To the Graduate Council,

I am submitting herewith a dissertation written by John S. Placyk, Jr., entitled "Historical Processes, Evolutionary Change, and Phenotypic Plasticity: Geographic Variation in Behavior, Morphology, and Life-History Traits of Common Gartersnake, *Thamnophis sirtalis*, Populations." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

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HISTORICAL PROCESSES, EVOLUTIONARY CHANGE, AND PHENOTYPIC PLASTICITY: GEOGRAPHIC VARIATION IN BEHAVIOR, MORPHOLOGY, AND LIFE-HISTORY TRAITS OF COMMON GARTERSNAKE, *THAMNOPHIS SIRTALIS*, POPULATIONS

A Dissertation Presented for the
Doctor of Philosophy Degree
The University of Tennessee, Knoxville

John S. Placyk, Jr.
August 2006

DEDICATION

This dissertation is dedicated to Fuzzy and Sunny, two faithful and loving friends that were lost along the way. May they be frolicking and napping in the sun of another place.

ACKNOWLEDGMENTS

I'd like to start by saying that it will be impossible for me to remember the name of every person that helped me with this project in the last six years, so as you read on and notice that your name is absent, I apologize in advance for my poor memory and thank you for whatever it was you assisted me with.

To begin, I must thank my doctoral committee chair, Dr. Gordon M. Burghardt, for providing guidance, lab space, funding, patience, and both solicited and unsolicited advice. Gordon is like a walking behavior, evolution, and reptile encyclopedia and without his knowledge and advisement, this dissertation would be worth much less. Thanks also to my committee members, Dr. Chris Boake, Dr. Sandy Echternacht, and Dr. Randy Small. Chris for her no nonsense style and for taking on yet "another snake project," Sandy for being a role model of a herpetologist, and Randy for helping me to become a gel jockey, a skill that was missing from my toolbox. Drs. Paul and Hazel Delcourt also provided their expertise early on in my doctoral research and helped me to better understand the history of my study sites and of Michigan in general.

Special thanks to all of those that have helped facilitate my research by helping to collect data and catch snakes, providing research and, sometimes, living space, assisting with my thought processes and helping me to understand where I wanted to go with this work. The greatest help came in the form of Dr. Jim Gillingham and the researchers and staff of the Central Michigan University Biological Station on Beaver Island. Jim provided me with lab space, a roof over my head (even though it was most likely made of

OSB), research supplies, transportation to and from islands, vehicles, and other types of support. If it were not for "his" field station, much of this work would not have been possible. While spending two summers on Beaver Island, I made some great friendships and was assisted by some great folks including Adam Nelson, Jamie Pejza, Mike Seider, Jayme Rogers, Sarah Davidson, Kerry Hansknecht, Lisa McDonald and her parents Ron and Bert, Stephanie Schlitler, Dr. John Rowe, Dr. Beth Leuck, Kenny Bowen, Amy Boetcher, Josh Picotte, Dr. Dave Clark, and Theresa Barber. Dr. Brent Graves provided me with lab and living space during my stays in the upper peninsula of Michigan and has always been a role model when it comes to being a great scientist. I thank Brent for not only helping with my doctoral research but for being a great advisor well after I finished my M.S. with him. Also thanks to Jon Lobello, Denise Seubert, Dave Pickering, Nathan Forst, Joe Werner, Kari Stanley, Dr. Mac Strand, and Joanne and Jack Wofford for providing entertainment, places to crash, and camaraderie during my stays in da' UP. Of course, the field work was only half of what needed to be done for my research, the other half involved tending to pregnant female snakes and their offspring, as well as testing offspring. This component of my research was all conducted in Gordon's lab at the University of Tennessee and involved an army of undergraduates, most of whose names evade me. Those I do remember include Michael Boling, Sam and Richard, John Paul, and especially Stephanie Drumheller for counting ventral scales on stinky, dead baby snakes.

In addition, special thanks to many past and present members of the Burghardt lab for providing support in a variety of ways, especially Dr. Paul Andreadis, Kerry

Hansknecht, Jennifer Manrod, Lynn Almli (even though she gave our couch to the Echternacht lab), Karen Davis, Dr. Mark Krause, Dr. Jesus Rivas, Dr. Julie Albright, Matthew Bealor, Dr. Harry Greene, and Dr. Matthew Kramer. Other folks at the University of Tennessee that have been helpful include Nadia Ayoub, Dr. Todd Campbell, Dr. Stan Guffey, Monica Beals, Rachel Goodman, Nathan Turnbough, Bridget Donaldson, Jason Leonard, Michael Doosey, Dr. Neil Greenberg, Drs. Florencia and Diego, Dr. Tad Fukami, and the Small lab including Dr. Joey Shaw, John Beck, Dwayne Estes, Wushen Lui, Charles Winder, and Dr. Ed Lickey. Of course, I must not forget the staff of the Department of Ecology & Evolutionary Biology, Linda, Cheryl, Phyllis, and Pearl, and the staff of the Department of Psychology, Connie, Louise, Madge, Karen, Sandy, and Anne, as without their support, my life as a graduate student could have been very miserable.

Thanks to the other researchers that I've encountered along the way that have shown interest in and offered assistance with this research including Dr. Gary Casper, Dr. Rich King, Jace Robinson, Dr. Frank Burbrink, Jim Harding, Dr. Ronald Gratz, Dr. Steve Beupre, Dr. Susan Foster, and Briar Howes. Also, special thanks to all that have supported my research financially including the Department of Ecology & Evolutionary Biology at the University of Tennessee, Gordon Burghardt, and the National Science Foundation.

One of the only reasons that I stayed sane throughout the entire journey that was my graduate career is the relaxation and downtime provided to me by the following

artists, performers, actors, authors, personalities, and fictional characters: Frank Sinatra, Dean Martin, Sammy Davis, Jr., Tony Bennett, Bing Crosby, Peter Lawler, Joey Bishop, Johnny Cash, Ozzy Osbourne, The Rolling Stones, B. B. King, Johnny Lee Hooker, Muddy Waters, Aretha Franklin, Michael Bublé, Tool, The John Corbett Band, Jake and Elwood Blues, Uncle Willie, Marcus, Thurman Merman, Bernie Mac, John Ritter, Jeffrey "The Dude" Lebowski, Walter, Donny, The Jesus, nihilists, marmots and amphibious rodents, the Tenenbaum family, Napoleon Dynamite and Pedro, Ernest Hemingway, John Steinbeck, F. Scott Fitzgerald, Lewis Carroll, J. R. R. Tolkien, C. S. Lewis, William Faulkner, J. K. Rowling, J. D. Salinger, George Orwell, Aldous Huxley, Peter Jackson, George Lucas, Tim Burton, Gene Roddenberry, Alan Rickman, Emma Thompson, Billy Bob Thornton, Steven Spielberg, Frank Oz, Hugh Grant, Billy Mack, William Shatner, Howard Stern, Eric "Mancow" Muller, Tammy Bruce, Dave Ramsey, Sir Ian McKellen, Vince Vaughn, Jon Favreau, Chris Farley, Jack Lemmon, Walter Matthau, Burgess Meredith, Humphrey Bogart, Kurt Vonnegut, The Trailer Park Boys (Julian, Ricky, Bubbles, Corey, Trevor, Randy, and Mr. Lahey), BBC America, Trey Parker and Matt Stone, Ethan and Joel Coen, Carrie, Miranda, Charlotte, Samantha, and Mr. Big, Lorali and Rori Gilmore, Kevin Smith (the writer, director, and actor, not the herpetologist), Silent Bob and Jay, the Sopranos, the Simpsons, Jerry Seinfield and company, Drew Carey, Kathy Griffin, Larry David, the creators of Lost, and last, but not least, Spongebob Squarepants. Also special thanks to Jack Daniels, Evan Williams, Elijah Craig, and Old Granddad for those especially hectic times.

