple genetic controls for the traits under selection, and narrow fitness landscapes due to pleiotropy from the biophysical constraints on Na_v loci may all have conspired to push adaptive evolution along parallel paths of least resistance. Hence, a number of factors probably impose limits on adaptive evolution in these snakes, and there may be a ceiling to escalating arms-races between snake predators and amphibian prey (but see Hanifin et al., 2008). The extent to which the traits behind evolutionary convergence are constrained remains to be seen, but it may be that natural selection is often tightly bound, leading diverse evolutionary lineages along predestined genetic and phenotypic responses (e.g., Cresko et al., 2004; Prud' homme et al., 2006; Segré et al., 2006; Zhang, 2006; Tishkoff et al., 2007).

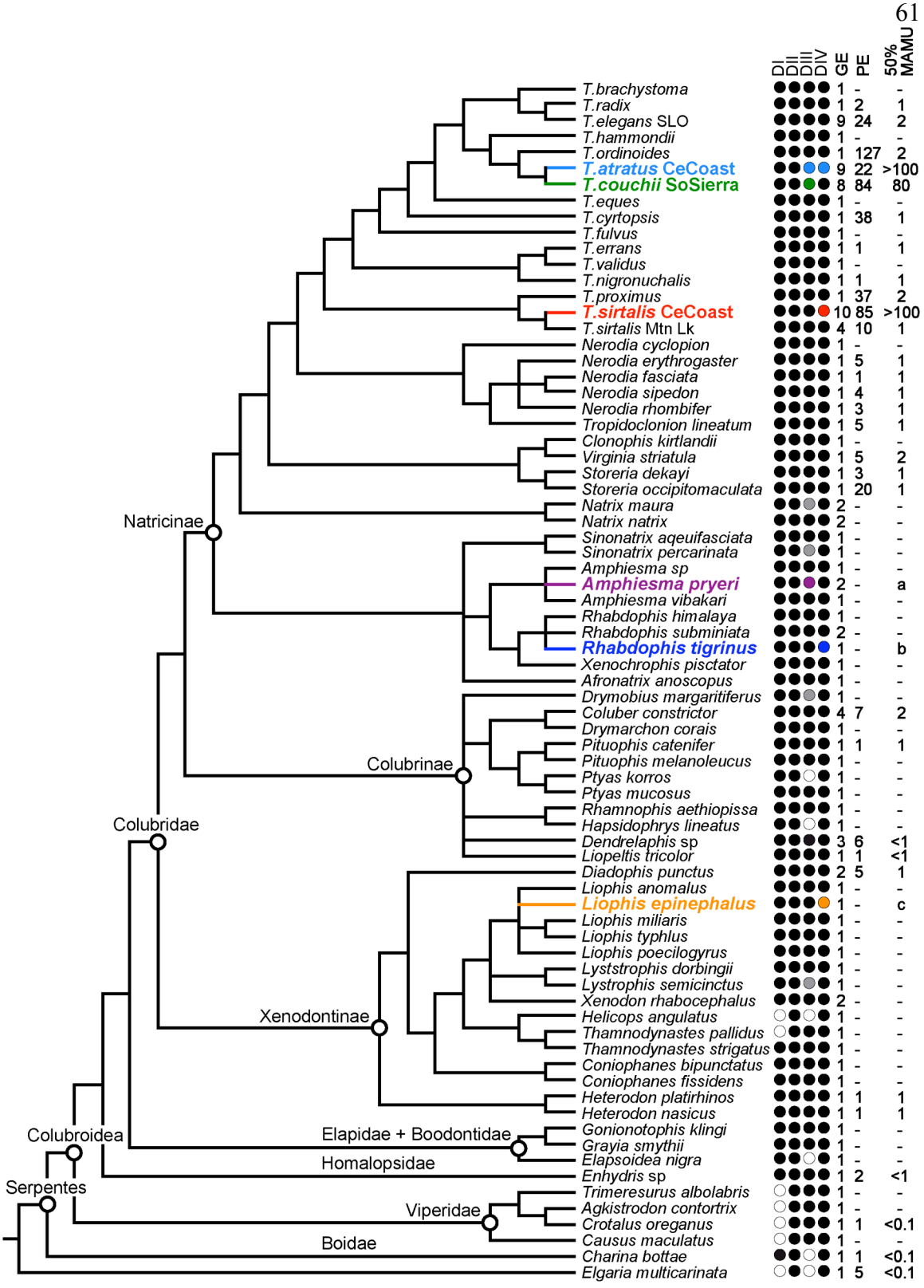

FIGURE 4.1. Phylogenetic distribution of snakes that prey on TTX bearing amphibians and also posses adaptive variation in $Na_v1.4$. *(Continued)*

FIGURE 4.1. *(Continued)* Phylogeny of colubroid snakes and relatives based on relationships presented by Cadle (1984a, 1984b, 1994), Vidal et al. (2000), Alfaro and Arnold (2001), de Queiroz et al. (2002), Parkinson et al. (2002), Pinou et al. (2004), Lawson et al. (2005), Burbrink and Lawson (2007), Jiang et al. (2007), Mulcahy (2007), and Wiens et al. (2008). Snakes that prey on TTX laden frogs or salamanders (colored taxa and branches) show derived variation in the P-loops of DIII and DIV (colored circles), while P-loops in other domains (and other taxa) lack adaptive variation (black circles). In a few cases I were unable to obtain P-loop sequences (white circles) or generated incomplete P-loop sequences (grey circles). Numbers of individuals sequenced (GE) and assayed for TTX resistance (PE) alongside measures of TTX resistance (50% MAMU). Direct measures of whole animal resistance to TTX come from Brodie (1968), Motychak et al. (1999), Brodie et al. (2005), Chapter 2, and this study. Elevated levels of TTX resistance are inferred for three taxa based on the measures of TTX recorded in prey items: (a) prey *Cynops ensicauda* possess 60–7000 Mouse Units (MU) of TTX (Mosher et al., 1964; Wakely et al., 1966; Yotsu et al., 1990; MU estimates from Daly, 2004); (b) prey *Polypedates* sp. possess 30–920 MU of TTX (Tanu et al., 2001); (c) prey *Atelopus* sp. possess 10-100 MU of TTX (Kim et al., 1975; Daly et al., 1994; MU estimate from Daly, 2004).

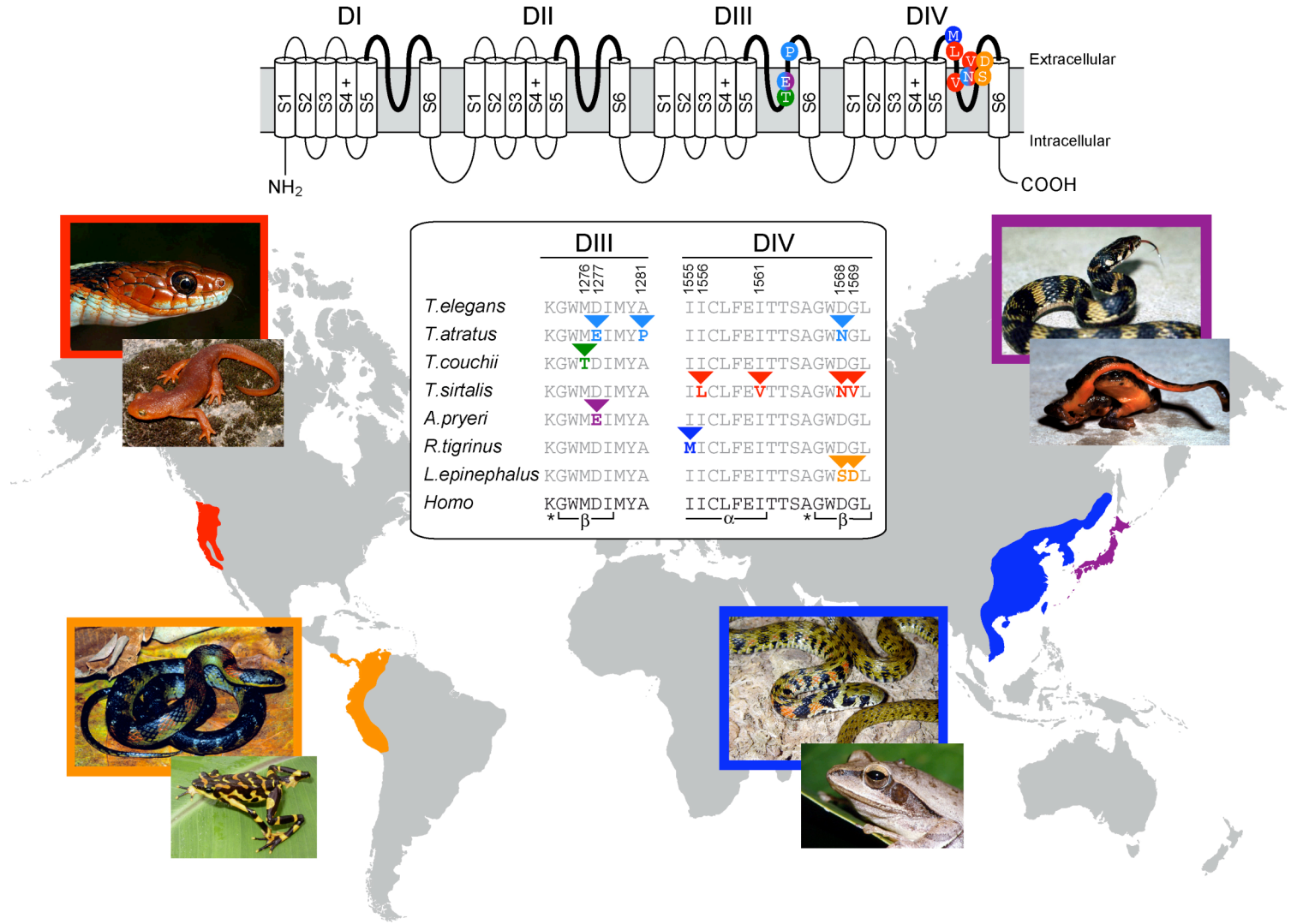

FIGURE 4.2. Ecological and molecular convergence in TTX resistant snakes from across the globe. *(Continued)*

FIGURE 4.2. *(Continued)* In parts of North America, *Thamnophis sirtalis* (red), *T. atratus* (light blue), and *T. couchii* (green) prey on *Taricha* newts (e.g., Brodie et al., 2002, 2005; Chapter 2); Central American *Liophis epinephalus* consume several *Atelopus* toads (Myers et al., 1978; Greene, 1997); Asian *Rhabdophis tigrinus* prey on the treefrog *Polypedates leucomystax* (Zhao et al., 1998); Japanese *Amphiesma pryeri* take the newt *Cynops ensicauda* (Mori and Moriguchi, 1988; Goris and Maeda, 2004). Structure of the α-subunit of $Na_v1.4$ showing the four domains (DI-DIV), their six transmembrane segments (S1-S6), and the linkers that connect segments (Hille, 2001). The four polypeptide chains that link S5 to S6 (bold) form the outer pore of the channel by folding back into the membrane to create a funnel at the base of which lies a narrow selectivity filter (Lipkind and Fozzard, 1994, 2000; Hille, 2001) that preferentially conducts Na^+ ions. A number of residues that form the external mouth, lining, and selectivity filter of the pore bind strongly to TTX (Noda et al., 1989; Terlau et al., 1991; Kontis and Goldin, 1993; Pérez-García et al., 1996; Chen et al., 1997; Penzotti et al., 1998; Yamagishi et al., 2001; Choudhary et al., 2003), which occludes the pore and halts Na^+ movement (Hille, 2001). Though a number of residues in each P-loop are known to be important in TTX ligation, all the adaptive variation in snakes is clustered in DIII and DIV, and often at the same site. Coincident substitutions occur in one or both of the two copies of $Na_v1.4$ in TTX bearing pufferfish and generally involve identical replacements (Yotsu-Yamashita et al., 2000; Venkatesh et al., 2005; Soong and Venkatesh, 2006). Human sequence given for comparison (M81758), but positions follow $Na_v1.4$ CDS from *T. sirtalis* AY851746; structures of the pore labeled below human sequence (✶, selectivity filter; α, α-helix; β, β-strand). Geographic distributions follow Campden-Main (1970), Dixon (1980, 1989), Zhao and Adler (1993), Stebbins (2003), Szczerbak (2003), Ota and Iwanaga (1997); *A.pryeri* is actually confined to the Ryukyu archipelago (Ota and Iwanaga, 1997) but is shown here across all of Japan for ease of illustration.

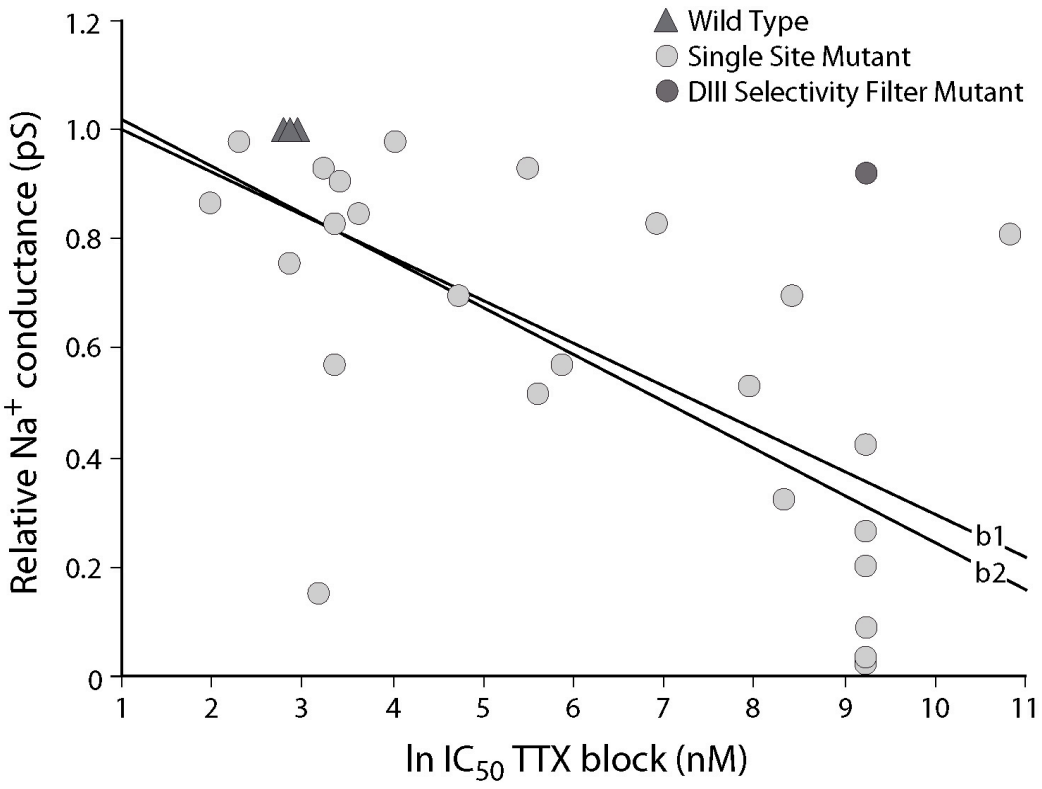

FIGURE 4.3. Regression of inhibitory concentrations of TTX against single channel Na^+ conductance. Relationship between the amount of TTX (nM) required to inhibit peak Na^+ current by 50% (IC_{50}) against single channel conductance (pS) in wild type (triangles) and single site Na_v mutants (circles); simple regression on all data (b1: r^2 = 0.417, $P < 0.0001$) and with a mutant critical to ion selectivity removed (b2: $r^2 = 0.497$, $P < 0.0001$) (see text for details); data from Terlau et al. (1991), Backx et al. (1992) and Yamagishi et al. (2001). The tradeoff between TTX sensitivity and sodium channel performance is readily apparent at the molecular level where mutations that significantly improve TTX resistance generally reduce Na^+ permeability (and often selectivity). Thus, intramolecular pleiotropy may have restricted the independent evolution of TTX resistance in snakes.

CHAPTER 5

SUMMARY

One of the chief aims of evolutionary biology is to understand the genetics of adaptive evolution (Futuyma, 1998; Feder, 2007; Ellegren and Sheldon, 2008). We can certainly measure the origins, evolution, and persistence of consequential mutations in controlled laboratory settings, and place these data into a well-developed framework of adaptive evolution (Orr, 2005a, 2005b; Phillips, 2005; e.g., Wichman et al., 1999; Rokyta et al., 2005; Miller et al., 2006; Weinreich et al., 2006). Yet our ability to document and explain the effects of analogous genetic changes on the rate and direction of phenotypic evolution in real communities is only now emerging (Feder and Mitchell-Olds, 2003; Ellegren and Sheldon, 2008; Stinchcombe and Hoekstra, 2008; e.g., Gompel et al., 2005; Hoekstra et al., 2006; Joron et al., 2006; Storz et al., 2007).

The coevolutionary interactions between newts (*Taricha*) that possess the potent neurotoxin, tetrodotoxin, (TTX) and their resistant garter snake (*Thamnophis*) predators, forms an ideal setting for studying adaptation because of the well-defined ecological context and selection pressures (Brodie, 1968; Brodie and Brodie 1999; Hanafin et al., 2008), and the partially defined genetic basis of the predatory adaptation (Geffeney et al., 2002, 2005). Here, I focus on patterns of phenotypic and genetic variation in natural populations of garter snakes (and relatives) as a model for the study of adaptive evolution.

A fundamental question surrounding adaptive evolution is to understand the roles that novel mutations, standing genetic variation, and introgression play in the adaptive

process (Orr and Betancourt, 2001; Hermisson and Pennings, 2005; Barrett and Schluter, 2008). I focus on parallel adaptations seen in three species of *Thamnophis* (*T. sirtalis*, *T. couchii*, and *T. atratus*) that experience common patterns of reciprocal selection to determine whether adaptation has evolved through novel mutations, recruitment of standing adaptive variation, or introgression of beneficial alleles (Chapter 2). I describe the genetic basis and evolutionary history of elevated TTX resistance in *Thamnophis* and show that TTX resistance has evolved independently through amino acid changes at critical sites in a voltage-gated sodium channel protein ($Na_v1.4$) that is the specific target of the TTX. These results demonstrate that adaptive evolution has occurred multiple times in garter snakes via the *de novo* acquisition of beneficial mutations.

Determining the underlying genetic architecture of adaptations is essential in underatnding the tempo and mode of phenotypic evolution and patterns of coevolution (Falconer and MacKay, 1995; Kopp and Gavrilets, 2006). I evaluate the contribution of $Na_v1.4$ alleles to TTX resistance in *T. atratus* and *T. sirtalis* populations along the coast of central California (Chapter 3). Allelic variation in $Na_v1.4$ explains 29% and 98% of the variation in TTX resistance in *T. atratus* and *T. sirtalis*, respectively, demonstrating that $Na_v1.4$ is a major effect locus. The simple genetic architecture of TTX resistance in garter snakes may significantly impact the dynamics of trait change and coevolution.