Finally and most importantly, thanks to my immediate and extended family, and my wife, Lana, and our furry companions. These are the individuals that have sacrificed more than any other. Some saw a son and brother move far away from home, others saw a husband spend long nights and weekends studying, reading, and writing, while others have known me only as a graduate student constantly trying to finish my degree. Some thought I'd never stop being a student, and in a sense that is still true, but it's finally time for me to stop accumulating student loan debt, be rewarded with a terminal degree, and get a "real" job. I apologize for being away for so long, but this part of my journey is now complete and the next stage is about to begin. I hope many of the nights and weekends that were lost for the sake of finishing my M.S. and Ph.D. can be reclaimed and I look forward to being more of who I was before I buried my mind, body, and soul in eight years of graduate school. I love all of you and apologize if that wasn't evident during the almost ten years I've been working to get where I am.

ABSTRACT

Islands have long been of interest to biologists, as they are often home to unusual species or populations of species that are characterized by unique behavior, morphology, and gene pools. My research explores the mechanisms driving and maintaining phenotypic variation in the common gartersnake, *Thamnophis sirtalis*, populations of the Beaver Archipelago. Specifically, I focused on antipredator-related traits, foraging/feeding-related traits, and reproductive life-history traits, as all are known to vary with differences in predator composition and resource availability, which vary among the islands of the Beaver Archipelago and between the islands and the surrounding mainland. Since the exact origin of Beaver Archipelago fauna is debated, I began this study by using mtDNA sequences to determine the underlying genetics of the island populations and their most likely source. The results of this part of my dissertation indicate that the island populations are derived from both the lower and upper peninsula of Michigan and that the upper peninsula is more closely related to island populations than the lower peninsula populations, contradicting previous hypotheses that suggested the lower peninsula was the most likely point of origin for the islands and that the upper peninsular populations played little or no role in the system. This knowledge proved to be invaluable in understanding observed variation in phenotypic traits, all of which appear to be the result of some combination of their underlying genetics, as related to the mtDNA data, and plastic responses to selection with little if any evolutionary change resulting from selection. In terms of antipredator-related traits, populations that occur with fewer predators appear to be less reactive than those that occur with a greater

number of predators as the result of plasticity, although there was no clearcut island/mainland trend. Ventral scale counts for island populations correspond best with the underlying genetics of the populations resulting from historical/colonization processes and do not appear to have changed as the result of current predator pressures. In terms of feeding/foraging-related traits, geographic variation in the head morphology of island snakes appears to be primarily the result of phenotypic plasticity with some evidence of genetic influence from historical/colonization processes. Behavioral responses to prey chemical extracts varied little between populations, with observed differences potentially being due to evolutionary change resulting from current selection pressures. Finally, reproductive life-history traits also appear to be mostly the result of underlying genetics, but some influence of plasticity and microevolution may also play a role. Combined, the genetic and phenotypic traits studied provide researchers with one of the more complete studies of variation in the traits of insular faunas to date and should provide a framework for future studies centered in island systems.

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Part I

INTRODUCTION: OVERVIEW AND OBJECTIVES

INTRODUCTION

It was Charles Darwin that stated "… the zoology of archipelagos will be well worth examination …" (Barlow, 1963) and he was correct. Studies of insular flora and fauna have resulted in major contributions to biological thinking (e.g., MacArthur and Wilson, 1967), especially evolutionary theory (Darwin, 1859). Many islands interest biologists because they act as simple, discrete, geographical units when compared to vast continents and oceans, and since many islands are found in groups (archipelagos), they often provide researchers with natural replicates for use in their studies. Given the size of many islands, especially those in the northern hemisphere, insular flora and fauna residing on them are usually easily identified and accounted for in a fraction of the time it would take to determine such biotic characteristics in larger systems. Abiotic factors (e.g., temperature, climate, habitat availability) of many island ecosystems often do not vary as much across an island when compared to such characteristics across mainland landmasses. Finally, we often know the exact starting point particular islands came into existence, before which they did not exist and the flora and fauna that currently reside on them did not exist. Of course, some of the characteristics mentioned above do not apply to all islands, especially many tropical oceanic islands, but, in general, they can be applied to a variety of island systems. As a result of all of these attributes, the biology of organisms living on islands has drawn a considerable amount of attention from researchers.

In particular, variation in the phenotypic traits of organisms living on islands has interested biologists at least since Charles Darwin's and Alfred Russel Wallace's

respective explorations of the Galapagos and Malay archipelagos led them to independently develop the theory of natural selection (Darwin, 1839, 1859; Wallace, 1860, 1869, 1880). One reason island systems are ideal for studying phenotypic variation is due to the unique genetic attributes of insular flora and fauna. Many insular faunas, especially thos on "oceanic" islands, are typically the result of rare founder events and, once established, the opportunities for gene flow are often limited (MacArthur and Wilson, 1967; Grant, 1998; Johnson et al., 2000). As a result, the possibility of biological divergence via a founder effect, genetic drift, and mutation, both between island and mainland/continental populations and among island populations, is enhanced. The environmental attributes of islands may also drive and maintain phenotypic variation. For example, since the flora and fauna of many islands often represent only a subset of the organisms found on the nearest mainland/continental (MacArthur and Wilson, 1967; Hecnar et al., 2002), predator/prey relationships, among other biotic interactions, may differ substantially between islands and the nearest landmass. In addition, abiotic factors (e.g., temperature, habitat availability) are also usually different on islands when compared to the surrounding mainland or closest continental landmass. The result of less genetically diverse populations, restricted gene flow, and different environmental conditions experienced by island faunas allows populations of the same species to evolve independently (e.g., Berry et al., 1987; Miller et al., 2000). This independent evolution is often characterized by variation in phenotypes and genotypes. However, many of the ecologically important differences in phenotypic characters that develop between individuals of the same species in heterogeneous environments (e.g., island systems) may often result from phenotypic plasticity (Via, 1994).

When environments within the range of a species differ, it is unlikely that any single phenotype will confer high fitness in all situations. Therefore, a change in the phenotype that depends on the environment can provide increased environmental tolerance. Therefore, phenotypic plasticity is one solution to the problem of adaptation to heterogeneous environments (Via et al., 1995), and it may be easily confused with genetic-based evolutionary change. For example, Losos et al. (1994, 1997) found that lizards from the genus *Anolis* (Polychrotidae) differed morphologically within species throughout the Caribbean islands and initially attributed these differences to rapid evolutionary change. However, upon closer examination, it was determined that phenotypic plasticity might be important at the onset of the observed intraspecific differences (Losos et al., 2000). To further confuse matters, phenotypic plasticity, itself, may be inherited, and the Baldwin effect and related processes, which involve interactions between plasticity and genetic change may also come into play (Baldwin, 1896; Burghardt, 2004).

The Baldwin effect involves differences in phenotypic characters beginning as the result of phenotypic plasticity and ending up being genetically fixed (Weber and Depew, 2003; Burghardt, 2004). Essentially, many animals can respond to their environments via modifications of their morphology, physiology, or behavior. This capacity to adapt is, itself, congenital, and represents an animal's innate plasticity. Not only are the animals that undergo change in response to environmental variables more likely to survive and breed, they may also accumulate random genetic changes that support the phenotypic

changes that resulted from plasticity. Genetic changes that enhance the fitness of an individual beyond that already provided by plasticity and that are favored by natural selection will allow phenotypes that were once noninheritable to become heritable as more genes supporting the trait accumulate. The related Waddington effect relies on uncovering "hidden" genetic variation supporting the environmentally induced trait (Burghardt, 2004). Thus, a character that was once developed over the life of an organism may eventually become genetically fixed. While Baldwin and others believed that advanced cognitive abilities, sociality, imitation, and consciousness could have evolved this way, this process has not been strongly supported by empirical studies (Burghardt, 2004). Critics of the Baldwin effect tend to dispute that acquired traits can become heritable indirectly via natural selection; however, Koegh et al. (2005) recently suggested that it is responsible for the occurrence of gigantism and dwarfism in insular is populations of tiger snakes (*Notechis* spp., Elapidae).

Variation in phenotypic characters may result from a variety of factors including 1) the underlying genetics of source populations, 2) abiotic natural selection (e.g., temperature regimes, habitat availability), 3) biotic natural selection (predation, diet), 4) genetic drift, and 5) phenotypic plasticity due to varying environmental factors. Since phenotypic plasticity and genetic factors may interact with each other, detailed studies of phenotypic variation are required to fully understand its underlying processes. My research examines variation in various phenotypic traits in an insular fauna and investigates the roles that genetic change and phenotypic plasticity play in creating and maintaining any such variation. I choose to focus my research on the Beaver

Archipelago of northeastern Lake Michigan, as this system offers an excellent opportunity to study the basis of intraspecific variation in phenotypic traits exhibited in island populations.

The Beaver Archipelago: A model system—The Beaver Archipelago consists of four main islands (i.e., Beaver, Garden, High, and Hog), each $\geq$ 6.2 km^2, and six smaller islands (i.e., Hat, Pismire, Shoe, Squaw, Trout, and Whiskey), each $\leq$ 0.64 km^2, situated in northeastern Lake Michigan. The islands are separated from the lower and upper peninsula of Michigan by approximately 30 and 25 km of open water, respectively (Fig. 1.1; all tables and figures for Part I are located in Appendix I). The recent geological history of the archipelago is marked with drastic lake level fluctuations due, in part, to complex interactions between the retreating Laurentide Ice Sheet, differential isotatic rebound, and regional climate change (Delcourt et al., 1996; Petty et al., 1996). Specifically, 11,000 to 8,000 years before present (ybp), directly following the last glaciation of the Holocene, the largest and latest proglacial lake (i.e., Lake Algonquin) occupied the three upper Great Lakes basins (Larsen, 1987). During this time, the lake level around the archipelago stood at ca. 250 m with only the higher elevation islands (i.e., Beaver Island and High Island) remaining emerged (Kapp et al., 1969). Starting ca. 8,000 ybp, lake levels dropped allowing the archipelago to be connected with the lower peninsula of Michigan and separated from the upper peninsula by the Mackinac River, which may have only been 1.6 km wide in some areas (Hough, 1958; Kapp et al., 1969; Dietrich, 1978). This low lake level persisted until ca. 6,900 to 5,500 ybp when a drastic increase in the lake level once again caused all but the higher elevation islands to become

submerged (Hough, 1958; Kapp et al., 1969; Dietrich, 1978; Petty et al., 1996). Recently (i.e., 4,000 ybp to present day), islands of low elevation have reemerged (Hough, 1958; Kapp et al., 1969; Dietrich, 1978). As a result of this well-documented recent geological history, any phenotypic variation present in the island populations can be related, if necessary, to the recent colonization, extinction, and recolonization events that may have occurred there.

It has been hypothesized that since the archipelago was at one time connected by a land bridge to the lower peninsula of Michigan (Kapp et al., 1969; Hough, 1958; Dietrich, 1988), the taxa found on the islands are descendents of lower peninsula populations. Further support for this view is that some species found in the Beaver Archipelago are rare (eastern milksnake, *Lampropeltis t. triangulum*, Colubridae) or absent (northern ribbonsnake, *T. sauritus septentrionalis*, Colubridae) from the upper peninsula of Michigan but common in the lower peninsula. However, some terrestrial animals that reside on the islands, especially many of the reptiles and amphibians are adept swimmers and may have been able to colonize the archipelago at any point (as indicated by their presence on oceanic islands in the upper Great Lakes, King, 1988). In fact in the last 10,000 years the archipelago was separated from Michigan's upper peninsula by the Mackinac River, which may have only been 1.6 km wide in some areas (Hough, 1958). As a result, part of my doctoral research (see Objectives section below) will involve mitochondrial DNA (mtDNA) sequencing to determine the underlying genetics of Beaver Archipelago populations and to establish if the island populations may have undergone any molecular genetic change resulting from random processes (drift,

founder effect). Based on the above evidence, I hypothesize that the islands were colonized by both lower and upper peninsula populations, but that lower peninsula populations played a more substantial role, and, thus, will account for more of the genetic variation in the island populations than the upper peninsula populations. I also hypothesize that given the current distance between the mainland and the islands, and the fact that the island populations are smaller than the mainland populations, some genetic divergence resulting from drift or a founder effect has occurred.

To accomplish the objectives of my study, it was essential to utilize an organism in which phenotypic plasticity and evolutionary change could be discriminated. I chose the common gartersnake (*Thamnophis sirtalis*, Colubridae) for the focus of this work, in part, because it offers excellent opportunities to distinguish between the genetic factors and environmental influences underlying phenotypic characters.

The common gartersnake: A model species—The common gartersnake, *Thamnophis sirtalis*, is the most widespread snake species in North America, occurring in nearly every natural habitat found throughout its range, which extends from the Atlantic to the Pacific Coasts and much farther northward than any other snake in the Western Hemisphere (Rossman et al., 1996). The research described herein focused on the eastern gartersnake, *Thamnophis s. sirtalis*, which ranges from southern New England and south-central Canada southward to Florida and southeastern Texas (Rossman et al., 1996). The largest populations of *T. sirtalis* can be found in moist grassy places, particularly near the edges of ponds, lakes, ditches, and streams, and in vacant urban and suburban lands with

abundant cover in the form of scattered boards, metal sheeting, and piles of rocks or construction debris (Harding, 1996). The success of this primarily diurnal predator in North America may be attributed to its generalist feeding nature, as it preys upon a wide variety of taxa (e.g., terrestrial and aquatic insects, fish, amphibians and their larvae, mammals and birds). Therefore, one reason that *T. sirtalis* is being used for this work is due to its wide distribution and utilization of variable habitats that may lead to geographic variation in phenotypically expressed traits. In addition, given its generalist nature, *T. sirtalis* are easily maintained in a laboratory setting and make excellent test subjects for common garden experiments in which environmental conditions need to be varied. Furthermore, *T. sirtalis* occurs in Michigan and throughout the Beaver Archipelago. In fact, Harding (1996) lists *T. sirtalis* as being the most familiar and frequently encountered snake in the Great Lakes region, and fossil evidence suggests that *T. sirtalis* have most likely existed in the region since the late Pleistocene (ca. 150,000 $\pm$ 25,000 to 10,000 ybp) (Holman, 2000). Therefore, given the geological history of the region, this taxon has had the opportunity to evolve independently throughout the entire Beaver Archipelago for at least the last 4,000 years.