Patterns of convergent evolution are cited as some of the most compelling examples of the strength of natural selection in shaping organismal diversity. Yet repeated patterns may tell us as much about the constraints that restrict evolution as about the importance of natural selection. I describe convergent molecular adaptations in parallel ecological arms-races between diverse snakes and amphibians from across the

globe (Chapter 4). Six snake species from three separate clades have independently acquired amino acid changes in $Na_v1.4$, the target of the TTX contained in their amphibian prey. The derived mutations are clustered in two portions of the gene and most of the replacements (seven out of twelve) are confined to the same three sites, two of which involve substitutions to the same amino acid. While a number of amino acid changes are known to make $Na_v1.4$ insensitive to TTX and might be favored by selection in some environments, most of these substitutions negatively impact or even abolish the ion-conducting function of the protein. Indeed, a functional tradeoff between channel function and TTX resistance is evident in data taken from the literature (Terlau et al., 1991; Backx et al., 1992; Yamagishi et al., 2001). Thus, intramolecular pleiotropy appears to have restricted the variation accessible to natural selection and lead to a relatively narrow genetic response across TTX resistant taxa. Natural selection appears to have been bounded, repeatedly moving independent snake species up the same few adaptive peaks.

Finally, it is worth noting that I have only identified an intriguing correlation between the physiological ability of several snake species to consume deadly prey and derived genetic changes in those snakes at a candidate locus affected by the prey toxin. The case is strong: several prey species share high levels of the neurotoxin TTX (selective agent) which binds selectively to the pore of the $Na_v1.4$ protein (target of selection) involved in muscle activity and coordination; only predators known to eat the prey possess derived allelic variation in $Na_v1.4$ at sites critical to TTX ligation. Future work should directly link organismal performance (or feeding ability) to the allelic variation at $Na_v1.4$ through functional expression of the derived alleles in heterologous

cells and subsequent measures of TTX binding affinity to the protein. Future efforts should also seek to uncover adaptive variation across all TTX sensitive Na_v members (peripheral and central nerve sodium channels) in TTX resistant snakes. It seems likely that all TTX sensitive Na_v paralogs have changed in concert with $Na_v1.4$ to produce the final TTX resistant phenotype. Establishing the number and expression patterns of all the Na_v members in snakes will be a first step towards understanding adaptation in an entire gene family in what is emerging as a model system for the study of adaptive evolution.

REFERENCES

Abzhanov, A., M. Protas, B. R. Grant, P. R. Grant, and C. J. Tabin. 2004. *Bmp4* and morphological variation of beaks in Darwin's Finches. Science 305:1462-1465.

Agrawal, A. A., and C. M. Lively. 2002. Infection genetics: gene-for-gene versus matching-alleles models and all points in between. Evol. Ecol. Res. 4:79–90.

Agrawal, A. A., and C. M. Lively. 2003. Modelling infection as a two-step process combining gene-for-gene and matching-allele genetics. Proc. R. Soc. Biol. Sci. Ser. B 270:323–334.

Albertson, R. C., and T. D. Kocher. 2006. Genetic and developmental basis of cichlid trophic diversity. Heredity 97:211–221.

Albertson, R. C., J. T. Streelman, T. D. Kocher, and P. C. Yelick. 2005. Integration and evolution of the cichlid mandible: the molecular basis of alternate feeding strategies. Proc. Natl. Acad. Sci. U.S.A. 102:16287-16292.

Alfaro, M. E., and S. J. Arnold. 2001. Molecular systematics and evolution of *Regina* and the thamnophiine snakes. Mol. Phylogenet. Evol. 21:408-423.

Allendorf, F. W. 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo. Biol. 5:181-190.

Arendt, J., and D. Reznick. 2008. Convergence and parallelism reconsidered: what have we learned about the genetics of adaptation? Trends Ecol. Evol. 23:26-32.

Arnold, E. N., and D. W. Ovenden. 2002. Reptiles and amphibians of Europe, 2nd edition. Princeton University Press, Princeton.

Arnold, M. L. 1997. Natural hybridization and evolution. Oxford University Press, New York.

Arnold, S. J. 1992. Constraints on phenotypic evolution. Am. Nat. 140:S85-S107.

Arnold, S. J., M. E. Pfrender, and A. G. Jones. 2001. The adaptive landscape as a conceptual bridge between micro and macroevolution. Genetica 112-113:9-32.

Backx, P. H., D. T. Yue, J. H. Lawrence, E. Marban, and G. F. Tomaselli. 1992. Molecular localization of an ion-binding site within the pore of mammalian sodium channels. Science 257:248-251.

Barrett, R. D. H., and D. Schluter. 2008. Adaptation from standing genetic variation.

Trends Ecol. Evol. 23:38-44.

Barton, N. H. 2001. The role of hybridization in evolution. Mol. Ecol. 10:551–568.

Beckh, S., M. Noda, H. Lübbert, and S. Numa. 1989. Differential regulation of three sodium channel messenger RNAs in the rat central nervous system during development. EMBO J. 8:3611–3616.

Beerli, P. 2006. Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. Bioinformatics 22:341–345.

Beldade, P., K. Koops, and P. M. Brakefield. 2002. Developmental constraints versus flexibility in morphological evolution. Nature 416:844–847.

Bohannan, B. J. M., and R. E. Lenski. 2000. Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage. Ecol. Lett. 3:362-377.

Brakefield, P. M. 2007. Evo-devo and constraints on selection. Trends Ecol. Evol. 21:361-368.

Brakefield, P. M., and J. C. Roskam. 2006. Exploring evolutionary constraints is a task for an integrative evolutionary biology. Am. Nat. 168:S1-S13.

Brodie, E. D., Jr. 1968. Investigations on the skin toxin of the adult Rough-skinned Newt, *Taricha granulosa*. Copeia 1968:307-313.

Brodie, E. D., Jr. 1983. Antipredator adaptations of salamanders: evolution and convergence among terrestrial species. Pages 109-133 *in* Plant, animal, and microbial adaptations to terrestrial environment (N. S. Margaris, M. Arianoutsou-Faraggitaki, and R. J. Reiter, eds.). Plenum Publishing, New York.

Brodie, E. D., III, and E. Brodie, D., Jr. 1990. Tetrodotoxin resistance in garter snakes: An evolutionary response of predators to dangerous prey. Evolution 44:651-659.

Brodie, E. D., III, and E. D. Brodie, Jr. 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.

Brodie, E. D., III, and E. D. Brodie, Jr. 1999. Predator-prey arms races: asymmetrical selection on predators and prey may be reduced when prey are dangerous. Bioscience 49:557-568.

Brodie, E. D., III, and B. J. Ridenhour. 2003. Reciprocal selection at the phenotypic interface of coevolution. Integr. Comp. Biol. 43:408-418.

Brodie, E. D., III, C. R. Feldman, C. T. Hanifin, J. E. Motychak, D. G. Mulcahy, B. L. Williams, and E. D. Brodie, Jr. 2005. Parallel arms races between garter snakes and newts involving tetrodotoxin as the phenotypic interface of coevolution. J. Chem. Ecol. 31:343-356.

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., Jr., B. J. Ridenhour, and E. D. Brodie, III. 2002. The evolutionary response of predators to dangerous prey: hotspots and coldspots in the geographic mosaic of coevolution between garter snakes and newts. Evolution 56:2067-2082.

Budd, G. E. 2006. On the origin and evolution of major morphological characters. Biol. Rev. 81:609-628.

Burbrink, F. T., and R. Lawson. 2007. How and when did Old World rat snakes disperse into the New World? Mol. Phylogenet. Evol. 43:173-189.

Cadle, J. E. 1984a. Molecular systematics of neotropical xenodontine snakes: I. South American xenodontines. Herpetologica 40:8-20.

Cadle, J. E. 1984b. Molecular systematics of neotropical xenodontine snakes: II. Central American xenodontines. Herpetologica 40:21-30.

Cadle, J. E. 1994. The colubrid radiation in Africa (Serpentes: Colubridae): phylogenetic relationships and evolutionary patterns based on immunological data. Zool. J. Linn. Soc. 110:103-140.

Cahan, S. H., and L. Keller. 2003. Complex hybrid origin of genetic caste determination in harvester ants. Nature 424:306-309.

Campden-Main, S. M. 1970. A field guide to the snakes of south Vietnam. United States National Museum, Washington, DC.

Carbonneau, E., K. Vijayaragavan, and M. Chahine. 2002. A tryptophan residue (W736) in the amino-terminus of the P-segment of domain II is involved in pore formation in $Na_v1.4$ voltage-gated sodium channels. Eur. J. Physiol. 445:18–24.

Cardall, B. L., E. D. Brodie, Jr, E. D. Brodie, III, and C. T. Hanifin. 2004. Secretion and regeneration of tetrodotoxin in the rough-skin newt (*Taricha granulosa*). Toxicon 44:933–938.

Carroll, S. B. 2005. Evolution at two levels: on genes and form. Public Libr. Sci. Biol. 3:1159–1166.

Chen, S.-F., H. A. Hartmann, and G. E. Kirsche. 1997. Cysteine mapping in the ion selectivity and toxin binding region of the cardiac Na^+ channel pore. J. Membr. Biol. 155:11-25.

Chiamvimonvat, N., M. T. Perez-Garcia, G. F. Tomaselli, and E. Marban. 1996. Control of ion flux and selectivity by negatively charged residues in the outer mouth of rat sodium channels. J. Physiol. 491:51-59.

Choudhary, G., M. Yotsu-Yamashita, L. Shang, T. Yasumoto, and S. C. Dudley, Jr. 2003. Interactions of the C-11 hydroxyl of tetrodotoxin with the sodium channel outer vestibule. Biophys. J. 84:287-294.

Cohn, M. J., and C. Tickle. 1999. Developmental basis of limblessness and axial patterning in snakes. Nature 399:474-479.

Colosimo, P. F., K. E. Hosemann, S. Balabhadra, G. J. Villarreal, M. Dickson, J. Grimwood, J. Schmutz, R. M. Myers, D. Schluter, and D. M. Kingsley. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. Science 307:1928-1933.

Cooper, T. F., D. E. Rozen, and R. E. Lenski. 2003. Parallel changes in gene expression after 20,000 generations of evolution in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 100:1072–1077.

Cowen, R. 2004. History of life, 4th edition. Blackwell Publishing, Malden.

Creer, S., W.-H. Chou, A. Malhotra, and R. S. Thorpe. 2002. Offshore insular variation in the diet of the Taiwanese bamboo viper *Trimeresurus stejnegeri* (Schmidt). Zool. Sci. 19:907-913.

Cresko, W. A., A. Amores, C. Wilson, J. Murphy, M. Currey, P. Phillips, M. A. Bell, C. B. Kimmel, and J. H. Postlethwait. 2004. Parallel genetic basis for repeated evolution of armor loss in Alaskan threespine stickleback populations. Proc. Natl. Acad. Sci. U.S.A. 101:6050–6055.

Daly, J. W. 2004. Marine toxins and nonmarine toxins: convergence or symbiotic organisms? J. Nat. Prod. 67:1211-1215.

Daly, J. W., F. Gusovsky, C. W. Myers, M. Yotsu-Yamashita, and T. Yasumoto. 1994. First occurrence of tetrodotoxin in a dendrobatid frog (*Colostethus inguinalis*), with further reports for the bufonid genus *Atelopus*. Toxicon 32:279-285.

Darwin, C. 1859. On the origin of species by means of natural selection, 1st edition. Harvard University Press, Cambridge.

Deng, H.-W. 1998. Estimating within-locus nonadditive coefficient and discriminating dominance versus overdominance as the genetic cause of heterosis. Genetics 148:2003–2014.

DePristo, M. A., D. M. Weinreich, and D. L. Hartl. 2005. Missense meanderings in sequence space: a biophysical view of protein evolution. Nat. Rev. Genet. 6:678-687.

de Queiroz, A., R. Lawson, and J. A. Lemos-Espinal. 2002. Phylogenetic relationships of North American garter snakes (*Thamnophis*) based on four mitochondrial genes: how much DNA sequence is enough? Mol. Phylogenet. Evol. 22:315-329.

Dixon, J. R. 1980. The neotropical colubrid snake genus *Liophis*: the generic concept. Milw. Public Mus. Contrib. Biol. Geol. 31:1-40.

Dixon, J. R. 1989. A key and checklist to the neotropical snake genus *Liophis* with country lists and maps. Smithson. Herpetol. Inf. Serv. 1989:1-40.

Doebley, J., and A. Stec. 1991. Genetic analysis of the morphological differences between maize and teosinte. Genetics 129:285-295.

Duellman, W. E., and L. Trueb. 1986. Biology of the amphibians. McGraw-Hill Co., New York.

Ellegren, H., and B. C. Sheldon. 2008. Genetic basis of fitness differences in natural populations. Nature 452:169-175.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics, 4th edition. Addison-Wesley Longman, Harlow.

Farris, J. S. 1983. The logical basis of phylogenetic analysis. Pages 7-36 *in* Advances in cladistics (N. Platnick and V. A. Funk, eds.). Columbia University Press, New York.

Favre, I., E. Moczydlowski, and L. Schild. 1996. On the structural basis for ionic selectivity among Na^+, K^+, and Ca^{2+} in the voltage-gated sodium channel. Biophys. J. 71:3110-3125.

Feder, M. E. 2007. Evolvability of physiological and biochemical traits: evolutionary mechanisms including and beyond single-nucleotide mutation. J. Exp. Biol. 210:1653-1660.

Feder, M. E., and T. Mitchell-Olds. 2003. Evolutionary and ecological functional genomics. Nat. Rev. Genet. 4:649-655.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17:368-376.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.

ffrench-Constant, R. H. 1994. The molecular and population genetics of Cyclodiene insecticide resistance. Insect Biochem. Mol. Biol. 24:335-345.

ffrench-Constant, R. H., P. J. Daborn, and G. Le Goff. 2004. The genetics and genomics of insecticide resistance. Trends Genet. 20:163-170.

Filippi, E., and L. Luiselli. 2002. Crested newts, *Triturus carnifex* (Laurenti, 1768) form the bulk of the diet in high-altitude Grass snakes *Natrix natrix* (Linnaeus, 1758) of the central Apennines. Herpetozoa 15:83-85.

Fox, W. 1951. Relationships among the garter snakes of *Thamnophis elegans* rassenkreis. University of California Publications in Zoology 50:485-530.

Frankino, W. A., B. J. Zwaan, D. L. Stern, and P. M. Brakefield. 2005. Natural selection and developmental constraints in the evolution of allometries. Science 307:718–720.

Frankino, W. A., B. J. Zwaan, D. L. Stern, and P. M. Brakefield. 2007. Internal and external constraints in the evolution of morphological allometries in a butterfly. Evolution 61:2958–2970.

Futuyma, D. J. 1998. Evolutionary biology, 3rd edition. Sinauer Associates, Sunderland.

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

Geffeney, S. L., E. Fujimoto, E. D. Brodie, III, E. D. Brodie, Jr., and P. C. Ruben. 2005. Evolutionary diversification of TTX-resistant sodium channels in a predator-prey interaction. Nature 434:759-763.

Geffeney, S. L., and P. C. Rubin. 2006. The structural basis and functional consequences of interactions between tetrodotoxin and voltage-gated sodium channels. Mar. Drugs 4:143-156.

George, A. L., Jr,, G. S. Iyer, R. Kleinfield, R. G. Kallen, and R. L. Barchi. 1993. Genomic organization of the human skeletal muscle sodium channel gene. Genomics 15:598-606.

Goldin, A. L. 2001. Resurgence of sodium channel research. Annu. Rev. Physiol. 63:871-894.

Goldin, A. L. 2002. Evolution of voltage-gated Na^+ channels. J. Exp. Biol. 205:575-584.

Gompel, N., B. Prud'homme, P. J. Wittkopp, V. A. Kassner, and S. B. Carroll. 2005. Chance caught on the wing: *cis*-regulatory evolution and the origin of pigment patterns in *Drosophila*. Nature 433:481-487.

Goris, R. C., and N. Maeda. 2004. Guide to the amphibians and reptiles of Japan. Krieger, Malabar.

Gould, S. J. 1989a. Wonderful life: the Burgess Shale and the nature of history. Norton, New York.

Gould, S. J. 1989b. A developmental constraint in *Cerion*, with comments on the definition and interpretation of constraint in evolution. Evolution 43:516-539.

Gould, S. J., and R. C. Lewontin. 1979. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. Proc. R. Soc. Biol. Sci. Ser. B 205:581-598.

Gould, S. J., and E. S. Vrba. 1982. Exaptation: a missing term in the science of form. Paleobiology 8:4-15.

Grant, P. R., B. R. Grant, J. A. Markert, L. F. Keller, and K. Petren. 2004. Convergent evolution of Darwin's Finches caused by introgressive hybridization and selection. Evolution 58:1588–1599.

Greene, H. W. 1997. Snakes: The evolution of mystery in nature. University of California Press, Berkeley.

Greene, R. R., and C. R. Feldman. 2008. *Thamnophis atratus atratus*. Diet. Herpetol. Rev. *In Press*.

Gu, X., Y. Fu, and W.-H. Li. 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. Mol. Biol. Evol. 12:546-557.

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.

Hanifin, C. T., E. D. Brodie, III, and E. D. Brodie, Jr. 2003. Tetrodotoxin levels in eggs of the rough-skin newt,*Taricha granulosa*, are correlated with female toxicity. J. Chem. Ecol. 29:1729-1739.

Hanifin, C. T., E. D. Brodie, Jr., and E. D. Brodie, III. 2008. Phenotypic mismatches reveal escape from arms-race coevolution. Public Libr. Sci. Biol. 6:e60.

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.

Hartley, C. J., R. D. Newcomb, R. J. Russell, C. G. Yong, J. R. Stevens, D. K. Yeates, J. La Salle, and J. G. Oakeshott. 2006. Amplification of DNA from preserved specimens shows blowflies were preadapted for the rapid evolution of insecticide resistance. Proc. Natl. Acad. Sci. U.S.A. 103:8757–8762.

Hasegawa, M., K. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 21:160-174.

Hawthorne, D. J., and S. Via. 2001. Genetic linkage of ecological specialization and reproductive isolation in pea aphids. Nature 412:904-907.

Heinemann, S. H., H. Terlau, W. Stühmer, K. Imoto, and S. Numa. 1992. Calcium channel characteristics conferred on the sodium channel by single mutations. Nature 356:441-443.

Herder, F., A. W. Nolte, J. Pfaender, J. Schwarzer, R. K. Hadiaty, and U. K. Schliewen. 2006. Adaptive radiation and hybridization in Wallace's Dreamponds: evidence from sailfin silversides in the Malili Lakes of Sulawesi. Proc. R. Soc. Biol. Sci. Ser. B 273:2209-2217.

Hermisson, J., and P. S. Pennings. 2005. Soft sweeps: molecular population genetics of adaptation from standing genetic variation. Genetics 169:2335–2352.

Hey, J., and R. Nielsen. 2004. Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimili*s. Genetics 167:747-760.

Hille, B. 2001. Ion channels of excitable membranes, 3rd edition. Sinauer Associates, Sunderland.

Hirai, T. 2004. Dietary shifts of frog eating snakes in response to seasonal changes in prey availability. J. Herpetol. 38:455-460.

Hoekstra, H. E., and J. A. Coyne. 2007. The locus of evolution: evo devo and the genetics of adaptation. Evolution 61:995–1016.

Hoekstra, H. E., R. J. Hirschmann, R. A. Bundey, P. A. Insel, and J. P. Crossland. 2006. A single amino acid mutation contributes to adaptive beach mouse color pattern. Science 313:101-107.