Another major reason this natricine snake is being used is because representatives from this subfamily are known to exhibit geographic variation in phenotypic traits both within and between species (Burghardt and Schwartz, 1999), making it an ideal taxon in which to study the divergence of phenotypic characters. In addition, snakes, in general, have large litter sizes (*T. sirtalis* averages 10-15 (Rossman et al., 1996)), which can be beneficial in several ways. For example, large families provide an opportunity to split

litters among environmental treatments to address concepts empirically such as genotype-by-environment interactions and common family environment (including maternal) effects (e.g., Brodie and Garland, 1993; Burghardt and Schwartz, 1999; King et al., 2001; Badyaev et al., 2002; King, 2002). Snakes are also excellent candidates for studying the role of genetics and environment on behavior, as they have highly precocial young that are naïve to the external stimuli that elicit behavioral responses. Therefore, neonate snakes can be used to determine if the behaviors exhibited by different populations have a genetic basis. In fact, geographic variation in foraging behavior and antipredator behavior is known to occur in *Thamnophis* (e.g., Burghardt and Schwartz, 1999), and is influenced by both genetic factors and phenotypic plasticity (Arnold, 1981a, 1981b; Schwartz, 1989; Burghardt and Krause, 1999). Thus, given the abundance and natural history of *T. sirtalis* in Michigan, as well as the other attributes listed above, the eastern gartersnake provides a model organism for this work.

Why would geographic variation in phenotypic traits exist in the fauna of the Beaver Archipelago?—Most of the phenotypic differences between island and mainland populations of animals are attributed to differences in predator pressures and resource availability (see below) (e.g., Case, 1978a; Heaney, 1978; King and Lawson, 1997) and differences resulting from these factors were the focus of my study. *Thamnophis sirtalis* predator and prey composition differs between the islands of the Beaver Archipelago (e.g., Hatt et al., 1948; Placyk and Burghardt, 2005; Tables 1.1 & 1.2), as well as between the islands and both the upper and lower peninsula of Michigan; thus, it is likely that

phenotypic differences exist between populations inhabiting the various islands and peninsulas.

In the Great Lakes region *T. sirtalis* is preyed upon by large fish, bullfrogs, snapping turtles, various snakes (e.g., milksnakes, racers, and massasaugas), birds (e.g., crows, turkeys, hawks, and herons), and many mammals, from shrews and rodents to foxes, raccoons, and bears (Harding, 1996). In addition, domestic animals such as dogs, cats, hogs, and chickens also prey upon gartersnakes in areas where these predators have been introduced. Accidental and purposeful killing by humans is also a significant cause of mortality for *T. sirtalis*. All of these potential predators occur sympatrically with *T. sirtalis* populations on the lower peninsula of Michigan; however, the upper peninsula of Michigan is lacking in any ophiophagous (i.e., snake-eating) snakes (Table 1.1). Furthermore, only the largest island in the Beaver Archipelago (i.e., Beaver Island) is currently inhabited by humans, and racers, massasaugas, and bears do not occur on any of the islands in the archipelago. In addition, milksnakes have only been reported on four of the islands (Hatt et al., 1948; Bowen, pers. obs.), and several islands that are lacking inland bodies of water do not support populations of bullfrogs and other large amphibians or snapping turtles. Evidence of variation in predation pressures in this system comes from recent work completed on tail stub frequencies conducted during this study (Placyk and Burghardt, 2005), which indicates that populations occurring with more predators exhibit significantly greater frequencies of tail stubs and other wounds caused by failed predatory attacks.

Resource availability also varies both among the islands and between the islands and the mainland (Table 1.2). For example, islands with inland lakes and ponds may have amphibians and their larvae, aquatic invertebrates, and certain small fish as potential prey that do not occur on islands lacking inland bodies of water. In addition, potential prey may not be available because they may have never migrated to or colonized particular islands. For example, viable populations of gray treefrogs (*Hyla versicolor*, Anura: Hylidae), which occur in the diet of Beaver Island *T. sirtalis* (Krause, 2000; Placyk, pers. obs.), are not known to occur on Garden, Gull, Hat, High, Hog, Pismire, Shoe, Squaw, or Whiskey Island. Thus, snake populations on some of the islands may have more varied diets and prey on different prey items than snake populations on other islands. Therefore, it is also likely that geographic variation in phenotypic traits may be correlated with the availability of different prey items. In fact, geographic variation in prey preference is commonly seen in gartersnakes and snakes in general and can vary among populations that have only recently been separated and/or that are in close proximity to each other (e.g., Burghardt, 1969, 1970, 1975; Cooper et al., 2000; Mori and Burghardt, 2000; Krause and Burghardt, 2001; Aubret et al., in press).

Based on the above information, several predictions can be made regarding the behavioral, morphological, and life-history traits that characterize *T. sirtalis* populations of the Beaver Archipelago. To begin, populations of the Beaver Archipelago islands have only been separarted from their mainland source populations for a relatively short time (i.e., 4,000 – 8,000 years). As a result, I predict that most variation in phenotypic traits found in this system will either be the result of phenotypic plasticity or historical

processes. For morphological traits that are discrete/meristic in nature (e.g., scale counts), I predict that historical processes alone will account for any variation in such traits, while for behavior and continuous morphological traits (e.g., size, head morphology), some combination of historical processes and plasticity will most likely be at work. While genetically-based phenotypic divergence resulting from natural selection has been known to occur relatively rapidly in some taxa, I believe that the generalist nature of *T. sirtalis* would require strong selection pressures and very limited or no gene flow to have operated in this system in the last 10,000 years. While predator and prey species vary from island to island and betweent the islands and the mainland, all sites are characterized by earthworms, which are a staple in the diet of many gartersnake populations (e.g., Harding, 1996), and all populations are preyed upon by birds such as crows, which are considered a primary predator of *T. sirtalis* (e.g., Harding, 1996). Therefore, while, I expect phenotypic traits to vary from site to site based on the biotic and abiotic characteristics of each site, it is my opinion that differences in selection pressures may not be strong enough to warrant evolutionary change via natural selection. Regardless of the mechanisms that may be driving and maintaining phenotypic trait variation in th Beaver Archipelago, further support that phenotypic variation is possible in the Beaver Archipelago comes from a number of studies indicating that such variation is common in insular systems.

Morphological variation in insular systems—Some of the most common differences seen between island and mainland populations are differences in body size and these differences are usually attributed to differences in prey availability and predator

pressures; however, competition and physiological characteristics may also play a role in some cases. In a major early review of vertebrate size shifts in insular systems, Case (1978a) found that lagomorphs, bats, artiodactyls, elephants, foxes, raccoons, snakes, and teiid and lacertid lizards tend to be smaller on islands, while cricetid rodents, iguanid lizards, tortoises, and bears tend to be larger on islands. In addition, in general, large mammals have been found to be smaller in size on islands, whereas small mammals are larger in size on islands (e.g., Foster, 1964; Heaney, 1978; Lomolino, 1985; Brown and Lomolino, 1998). Small insects (Zimmerman, 1972) and small snails (Vagvolgyi, 1975) also become significantly larger on islands. However, those relying on broad sweeping predictions for any one taxonomic group based on the literature need to be cautious, as trends in size shifts often vary from species to species and from system to system. For example, while insular populations of Japanese keelback snakes, *Amphiesma vibakari* (Colubridae), tend to be smaller than individuals from mainland populations (Hasegawa, 1990), insular Japanese ratsnakes, *Elaphe climacophora* (Colubridae), and black tiger snakes, *Notechis ater niger* (Elapide), are much larger than mainland conspecifics (Schwaner, 1985; Shine, 1987; Schwaner and Sarre, 1988; Mori, 1994). Regardless of the direction the size shift occurs, all of the size differences detailed above are linked to differences in predation, food limitation, competition, or physiological efficiency. However, explaining such trends is not easy, as interactions between all of these factors are possible. In addition, in only a few of these cases are the roles of phenotypic plasticity, genetic change, and interactions between the two examined, further confusing attempts at interpreting such shifts. Other morphological traits (e.g., head morphology in snakes (e.g., Grudzien et al., 1992; Aubret et al., 2004)) are known to vary in insular

systems, but none are as well-documented as size shifts. In addition to morphological differences, variation in behavioral and life-history traits is also common between island and mainland populations.

Behavioral variation in insular systems—While there are many studies on size shifts in insular systems, studies of behavioral and life-history trait variation in such systems are less common. The majority of research on behavioral variation in island systems reports on variation due to differences in predator pressures (e.g., Grant, 1998). In general, it is believed that most vertebrates inhabiting islands are characterized by reduced antipredator responses (less flight, etc.) as a result of fewer predators (e.g., Pimm, 1987; Atkinson, 1989; Case et al., 1992) and relaxed selection (see review in Coss, 1999); however, McLaughling and Roughgarden (1989) and Alcover and McMinn (1994) caution that this assumption can be misleading. For example, Galapagos finches retain alarm responses to snakes, owls, and hawks even on islands from which these predators have been missing for 3-4 million years (Curio 1964, 1966, 1969, 1976). Furthermore, this trend of fearlessness and tameness in island species is mainly based on endemic island species rather than species that occur both on the mainland and in insular habitats (e.g., Stone et al., 1994). In fact, Scharf (1973) observed that gartersnakes, *Thamnophis sirtalis* (Colubridae), found on South Manitou Island in northern Lake Michigan are seemingly more "aggressive" than mainland *T. sirtalis* that occur with more predators, which does not support the island tameness hypothesis. However, more recent research does support such fearlessness/tameness trends at the intraspecific level. For example, insular spiny-tailed iguanas (*Ctenosaura hemilopha*, Iguanidae) are believed to

have modified their behavior in response to a decline in predation risk by basking at greater distances from their refuges and taking flight at shorter distances than *C. hemilopha* from mainland populations (Blazquez et al., 1997). Similarly, insular tiger snakes (*Notechis scutatus occidentalis*) were found to be more placid than mainland populations of the same species (Bonnet et al., 2005). While Bonnet et al. (2005) attribute the differences they found to phenotypic plasticity, the cause of the differences exhibited by spiny-tailed iguanas was not examined. Despite the support these studies provide the hypothesis that island animals are less reactive than mainland animals, caution should still be taken, as a variety of factors (both intrinsic and extrinsic) need to be taken into account when examining such geographic variation in behavior. Studies focusing on other types of behavioral variation in insular systems are generally lacking; however, it should be expected that traits related to differences in prey availability should also be common in such systems. For example, the very recent work of Aubret et al. (in press) indicates that insular tiger snake (*Notechis scutatus*) populations prefer prey that they encounter more frequently and that this preference has a genetic basis; however, island and mainland populations also prefer similar prey even if they do not currently occur with them, indicating historical influences on the behavior of these insular populations.

Life-history trait variation in insular systems—While size shifts and variation in antipredator behavior have been relatively well-documented in island systems, variation in life-history traits is less well known. Stearns (1992) considers 1) size at birth, 2) patterns of growth, 3) age at maturity, 4) size at maturity, 5) number, size, and sex of

offspring, 6) age and size-specific reproductive investment, 7) age and size-specific mortality schedules, and 8) length of life as the principle life-history traits. Some of the best examples of geographic variation in the life-history traits of insular populations come from studies on growth rate, as growth rate is often intimately connected to prey availability (Case, 1978b), which can vary greatly in island systems. For example, insular slider turtles, *Chrysemys scripta* (Emydidae), have very rapid growth rates on Atlantic Coast islands when compared to mainland individuals as a result of higher quality diets (Gibbons et al., 1978), while island watersnakes, *Nerodia sipedon insularum* (Colubridae), (King, 1986) from the Lake Erie area and juvenile *Anolis* lizards on islands in the West Indies (Andrews, 1976) have lower growth rates than mainland individuals of similarly sized species, respectively, most likely due to poorer diets. In addition to differences in growth rates, other differences in life-history traits between insular and mainland populations have also been noted and, as with growth rate and the morphological and behavioral differences already noted, are often connected to differences in prey availability and predator pressures. For example, there appears to be a general trend towards reduced birth rates on islands, at least for terrestrial vertebrates (e.g., Case, 1983; Hasegawa, 1990; Grant, 1998) and birds (e.g., Grant and Grant, 1989; Martin, 1992; Grant, 1998); however, Grant (1998) notes that systematic and broad-scale analyses of insular clutch size differences are lacking. Other types of life-history trait variation documented in insular systems include differences in survivorship (e.g., Lister and Aguayo, 1992), degree of sexual dimorphism (e.g., Andrews, 1979; Schwaner, 1985), and sex ratios (e.g., Schwaner, 1985; Shine, 1987).

What's missing from studies of phenotypic variation in insular systems?—It is clear that variation in the phenotypic traits of insular populations is common. However, besides work on size shifts and antipredator behavior, little is known about variation in many other phenotypic traits. In addition, all of the studies mentioned above are limited and incomplete in that they do not include comparisons among morphological, behavioral, and life-history trait variation or explore the role that phenotypic plasticity and evolutionary change play in their systems. In addition, many do not take into account the geological and phylogeographical nature of the populations in question. My research includes all of these aspects and this makes it one of the more complete studies of variation in phenotypic traits of a vertebrate in an insular system.

OBJECTIVES

The main goals of this research were to document geographic variation in phenotypic traits both among insular populations and between island and mainland populations and to determine the role that genetics and phenotypic plasticity play in the expression and maintenance of any such variation when possible. Nested within this goal was a variety of objectives that helped to determine how much and what kind of variation existed in this system, what the root of that variation is, and how that variation is being maintained. To accomplish these objectives a variety of methodologies were used.

To begin, in addition to using data on the geological history of the Beaver Archipelago to understand the possible origins of the populations in question, mtDNA

sequencing, as discussed above, was utilized to provide a more accurate and empirical data set from which to form hypotheses on the phylogeography and origins of Beaver Archipelago populations of *T. sirtalis*. The sequence data, along with the geological history of the archipelago, helped to fully understand variation in phenotypic traits based on historical processes in this system. In addition, while some have studied the phylogeography of *T. sirtalis* populations (Rye, 2000; Janzen et al., 2002), and some have studied the phylogeography of other Great Lakes region taxa (Austin et al., 2002; Brant and Orti, 2003; Zamudio and Savage, 2003), none of the past *T. sirtalis* studies have focused on this region and none that have been conducted in this region have been as complete as the data collected for this work. Therefore, in addition to helping interpret phenotypic variation in the system of question for this study, the mtDNA sequence data collected for this work is the most complete phylogeographical study conducted in the Great Lakes region to date. The molecular phylogeographical component of this study is discussed in the second part of this dissertation, as understanding this aspect of the system in question is key to understanding much of the variation that is discussed in the parts that follow.

Parts 3, 4, and 5 all document the amount of and different types of variation exhibited by *T. sirtalis* populations in this system with Part 3 focusing on traits related to predator pressures and Parts 4 and 5 focusing on traits related to prey availability. Part 4 focuses on head morphology and prey preferences, while Part 5 focuses on adult size and reproductive life-history traits.

As suggested above, varying predator pressures and resource availability are the main influences on differences in phenotypic traits in many snakes (e.g., Case, 1978a; Forsman, 1991; King, 1993b), and taxonomically diverse systems suggest the same (e.g., funnel-web spiders (*Agelenopsis aperta*, Agelenidae), (Riechert, 1999); Trinidadian guppies (*Poecilia reticulate*, Poeciliidae), (Magurran, 1999); California ground squirrels (*Spermophilus beecheyi*, Sciuridae), (Coss, 1999)). These and other studies show that predator pressures and prey availability may be extremely influential on the behavior of a variety of taxa, including insular snake populations. While population level studies on various species of *Thamnophis* and other natricines have been conducted in the past, none have combined behavioral, morphological, phylogeographical, and geological data along with field data on current predator pressures and prey availability, making this work more complete than past studies (e.g., Arnold 1981a, 1981b, 1990, 1992; Brodie 1989, 1992, 1993; Burghardt and Schwartz, 1999; King, 1986, 1993a, 1997; King and Lawson, 1997, 2001; King et al., 2001).