How, C. K., C. H. Chern, Y. C. Huang, L. M. Wang, and C. H. Lee. 2003. Tetrodotoxin poisoning. Am. J. Emerg. Med. 21:51-54.

Huelsenbeck, J. P., and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754-755.

Isbister, G. K., and M. C. Kiernan. 2005. Neurotoxic marine poisoning. Lancet Neurol. 4:219-228.

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.

Jiang, Z. J., T. A. Castoe, C. C. Austin, F. T. Burbrink, M. D. Herron, J. A. McGuire, C. L. Parkinson, and D. D. Pollock. 2007. Comparative mitochondrial genomics of snakes: extraordinary substitution rate dynamics and functionality of the duplicate control region. BMC Evol. Biol. 7:123.1-123.14.

Joron, M., R. Papa, M. Beltrán, N. Chamberlain, J. Mavárez, S. Baxter, S. Abanto, E. Bermingham, S. J. Humphray, J. Rogers, H. Beasley, K. Barlow, R. H. ffrench-Constant, J. Mallet, W. O. McMillan, and C. D. Jiggins. 2006. A conserved supergene locus controls colour pattern diversity in *Heliconius* butterflies. Public Libr. Sci. ONE 4:1831-1840.

Kao, C. Y., and S. R. Levinson (eds) 1986. Tetrodotoxin, saxitoxin, and the molecular biology of the sodium channel. Ann. N.Y. Acad. Sci. 479: 1-448.

Kawakita, A., T. Sota, J. S. Ascher, M. Ito, H. Tanaka, and M. Kato. 2003. Evolution and phylogenetic utility of alignment gaps within intron sequences of three nuclear genes in bumble bees (*Bombus*). Mol. Biol. Evol. 20:87-92.

Kim, Y. H., G. B. Brown, H. S. Mosher, and F. A. Fuhrman. 1975. Tetrodotoxin: occurrence in atelopid frogs of Costa Rica. Science 189:151–152.

Knowles, L. L., and B. C. Carstens. 2007. Estimating a geographically explicit model of population divergence. Evolution 61:477-493.

Kontis, K. J., and A. L. Goldin. 1993. Site-directed mutagenesis of the putative pore region of the rat IIA sodium channel. Mol. Pharmacol. 43:635-644.

Kopp, M., and S. Gavrilets. 2006. Multilocus genetics and the coevolution of quantitative traits. Evolution 60:1321–1336.

Krebs, C. J. 1999. Ecological methodology, 2nd edition. Benjamin-Cummings, Menlo Park.

Larget, B., and D. L. Simon. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol. Biol. Evol. 16:750–759.

Lawson, R., J. B. Slowinski, B. I. Crother, and F. T. Burbrink. 2005. Phylogeny of the Colubroidea (Serpentes): new evidence from mitochondrial and nuclear genes. Mol. Phylogenet. Evol. 37:581-601.

Lehman, E. M., E. D. Brodie, Jr., and E. D. Brodie, III. 2004. No evidence for an endosymbiotic bacterial origin of tetrodotoxin in the newt *Taricha granulosa*. Toxicon 44:243-249.

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.

Lipkind, G. M., and H. A. Fozzard. 2000. KcsA crystal structure as framework for a molecular model of the Na^+ channel pore. Biochemistry 39:8161-8170.

Liu, Z., W. Song, and K. Dong. 2004. Persistent tetrodotoxin-sensitive sodium current resulting from U-to-C RNA editing of an insect sodium channel. Proc. Natl. Acad. Sci. U.S.A. 101:11862-11867.

Losos, J. B., T. R. Jackman, A. Larson, K. de Queiroz, and L. Rodríguez-Schettino. 1998. Contingency and determinism in replicated adaptive radiations of island lizards. Science 279:2115-2118.

Losos, J. B., M. Leal, R. Glor, K. de Queiroz, P. E. Hertz, L. R. Schettino, A. C. Lara, T. R. Jackman, and A. Larson. 2003. Nich lability in the evolution of a Caribbean lizard community. Nature 424:542-545.

Luiselli, L., M. Capula, and R. Shine. 1997. Food habits, growth rates, and reproductive biology of grass snakes, *Natrix natrix* (Colubridae) in the Italian Alps. J. Zool. 241:371-380.

Luiselli, L., E. Filippi, and M. Capula. 2005. Geographic variation in diet composition of the grass snake (*Natrix natrix*) along the mainland and an island of Italy: The effects of habitat type and interference with potential competitors. Herpetol. J. 15:221-230.

Maddison, D. R., and W. P. Maddison. 2005. MacClade: analysis of phylogeny and character evolution. Version 4.08. Sinauer Associates, Sunderland.

Maruta, S., K. Yamaokab, and M. Yotsu-Yamashita. 2008. Two critical residues in p-loop regions of puffer fish Na^+ channels on TTX sensitivity. Toxicon 51:381–387.

Maynard Smith, J., R. Burian, S. Kauffman, P. Alberch, J. Campbell, B. Goodwin, R. Lande, D. Raup, and L. Wolpert. 1985. Developmental constraints and evolution. Q. Rev. Biol. 60:265-287.

Mayr, E., and W. B. Provine. 1998. The evolutionary synthesis: perspectives on the

unification of biology, 2nd edition. Harvard University Press, Cambridge, MA.

McGaugh, S., E. A. Alacs, S. V. Edwards, C. R. Feldman, A. Georges, J. W. Sites, Jr., and N. Valenzuela. 2007. Molecules to organisms: research applications of modern genetic tools for turtle biology and conservation. Pages 47-72 *in* Defining turtle diversity: proceedings of a workshop on genetics, ethics, and taxonomy of tortoises and freshwater turtles (H. B. Shaffer, N. N. FitzSimmons, A. Georges, and A. G. J. Rhodin, eds.). Chelonian Research Foundation, Lunenburg.

Medinsky, M. A., and C. D. Klaassen. 1996. Toxicokinetics. Pages 187-198 *in* Casarett and Doull's toxicology: the basic science of poisons (C. D. Klaassen, ed.) McGraw-Hill, New York.

Meyer, A. 1993. Phylogenetic relationships and evolutionary processes in East African ciclid fishes. Trends Ecol. Evol. 8:279-284.

Meyer, A., T. D. Kocher, P. Basasibwaki, and A. C. Wilson. 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. Nature 347:550-553.

Miller, S. P., M. Lunzer, and A. M. Dean. 2006. Direct demonstration of an adaptive constraint. Science 314:459-461.

Moore, W. S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution 49:718-726.

Mori, A., and H. Moriguchi. 1988. Food habits of the snakes in Japan: a critical review. Snake 20:98-113.

Mori, A., M. Toda, S. Kadowaki, and H. Moriguchi. 1992. Lying in ambush for nocturnal frogs: field observations on the feeding behavior of three colubrid snakes, *Elaphe quadrivirgata, E. climacophora,* and *Rabdophis tigrinus*. Jpn. J. Herpetol. 14:107-115.

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.

Mulcahy, D. G. 2007. Molecular systematics of neotropical cat-eyed snakes: a test of the monophyly of Leptodeirini (Colubridae: Dipsadinae) with implications for character evolution and biogeography. Biol. J. Linn. Soc. 92:483–500.

Mundy, N. I., N. S. Badcock, T. Hart, K. Scribner, K. Janssen, and N. J. Nadeau. 2004.

Conserved genetic basis of a quantitative plumage trait involved in mate choice. Science 303:1870-1873.

Myers, C., W., J. W. Daly, and B. Malkin. 1978. A dangerously toxic new frog (*Phyllobates*) used by Embera Indians of Western Columbia, with discussion of blowgun fabrication and dart poisoning. Bull. Am. Mus. Nat. Hist. 161:309-365.

Nachman, M. W., H. E. Hoekstra, and S. L. D'Agostino. 2003. The genetic basis of adaptive melanism in pocket mice. Proc. Natl. Acad. Sci. U.S.A. 100:5268-5273.

Nei, M., T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1-10.

Noda, M., H. Suzuki, S. Numa, and W. Stuhmer. 1989. A single point mutation confers tetrodotoxin and saxitoxin insensitivity on the sodium channel II. FEBS Lett. 259:213-216.

Novak, A. E., M. C. Jost, Y. Lu, A. D. Taylor, H. H. Zakon, and A. B. Ribera. 2006. Gene duplications and evolution of vertebrate voltage-gated sodium channels. J. Mol. Evol. 63:208-221.

Nuismer, S. L., B. J. Ridenhour, and B. P. Oswald. 2007. Antagonistic coevolution mediated by phenotypic differences between quantitative traits. Evolution 61:1823-1834.

Orr, H. A. 2005a. The genetical theory of adaptation: a brief history. Nat. Rev. Genet. 6:119-127.

Orr, H. A. 2005b. Theories of adaptation: what they do and do not say. Genetica 123:2-13.

Orr, H. A., and A. J. Betancourt. 2001. Haldane's seive and adaptation from the standing genetic variation. Genetica 157:875-884.

Orr, H. A., and J. A. Coyne. 1992. The genetics of adaptation: a reassessment. Am. Nat. 140:724-742.

Ota, H., and S. Iwanaga. 1997. A systematic review of the snakes allied to *Amphiesma pryeri* (Boulenger) (Squamata: Colubridae) in the Ryukyu Archipelago, Japan. Zool. J. Linn. Soc. 121:339-360.

Palumbi, S. R., F. Cipriano, and M. P. Hare. 2001. Predicting nuclear gene coalescence from mitochondrial data: the three times rule. Evolution 55:859-868.

Parkinson, C. L., J. A. Campbell, and P. T. Chippindale. 2002. Multigene phylogenetic

analysis of pitvipers, with comments on their biogeography. Pages 93-110 *in* Biology of the vipers (G. W. Schuett, M. Höggren, M. E. Douglas, and H. W. Greene, eds.). Eagle Mountain Publishing, Eagle Mountain.

Peichel, C. L., K. S. Nereng, K. A. Ohgi, B. L. E. Cole, P. F. Colosimo, C. A. Buerkle, D. Schluter, and D. M. Kingsley. 2001. The genetic architecture of divergence between threespine stickleback species. Nature 414:901-905.

Pennisi, E. 2008. Deciphering the genetics of evolution. Science 321:760-763.

Penzotti, J. L., H. A. Fozzard, G. M. Lipkind, and S. C. Dudley, Jr. 1998. Differences in saxitoxin and tetrodotoxin binding revealed by mutagenesis of the Na^+ channel outer vestibule. Biophys. J. 75:2647-2657.

Pérez-García, M. T., N. Chiamvimonvat, E. Marban, and G. F. Tomaselli. 1996. Structure of the sodium channel pore revealed by serial cysteine mutagenesis. Proc. Natl. Acad. Sci. U.S.A. 93:300-304.

Phillips, P. C. 2005. Testing Hypotheses regarding the genetics of adaptation. Genetica 123:15-24.

Pigliucci, M., and J. Kaplan. 2000. The fall and rise of Dr Pangloss: adaptationism and the Spandrels paper 20 years later. Trends Ecol. Evol. 15:66-70.

Pinou, T., S. Vicario, M. Marschner, and A. Caccone. 2004. Relict snakes of North America and their relationships within Caenophidia, using likelihood-based Bayesian methods on mitochondrial sequences. Mol. Phylogenet. Evol. 32:563–574.

Pires, O. R., Jr., A. Sebbena, E. F. Schwartza, R. A. V. Moralesb, C. J. Bloch, and C. A. Schwart. 2005. Further report of the occurrence of tetrodotoxin and new analogues in the Anuran family Brachycephalidae. Toxicon 45:73-79.

Plummer, N. W., and M. H. Meisler. 1999. Evolution and diversity of mammalian sodium channel genes. Genomics 57:323-331.

Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817-818.

Prud'homme, B., N. Gompel, A. Rokas, V. A. Kassner, T. M. Williams, S. D. Yeh, J. R. True, and S. B. Carroll. 2006. Repeated morphological evolution through *cis*-regulatory changes in a pleiotropic gene. Nature 440:1050-1053.

Ridenhour, B. J., E. D. Brodie, III , and E. D. Brodie, Jr. 2004. Resistance of neonates and field-collected garter snakes (*Thamnophis* spp.) to tetrodotoxin. J. Chem. Ecol. 30:143-154.

Ridenhour, B. J., E. D. Brodie, Jr., and E. D. Brodie, III. 2007. Patterns of genetic differentiation in *Thamnophis* and *Taricha* from the Pacific Northwest. J. Biogeogr. 34:724-735.

Rieseberg, L. H., O. Raymond, D. M. Rosenthal, Z. Lai, K. Livingstone, T. Nakazato, J. L. Durphy, A. E. Schwarzbach, L. A. Donovan, and C. Lexer. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. Science 301:1211-1216.

Rokas, A., and W. H. Holland. 2000. Rare genomic changes as a tool for phylogenetics. Trends Ecol. Evol. 15:454-459.

Rokyta, D. R., P. Joyce, S. B. Caudle, and H. A. Wichman. 2005. An empirical test of the mutational landscape model of adaptation using a single-stranded DNA virus. Nat. Genet. 37:441-444.

Ronquist, F., and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574.

Rose, M. R., and G. V. Lauder (eds.). 1996. Adaptation. Academic Press, San Diego.

Rosenblum, E. B., M. J. Hickerson, and C. Moritz. 2007. A multilocus perspective on colonization accompanied by selection and gene flow. Evolution 61:2971–2985.

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

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. G. Higuchi, T. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487-491.

Santarelli, V. P., A. L. Eastwood, D. A. Dougherty, R. Horn, and C. A. Ahern. 2007. A cation-(π) interaction discriminates among sodium channels that are either sensitive or resistant to tetrodotoxin block. J. Biol. Chem. 282:8044–8051.

Sasaki, A. 2000. Host–parasite coevolution in a multilocus gene-for-gene system. Proc. R. Soc. Biol. Sci. Ser. B 267:2183-2188.

Scheib, H., I. McLay, N. Guex, J. J. Clare, F. E. Blaney, T. J. Dale, S. N. Tate, and G. M. Robertson. 2006. Modeling the pore structure of voltage-gated sodium channels in closed, open, and fast-inactivated conformation reveals details of site 1 toxin and local anesthetic binding. J. Mol. Model. 12:813–822.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766-1774.

Schluter, D., E. A. Clifford, M. Nemethy, and J. S. McKinnon. 2004. Parallel evolution and inheritance of quantitative traits. Am. Nat. 163:809–822.

Seehausen, O. 2004. Hybridization and adaptive radiation. Trends Ecol. Evol. 19:198-207.

Segré, A. V., A. W. Murray, and J.-Y. Leu. 2006. High resolution mutation mapping reveals parallel experimental evolution in yeast. Public Libr. Sci. Biol. 4:e256.

Shimodaira, H., and M. Hasegawa. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16:1114-1116.

Shine, R., B. Phillips, H. Wayne, M. Lemaster, and R. T. Mason. 2004. Species-isolating mechanisms in a mating system with male mate choice (garter snakes, *Thamnophis* spp.). Can. J. Zool. 82:1091-1098.

Song, W., Z. Liu, J. Tan, Y. Nomura, and K. Dong. 2004. RNA editing generates tissue-specific sodium channels with distinct gating properties. J. Biol. Chem. 279:32554-32561.

Soong, T. W., and B. Venkatesh. 2006. Adaptive evolution of tetrodotoxin resistance in animals. Trends Genet. 22:621-626.

Stebbins, R. C. 2003. A field guide to the western reptiles and amphibians, 3rd edition. Houghton Mifflin Co., Boston.

Steiner, C. C., J. N. Weber, and H. E. Hoekstra. 2007. Adaptive variation in beach mice produced by two interacting pigmentation genes. Public Libr. Sci. Biol. 5:1880-1889.

Stewart, C. B., J. W. Schilling, and A. C. Wilson. 1987. Adaptive evolution in the stomach lysozymes of foregut fermenters. Nature 330:401-404.

Stinchcombe, J. R., and H. E. Hoekstra. 2008. Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. Heredity 100:158-170.

Storz, J. F., S. J. Sabatino, F. G. Hoffmann, E. J. Gering, H. Moriyama, N. Ferrand, B. Monteiro, and M. W. Nachman. 2007. The molecular basis of high-altitude adaptation in deer mice. Public Libr. Sci. Genet. 3:448-459.

Sun, Y.-M., I. Favre, L. Schild, and E. Moczydlowski. 1997. On the structural basis for size-selective permeation of organic cations through the voltage-gated sodium channel: effect of alanine mutations at the DEKA locus on selectivity, inhibition by Ca^{2+} and H^{+}, and molecular sieving. J. Gen. Physiol. 110:693-715.

Sutter, N. B., C. D. Bustamante, K. Chase, M. M. Gray, K. Zhao, L. Zhu, B. Padhukasahasram, E. Karlins, S. Davis, P. G. Jones, P. Quignon, G. S. Johnson, H. G. Parker, N. Fretwell, D. S. Mosher, D. F. Lawler, E. Satyaraj, M. Nordborg, K. G. Lark, R. K. Wayne, and E. A. Ostrander. 2007. A single IGF1 allele is a major determinant of small size in dogs. Science 316:112-115.

Swofford, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Sunderland.

Szczerbak, N. N. 2003. Guide to the reptiles of the eastern Palearctic. Krieger, Malabar.

Tanu, M. B., Y. Mahmud, K. Tsuruda, O. Arakawa, and T. Noguchi. 2001. Occurrence of tetrodotoxin in the skin of a rhacophoridid frog *Polypedates* sp. from Bangladesh. Toxicon 39:937-941.

Templeton, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. Evolution 37:221-244.

Teotónio, H., and M. R. Rose. 2000. Variation in the reversibility of evolution. Nature 408:463-466.

Terlau, H., S. H. Heinemann, W. Stuhmer, M. Pusch, F. Conti, K. Imoto, and S. Numa. 1991. Mapping the site of block by tetrodotoxin and saxitoxin of sodium channel II. FEBS Lett. 293:93-96.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positive-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

Thompson, J. N. 1994. The coevolutionary process. University of Chicago Press, Chicago.

Thompson, J. N. 2005. The geographic mosaic of coevolution. University of Chicago Press, Chicago.

Tikhonov, D. B., and B. S. Zhorov. 2005. Modeling P-Loops domain of sodium channel: homology with potassium channels and interaction with ligands. Biophys. J. 88:184-197.

Tishkoff, S. A., F. A. Reed, A. Ranciaro, B. F. Voight, C. C. Babbitt, J. S. Silverman, K. Powell, H. M. Mortensen, J. B. Hirbo, M. Osman, M. Ibrahim, S. A. Omar, G. Lema, T. B. Nyambo, J. Ghori, S. Bumpstead, J. K. Pritchard, G. A. Wray, and P. Deloukas.

2007. Convergent adaptation of human lactase persistence in Africa and Europe. Nat. Genet. 39:31-40.

Toledo, L. F., R. S. Ribeiro, and C. F. B. Haddad. 2007. Anurans as prey: an exploratory analysis and size relationships between predators and their prey. J. Zool. 271:170-177.

Travisano, M., J. A. Mongold, A. F. Bennett, and R. E. Lenski. 1995. Experimental tests of the roles of adaptation, chance, and history in evolution. Science 267:87-90.

Trimmer, J. S., S. S. Cooperman, W. S. Agnew, and G. Mandel. 1990. Regulation of muscle sodium channel transcripts during development and in response to denervation. Devel. Biol. 142:360-367.