Both Part 3 and Part 4 combine behavioral data with morphological data in examinations of the influence of varying prey availability (Part 4) and predator pressures (Part 3) on geographic variation in phenotypic traits. Both parts utilize data collected both from adult animals and neonates in an attempt to understand the genetic basis of variation and the possible role that experience and environmental influences play throughout the life of individuals. In addition, both parts employ experiments designed to elucidate the role of phenotypic plasticity on foraging- and antipredator- related traits.

The overall goals of Part 3 and 4 were to determine (1) if variation in antipredator- and foraging- related traits exists between populations, (2) if variation between behavioral and morphological traits for each part correspond with each other, (3) if any such variation is related to local selection pressures, (4) the influence of genetics and phenotypic plasticity on any such variation, and (5) the role of historical/geological processes on any phenotypic variation.

More specifically, for Part 3, variation in antipredator-related traits including morphology and behavior were surveyed. In snakes, very few morphological characters are related to predator pressures with many only being assumed to respond to differences in such selection pressures. The most support that any of these traits relate to differences in predator pressures exists for the number of ventral scales and this is the morphological character examined for this study. Ventral scales relate to the number of muscles that help with locomotion and intraspecific variation in this trait has been observed with populations under high predation pressures exhibiting significantly greater number of ventral scales than populations under lower predation pressures (Arnold and Bennett, 1988). As for differences in antipredator behavior, this was surveyed in both neonates and adults via a standardized antipredator test (Herzog and Burghardt, 1986; Mori et al., 1996) and an ophiophage antipredator test (Placyk, 2006). During the standardized antipredator test, a predatory attack was simulated and a variety of antipredator behaviors were recorded, while the ophiophage antipredator test was specifically concerned with how individuals respond to chemical cues from ophiophagous snakes (i.e., milksnakes). Data from adults and neonates were then compared for both tests to determine if any site

differences may be the result of genetic change due to recent selection pressures or phenotypic plasticity. The sequence data collected in Part 2 and the geological history of the system was also used to help explain observed differences among sites and between adults and neonates. In addition, to somewhat better understand the role phenotypic plasticity plays in shaping antipredator behaviors, two plasticity experiments were conducted. One involved exposing snakes to simulated predatory attacks once a week for six weeks, while other groups were only handled gently or handled only to clean their cages. After the treatment was completed, behaviors were surveyed again using the standardized antipredator test. The second plasticity experiment involved exposing neonates to chemical cues emitted from milksnakes on a weekly basis over a six week period, while another group was not exposed to milksnake chemical cues. Gutzke et al. (1993) suggested that snakes not confronted by such predators periodically, lose their antipredator responses to such stimuli; however, as discussed above, Galapagos finches retain their antipredator responses to predators they have not occurred with for millions of years (Curio 1964, 1966, 1969). At the end of the treatment period, neonates were tested again using the ophiophagous antipredator test to determine if differences in their responses to ophiophagous snakes resulted.

For Part 4, both variation in morphology and behavior were surveyed again, but with an emphasis on traits related to feeding or foraging behavior. Since snakes have often been described as "gape-limited" predators (e.g., Forsman and Shine, 1997), the best morphological characters to examine when looking for differences related to prey availability are related to head morphology, and those are the characters examined for

this study. In particular, measurements such as jaw length, head length, head width and a variety of others were taken for both adults and neonates. Site differences were then attributed to either recent genetic change or phenotypic plasticity, with historical evolutionary influences being determined using the mtDNA sequence data from Part 2 and the geological history of the system. Variation in foraging behavior was observed in the adults by physically determining the prey being taken by individuals from each site via palpation and surveyed in the neonates using prey chemical extract tests (Burghardt, 1969). As with Part 3, plasticity of foraging-related traits was tested, but this time with the morphological traits rather than the behavioral traits for two reasons. First, plasticity of foraging behavior has been well-documented with specific studies on plasticity in eastern gartersnake (*T. sirtalis*) foraging behavior being examined very recently (Krause and Burghardt, 2001). Second, since the morphological character examined in Part 3 is a discrete character that does not change throughout the lifetime of snakes, I wanted to examine a morphological character(s) that would potentially react to environmental stimuli. The head morphology of *T. sirtalis* has been examined in this system before with differences being noted (Grudzien et al., 1992), and Part 3 examined similarities and differences in my two data sets. While some have demonstrated that phenotypic plasticity in head morphology plays a big role in intraspecific variation (e.g., Queral-Regil and King, 1998), others suggest such differences are solely due to genetic change (e.g., Forsman, 1991). However, none of the more detailed studies have examined the phenomenon in a generalist predator such as *T. sirtalis*, which may vary from data collected from specialist predators (e.g., those that only eat endothermic prey).

Part 5 of this dissertation focuses on variation in some reproductive life-history traits and population demographics of the populations from the Beaver Archipelago and surrounding mainland and the mechanisms that may be causing and maintaining such variation. Insular populations often differ in size and reproductive life-history traits from mainland populations of the same species (e.g., Andrews, 1979; Andren and Nilson, 1983; Schwaner, 1985; Dobson and Murie, 1987; Ford and Seigel, 1989; Hasegawa, 1990; Lister and Aguayo, 1992; Madsen and Shine, 1993), and a number of studies have indicated that this variation, which is thought to depend largely on phenotypically plastic responses to local environmental conditions, is a result of proximate environmental factors (e.g., temperature, prey availability). *Thamnophis sirtalis* is known to vary in clutch size, offspring size and mass, and reproductive frequency throughout its range, and at least variation in clutch size is known to result from changes in prey availability (Rossman et al., 1996). Therefore, most of the variation exhibited by different populations of *T. sirtalis* may be the result of phenotypically plastic responses to local environmental conditions; however, the causes of such variation have not been fully examined.

Finally, Part 6 summarizes and integrates Parts 2-5 and closes with future directions and further implications.

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APPENDIX I

TABLES AND FIGURES

Table 1.1. Potential predators of common gartersnakes, *Thamnophis sirtalis*, from the lower peninsula (LP), upper peninsula (UP), and Beaver Island (BI), Garden Island (GI), High Island (HI), Hog Island (HgI), Squaw Island (SI), Trout Island (TI), and Whiskey Island (WI) of the Beaver Archipelago of Michigan, USA. An **X** indicates the presence of a predator (Bowen and Gillingham, 2002a, 2002b; Hatt et al., 1948; Harding, 1996; Phillips et al., 1965; Placyk and Gillingham, 2002; Placyk and Burghardt, 2005). Raptors and other large birds are found at all locations.