Trimmer, J. S., S. S. Cooperman, S. A. Tomiko, J. Zhou, S. M. Crean, M. B. Boyle, R. G. Kallen, Z. Sheng, R. L. Barchi, F. J. Sigworth, R. H. Goodman, W. S. Agnew, and G. Mandel. 1989. Primary structure and functional expression of a mammalian skeletal muscle sodium channel. Neuron 3:33-49.

True, J. R., and E. S. Haag. 2001. Developmental system drift and flexibility in evolutionary trajectories. Evol. Devel. 3:109-119.

Tsuruda, K., O. Arakawa, K. Kawatsu, Y. Hamano, T. Takatani, and T. Noguchi. 2002. Secretory glands of tetrodotoxin in the skin of the Japanese newt *Cynops pyrrhogaster*. Toxicon 40:131-136.

Tsushima, R. G., R. A. Li, and P. H. Backx. 1997. Altered ionic selectivity of the sodium channel revealed by cysteine mutations within the pore. J. Gen. Physiol. 109:463-475.

Tuffley, C., and M. Steel. 1997. Links between maximum likelihood and maximum parsimony under a simple model of site substitution. Bull. Math. Biol. 59:581-607.

Venkatesh, B., S. Q. Lu, N. Dandona, S. L. See, S. Brenner, and T. W. Soong. 2005. Genetic basis of tetrodotoxin resistance in pufferfishes. Curr. Biol. 15:2069-2072.

Vidal, N., S. K. Kindl, A. Wong, and S. B. Hedges. 2000. Phylogenetic relationships of xenodontine snakes inferred from 12S and 16S ribosomal RNA sequences. Mol. Phylogenet. Evol. 14:389-402.

Wade, M. J. 2007. The co-evolutionary genetics of ecological communities. Nat. Rev. Genet. 8:185-195.

Wake, D. B. 1991. Homoplasy: the result of natural selection, or evidence of design limitations? Am. Nat. 138:543-567.

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 the Newts (*Taricha*). Toxicon 3:195-203.

Weinreich, D. M., N. F. Delaney, M. A. DePristo, and D. L. Hartl. 2006. Darwinian evolution can follow only very few mutational paths to fitter proteins. Science 312:111-114.

Wichman, H. A., M. R. Badgett, L. A. Scott, C. M. Boulianne, and J. J. Bull. 1999. Different trajectories of parallel evolution during viral adaptation. Science 285:422-424.

Wiens, J. J., C. A. Kuczynski, S. A. Smith, D. G. Mulcahy, J. W. Sites, Jr., T. M. Townsend, and T. W. Reeder. 2008. Branch length, support, and congruence: testing the phylogenomic approach with 20 nuclear loci in snakes. Syst. Biol. 57:420-431.

Williams, B. L., E. D. Brodie, Jr, and E. D. Brodie, III. 2003. Coevolution of deadly toxins and predator resistance: self-assessment of resistance by garter snakes leads to behavioral rejection of toxic newt prey. Herpetologica 59:155-163.

Williams, G. 2002. Tranalign. Genome Campus, Hinxton.

Wiseman, K. D., and A. C. Pool. 2007. *Thamnophis couchii* (Sierra garter snake): Predator-prey interaction. Herpetol. Rev. 38:344.

Wood, T. E., J. M. Burke, and L. H. Rieseberg. 2005. Parallel genotypic adaptation: when evolution repeats itself. Genetica 123:157-170.

Woods, R., D. Schneider, C. L. Winkworth, M. A. Riley, and R. E. Lenski. 2006. Tests of parallel molecular evolution in a long-term experiment with *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 103:9107-9112.

Wray, G. A. 2007. The evolutionary significance of *cis*-regulatory mutations. Nat. Genet. 8:206-218.

Yamagishi, T., R. A. Li, K. Hsu, E. Marbán, and G. F. Tomaselli. 2001. Molecular architecture of the voltage-dependent Na channel: functional evidence for helices in the pore. J. Gen. Physiol. 118:171-181.

Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. J. Mol. Evol. 39:306-314.

Yoshida, T., S. P. Ellner, L. E. Jones, B. J. M. Bohannan, R. E. Lenski, and N. G. Hairston. 2007. Cryptic population dynamics: rapid evolution masks trophic interactions. Public Libr. Sci. Biol. 5:1868-1879.

Yotsu, M., M. Iorizzi, and T. Yasumoto. 1990. Distribution of tetrodotoxin, 6-epitetrodotoxin, and 11-deoxytetrodotoxin in newts. Toxicon 28:238-41.

Yotsu-Yamashita, M., K. Nishimori, Y. Nitanai, M. Isemura, S. A., and T. Yasumoto. 2000. Binding properties of 3H-PbTx-3 and 3H-saxitoxin to brain membranes and to skeletal muscle membranes of puffer fish *Fugu pardalis* and the primary structure of a voltage-gated Na^+ channel α-subunit (fMNa1) from skeletal muscle of *F. pardalis*. Biochem. Biophys. Res. Commun. 267:403-412.

Yotsu-Yamashita, M., A. Sugimoto, A. Takai, and T. Yasumoto. 1999. Effects of specific modifications of several hydroxyls of tetrodotoxin on its affinity to rat brain membrane. J. Pharmacol. Exp. Ther. 289:1688-1696.

Zhang, J. 2006. Parallel adaptive origins of digestive RNases in Asian and African leaf monkeys. Nat. Genet. 38:819-823.

Zhang, J., and S. Kumar. 1997. Detection of convergent and parallel evolution at the amino acid sequence level. Mol. Biol. Evol. 14:527-536.

Zhao, E., and K. Adler. 1993. Herpetology of China. Society for the Study of Amphibians and Reptiles, St. Louis.

Zhao, E., M. Huang, and Y. Zong (eds.). 1998. Fauna sinica: reptilia volume 3 Squamata, Serpentes. Science Press, Beijing, PRC. [in Chinese]

APPENDICES

APPENDIX A

Specimens examined

TABLE A.1. List of specimens examined for TTX bioassay (PE) and $Na_v1.4$ sequence variation (GE). General locality, laboratory run number for phenotypic assay, and voucher number also provided. Specimens without voucher numbers are captive snakes still maintained in the USU live collection; CAS: California Academy of Sciences; LSUMZ: Louisiana State University Museum of Natural Science; MVZ: Museum of Vertebrate Zoology; SDSNH: San Diego Natural History Museum; UMMZ: University of Michigan Museum of Zoology; UTACV: University of Texas, Arlington; ZVC: Departmento de Zoologia Vertebrados, Facultad de Ciencias, Universidad de la República Uruguay; AdQ: A. de Queiroz; CRF: C.R. Feldman (deposited at CAS); EDB jr: E.D. Brodie jr (deposited at CAS or UTACV); SJA ts: S.J. Arnold (tissue collection).

Species	Voucher #	Run #	Locality	PE	GE
Afronatrix anoscopus	MVZ 245370		Togo Hills, Kyobobo National Park, Ghana		GE
Agkistrodon contortrix	MVZ 137595		SE Highlands, Macon Co., NC		GE
Amphiesma	CAS 233242		Laiva Dam, Falam, Chin, Myanmar		GE
Amphiesma pryeri	LSUMZ 8162		locality unknown, Japan		GE
Amphiesma pryeri	LSUMZ 8163		locality unknown, Japan		GE
Amphiesma vibakari	LSUMZ 377		Izugo, Oyamada Mura, Japan		GE
Causus maculatus	MVZ 249820		Nkwanta, Volta, Ghana		GE
Charina bottae	CRF 1979		Willow Creek, Sonoma Co., CA		GE
Clonophis kirtlandii	CAS 184363		Indianapolis, Indianapolis Co., IN		GE
Coluber constrictor	EDB jr 22864		Dogtown Creek, El Dorado Co., CA		GE
Coluber constrictor	EDB jr 23522	7.040	Mink Creek, Pocatello, Bannock Co., ID	PE	
Coluber constrictor	EDB jr 23491	7.001	Ross Park, Pocatello, Bannock Co., ID	PE	GE
Coluber constrictor	EDB jr 23523	7.032	West Bench, Pocatello, Bannock Co., ID	PE	
Coluber constrictor	EDB jr 21524	5.26	Willow Creek, Sonoma Co., CA	PE	GE
Coluber constrictor	EDB jr 21525	5.27	Willow Creek, Sonoma Co., CA	PE	GE
Coluber constrictor	EDB jr 21526	5.28	Willow Creek, Sonoma Co., CA	PE	GE
Coluber constrictor	EDB jr 21527	5.29	Willow Creek, Sonoma Co., CA	PE	GE
Coniophanes bipunctatus	LSUMZ 6791		Sandy Bay, Isle Roatan, Honduras		GE
Coniophanes fissidens	LSUMZ 6448		locality unknown		GE
Coniophanes fissidens	MVZ 180410		Sinca San Jeronimo, Chiapas, Mexico		GE
Crotalus oreganus	MVZ 229157		Bitterwater, San Benito Co., CA		GE
Dendrelaphis sp.	EDB jr 23485	7.007	locality unknown	PE	GE
Dendrelaphis sp.	EDB jr 23486	7.008	locality unknown	PE	
Dendrelaphis sp.	EDB jr 23487	7.009	locality unknown	PE	GE
Dendrelaphis sp.	EDB jr 23488	7.01	locality unknown	PE	
Dendrelaphis sp.	EDB jr 23489	7.011	locality unknown	PE	GE
Dendrelaphis sp.	EDB jr 23490	7.012	locality unknown	PE	
Diadophis punctatus	MVZ 233490		30 mi SW of Omaha, Cass Co., NE		GE

Diadophis punctatus	EDB jr 23498	7.023	Luray, Russel Co., KS	PE	
Diadophis punctatus	EDB jr 23499	7.024	Luray, Russel Co., KS	PE	
Diadophis punctatus	SDSNH 68750		Palomar Mountain, San Diego Co., CA		GE
Drymarchon corais	CAS 198327		Citrus Springs, Citrus Co., FL		GE
Drymobius margaritiferus	MVZ 233289		Tortuguero, Limon Prov, Costa Rica		GE
Elapsoidea nigra	CAS 168978		Usambara Mtns, Lushoto, Tanga, Tanzania		GE
Elgaria multicarinata	EDB jr 21660	5.136	Grizzley Flat, El Dorado Co., CA	PE	
Elgaria multicarinata	EDB jr 21661	5.137	Grizzley Flat, El Dorado Co., CA	PE	
Elgaria multicarinata	EDB jr 21658	5.135	Manzanas Creek Reservoir, Sonoma Co., CA	PE	
Elgaria multicarinata	EDB jr 21659	5.138	Manzanas Creek Reservoir, Sonoma Co., CA	PE	
Elgaria multicarinata	EDB jr 21657	5.134	Sebastapol, Sonoma Co., CA	PE	
Elgaria multicarinata	CAS 236534		Spring Lake County Park, Sonoma Co., CA		GE
Enhydris sp.	EDB jr 23481	7.005	locality unknown	PE	GE
Enhydris sp.	EDB jr 23482	7.006	locality unknown	PE	
Gonionotophis klingi	MVZ 245374		Ankasa National Park, Ghana		GE
Grayia smithii	LSUMZ 9143		locality unknown, Togo		GE
Hapsidophrys lineatus	CAS 201746		Bwindi National Prk, Rukungiri, Uganda		GE
Helicops angulatus	LSUMZ 3346		locality unknown, Trinidad and Tobago		GE
Heterodon nasicus	UTA R56287	Het 7.40	locality unknown	PE	GE
Heterodon platirhinos	UTA R55382	Het 7.39	Lake Whitney, Bosque Co., TX	PE	GE
Liopeltis tricolor	EDB jr 23483	7.004	locality unknown	PE	GE
Liophis anomalus	LSUMZ 6970		locality unknown, Argentina		GE
Liophis epinephalus	MVZ 204111		Cartago, Cartago, Costa Rica		GE
Liophis miliaris	LSUMZ 6560		locality unknown, Peru		GE
Liophis poecilogyrus	LSUMZ 6972		locality unknown, Argentina		GE
Liophis typhlus	LSUMZ 6456		locality unknown		GE
Lystrophis dorbingyii	ZVC (pending)		Villa Dolores, Uruguay		GE
Lystrophis semicinctus	LSUMZ 6363		locality unknown		GE
Natrix maura	MVZ 200533		NE Balenya, Catalonia, Spain		GE
Natrix maura	MVZ 235726		Skikda Wilayat, Algeria		GE
Natrix natrix	MVZ 232105		Facinas, Andalusia, Spain		GE
Natrix natrix	MVZ 200534		NE Balenya, Catalonia, Spain		GE
Nerodia clarkii	EDB jr 22734	6.120	Galveston, Galveston Co., TX	PE	GE
Nerodia cyclopion	CAS 184361		near Florida City, Dade Co., FL		GE
Nerodia erythrogaster	EDB jr 22802	6.170	Auburn, Lee Co., AL	PE	GE
Nerodia erythrogaster	EDB jr 22803	6.171	Auburn, Lee Co., AL	PE	
Nerodia erythrogaster	EDB jr 22804	6.172	Auburn, Lee Co., AL	PE	
Nerodia erythrogaster	EDB jr 22805	6.173	Auburn, Lee Co., AL	PE	
Nerodia erythrogaster	EDB jr 22806	6.179	near Frenier, St. John Baptist Parish, LA	PE	

Nerodia fasciata	EDB jr 22810	6.178	near Frenier, St. John Baptist Parish, LA	PE	GE
Nerodia rhombifer	EDB jr 22799	6.167	Auburn, Lee Co., AL	PE	GE
Nerodia rhombifer	EDB jr 22800	6.168	Auburn, Lee Co., AL	PE	
Nerodia rhombifer	EDB jr 22801	6.169	Auburn, Lee Co., AL	PE	
Nerodia sipedon	EDB jr 22806	6.174	Auburn, Lee Co., AL	PE	GE
Nerodia sipedon	EDB jr 22807	6.175	Auburn, Lee Co., AL	PE	
Nerodia sipedon	EDB jr 22808	6.176	Auburn, Lee Co., AL	PE	
Nerodia sipedon	EDB jr 22809	6.177	Auburn, Lee Co., AL	PE	
Pituophis catenifer	MVZ 229148		Tassajara Rd., Monterey Co., CA		GE
Pituophis catenifer	EDB jr 23524	7.033	West Bench, Pocatello, Bannock Co., ID	PE	
Pituophis melanoleucus	MVZ 150219		near Southport, Brunswick Co., NC		GE
Protobothrops flavoviridis	UMMZ 199973		Tokunoshima, Ryukyu Island, Japan		GE
Protobothrops mucrosquamatus	MVZ 230461		Hainan Island, Hainan, China		GE
Ptyas korros	CAS 205259		Pyin Si, Nwahtogyi, Mandalay, Myanmar		GE
Ptyas mucosus	CAS 208434		Po Ywa, Kyaukse Pyaw Bwe, Mandalay, Myanmar		GE
Rhabdophis himalaya	CAS 222906		Gwa, Rakhine, Myanmar		GE
Rhabdophis subminiata	CAS 212025		Mwe Huak, Ayeyarwade, Myanmar		GE
Rhabdophis subminiata	MVZ 226583		Tam Dao, Vinh Yen, Vietman		GE
Rhabdophis tigrinus	LSUMZ 6808		locality unknown, South Korea		GE
Rhamnophis aethiopissa	MVZ 245384		Ankasa National Park, Ghana		GE
Sinonatrix aequifasciata	MVZ 224258		Tam Dao, Vinh Phu, Vietman		GE
Sinonatrix percarinata	MVZ 224256		Tam Dao, Vinh Phu, Vietman		GE
Storeria dekayi	EDB jr 21928	5.235	Onondaga Lake, Geddes, Onondaga Co., NY	PE	GE
Storeria dekayi	EDB jr 23509	7.038	Richards, Montgomery Co., TX	PE	
Storeria dekayi	EDB jr 23510	7.039	Richards, Montgomery Co., TX	PE	
Storeria occipitomaculata	EDB jr 21909	WE1	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21910	WE2	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21911	WE3	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21912	WE4	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21913	WE5	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21914	WE6	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21915	WE7	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21916	WE8	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21917	5.228	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21918	WF1	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21919	WF2	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21920	WF3	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21921	WF4	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21922	5.229	Cincinnatus, Cortland Co., NY	PE	

Storeria occipitomaculata	EDB jr 21923	5.230	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21924	5.231	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21925	5.232	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21926	5.233	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21927	5.234	Cincinnatus, Cortland Co., NY	PE	
Storeria occipitomaculata	EDB jr 21908	5.227	Cincinnatus, Cortland Co., NY	PE	GE
Thamnodynates pallidus	LSUMZ 9375		Chuspipata, La Paz, Bolivia		GE
Thamnodynates strigatus	LSUMZ 6563		unknown (likely Peru)		GE
Thamnophis atratus	EDB jr 22040	5.208	Bald Hills, Sonoma Co., CA	PE	GE
Thamnophis atratus	EDB jr 22041	5.209	Bald Hills, Sonoma Co., CA	PE	GE
Thamnophis atratus	EDB jr 22042	5.210	Bald Hills, Sonoma Co., CA	PE	GE
Thamnophis atratus	EDB jr 22043	5.211	Bald Hills, Sonoma Co., CA	PE	GE
Thamnophis atratus	EDB jr 22044	5.212	Bald Hills, Sonoma Co., CA	PE	GE
Thamnophis atratus	EDB jr 22058	5.226	Coleman Valley, Sonoma Co., CA	PE	GE
Thamnophis atratus		TaC TL	Coquille, Coos Co., OR	PE	GE
Thamnophis atratus	EDB jr 22055	5.223	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis atratus	EDB jr 20933	TaM TG	Molino Creek, Santa Cruz Co., CA	PE	GE
Thamnophis atratus	EDB jr 21486	4.109	Molino Creek, Santa Cruz Co., CA	PE	GE
Thamnophis atratus	EDB jr 21650	5.19	Molino Creek, Santa Cruz Co., CA	PE	GE
Thamnophis atratus	EDB jr 21651	5.20	Molino Creek, Santa Cruz Co., CA	PE	GE
Thamnophis atratus	EDB jr 21652	5.21	Molino Creek, Santa Cruz Co., CA	PE	GE
Thamnophis atratus	EDB jr 21653	5.22	Molino Creek, Santa Cruz Co., CA	PE	
Thamnophis atratus	EDB jr 21654	5.23	Molino Creek, Santa Cruz Co., CA	PE	
Thamnophis atratus	EDB jr 21655	5.60	Molino Creek, Santa Cruz Co., CA	PE	GE
Thamnophis atratus	EDB jr 21656	5.61	Molino Creek, Santa Cruz Co., CA	PE	GE
Thamnophis atratus	EDB jr 22309	5.24	Molino Creek, Santa Cruz Co., CA	PE	GE
Thamnophis atratus	EDB jr 21323	2.46	Navarro Beach, Mendocino Co., CA	PE	GE
Thamnophis atratus	EDB jr 21324	2.47	Navarro Beach, Mendocino Co., CA	PE	GE
Thamnophis atratus	EDB jr 21329	2.45	Navarro Beach, Mendocino Co., CA	PE	GE
Thamnophis atratus	EDB jr 21336	2.44	Navarro Beach, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 22371	6.002	Pillar Point Harbor, San Mateo Co., CA	PE	GE
Thamnophis atratus	EDB jr 21634	5.112	Redwood Creek, Humboldt Co., CA	PE	GE
Thamnophis atratus	EDB jr 21635	5.113	Redwood Creek, Humboldt Co., CA	PE	GE
Thamnophis atratus	EDB jr 21636	5.115	Redwood Creek, Humboldt Co., CA	PE	GE
Thamnophis atratus	EDB jr 21355	4.25	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21356	4.26	Redwood River Resort, Mendocino Co., CA	PE	GE
Thamnophis atratus	EDB jr 21357	4.28	Redwood River Resort, Mendocino Co., CA	PE	GE
Thamnophis atratus	EDB jr 21358	4.30	Redwood River Resort, Mendocino Co., CA	PE	GE
Thamnophis atratus	EDB jr 21359	4.31	Redwood River Resort, Mendocino Co., CA	PE	

Thamnophis atratus	EDB jr 21360	4.32	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21361	4.33	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21362	4.34	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21363	4.35	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21364	4.36	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21401	VA1	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21402	VA2	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21403	VA3	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21404	VA4	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21405	VA5	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21406	VA6	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21407	VA7	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21408	VA8	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21409	VB1	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21410	VB2	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21421	4.29	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21422	4.27	Redwood River Resort, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21380	4.03	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 21387	4.10	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 22273	XC1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22274	XC2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22275	XC3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22276	XC4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22277	XC5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22278	XC6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22279	XC7	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22280	XC8	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22302	XF1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22303	XF2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22304	XF3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22305	XF4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22306	XF5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22307	XF6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22308	XF7	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 22344	5.260	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 22361	5.253	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 22363	5.262	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 22364	5.264	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 22365	5.265	Santa Lucia Preserve, Monterey Co., CA	PE	GE

Thamnophis atratus	EDB jr 22369	5.271	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23092	XZ1	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23093	XZ2	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23094	XZ3	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23095	XZ4	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23096	XZ5	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23097	XZ6	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23098	XZ7	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23099	XZ8	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23100	XZ9	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23146	YD1	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23147	YD2	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23148	YD3	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23149	YD4	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23150	YD5	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23151	YD6	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23152	YD7	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23153	YD8	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23154	YD9	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23155	YD10	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23156	YD11	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23157	YD12	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23189	YH1	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23190	YH2	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23191	YH4	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23192	YH5	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23203	YK1	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23204	YK2	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23205	YK3	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23206	YK4	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23207	YK5	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23208	YK6	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23209	YK7	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23210	YK8	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23211	YL1	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23212	YL2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23213	YL3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23214	YL4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23215	YL5	Santa Lucia Preserve, Monterey Co., CA	PE	

Thamnophis atratus	EDB jr 23216	YL6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23227	YP1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23228	YR1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23229	YT1	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23230	YT2	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23231	YT3	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23232	YT4	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23233	YT5	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23234	YT6	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23235	YT7	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23236	YT8	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23237	YV1	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23238	YV2	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23239	YV3	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23240	YV4	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23241	YV5	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23242	YV6	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23243	YV7	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23244	YV8	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23245	YV9	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23246	YV10	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23247	YV11	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23248	YV12	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23249	YV13	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23277	6.240 (YD)	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23278	6.241 (YL)	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23279	6.242 (XZ)	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23280	6.249 (YT)	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23281	6.110 (YR)	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23282	6.244 (YY)	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23288	YY1	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23289	YY2	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23290	YY3	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 23546	ZD1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23547	ZD2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23548	ZD3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23549	ZD4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23550	ZD5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23551	ZD6	Santa Lucia Preserve, Monterey Co., CA	PE	

Thamnophis atratus	EDB jr 23552	ZD7	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23553	ZD8	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23554	ZD9	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23555	ZD10	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23556	ZD11	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23557	ZD12	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23558	ZD13	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23559	ZD14	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23560	ZD15	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23561	ZD16	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23562	ZD17	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23563	ZD18	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23564	ZD19	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23565	ZD20	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23566	ZD21	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23567	ZD22	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23568	ZD23	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23569	ZE1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23570	ZE2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23571	ZE3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23572	ZE4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus	EDB jr 23573	ZE5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis atratus		6.243 (YS)	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus		6.245 (YP)	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus		6.250 (YX)	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus		6.252 (YH)	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus		6.256 (YK)	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus		6.257 (YV)	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus		YX2	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus		YX3	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis atratus	EDB jr 21606	5.82	Smith River, Del Norte Co., CA	PE	GE
Thamnophis atratus	EDB jr 21607	5.83	Smith River, Del Norte Co., CA	PE	GE
Thamnophis atratus	EDB jr 21608	5.84	Smith River, Del Norte Co., CA	PE	GE
Thamnophis atratus	EDB jr 21609	5.85	Smith River, Del Norte Co., CA	PE	GE
Thamnophis atratus	EDB jr 21610	5.86	Smith River, Del Norte Co., CA	PE	
Thamnophis atratus	EDB jr 22370	5.81	Smith River, Del Norte Co., CA	PE	
Thamnophis atratus	EDB jr 21309	2.28	Tomki Creek, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21310	2.21	Tomki Creek, Mendocino Co., CA	PE	GE
Thamnophis atratus	EDB jr 21311	2.27	Tomki Creek, Mendocino Co., CA	PE	GE

Thamnophis atratus	EDB jr 21312	2.24	Tomki Creek, Mendocino Co., CA	PE	GE
Thamnophis atratus	EDB jr 21313	2.25	Tomki Creek, Mendocino Co., CA	PE	GE
Thamnophis atratus	EDB jr 21314	2.26	Tomki Creek, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21315	2.29	Tomki Creek, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21317	2.30	Tomki Creek, Mendocino Co., CA	PE	
Thamnophis atratus	EDB jr 21318	2.22	Tomki Creek, Mendocino Co., CA	PE	GE
Thamnophis atratus	EDB jr 21319	2.23	Tomki Creek, Mendocino Co., CA	PE	GE
Thamnophis atratus	EDB jr 20700	RS	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis atratus	EDB jr 20701	RU	Willow Creek, Sonoma Co., CA		
Thamnophis atratus	EDB jr 20926	RV	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis atratus	EDB jr 21376	4.57	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis atratus	EDB jr 21373	4.46	Yager Creek, Humboldt Co., CA	PE	GE
Thamnophis atratus	EDB jr 21374	4.47	Yager Creek, Humboldt Co., CA	PE	GE
Thamnophis atratus	EDB jr 21375	4.48	Yager Creek, Humboldt Co., CA	PE	GE
Thamnophis brachystoma	MVZ 137702		near Kellettville, Forest Co., PA		GE
Thamnophis couchii	EDB jr 23520	7.046	Akin Powerhouse, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 21337	3.40	Antelope Creek, Tehama Co., CA	PE	
Thamnophis couchii	EDB jr 21338	3.39	Antelope Creek, Tehama Co., CA	PE	
Thamnophis couchii	EDB jr 21339	3.37	Antelope Creek, Tehama Co., CA	PE	
Thamnophis couchii	EDB jr 22944	6.226	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22945	6.227	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 20702	SE	Cold Springs Creek, Tulare Co., CA	PE	GE
Thamnophis couchii	EDB jr 20703	SS	Cold Springs Creek, Tulare Co., CA	PE	GE
Thamnophis couchii	EDB jr 20704	SZ	Cold Springs Creek, Tulare Co., CA	PE	GE
Thamnophis couchii	EDB jr 21416	4.52	Coy Flat, Tulare Co., CA	PE	GE
Thamnophis couchii	EDB jr 21589	5.64	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 21590	5.65	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 21591	5.66	Deep Creek, Fresno Co., CA	PE	GE
Thamnophis couchii	EDB jr 21592	5.67	Deep Creek, Fresno Co., CA	PE	GE
Thamnophis couchii	EDB jr 21593	5.68	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 21594	5.69	Deep Creek, Fresno Co., CA	PE	GE
Thamnophis couchii	EDB jr 21595	5.70	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 21596	5.71	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 21597	5.72	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 21598	5.73	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 21599	5.74	Deep Creek, Fresno Co., CA	PE	GE
Thamnophis couchii	EDB jr 21600	5.75	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 21601	5.76	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 21602	5.77	Deep Creek, Fresno Co., CA	PE	GE

Thamnophis couchii	EDB jr 21603	5.78	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 21604	5.79	Deep Creek, Fresno Co., CA	PE	GE
Thamnophis couchii	EDB jr 21605	5.80	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22284	XD1	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22285	XD2	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22286	XD3	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22287	XD4	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22288	XD5	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22289	XD6	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22290	XD7	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22291	XD8	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22292	XD9	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22293	XD10	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22294	XD11	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22295	XD12	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22296	XD13	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22297	XD14	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22298	XD15	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22299	XD16	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22300	XD17	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 22301	XD19	Deep Creek, Fresno Co., CA	PE	
Thamnophis couchii	EDB jr 21341	3.35	Deer Creek and Beaver Creek, Tehama Co., CA	PE	
Thamnophis couchii	EDB jr 21342	3.34	Deer Creek and Beaver Creek, Tehama Co., CA	PE	
Thamnophis couchii	EDB jr 21945	5.139	Devil's Corral, Lassen Co., CA	PE	
Thamnophis couchii	EDB jr 21946	5.140	Devil's Corral, Lassen Co., CA	PE	
Thamnophis couchii	EDB jr 21947	5.141	Devil's Corral, Lassen Co., CA	PE	
Thamnophis couchii	EDB jr 21948	5.142	Devil's Corral, Lassen Co., CA	PE	
Thamnophis couchii	EDB jr 21949	5.143	Devil's Corral, Lassen Co., CA	PE	
Thamnophis couchii	EDB jr 21950	5.144	Devil's Corral, Lassen Co., CA	PE	
Thamnophis couchii	EDB jr 21951	5.145	Devil's Corral, Lassen Co., CA	PE	
Thamnophis couchii	EDB jr 21952	5.146	Devil's Corral, Lassen Co., CA	PE	
Thamnophis couchii	EDB jr 21953	5.147	Devil's Corral, Lassen Co., CA	PE	
Thamnophis couchii	EDB jr 21954	5.148	Devil's Corral, Lassen Co., CA	PE	
Thamnophis couchii	EDB jr 21955	5.149	Devil's Corral, Lassen Co., CA	PE	
Thamnophis couchii	EDB jr 21956	5.150	Devil's Corral, Lassen Co., CA	PE	
Thamnophis couchii	EDB jr 21957	5.151	Devil's Corral, Lassen Co., CA	PE	
Thamnophis couchii	EDB jr 21958	5.152	Devil's Corral, Lassen Co., CA	PE	
Thamnophis couchii		TcD UC	Devil's Corral, Lassen Co., CA	PE	GE
Thamnophis couchii	EDB jr 21627	5.106	Dogtown Creek, El Dorado Co., CA	PE	

Thamnophis couchii	EDB jr 21628	5.107	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 21629	5.108	Dogtown Creek, El Dorado Co., CA	PE	GE
Thamnophis couchii	EDB jr 21630	5.109	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22069	5.202	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22070	5.203	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22071	5.204	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22072	5.205	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22073	5.206	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22074	5.207	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22866	6.182	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22867	6.183	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23294	ZA1	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23295	ZA2	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23296	ZA3	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23297	ZA4	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23298	ZA5	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23299	ZA6	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23300	ZA7	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23301	ZA8	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23302	ZB1	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23303	ZB2	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23304	ZB3	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23305	ZB4	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23306	ZB5	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23307	ZB6	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23308	ZB7	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23309	ZB8	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23310	ZB9	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23311	ZB10	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23312	ZB11	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 21417	4.53	Grizzly Flat, El Dorado Co., CA	PE	GE
Thamnophis couchii	EDB jr 21418	4.54	Grizzly Flat, El Dorado Co., CA	PE	GE
Thamnophis couchii	EDB jr 21419	4.55	Grizzly Flat, El Dorado Co., CA	PE	GE
Thamnophis couchii	EDB jr 21420	4.56	Grizzly Flat, El Dorado Co., CA	PE	GE
Thamnophis couchii	EDB jr 21343	3.33	Hat Creek, Shasta Co., CA	PE	
Thamnophis couchii	EDB jr 21344	3.32	Hat Creek, Shasta Co., CA	PE	
Thamnophis couchii	EDB jr 21345	3.31	Hat Creek, Shasta Co., CA	PE	
Thamnophis couchii	EDB jr 21346	3.30	Hat Creek, Shasta Co., CA	PE	
Thamnophis couchii	EDB jr 21340	3.36	Indian Creek, Tehama Co., CA	PE	

Thamnophis couchii	EDB jr 21388	4.14	Keleher, Nevada Co., CA	PE	GE
Thamnophis couchii	EDB jr 21389	4.15	Keleher, Nevada Co., CA	PE	GE
Thamnophis couchii	EDB jr 21390	4.16	Keleher, Nevada Co., CA	PE	GE
Thamnophis couchii	EDB jr 21391	4.17	Keleher, Nevada Co., CA	PE	GE
Thamnophis couchii	EDB jr 21392	4.18	Keleher, Nevada Co., CA	PE	GE
Thamnophis couchii	EDB jr 21393	4.19	Keleher, Nevada Co., CA	PE	GE
Thamnophis couchii	EDB jr 21394	4.20	Keleher, Nevada Co., CA	PE	GE
Thamnophis couchii	EDB jr 21395	4.21	Keleher, Nevada Co., CA	PE	GE
Thamnophis couchii	EDB jr 21396	4.22	Keleher, Nevada Co., CA	PE	GE
Thamnophis couchii	EDB jr 21397	4.23	Keleher, Nevada Co., CA	PE	GE
Thamnophis couchii	EDB jr 22063	5.195	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22067	5.200	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22068	5.201	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22095	5.228a	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22736	6.126	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22737	6.130	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22738	6.131	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22739	6.132	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22740	6.133	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22786	6.124	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22787	6.128	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22788	6.129	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 21365	4.37	North Battle Creek, Shasta Co., CA	PE	GE
Thamnophis couchii	EDB jr 21366	4.38	North Battle Creek, Shasta Co., CA	PE	GE
Thamnophis couchii	EDB jr 21367	4.39	North Battle Creek, Shasta Co., CA	PE	GE
Thamnophis couchii	EDB jr 21368	4.40	North Battle Creek, Shasta Co., CA	PE	GE
Thamnophis couchii	EDB jr 21369	4.41	North Battle Creek, Shasta Co., CA	PE	GE
Thamnophis couchii	EDB jr 21370	4.42	North Battle Creek, Shasta Co., CA	PE	GE
Thamnophis couchii	EDB jr 23516	7.037	North Fork Consumnes River, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23506	7.013	North Fork Feather River, Plumas Co., CA	PE	
Thamnophis couchii	EDB jr 23526	7.049	North Fork Feather River, Plumas Co., CA	PE	
Thamnophis couchii	EDB jr 23513	7.034	Oligby Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23515	7.036	Oligby Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23517	7.043	Oligby Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23518	7.044	Oligby Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22329	5.248	Pollock Pines, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 22350	5.273	Pollock Pines, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 21371	4.44	Rock Creek, Shasta Co., CA	PE	GE
Thamnophis couchii	EDB jr 21372	4.45	Rock Creek, Shasta Co., CA	PE	GE

Thamnophis couchii	EDB jr 22096	4.43	Rock Creek, Shasta Co., CA	PE	
Thamnophis couchii	EDB jr 23519	7.045	Silver Creek, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 23525	7.048	South Fork American River, El Dorado Co., CA	PE	
Thamnophis couchii	EDB jr 20104	1.19	Tyler Creek, Tulare Co., CA	PE	GE
Thamnophis couchii	EDB jr 20105	1.20	Tyler Creek, Tulare Co., CA	PE	GE
Thamnophis couchii	EDB jr 21414	4.49	Wishon, Tulare Co., CA	PE	GE
Thamnophis couchii	EDB jr 21415	4.50	Wishon, Tulare Co., CA	PE	GE
Thamnophis couchii	EDB jr 21489	VC1	Wishon, Tulare Co., CA	PE	
Thamnophis couchii	EDB jr 21490	VC2	Wishon, Tulare Co., CA	PE	
Thamnophis couchii	EDB jr 21491	VC3	Wishon, Tulare Co., CA	PE	
Thamnophis couchii	EDB jr 21492	VC4	Wishon, Tulare Co., CA	PE	
Thamnophis couchii	EDB jr 21493	VC5	Wishon, Tulare Co., CA	PE	
Thamnophis couchii	EDB jr 21494	VC6	Wishon, Tulare Co., CA	PE	
Thamnophis couchii	EDB jr 21495	VC7	Wishon, Tulare Co., CA	PE	
Thamnophis couchii	EDB jr 21496	VC8	Wishon, Tulare Co., CA	PE	
Thamnophis couchii	EDB jr 21497	VC9	Wishon, Tulare Co., CA	PE	
Thamnophis couchii	EDB jr 21498	VC10	Wishon, Tulare Co., CA	PE	
Thamnophis couchii	EDB jr 21499	VC11	Wishon, Tulare Co., CA	PE	
Thamnophis couchii	EDB jr 21500	VC12	Wishon, Tulare Co., CA	PE	
Thamnophis couchii	EDB jr 21546	4.51 (VC)	Wishon, Tulare Co., CA	PE	
Thamnophis couchii	EDB jr 21587	5.62	Wishon, Tulare Co., CA	PE	
Thamnophis cyrtopsis	EDB jr 21443	4.77	Artesia Well, Ladder Ranch, Sierra Co., NM	PE	GE
Thamnophis cyrtopsis	EDB jr 21444	4.78	Artesia Well, Ladder Ranch, Sierra Co., NM	PE	
Thamnophis elegans	EDB jr 23514	7.035	Alder Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 22000	5.185	Bodega Marine Lab, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22030	5.186	Bodega Marine Lab, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22031	5.187	Bodega Marine Lab, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22320	5.237 (WJ)	Bodega Marine Lab, Sonoma Co., CA	PE	
Thamnophis elegans	MVZ 241624	TeCV	Cache Valley, Cahe Co., UT	PE	GE
Thamnophis elegans	EDB jr 21995	5.179	Dillon Beach, Marin Co., CA	PE	
Thamnophis elegans	EDB jr 21996	5.180	Dillon Beach, Marin Co., CA	PE	
Thamnophis elegans	EDB jr 21997	5.181	Dillon Beach, Marin Co., CA	PE	
Thamnophis elegans	EDB jr 21998	5.182	Dillon Beach, Marin Co., CA	PE	
Thamnophis elegans	EDB jr 21999	5.183	Dillon Beach, Marin Co., CA	PE	
Thamnophis elegans	EDB jr 21631	5.110	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 21632	5.111	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 22064	5.197	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 22065	5.198	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 22066	5.199	Dogtown Creek, El Dorado Co., CA	PE	GE