Predator	LP	UP	BI	GI	HI	HgI	SI	TI	WI
Large fish	**X**	**X**	**X**						
Bullfrog (*Rana catesbeiana*)	**X**	**X**	**X**						
Snapping turtle (*Chelydra serpentina*)	**X**	**X**	**X**	**X**					
Massasauga (*Sistrurus catenatus*)	**X**								
Blue racer (*Coluber constrictor foxii*)	**X**								
Milksnake (*Lampropeltis triangulum*)	**X**		**X**	**X**	**X**				**X**
Raccon (*Procyon lotor*)	**X**	**X**	**X**						
Red fox (*Vulpes fulva*)	**X**	**X**	**X**				**X**		**X**
Bobcat (*Lynx rufus*)	**X**	**X**	**X**						
Bear (*Ursus americanus*)	**X**	**X**							
Coyote (*Canis latrans*)	**X**	**X**	**X**	**X**		**X**			
Gray wolf (*Canis lupus*)	**X**	**X**							
House cat (*Felis catus*)	**X**	**X**	**X**						
Domestic dog (*Canis domesticus*)	**X**	**X**	**X**						
Human (*Homo s. sapiens*)	**X**	**X**	**X**						

Table 1.2. Prey available to common gartersnakes, *Thamnophis sirtalis*, from the lower peninsula (LP), upper peninsula (UP), and Beaver Island (BI), Garden Island (GI), High Island (HI), Hog Island (HgI), Squaw Island (SI), Trout Island (TI), and Whiskey Island (WI) of the Beaver Archipelago of Michigan, USA. Earthworms, American toads (*Bufo americanus*), and eastern red-backed salamanders (*Plethodon cinereus*) occur in both the LP and UP, as well as on all/most of the islands. An **X** indicates the presence of a prey item (Hatt et al., 1948; Phillips et al., 1965; Harding, 1996; Bowen and Gillingham, 2002a, 2002b; Placyk and Gillingham, 2002; Placyk et al., 2002; Placyk, 2006).

Prey	LP	UP	BI	GI	HI	HgI	SI	TI	WI
Small pond fish (e.g., Cyprinidae)	**X**	**X**	**X**		**X**				
Green frog (*Rana clamitans*)	**X**	**X**	**X**	**X**	**X**				
Mink frog (*Rana septentrionalis*)		**X**							
Leopard frog (*Rana pipiens*)	**X**	**X**	**X**						
Bullfrog (*Rana catesbeiana*)	**X**	**X**	**X**						
Gray treefrog (*Hyla versicolor*)	**X**	**X**	**X**					**X**	
Spring peeper (*Pseudacris crucifer*)	**X**	**X**	**X**					**X**	
Eastern newt (*Notophthalmus viridescens*)	**X**	**X**	**X**	**X**	**X**				

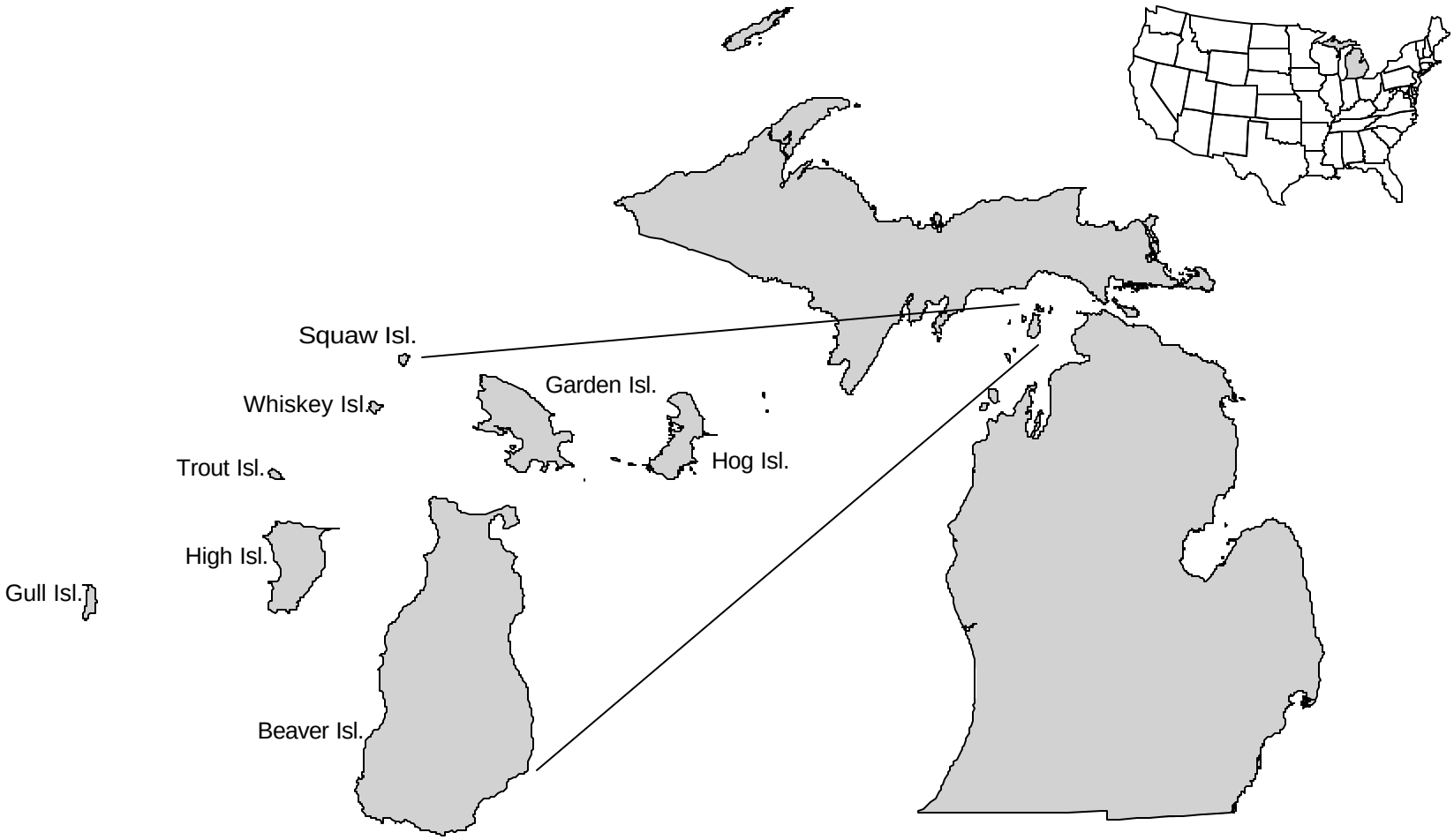

Fig. 1.1. The Beaver Archipelago of Lake Michigan located in Charlevoix County, Michigan, USA (modified from Hansknecht, 2003).

Part II

POST-GLACIAL RECOLONIZATION OF MICHIGAN BY THE COMMON GARTERSNAKE, *THAMNOPHIS SIRTALIS*, INFERRED FROM MTDNA SEQUENCES

INTRODUCTION

Thamnophis sirtalis (Colubridae) is the widest ranging reptile in North America (Rossman et al., 1996) due, in part, to its generalist nature. Populations of *T. sirtalis* are known from nearly every natural habitat across its range, except for deserts, as well as from many urban areas. Within those habitats *T. sirtalis* preys on earthworms, leeches, fish, amphibians, small mammals and birds, and even roadkill (Harding, 1996; Rossman et al., 1996). *Thamnophis sirtalis* is arguably the most researched snake in the world, being used for short- and long- term studies on behavior, physiology, ecology, and evolution for more than fifty years (e.g., Carpenter, 1952; Fitch, 1965; Burghardt, 1969; Seigel et al., 1987; Seigel and Collins, 1993; Rossman et al., 1996; de Queiroz et al., 2002; Janzen et al., 2002). It is also the only snake species to be nominated to have its whole genome sequenced. While phylogeographic work has been performed with *T. sirtalis* subspecies from the western USA (Janzen et al., 2002) and with some Canadian populations (Rye, 2000), virtually nothing is known about the phylogeography of *T. sirtalis* distributed east of the Rocky Mountains in the United States or for the genus in general (Alfaro and Arnold, 2001). Given the research popularity of this species, it seems timely to examine the phylogeography of *T. sirtalis* in the more central and eastern parts of its vast range. I chose to examine the phylogeography of Great Lakes region populations of *T. sirtalis* with an emphasis on the recolonization of Michigan and the Beaver Archipelago of northeastern Lake Michigan following the last glaciation of the Pleistocene.