Thamnophis elegans	EDB jr 22865	6.181	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23217	YN1	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23218	YN2	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23219	YN3	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23220	YN4	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23221	YN5	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23222	YN6	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23224	YO1	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23225	YO2	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23226	YO3	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23250	YW1	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23251	YW2	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23252	YW3	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23253	YW4	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23254	YW5	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23255	6.263 (YZ)	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23256	6.264 (YO)	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23257	6.265 (YW)	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23258	6.266 (YN)	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23291	YZ1	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23292	YZ2	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23293	YZ3	Dogtown Creek, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 21968	5.162	Feather Lake, Lassen Co., CA	PE	
Thamnophis elegans	EDB jr 22053	5.221	Gilroy, Santa Clara Co., CA	PE	
Thamnophis elegans	EDB jr 22054	5.222	Gilroy, Santa Clara Co., CA	PE	
Thamnophis elegans	EDB jr 22056	5.224	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis elegans	EDB jr 22903	6.233	Grizzley Lake, Madera Co, CA	PE	
Thamnophis elegans	EDB jr 22948	6.230	Grizzley Lake, Madera Co, CA	PE	
Thamnophis elegans	EDB jr 22949	6.231	Grizzley Lake, Madera Co, CA	PE	
Thamnophis elegans	EDB jr 22950	6.232	Grizzley Lake, Madera Co, CA	PE	
Thamnophis elegans	EDB jr 22951	6.234	Grizzley Lake, Madera Co, CA	PE	
Thamnophis elegans	EDB jr 22725	6.090	Half Moon Bay, San Mateo Co., CA	PE	
Thamnophis elegans	EDB jr 22726	6.091	Half Moon Bay, San Mateo Co., CA	PE	
Thamnophis elegans	EDB jr 22727	6.092	Half Moon Bay, San Mateo Co., CA	PE	
Thamnophis elegans	EDB jr 22728	6.094	Half Moon Bay, San Mateo Co., CA	PE	
Thamnophis elegans	EDB jr 22729	6.095	Half Moon Bay, San Mateo Co., CA	PE	
Thamnophis elegans	EDB jr 22730	6.096	Half Moon Bay, San Mateo Co., CA	PE	
Thamnophis elegans	EDB jr 22731	6.097	Half Moon Bay, San Mateo Co., CA	PE	
Thamnophis elegans	EDB jr 22732	6.098	Half Moon Bay, San Mateo Co., CA	PE	

Thamnophis elegans	EDB jr 22733	6.099	Half Moon Bay, San Mateo Co., CA	PE	
Thamnophis elegans	EDB jr 22741	6.134	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 22742	6.138	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 22743	6.139	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 22744	6.140	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 22745	6.141	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis elegans	EDB jr 23316	6.135 (YQ)	Leoni Meadows, El Dorado Co., CA	PE	GE
Thamnophis elegans	EDB jr 23317	6.137	Leoni Meadows, El Dorado Co., CA	PE	GE
Thamnophis elegans	EDB jr 21517	5.120	Manzanas Creek Reservoir, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21487	4.110	Molino Creek, Santa Cruz Co., CA	PE	
Thamnophis elegans	EDB jr 21503	4.113	Molino Creek, Santa Cruz Co., CA	PE	
Thamnophis elegans	EDB jr 21518	5.13	Molino Creek, Santa Cruz Co., CA	PE	GE
Thamnophis elegans	EDB jr 21519	5.14	Molino Creek, Santa Cruz Co., CA	PE	
Thamnophis elegans	EDB jr 21520	5.15	Molino Creek, Santa Cruz Co., CA	PE	GE
Thamnophis elegans	EDB jr 21521	5.17	Molino Creek, Santa Cruz Co., CA	PE	
Thamnophis elegans	EDB jr 21522	5.18	Molino Creek, Santa Cruz Co., CA	PE	
Thamnophis elegans	EDB jr 22641	5.16	Molino Creek, Santa Cruz Co., CA	PE	
Thamnophis elegans	EDB jr 22869	6.184	Mud Lake, Amador Co., CA	PE	GE
Thamnophis elegans	EDB jr 22870	6.185	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22871	6.186	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22872	6.187	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22873	6.188	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22874	6.189	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22875	6.190	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22876	6.191	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22877	6.192	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22878	6.193	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22879	6.194	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22880	6.195	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22881	6.196	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22882	6.197	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22883	6.198	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22884	6.199	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22885	6.200	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22886	6.201	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22887	6.202	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22888	6.203	Mud Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22759	6.087	Nacimiento Creek, Monterey Co., CA	PE	GE
Thamnophis elegans	EDB jr 22760	6.088	Nacimiento Creek, Monterey Co., CA	PE	GE

Thamnophis elegans	EDB jr 23521	7.047	North Fork Feather River, Butte Co., CA	PE	
Thamnophis elegans	EDB jr 23521	7.047	North Fork Feather River, Butte Co., CA	PE	
Thamnophis elegans	EDB jr 23574	ZF1	North Fork Feather River, Butte Co., CA	PE	
Thamnophis elegans	EDB jr 23575	ZF2	North Fork Feather River, Butte Co., CA	PE	
Thamnophis elegans	EDB jr 23576	ZF3	North Fork Feather River, Butte Co., CA	PE	
Thamnophis elegans	EDB jr 23577	ZF4	North Fork Feather River, Butte Co., CA	PE	
Thamnophis elegans	EDB jr 23578	ZF5	North Fork Feather River, Butte Co., CA	PE	
Thamnophis elegans	EDB jr 23579	ZF6	North Fork Feather River, Butte Co., CA	PE	
Thamnophis elegans	EDB jr 23580	ZF7	North Fork Feather River, Butte Co., CA	PE	
Thamnophis elegans	EDB jr 23581	ZF8	North Fork Feather River, Butte Co., CA	PE	
Thamnophis elegans	EDB jr 23582	ZF9	North Fork Feather River, Butte Co., CA	PE	
Thamnophis elegans	EDB jr 23507	7.015	Opapee Creek, Plumas Co., CA	PE	
Thamnophis elegans	EDB jr 22034	5.190	Podatti Ranch, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22035	5.191	Podatti Ranch, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22036	5.192	Podatti Ranch, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22037	5.193	Podatti Ranch, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22038	5.194	Podatti Ranch, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22359	6.085 (XQ)	Prewitt Creek, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 22754	6.078	Prewitt Creek, San Luis Obispo Co., CA	PE	GE
Thamnophis elegans	EDB jr 22755	6.079	Prewitt Creek, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 22756	6.080	Prewitt Creek, San Luis Obispo Co., CA	PE	GE
Thamnophis elegans	EDB jr 22757	6.081	Prewitt Creek, San Luis Obispo Co., CA	PE	GE
Thamnophis elegans	EDB jr 22758	6.082	Prewitt Creek, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 22904	6.086	Prewitt Creek, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 22906	6.083	Prewitt Creek, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 21633	5.114	Redwood Creek, Humboldt Co., CA	PE	
Thamnophis elegans	EDB jr 22749	6.076	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 22750	6.067	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	GE
Thamnophis elegans	EDB jr 22751	6.068	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 22752	6.072	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 22753	6.073	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 22775	6.075	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 22776	6.062	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 22777	6.063	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	GE
Thamnophis elegans	EDB jr 22778	6.066	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	GE
Thamnophis elegans	EDB jr 22779	6.077	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 22780	6.070	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 22781	6.065	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	GE
Thamnophis elegans	EDB jr 22783	6.064	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	GE

Thamnophis elegans	EDB jr 22784	6.069	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 22785	6.074	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	
Thamnophis elegans	EDB jr 22339	5.250	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis elegans	EDB jr 22343	5.258	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis elegans	EDB jr 22359	5.251	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis elegans	EDB jr 22362	5.257	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis elegans	EDB jr 22368	5.268	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis elegans	EDB jr 22918	XN1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22919	XN7	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22920	XN9	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22921	XN10	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22922	XP1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22923	XP4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22924	XP6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22925	XP10	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22927	XS5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22928	XS7	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22929	XS9	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22930	XS11	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22931	XS13	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22932	XS16	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22933	XS17	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22935	XU10	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22990	XN3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22991	XN4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22992	XN5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22993	XN6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22994	XN7a	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22995	XN8	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22996	XN11	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22997	XN12	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22998	XN13	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 22999	XN14	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23000	XN15	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23003	XP2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23004	XP3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23005	XP5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23006	XP7	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23007	XP8	Santa Lucia Preserve, Monterey Co., CA	PE	

Thamnophis elegans	EDB jr 23008	XP9	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23009	XP11	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23010	XP12	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23011	XP13	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23012	XP14	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23021	XS1	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23022	XS2	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23023	XS3	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23024	XS4	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23025	XS6	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23026	XS8	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23027	XS10	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23028	XS12	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23029	XS14	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23030	XS15	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23034	XU1	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23035	XU2	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23036	XU3	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23037	XU4	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23038	XU5	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23039	XU6	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23040	XU7	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23041	XU8	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23042	XU9	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23043	XU11	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23047	XW1	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23048	XW2	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23049	XW3	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23050	XW4	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23051	XW5	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23052	XW6	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23053	XW8	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23054	XW9	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23066	YG3	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23067	YA4	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23068	YA5	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23069	YA7	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23070	YA9	Santa Lucia Preserve, Monterey Co., CA	PE
Thamnophis elegans	EDB jr 23072	XX1	Santa Lucia Preserve, Monterey Co., CA	PE

Thamnophis elegans	EDB jr 23073	XX2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23074	XX3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23075	XX4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23076	XX5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23077	XX6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23078	XX7	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23079	XX8	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23080	XX9	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23081	XX10	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis elegans	EDB jr 23101	YA1	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis elegans	EDB jr 23102	YA2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23103	YA3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23104	YA6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23105	YA8	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23106	YA10	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23107	YA11	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23108	YA12	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23109	YA13	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23110	YA14	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23128	YC1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23129	YC2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23130	YC3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23131	YC4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23132	YC5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23133	YC6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23134	YC7	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23135	YC8	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23136	YC9	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23137	YC10	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23138	YC11	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23139	YC12	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23140	YC13	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23141	YC14	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23142	YC15	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23143	YC16	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23144	YC17	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23145	YC18	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis elegans	EDB jr 23170	YF1	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis elegans	EDB jr 23171	YF2	Santa Lucia Preserve, Monterey Co., CA	PE	

Thamnophis elegans	EDB jr 23172	YF3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23173	YF4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23174	YF5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23175	YF6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23176	YF7	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23177	YF8	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23178	YF9	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23179	YF10	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23180	YF11	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23181	YF12	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23182	YF13	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23183	YG1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23184	YG2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23185	YG4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23186	YG5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23187	YG6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23188	YG7	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23194	YJ1	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis elegans	EDB jr 23195	YJ2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23196	YJ3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23197	YJ4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23198	YJ5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23199	YJ6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23200	YJ7	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23201	YJ8	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23202	YJ9	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23260	6.234 (YC)	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23261	6.102	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23262	6.235 (YF)	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23263	6.236 (YA)	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23264	6.237 (YG)	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23265	6.104	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23266	6.238 (YJ)	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23267	6.105	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23268	6.239 (XX)	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23269	6.253 (XP)	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23270	6.246	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23271	6.106	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23272	6.103	Santa Lucia Preserve, Monterey Co., CA	PE	

Thamnophis elegans	EDB jr 23273	6.107	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23274	6.108	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23275	6.259	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23276	6.109	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23283	6.254 (XN)	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23284	6.247 (XU)	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23285	6.258	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23286	6.248 (XS)	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 23287	6.255 (XW)	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis elegans	EDB jr 21617	5.96	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21618	5.97	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21619	5.98	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21620	5.99	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21621	5.100	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21622	5.101	Sebastapol, Sonoma Co., CA	PE	GE
Thamnophis elegans	EDB jr 21623	5.102	Sebastapol, Sonoma Co., CA	PE	GE
Thamnophis elegans	EDB jr 21624	5.103	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21625	5.104	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21626	5.105	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21637	5.118	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21638	5.119	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21639	5.120	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21640	5.121	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21641	5.123	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21642	5.124	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21643	5.125	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21644	5.126	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21645	5.128	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21646	5.129	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21647	5.130	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21648	5.132	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21649	5.133	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22032	5.188	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22084	5.117	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22085	5.122	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22086	5.127	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22087	5.131	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22310	5.116	Sebastapol, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 22319	5.236 (WW)	Sebastapol, Sonoma Co., CA	PE	GE

Thamnophis elegans	EDB jr 22946	6.228	Silver Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 22947	6.229	Silver Lake, Amador Co., CA	PE	
Thamnophis elegans	EDB jr 21445	4.79	Siting Bull Falls, Eddy Co., NM	PE	GE
Thamnophis elegans	EDB jr 21446	4.80	Siting Bull Falls, Eddy Co., NM	PE	
Thamnophis elegans	EDB jr 22761	6.089	Strawberry Creek, Alameda Co., CA	PE	
Thamnophis elegans	EDB jr 22046	5.214	Tennessee Valley, Marin Co., CA	PE	
Thamnophis elegans	EDB jr 22047	5.215	Tennessee Valley, Marin Co., CA	PE	
Thamnophis elegans	EDB jr 22048	5.216	Tennessee Valley, Marin Co., CA	PE	
Thamnophis elegans	EDB jr 22049	5.217	Tennessee Valley, Marin Co., CA	PE	
Thamnophis elegans	EDB jr 22050	5.218	Tennessee Valley, Marin Co., CA	PE	
Thamnophis elegans	EDB jr 22051	5.219	Tennessee Valley, Marin Co., CA	PE	
Thamnophis elegans	EDB jr 21377	4.58	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis elegans	EDB jr 21423	4.57a	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis elegans	EDB jr 21424	4.58a	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis elegans	EDB jr 21425	4.59	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21426	4.60	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21427	4.61	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21428	4.62	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21429	4.63	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21430	4.64	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21431	4.65	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21432	4.66	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21433	4.67	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21434	4.68	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21435	4.69	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21436	4.70	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21437	4.71	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21438	4.72	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21439	4.73	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis elegans	EDB jr 21440	4.74	Willow Creek, Sonoma Co., CA	PE	
Thamnophis elegans	EDB jr 21501	4.111	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis elegans	EDB jr 21502	4.112	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis eques	MVZ 164782		Colonia Garcia, Chihahua, Mexico		GE
Thamnophis errans	AdQ 613		near Mil Diez, Durango, Mexico	PE	GE
Thamnophis fulvus	MVZ 143520		NW of Barillas, Huehuetenago, Guatemala		GE
Thamnophis hammondi	SJA ts 8196		San Carpford Creek, San Luis Obispo Co., CA		GE
Thamnophis marcianus	EDB jr 23511	7.041	Sitting Bull Falls, Eddy Co., NM	PE	
Thamnophis marcianus	EDB jr 23512	7.042	Sitting Bull Falls, Eddy Co., NM	PE	
Thamnophis nigronuchalis	AdQ 618		near Mil Diez, Durango, Mexico	PE	GE

Thamnophis ordinoides	CRF 2215		Beaverton, Washington Co., OR		GE
Thamnophis ordinoides	EDB jr 21263	3.82	Hunter Creek, Curry Co., OR	PE	GE
Thamnophis proximus	EDB jr 23497	7.021	Drennan Rd., Splendora, Montgomery Co., TX	PE	GE
Thamnophis proximus	EDB jr 23508	7.022	Spell Rd., Houston, Harris Co., TX	PE	GE
Thamnophis radix	EDB jr 21441	4.75	Ness Crossing, Corrumpa Creek, Union Co., NM	PE	GE
Thamnophis radix	EDB jr 21442	4.76	Ness Crossing, Corrumpa Creek, Union Co., NM	PE	
Thamnophis sirtalis	EDB jr 22889	6.204	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22890	6.205	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22891	6.206	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22892	6.207	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22893	6.208	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22894	6.209	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22896	6.211	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22897	6.212	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22898	6.213	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22899	6.214	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22900	6.215	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22901	6.217	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22902	6.218	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22936	6.216	Beauty Lake, El Dorado Co., CA	PE	
Thamnophis sirtalis		BCTs MO	Camp Adir, Benton Co., OR	PE	GE
Thamnophis sirtalis		BCTs OS	Camp Adir, Benton Co., OR	PE	GE
Thamnophis sirtalis		BCTs RI	Camp Adir, Benton Co., OR	PE	GE
Thamnophis sirtalis		BCTs RM	Camp Adir, Benton Co., OR	PE	GE
Thamnophis sirtalis	EDB jr 22649	OY1	captive cross (Warrenton/Bear Lake)	PE	GE
Thamnophis sirtalis	EDB jr 22650	OW3	captive cross (Willow Creek/Warrenton)	PE	GE
Thamnophis sirtalis	EDB jr 22651	PB9	captive cross (Willow Creek/Warrenton)	PE	GE
Thamnophis sirtalis	EDB jr 22652	PG2	captive cross (Willow Creek/Warrenton)	PE	GE
Thamnophis sirtalis	EDB jr 22653	PG3	captive cross (Willow Creek/Warrenton)	PE	GE
Thamnophis sirtalis	EDB jr 22654	PO2	captive cross (Willow Creek/Warrenton)	PE	GE
Thamnophis sirtalis	EDB jr 22937	6.219	Carson Spur, Amador Co., CA	PE	
Thamnophis sirtalis	EDB jr 22938	6.220	Carson Spur, Amador Co., CA	PE	
Thamnophis sirtalis	EDB jr 22939	6.221	Carson Spur, Amador Co., CA	PE	
Thamnophis sirtalis	EDB jr 22940	6.222	Carson Spur, Amador Co., CA	PE	
Thamnophis sirtalis	EDB jr 22941	6.223	Carson Spur, Amador Co., CA	PE	
Thamnophis sirtalis	EDB jr 22942	6.224	Carson Spur, Amador Co., CA	PE	
Thamnophis sirtalis	EDB jr 22943	6.225	Carson Spur, Amador Co., CA	PE	
Thamnophis sirtalis	EDB jr 21269	3.88	Cook Creek, Grays Harbor Co., WA	PE	GE
Thamnophis sirtalis	EDB jr 21506	5.01	Cornaz, Shasta Co., CA	PE	

Thamnophis sirtalis	EDB jr 21507	5.02	Cornaz, Shasta Co., CA	PE	
Thamnophis sirtalis	EDB jr 21508	5.03	Cornaz, Shasta Co., CA	PE	
Thamnophis sirtalis	EDB jr 21509	5.04	Cornaz, Shasta Co., CA	PE	
Thamnophis sirtalis	EDB jr 21510	5.05	Cornaz, Shasta Co., CA	PE	
Thamnophis sirtalis	EDB jr 21511	5.06	Cornaz, Shasta Co., CA	PE	
Thamnophis sirtalis	EDB jr 21512	5.07	Cornaz, Shasta Co., CA	PE	
Thamnophis sirtalis	EDB jr 21513	5.08	Cornaz, Shasta Co., CA	PE	
Thamnophis sirtalis	EDB jr 21514	5.09	Cornaz, Shasta Co., CA	PE	
Thamnophis sirtalis	EDB jr 21515	5.10	Cornaz, Shasta Co., CA		
Thamnophis sirtalis	EDB jr 21516	5.11	Cornaz, Shasta Co., CA		
Thamnophis sirtalis	EDB jr 21163	TsER	Elk River, Curry Co., OR	PE	GE
Thamnophis sirtalis	EDB jr 22352	5.275	Emilie Ruecker Wildlife Refuge, Newport Co., RI	PE	GE
Thamnophis sirtalis	EDB jr 21959	5.153	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21960	5.154	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21961	5.155	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21962	5.156	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21963	5.157	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21964	5.158	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21965	5.159	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21966	5.160	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21967	5.161	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21969	5.163	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21970	5.164	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21971	5.165	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21972	5.166	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21973	5.167	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21974	5.168	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21975	5.169	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21976	5.170	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21977	5.171	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21978	5.172	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21979	5.173	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21980	5.174	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21981	5.175	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21982	5.176	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21983	5.177	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21984	5.178	Feather Lake, Lassen Co., CA	PE	
Thamnophis sirtalis	EDB jr 21687	VF1	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21688	VF2	Gilroy, Santa Clara Co., CA	PE	

Thamnophis sirtalis	EDB jr 21689	VF3	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21690	VF4	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21691	VF5	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21692	VF6	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21693	VF7	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21694	VF8	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21695	VF9	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21696	VF10	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21697	VF11	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21698	VF12	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21699	VF13	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21700	VF14	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21701	VF15	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21702	VF16	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21703	VF17	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21704	VG1	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21705	VG2	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21706	VG3	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21707	VG4	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21708	VG5	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21709	VG6	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21710	VG7	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21711	VG8	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21712	VG9	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21713	VG10	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21714	VH1	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21715	VH2	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21716	VH3	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21717	VH4	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21718	VH5	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21719	VH6	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21720	VH7	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21721	VH8	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21722	VH9	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21723	VH10	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21724	VH11	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21725	VH12	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21726	VH13	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21727	VH14	Gilroy, Santa Clara Co., CA	PE	GE

Thamnophis sirtalis	EDB jr 21731	VH18	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21732	VH19	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21751	VL1	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21752	VL2	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21753	VL3	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21754	VL4	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21755	VL5	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21756	VL6	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21757	VL7	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21758	VL8	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21759	VL9	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21760	VL10	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21761	VL11	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21762	VL12	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21763	VL13	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21764	VL14	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21765	VL15	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21801	VQ1	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21802	VQ2	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21803	VQ3	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21804	VQ4	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21805	VQ5	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21898	WB1	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21899	WB2	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21900	WB3	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21901	WB4	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21902	WB5	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21903	WB6	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21904	WB7	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21905	WB8	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21906	WB9	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 21907	WB10	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 22052	5.220	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 22059	WG1	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 22060	WG2	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 22061	WG3	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 22062	WG4	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 22312	5.227a (VL)	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 22313	5.228b (VQ)	Gilroy, Santa Clara Co., CA	PE	

Thamnophis sirtalis	EDB jr 22314	5.229a (WB)	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 22315	5.230a (WG)	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 22316	5.231a (VF)	Gilroy, Santa Clara Co., CA	PE	
Thamnophis sirtalis	EDB jr 22317	5.232a (VG)	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22318	5.233a (VH)	Gilroy, Santa Clara Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21273	3.92	Hoh River, Jefferson Co., WA	PE	GE
Thamnophis sirtalis	EDB jr 21256	3.75	Hunter Creek, Curry, OR	PE	GE
Thamnophis sirtalis	EDB jr 21134	2.11	Inwood, Manitoba, Canada	PE	GE
Thamnophis sirtalis	EDB jr 21135	2.12	Inwood, Manitoba, Canada	PE	GE
Thamnophis sirtalis	EDB jr 21566	5.33	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21567	5.35	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21568	5.36	Ledson Marsh, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21569	5.37	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21570	5.39	Ledson Marsh, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21571	5.40	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21572	5.44	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21573	5.46	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21574	5.47	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21575	5.48	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21576	5.49	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21733	VI1	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21734	VI2	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21735	VI3	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21736	VI4	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21737	VI5	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21738	VI6	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21739	VI7	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21740	VI8	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21741	VI9	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21742	VI10	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21743	VI12	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21744	VJ1	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21745	VJ2	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21746	VJ3	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21747	VJ4	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21748	VJ5	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21749	VJ6	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21750	VJ7	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21766	VM1	Ledson Marsh, Sonoma Co., CA	PE	

Thamnophis sirtalis	EDB jr 21767	VM2	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21768	VM3	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21769	VM4	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21770	VM5	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21771	VM6	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21772	VM7	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21780	VO1	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21781	VO2	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21782	VO3	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21783	VO4	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21784	VO5	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21785	VO6	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21786	VO7	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21787	VO8	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21788	VO9	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21789	VO10	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21790	VO11	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21849	VV1	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21850	VV2	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21851	VV3	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21852	VV4	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21853	VV5	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22104	5.32 (VO)	Ledson Marsh, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22105	5.45 (VQ)	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22106	5.34 (VJ)	Ledson Marsh, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22107	5.30 (VI)	Ledson Marsh, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22108	5.42 (WI)	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22109	5.38 (VV)	Ledson Marsh, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22110	5.43 (VM)	Ledson Marsh, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22111	5.41 (WS)	Ledson Marsh, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22190	WS1	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22191	WS2	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22192	WS3	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22193	WS4	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22194	WS5	Ledson Marsh, Sonoma Co., CA	PE	
Thamnophis sirtalis		TsLM RX	Ledson Marsh, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22094	5.227	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22646	5.234a	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22647	5.235a	Leoni Meadows, El Dorado Co., CA	PE	

Thamnophis sirtalis	EDB jr 22735	6.123	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 22934	XT4	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23031	XT1	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23032	XT2	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23033	XT3	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23055	XY2	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23056	XY3	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23057	XY4	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23058	XY8	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23059	XY11	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23060	XY13	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23061	XY14	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23062	XY15	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23063	XY17	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23064	XY19	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23065	XY21	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23082	XY1	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23083	XY5	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23084	XY6	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23085	XY7	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23086	XY9	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23087	XY10	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23088	XY12	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23089	XY16	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23090	XY18	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23091	XY20	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23111	YB1	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23112	YB2	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23113	YB3	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23114	YB4	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23115	YB5	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23116	YB6	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23117	YB7	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23118	YB8	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23119	YB9	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23120	YB10	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23121	YB11	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23122	YB12	Leoni Meadows, El Dorado Co., CA	PE
Thamnophis sirtalis	EDB jr 23123	YB13	Leoni Meadows, El Dorado Co., CA	PE

Thamnophis sirtalis	EDB jr 23124	YB14	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23125	YB15	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23126	YB16	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23127	YB17	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23158	YE1	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23159	YE2	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23160	YE3	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23161	YE4	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23162	YE5	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23163	YE6	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23164	YE7	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23165	YE8	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23166	YE9	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23167	YE10	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23168	YE11	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23169	YE12	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23313	6.121 (YE)	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23314	6.122 (YB)	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 23315	6.124 (XT)	Leoni Meadows, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 15435	205 (BC)	Lofton Lake, Lake Co., OR	PE	GE
Thamnophis sirtalis		TsLL TV	Lost Lake, Linn Co., OR	PE	GE
Thamnophis sirtalis	EDB jr 16016	121 (CG)	Mahogany Lake, Lassen Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 16053	189 (AE)	McCumber, Shasta Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21164	TsMC	Mill City, Linn Co., OR	PE	GE
Thamnophis sirtalis	EDB jr 22643	5.288	Moss Landing, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22644	5.289	Moss Landing, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22645	5.290	Moss Landing, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23527	7.050	Mountain Lake Biological Station, Giles Co., VA	PE	
Thamnophis sirtalis	EDB jr 23528	7.051	Mountain Lake Biological Station, Giles Co., VA	PE	GE
Thamnophis sirtalis	EDB jr 23529	7.052	Mountain Lake Biological Station, Giles Co., VA	PE	GE
Thamnophis sirtalis	EDB jr 23530	7.053	Mountain Lake Biological Station, Giles Co., VA	PE	GE
Thamnophis sirtalis	EDB jr 23531	7.054	Mountain Lake Biological Station, Giles Co., VA	PE	
Thamnophis sirtalis	EDB jr 23532	7.055	Mountain Lake Biological Station, Giles Co., VA	PE	
Thamnophis sirtalis	EDB jr 23533	7.056	Mountain Lake Biological Station, Giles Co., VA	PE	
Thamnophis sirtalis	EDB jr 23534	7.057	Mountain Lake Biological Station, Giles Co., VA	PE	
Thamnophis sirtalis	EDB jr 23535	7.058	Mountain Lake Biological Station, Giles Co., VA	PE	
Thamnophis sirtalis	EDB jr 23536	7.059	Mountain Lake Biological Station, Giles Co., VA	PE	GE
Thamnophis sirtalis	EDB jr 21157	TsOR OmoB	Omo Ranch, El Dorado Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21160	TsOR 98-15	Omo Ranch, El Dorado Co., CA	PE	GE

Thamnophis sirtalis	EDB jr 21484	4.107	Omo Ranch, El Dorado Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21485	4.108	Omo Ranch, El Dorado Co., CA	PE	GE
Thamnophis sirtalis		TsOR OmoA	Omo Ranch, El Dorado Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21577	5.50	O'Neals Meadow, Mariposa Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21578	5.51	O'Neals Meadow, Mariposa Co., CA	PE	
Thamnophis sirtalis	EDB jr 21579	5.52	O'Neals Meadow, Mariposa Co., CA	PE	
Thamnophis sirtalis	EDB jr 21580	5.53	O'Neals Meadow, Mariposa Co., CA	PE	
Thamnophis sirtalis	EDB jr 21581	5.54	O'Neals Meadow, Mariposa Co., CA	PE	
Thamnophis sirtalis	EDB jr 21582	5.55	O'Neals Meadow, Mariposa Co., CA	PE	
Thamnophis sirtalis	EDB jr 21583	5.56	O'Neals Meadow, Mariposa Co., CA	PE	
Thamnophis sirtalis	EDB jr 21584	5.57	O'Neals Meadow, Mariposa Co., CA	PE	
Thamnophis sirtalis	EDB jr 21585	5.58	O'Neals Meadow, Mariposa Co., CA	PE	
Thamnophis sirtalis	EDB jr 21586	5.59	O'Neals Meadow, Mariposa Co., CA	PE	
Thamnophis sirtalis	CRF 943	TsM TF	Patuxent River State Park, Montgomery Co., MD	PE	GE
Thamnophis sirtalis	EDB jr 15795	139 (AH)	Peony Spring, Lassen Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22330	5.249	Pollock Pines, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 22351	5.274	Pollock Pines, El Dorado Co., CA	PE	
Thamnophis sirtalis	EDB jr 21162	TsPS	Potter Slough, Pacific Co., WA	PE	GE
Thamnophis sirtalis		TsPS TM	Potter Slough, Pacific Co., WA	PE	GE
Thamnophis sirtalis		TePC RZ	Pudding Creek, Mendocino Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22746	6.059	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22747	6.060	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22748	6.061	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22774	6.071	San Simeon Creek Ranch, San Luis Obispo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21411	4.11	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21412	4.12	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21413	4.13	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22232	VK1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22233	VK2	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22234	VK3	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22235	VK4	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22236	VK5	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22237	VK6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22238	VE2	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22239	VE4	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22240	VE5	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22241	VE7	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22242	VE9	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22243	VE10	Santa Lucia Preserve, Monterey Co., CA	PE	GE

Thamnophis sirtalis	EDB jr 22244	VE11	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22245	VE12	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22246	VE13	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22247	VE14	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22248	VE17	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22249	VE18	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22250	VE19	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22657	5.277	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22658	5.279	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22659	5.280	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22660	5.283	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22907	XL2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22908	XL3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22909	XL4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22910	XL7	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22911	XL12	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22912	XL14	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22913	XL17	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22914	XM8	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22915	XM9	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22916	XM10	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22917	XM13	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22926	XR1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22955	XK1	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22956	XK2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22957	XK3	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22958	XK5	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22959	XK6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22960	XK7	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22961	XK8	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22962	XK9	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22963	XK10	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22964	XK12	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22965	XK14	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22966	XK17	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22967	XK19	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22968	XK20	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22969	XK23	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22970	XK24	Santa Lucia Preserve, Monterey Co., CA	PE	GE

Thamnophis sirtalis	EDB jr 22971	XK25	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 22972	XK26	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22973	XL1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22974	XL5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22975	XL6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22976	XL11	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22977	XL13	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22978	XL15	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22979	XL16	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22980	XM1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22981	XM2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22982	XM3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22983	XM4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22984	XM5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22985	XM6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22986	XM7	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22987	XM11	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22988	XM12	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 22989	XM14	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23001	XO1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23002	XO2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23044	XV1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23045	XV2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23046	XV3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23223	6.161 (XM)	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23546	ZD1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23547	ZD2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23548	ZD3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23549	ZD4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23550	ZD5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23551	ZD6	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23552	ZD7	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23553	ZD8	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23554	ZD9	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23555	ZD10	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23556	ZD11	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23557	ZD12	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23558	ZD13	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23559	ZD14	Santa Lucia Preserve, Monterey Co., CA	PE	

Thamnophis sirtalis	EDB jr 23560	ZD15	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23561	ZD16	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23562	ZD17	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23563	ZD18	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23564	ZD19	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23565	ZD20	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23566	ZD21	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23567	ZD22	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23568	ZD23	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23569	ZE1	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23570	ZE2	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23571	ZE3	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23572	ZE4	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis	EDB jr 23573	ZE5	Santa Lucia Preserve, Monterey Co., CA	PE	
Thamnophis sirtalis		5.278	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis		5.279 (VK)	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis		5.282 (ZD)	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis		6.112 (ZD)	Santa Lucia Preserve, Monterey Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21223	3.41	Scott's Lake, Lane Co., OR	PE	GE
Thamnophis sirtalis	EDB jr 21612	5.87	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21613	5.89	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21614	5.91	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21615	5.92	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21616	5.93	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21869	VY1	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21870	VY2	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21871	VY3	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21872	VY4	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21873	VY5	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21874	VY6	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21875	VY7	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21876	VY8	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21877	VY9	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21878	VY10	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21879	VY11	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21880	VY12	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22033	5.189	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22039	5.196	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22088	5.95	Sebastapol, Sonoma Co., CA	PE	

Thamnophis sirtalis	EDB jr 22089	VY	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22090	5.88	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22091	WP	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22092	5.90	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22311	5.94	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22353	5.28	Sebastapol, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 20319	SU12	Stayton, Marion Co., OR	PE	GE
Thamnophis sirtalis	EDB jr 22863	6.180	Sunol Regional Park, Alameda Co., CA	PE	
Thamnophis sirtalis	EDB jr 23505	7.030	Sunol Regional Park, Alameda Co., CA	PE	
Thamnophis sirtalis	EDB jr 21232	3.50	Tahkenitch Lake, Douglas Co., OR	PE	GE
Thamnophis sirtalis	EDB jr 21165	Ts10 21165	Ten Mile, Lane Co., OR	PE	GE
Thamnophis sirtalis	EDB jr 21166	Ts10 21166	Ten Mile, Lane Co., OR	PE	GE
Thamnophis sirtalis	EDB jr 21167	Ts10 21167	Ten Mile, Lane Co., OR	PE	GE
Thamnophis sirtalis	EDB jr 21264	3.83	Threemile Creek, Douglas Co., OR	PE	GE
Thamnophis sirtalis	EDB jr 22045	5.213	Warm Springs Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21398	1.17	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21447	4.95	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21448	4.97	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21449	4.98	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21450	4.99	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21451	4.100	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21452	4.101	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21453	4.103	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21454	4.104	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21455	4.105	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21470	4.81	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21471	4.82	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21472	4.83	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21473	4.84	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21474	4.85	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21475	4.86	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21476	4.87	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21477	4.88	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21478	4.89	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21479	4.90	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21480	4.91	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21481	4.92	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21482	4.93	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21483	4.94	Willow Creek, Sonoma Co., CA	PE	

Thamnophis sirtalis	EDB jr 21819	VS1	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21820	VS2	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21821	VS3	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21865	VX1	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21866	VX2	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21867	VX3	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 21868	VX4	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22323	5.242	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22324	5.243	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22325	5.244	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22326	5.245	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22327	5.246 (VS)	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis	EDB jr 22328	5.247 (VX)	Willow Creek, Sonoma Co., CA	PE	
Thamnophis sirtalis		TsWC A3	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis sirtalis		TsWC A7	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis sirtalis		TsWC P1	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis sirtalis		WCTs 9465	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis sirtalis		WCTs A6	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis sirtalis		WCTs A9	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis sirtalis		WCTs JQ	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis sirtalis		WCTs N1	Willow Creek, Sonoma Co., CA	PE	GE
Thamnophis sirtalis	EDB jr 21662	VD1	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21663	VD2	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21664	VD3	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21665	VD5	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21666	VD7	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21667	VD10	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21668	VD11	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21669	VD12	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21670	VD14	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21671	VD16	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21672	VD18	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21673	VD20	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21674	VD21	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21675	VD22	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21676	VD23	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21677	VD24	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 21678	VD25	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22093	VD	Yolo Bypass, Yolo Co., CA	PE	

Thamnophis sirtalis	EDB jr 22322	5.239	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22762	6.100	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22763	6.101	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22812	XH1	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22813	XH2	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22814	XH3	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22815	XH4	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22816	XH5	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22817	XI1	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22818	XI2	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22819	XI3	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22820	XI4	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22821	XI5	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22822	XI6	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22823	XI7	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22824	XI8	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22825	XI9	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22826	XI10	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22827	XI11	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22952	6.153	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22953	6.154	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis sirtalis	EDB jr 22954	6.155	Yolo Bypass, Yolo Co., CA	PE	
Thamnophis validus	MVZ 236400		Agua Caliente, Baja California Sur, Mexico		GE
Trimeresurus albolabris	CAS 214112		Kyauk Pan Tawn, Mandalay, Myanmar		GE
Trimeresurus popeiorum	CAS 205847		Bagoa, Bagoa Yoma, Myanmar		GE
Trimeresurus purpureomaculatus	CAS 219783		Mein Ma Hla Kyun, Ayeyarwade, Myanmar		GE
Tropidoclonion lineatum	EDB jr 23500	7.025	Waldo Rd, Luray, Russel Co., KS	PE	
Tropidoclonion lineatum	EDB jr 23501	7.026	Waldo Rd, Luray, Russel Co., KS	PE	
Tropidoclonion lineatum	EDB jr 23502	7.027	Waldo Rd, Luray, Russel Co., KS	PE	
Tropidoclonion lineatum	EDB jr 23503	7.028	Waldo Rd, Luray, Russel Co., KS	PE	
Tropidoclonion lineatum	EDB jr 23504	7.029	Waldo Rd, Luray, Russel Co., KS	PE	GE
Virginia striatula	EDB jr 23492	7.016	North Houston, Harris Co., TX	PE	
Virginia striatula	EDB jr 23493	7.017	North Houston, Harris Co., TX	PE	
Virginia striatula	EDB jr 23494	7.018	North Houston, Harris Co., TX	PE	
Virginia striatula	EDB jr 23495	7.019	North Houston, Harris Co., TX	PE	
Virginia striatula	EDB jr 23496	7.020	North Houston, Harris Co., TX	PE	GE
Xenochrophis pisctator	CAS 233279		Vaar, Falam, Chin, Myanmar		GE
Xenodon rhabdocephalus	UTA R42297		Izabal, Guatemala		GE
Xenodon rhabdocephalus	LSUMZ 8942		locality unknown, Suriname		GE

APPENDIX B

Primers used to amplify and sequence $Na_v1.4$

TABLE B.1. Oligonucleotide primers used to amplify and sequence genomic DNA and messenger RNA (cDNA) in *Thamnophis* and other squamate reptiles.

Primer	Sequence (5'–3')	Position[a]
5'UTR.F Nav1.4	GTTTTCCCAGTCACGACGTT	5' UTR
SN46 1f[b]	GGGATATTGCTCATACCGTCA	5' UTR
e1.Fa Nav1.4	GGGATATTGCTCATACCGTCA	10
e3.F Nav1.4	AGCCAACTGTGTCTTCATGACT	420
e4.F Nav1.4	AAGGTACTTGCTCGGGGATT	517
e4.R Nav1.4	ACATTCCTGCGAGATCCTTG	553
e5.Ra Nav1.4	CGTAGACTTGGGCAACGTCT	621
e5.F Nav1.4	GGGCAACGTCTCTGCTCTAC	630
e5.R Nav1.4	ACATTCCGTGTTCTTCGAGC	652
e6.F Nav1.4	TCTGTGAAGAAGCTCGCTGA	730
e6.Ra Nav1.4	ACTGGGACCTTTGACTGGC	804
e6.R Nav1.4	ACTGTTCATGGGGAACTTGC	967
e8.F-PH Nav1.4	GTGTCCAGAAGGATTTCTCTGC	1074
e8.F Nav1.4	CGAAACCCAAATTACGGCTA	1108
DI.S6.1270.R	GGCTCTTTCTATCTCATCAATTTAATCCTGGC	1270
e9.R-PH Nav1.4	TTTAATCCTGGCTGTGGTGG	1290
e9.R Nav1.4	GCAGAACAGAATGATGCCAC	1321
e11.Fa Nav1.4	TGTTGTCCAGTTTGGGTGAA	1789
e11.F Nav1.4	GTCCTGGACCCTTTTGTTGA	1834
e11.R Nav1.4	TGAATGTGGGCAATCTGGTA	1940
e12.F Nav1.4	TTCACAGCTGAGATGGTGCT	1972
e12.R Nav1.4	GGATGGAACATCTTTGACAGC	2032
e13.F-PH Nav1.4	GCATGCAGCTATTTGGGAAG	2264
e13.R-PH Nav1.4	CCCGATGACCATGACCATTA	2459
e14.R Nav1.4	TTAGCTCCTTCAGTGCCGAT	2516
e14.F Nav1.4	AGCTCCTTCAGTGCCGATAG	2518
e14.Ra Nav1.4	CACAGGCCAGGATTTCAAGT	2748
DIII.S5.3584.F	TCTTCTGGCTCATCTTCAGCATTATGGG	3584
e19.F-PH Nav1.4	CGCTGTGTCAATACCACCAC	3640
e19.Fa-PH Nav1.4	TGAAAATGCCACTGATGTCC	3723
i19.R-PH Nav1.4	GCACATCCAGATCAACATGC	intron 19
i19.F-PH Nav1.4	GGGGTTTTCAAAAAGCACTTC	intron 19
e21.R-PH Nav1.4	TATTGAGGCTGTTCCTCCTG	3862
DIII.S6.3913.R	GTGAAGAATGACCCAAAGATAATAAAGATGAC	3913
e24.F-PH Nav1.4	CCGAACTCTGCTCTTTGCTT	4491
DIV.S5.4559.F	TGGTTATGTTCATTTATTCCATTTTTGG	4559
e24.R-PH Nav1.4	ATCTGGACAACCCTGGCAGT	4754
DIV.S6.4882.R	GAGAATTTTAATGTAGCCACAGAGGAGAG	4882
e24.Fb Nav1.4	GGAGAAATTTATGGCTGCAA	5193
3'UTR.R Nav1.4	GTTGGTGTATGGTTCCAAATGA	3' UTR

[a]positions follow $Na_v1.4$ CDS from *T. sirtalis* AY851746.
[b]from Geffeney et al (2005); all other primers designed herein.

APPENDIX C

Exon/intron structure of *Thamnophis* $Na_v1.4$

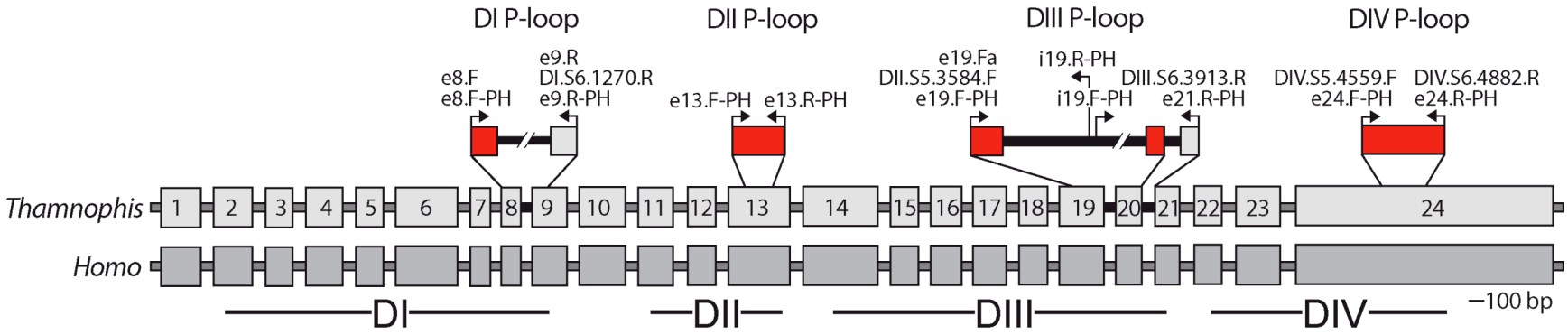

FIGURE C.1. Map of *Thamnophis* $Na_v1.4$ intron/exon structure emphasizing pore regions. The predicted exon/intron structure of *Thamnophis* $Na_v1.4$ is based on comparison to *Homo* (M81758) and *Rattus* (M26643). The 24 exons (large boxes) are drawn roughly to scale while untranslated regions (small boxes) are not; the four P-loops (red boxes) and primers used to capture these regions detailed above the locus; domain boundaries follow Trimmer et al. (1989) and George et al. (1993). I translated *Thamnophis* and mammal $Na_v1.4$ CDS in MacClade (Maddison and Maddison, 2005), aligned these with ClustalW (Thompson et al., 1994) and forced nucleotide sequences to follow resultant amino acid alignment in tranalign (Williams, 2002). I then created an exon/intron map of mammal reference sequences using GMAP 2 (Wu and Wanatabe, 2005). The introns sequenced herein (introns 8, 19, 20) match the assumed splicing sites exactly.

CURRICULUM VITAE

CHRIS R. FELDMAN
Department of Biology
Utah State University
Logan, UT 84322-5305

Education:

Ph.D. Biology. Utah State University, 2002-2008. Dissertation-"Evolutionary genetics of tetrodotoxin (TTX) resistance in snakes: tracking a feeding adaptation from populations through clades"

M.A. Ecology and Systematic Biology. San Francisco State University, 1997-2000. Thesis-"Comparative phylogeography of three Californian reptiles: *Contia tenuis*, *Diadophis punctatus*, *Elgaria multicarinata*"

B.A. Integrative Biology. University of California, Berkeley, 1992-1996. Minor Entomology. Minor Forestry & Resource Management.

Peer Review Publications:

Hoyer, R.F. & **C.R. Feldman**. *In Review*. A new species of snake from California and Oregon in the genus *Contia* (Squamata: Colubridae). Copeia.

Angielczyk, K.D., **Feldman, C.R.** & G. Miller. *In Review.* Geometric morphometric analysis of plastron shape in emydine turtles: the effects of phylogeny, habitat and function. Biological Journal of the Linnean Society.

Fontanella, F., **Feldman, C.R.** Siddall, M.E. & F.T. Burbrink. 2008. Phylogeography of *Diadophis punctatus*: extensive lineage diversity and repeated patterns of historical demography in a trans-continental snake. Molecular Phylogenetics and Evolution 46: 1049–1070.

McGaugh, S., Alacs, E.A., Edwards, S.V., **Feldman, C.R.**, Georges, A., Sites, J.W., jr., & N. Valenzuela. 2007. From molecules to organisms: research applications of modern genetic tools for turtle biology and conservation *in* Defining turtle diversity: proceedings of a workshop on genetics, ethics, and taxonomy of tortoises and freshwater turtles. Shaffer, H.B., FitzSimmons, N.N., Georges, A., & A.G.J. Rhodin, eds. Chelonian Research Monographs 4: 47-72.

Matocq, M.D., Shurtliff, Q.R. & **C.R. Feldman**. 2007. Phylogenetic relationships of the woodrat genus *Neotoma* (Rodentia: Muridae): implications for the evolution of phenotypic variation in male external genitalia. Molecular Phylogenetics and Evolution 42: 637-652.

Feldman, C.R. & G.S. Spicer. 2006. Comparative phylogeography of woodland reptiles in California: repeated patterns of cladogenesis and population expansion. Molecular Ecology 15: 2201-2222.

Parham, J.F., **Feldman, C.R.** & J.L. Boore. 2006. The complete mitochondrial genome of the enigmatic bigheaded turtle (*Platysternon*): Description of unusual genomic features and the reconciliation of phylogenetic hypotheses based on mitochondrial and nuclear DNA. BMC Evolutionary Biology 6: 11.1-11.11.

Parham, J.F., Macey, J.R., Papenfuss, T.J., **Feldman, C.R.**, Türkozan, O., Polymeni, R. & J.L. Boore. 2006. The phylogeny of Mediterranean tortoises and their close relatives (*Testudona* tax. nov.) based on complete mitochondrial genome sequences from museum specimens: with comments on mitochondrial genomic features,

phylogenetic taxonomy, and paleobiogeography. Molecular Phylogenetics and Evolution 38: 50-64.

Feldman, C.R. & K.E. Omland. 2005. Phylogenetics of the common raven complex (*Corvus*: Corvidae) and the utility of ND4, COI, and intron seven of the nuclear gene β-fibrinogen in avian molecular systematics. Zoologica Scripta 34: 145-156.

Buskirk, J.R., Parham, J.F. & **C.R. Feldman**. 2005. On the hybridisation between two distantly related Asian turtles (Testudines: *Sacalia* x *Mauremys*). Salamandra 41: 21-26.

Brodie, E.D., III, **Feldman, C.R.**, Hanifin, C.T, Motychak, J.E., Mulcahy, D.G., Williams, B.L. & E.D. Brodie, Jr. 2005. Parallel arms races between garter snakes and newts involving tetrodotoxin as the phenotypic interface of coevolution. Journal of Chemical Ecology 31: 343-356.

Feldman, C.R. & J.F. Parham. 2004. Molecular systematics of Old World stripe-necked turtles (Testudines: *Mauremys*). Asiatic Herpetological Research 10: 28-37.

Ashton K.G & **C.R. Feldman**. 2003. Bergmann's rule in non-avian reptiles: turtles follow it, lizards and snakes reverse it. Evolution 57: 1151-1163.

Feldman, C.R. & G.S. Spicer. 2002. Mitochondrial variation in sharp-tailed snakes (*Contia tenuis*): evidence of a cryptic species. Journal of Herpetology 36: 648-655.

Feldman, C.R. & J.F. Parham. 2002. Molecular phylogenetics of emydine turtles: taxonomic revision and the evolution of shell kinesis. Molecular Phylogenetics and Evolution 22: 388-398.

Parham, J.F., Simison, W.B., Kozak, K.H., **Feldman, C.R.** & S. Haitao. 2001. New Chinese turtles: endangered or invalid? A reassessment of two species using mitochondrial DNA, allozyme electrophoresis and known locality specimens. Animal Conservation 4: 357-367.*

*This research was profiled in Nature. Dalton R. 2003. Mock turtles. Nature. 423: 219-220.

Notes & Reports:

Greene, R.R. & **C.R. Feldman**. 2008. *Thamnophis atratus*. Diet. Herpetological Review. *In Press.*

Feldman, C.R., Vindum, J.V. & R.W. Hansen. 2007. *Thamnophis elegans*. Distribution. Herpetological Review 38: 222.

Feldman, C.R. & D.G. Mulcahy. 2003. *Ambystoma macrodactylum*. Distribution. Herpetological Review 34: 160.

Feldman, C.R., Mulcahy, D.G. & R.W. Hansen. 2003. *Xantusia vigilis*. Distribution. Herpetological Review 34: 167.

Parham, J.F. & **C.R. Feldman**. 2003. *Lampropeltis zonata.* Diet. Herpetological Review 34: 150.

Parham, J.F. & **C.R. Feldman**. 2002. Generic revisions of emydine turtles. Turtle and Tortoise Newsletter 6: 30-32.

Feldman, C.R. & J.F. Parham. 2001. Molecular systematics of emydine turtles. Chelonian Conservation and Biology 4: 224-228.

Feldman, C.R. & J.A. Wilkinson. 2000. *Rana muscosa*. Predation. Herpetological Review 31: 102.

Feldman, C.R. & J.A. Wilkinson. 2000. *Thamnophis sirtalis*. Diet. Herpetological Review 31: 248.

Presentations:

Is there more than one way to skin a newt? Parallel evolution in the newt-garter snake coevolutionary system. (talk) Annual Biology Department retreat, Utah State University, Aug. 2006.

Is there more than one way to skin a newt? Parallel evolution in the newt-garter snake

coevolutionary system. (talk) Annual joint Ichthyologist and Herpetologist meetings, Jul. 2006.
Phylogenetics of emydine turtles revisited. (invited talk) Western Pond Turtle Conference, Apr. 2005.
Comparative phylogeography of woodland reptiles in California. (poster) Annual Idaho NIH-BRIN Universities meetings, Jul. 2004.
Comparative phylogeography of three California Reptiles. (talk) Intermountain Herpetological Rendezvous, Nov. 2003.
Comparative phylogeography of three California Reptiles. (poster) Annual Society for the Study Evolution meetings, Jun. 2003.
Comparative phylogeography of three California reptiles. (poster) Annual International Society of Biogeographers meetings, Jan. 2003
New Chinese turtles: Endangered or invalid? (invited talk) Cornell Herpetology Society, Nov. 2001.
Molecular systematics of emydine turtles. (talk) Annual joint Ichthyologist and Herpetologist meetings, Jun. 2000.
Comparative phylogeography of three squamate reptiles in California: *Contia tenuis*, *Diadophis punctatus*, and *Elgaria multicarinata*. (talk) Annual joint Ichthyologist and Herpetologist meetings, Jun. 1999.
Evolution and natural history of snakes. (invited talk) San Francisco Naturalist Society, Apr. 1999.

Grants & Awards:
Robbins Award-Graduate Assistant Researcher of the Year, Utah State University, 2006.
School of Graduate Studies Dissertation Fellowship, Utah State University, 2006.
Graduate Student Senate Travel Fund, Utah State University, 2006.
Society for the Study of Amphibians Student Travel Award, 2006.
College of Science Graduate Student Researcher of the Year, Utah State University, 2006.
Dept. of Biology Graduate Student Researcher of the Year, Utah State University, 2006.
Charles Stearns Grant-in-Aid of Herpetological Research, California Academy of Sciences, 2000.
Henri Seibert Award-outstanding talk, Society for the Study of Amphibians and Reptiles, 2000.
Graduate Assistance in Areas of National Need Fellowship, U.S. Dept. of Education, 2000.
Henri Seibert Award-outstanding talk (honorable mention), Society for the Study of Amphibians and Reptiles, 1999.
Grants-in-Aid of Research, Sigma Xi, 1998.
Linnaeus Fund, Chelonian Research Foundation, 1997.

Journal Articles & Book Chapters Reviewed:
Asiatic Herpetological Research
Bulletin of the Southern California Academy of Sciences*
Conservation Genetics
Copeia*
Functional Ecology
Herpetologica*
Journal of Biogeography*
Molecular Ecology
Molecular Phylogenetics and Evolution*
Systematic Biology

Zoologica Scripta*
Biology of the Vipers (book)*
The Natural History of the UC Santa Cruz Campus (book)
*Multiple reviews

Societies:
American Association for the Advancement of Science, 2004-2007.
Society of Systematic Biologists, 2003-present.
American Society of Ichthyologists and Herpetologists, 2000-present.
Herpetologist's League, 2000-2006.
Chelonian Research Foundation, 1999-present.
San Francisco Naturalist Society, 1998-present.
Society for the Study of Amphibians and Reptiles, 1997-present.

Service & Committees:
Student committee member, Evolution faculty search, Utah State University, 2005.
Invited participant, Genetics, Ethics, and Systematics: A Workshop for Turtles (NSF funded workshop), Harvard University, 2005.
Student committee member, Graduate Student Policy Committee, Utah State University, 2004-2005.
Vice President, Biology Graduate Student Association, Utah State University, 2004-2005.
Treasurer, Biology Graduate Student Association, Utah State University, 2004-2005
Secretary, Biology Graduate Student Association, Utah State University, 2003-2004
Organizing committee member, Intermountain Herpetological Rendezvous (regional conference), Utah State University, 2003.
Student representative, Ecology faculty search, San Francisco State University, 1998-1999.

Professional Experience:
Teaching Experience:
Teaching Assistant, Aug. 2002 – June 2006.
Utah State University, Logan, UT
Courses:
- Evolution (Biol. 5250)*
- Herpetology (Biol. 5570)*
- Introductory Biology (Biol. 1210)

Contact: Dr. Mike Pfrender (USU)
*Also conducted class lectures

Teaching Assistant, Jan. 1998 – June 1999.
San Francisco State University, San Francisco, CA
Courses:
- Introductory Biology (Biol. 240)*
- Reptiles and Amphibians of the Sierra Nevada—one week summer course I designed and conducted for elementary students at SFSU Sierra Nevada Field Campus

Contact: Dr. Robert Patterson (SFSU)
*Also conducted class lectures

Laboratory Experience:
Research Assistant, Jan. 2003 – Dec. 2003; Jan. 2007 – May 2008.
Utah State University, Logan, UT
Contact: Dr. Mike Pfrender (USU)

Research Associate & Laboratory Manager, Feb. 2001 – May 2002.
University of Maryland Baltimore County, Baltimore, MD
Contact: Dr. Kevin Omland (UMBC)

Museum Experience:
Field Associate, Feb. 1999 – present.
California Academy of Sciences, San Francisco, CA
Contact: Jens Vindum (CAS)

Curatorial Assistant, Jan. 1997 – June 1997.
Museum of Vertebrate Zoology, University of California Berkeley, Berkeley, CA
Contact: Dr. James Patton (UCB)

Field Work:
2003-present. California, Oregon, Idaho: focused reptile collecting (PhD research)
1999-2002. California: reptile and amphibian inventory. Contact: Jens Vindum (CAS)
1997-present. California: focused small mammal collecting. Contact Dr. Marjorie Matocq (UNR)
1997-1999. California: focused reptile collecting (Master's research)
1996. California: marten and fisher habitat assessment. Contact: Dr. William Zielinski (CSUH)
1995. California: lizard population demography. Contact: Dr. Kelly Zamudio (Cornell)

References:
Dr. Mike E. Pfrender
Dept. of Biology
Utah State University
5305 Old Main Hill
Logan, UT 84322

Dr. Edmund D. Brodie, jr.
Dept. of Biology
Utah State University
5305 Old Main Hill
Logan, UT 84322

Dr. Greg S. Spicer
Dept. of Biology
San Francisco State University
1600 Holloway Ave.
San Francisco, CA 94132

Dr. Harry W. Greene
Dept. of Ecol. & Evol.
Corson Hall
Cornell University
Ithaca, NY 14853-2701