During the Pleistocene, climatic changes associated with glacial advances and retreats resulted in range reductions for a variety of taxa worldwide and most likely influenced the current genetic composition and diversification of those taxa (Hewitt, 1996, 2000, 2001). The Great Lakes region of North America, specifically, was marked by dramatic climate change, fluctuating lake levels, and isostatic rebound resulting from a series of glacial and interglacial oscillations (Petty et al., 1996). As recent as 18,000 years before present (ybp), the Wisconsinan glacier covered the majority of the area occupied by the Midwest region of the United States of America, not fully retreating until ca. 10,000 ybp (Larsen, 1987; Anderson and Lewis, 1992; Webb et al., 1993). These events had a dramatic effect on the distribution of taxa in this region, with the current inhabitants recolonizing only relatively recently after the retreat of the glaciers (e.g., Holman, 1992, 1998). Without taking into account these historical events, the current distributions and underlying genetics of taxa in this region and other areas influenced by the events of the Pleistocene cannot be fully understood. To date, most studies on the phylogeographic impact of Pleistocene events on North American taxa have been conducted on southern or western continental species, with few such studies focusing on taxa that have recolonized previously glaciated regions (Bernatchez and Dodson, 1991; Billington et al., 1992; Green et al., 1996; Hewitt, 1999, 2000, 2001; Austin et al., 2002; Janzen et al., 2002; Zamudio and Savage, 2003; Ayoub and Reichert, 2004; Fuerst and Austin, 2004). Therefore, my study not only adds to our knowledge of *T. sirtalis* ecology and evolution, but also provides us with one of few phylogeographic studies on a member of the Midwest fauna.

A hypothetical pattern of colonization in which primary amphibian and reptilian invaders closely followed the non-uniform retreat of the ice sheet into the areas surrounding the Great Lakes has been proposed based on geological data, paleobotanical and paleovertebrate assemblages, and ecological tolerances of modern reptilian and amphibian fauna (Holman, 1992). The ice sheet extended well into Illinois, Indiana, Ohio, and Wisconsin, and completely covered Michigan during its greatest extent (Fig. 2.1; all tables and figures for Part II are located in Appendix II). Since primary reptile and amphibian invaders are believed to have stayed close to the margins of the ice sheet, they probably never completely moved out of Illinois, Indiana, Ohio, and Wisconsin. However, Michigan populations must have either become locally extinct or retreated to more southerly states not affected by the ice sheet. As the ice sheet retreated and Michigan became exposed, populations are believed to have entered the lower peninsula of Michigan from Indiana and Ohio, and the upper peninsula of Michigan from Wisconsin. Populations are also believed to have colonized southern Ontario, Canada through the gap between Lake Erie and Lake Huron via Michigan. In addition to the hypothesized patterns of mainland recolonization, hypotheses regarding the recolonization of the archipelagos found in each lake have also been generated and vary from island system to island system. For this study, I not only sampled from mainland sites throughout the Great Lakes region, but also from an archipelago in Lake Michigan that may provide further support for the two-front Michigan recolonization hypothesis.

Great Lakes island age and mode of origin are variable, ranging from 2,000 – 10,000 years and including both land-bridge islands once part of the mainland and

'oceanic' islands that emerged as lake levels declined (Karrow and Calkin, 1985; Nuhfer and Dalles, 1987; Petty et al., 1996). For this work I focused on the Beaver Archipelago, located in northeastern Lake Michigan about 25-30 km from both the lower and upper peninsula of Michigan. As it is the largest archipelago in Lake Michigan and located in an area that may act as a secondary contact point for upper and lower peninsula *T. sirtalis* lineages, the origin of the fauna of the archipelago has been contentious. The archipelago consists of one main island (Beaver, 15130.0 hectares (ha)), three moderately large islands (Garden, High, and Hog, 1023.9 to 1989.0 ha), and six smaller islands (Hat, Pismire, Shoe, Squaw, Trout, and Whiskey, 1.0 to 52.3 ha). All but the smallest islands (i.e., Hat, Pismire, and Shoe) are thought to support, or historically to have supported, populations of *T. sirtalis* (Hatt et al., 1948; Placyk and Gillingham, 2002). It has been hypothesized that since the archipelago was at one time connected by a land bridge to the lower peninsula of Michigan (Hough, 1958; Kapp et al., 1969; Dietrich, 1988), the taxa found on the islands are descendents of lower peninsula populations. Further support for this view is that some species found in the Beaver Archipelago are rare (eastern milksnake, *Lampropeltis t. triangulum*, Colubridae) or absent (northern ribbonsnake, *T. sauritus septentrionalis*) from the upper peninsula of Michigan but common in the lower peninsula. However, many reptiles and amphibians, including gartersnakes, are adept swimmers and may have been able to colonize the archipelago at any point (as indicated by their presence on oceanic islands in the upper Great Lakes, King, 1988). In fact in the last 10,000 years the archipelago was separated from Michigan's upper peninsula by the Mackinac River, which may have only been 1.6 km wide in some areas (Hough, 1958). By sampling these islands, I hope to shed light on the evolutionary history of Beaver

Archipelago *T. sirtalis* populations and more fully understand the phylogeography of *T. sirtalis* in the Great Lakes region, especially in Michigan.

Thamnophis sirtalis is a key species for the examination of phylogeographic patterns in the Great Lakes region for several reasons. First, evidence from fossil deposits of ca. 15,000-14,000 ybp indicate that *T. sirtalis* was present in the Midwest during the last glaciation and that it stayed close to the margins of the retreating glacier (Holman, 2000). Holman (1992) considers it to be one of the primary vertebrate invaders of the postglacial Midwest. Second, *T. sirtalis* is currently considered the most common reptile species in the region, occurring in almost every county and on every Great Lakes archipelago (Harding, 1996) making the collection of specimens and tissue samples for this study relatively simple. Finally, molecular resources (e.g., mitochondrial DNA (mtDNA) and microsatellite primers) have been developed for this species and are readily available (e.g., Kumazawa et al., 1996, McCracken et al., 1999; Garner et al., 2002, 2004; Garner and Larsen, 2005).

While the hypotheses on the postglacial recolonization of the Great Lakes region discussed above are based on fossil and other geological data, they have not yet been tested with any other type of data. I used phylogeographic and population genetic analysis of mtDNA sequence data to test hypotheses regarding the recolonization of the Great Lakes region and specifically of Michigan and the Beaver Archipelago following the last glaciation. Michigan serves as the focal point for this work, because this study is part of a broader project that is examining geographic variation in the phenotypic traits of

Beaver Archipelago *T. sirtalis* populations and populations from the surrounding mainland. Using mtDNA sequences to reconstruct phylogenetic relationships among *T. sirtalis* from the Great Lakes region, I address several important evolutionary issues to help understand recolonization following the last glaciation. In particular, I was interested in (I) how populations are genetically structured within the Great Lakes region, especially in Michigan, and (II) if this structuring and the pattern of haplotypic variation are consistent with historical geography. In addition, this work will provide the first such study on a reptile from this region, as well as much needed data on recolonization of previously glaciated areas in the Great Lakes region in general.

MATERIALS AND METHODS

Taxon sampling—Mitochondrial haplotypic variation was examined in 148 individuals of *T. sirtalis* from 36 locations (Fig. 2.1, Table 2.1). Sampling was concentrated in sites surrounding and within Lake Michigan to examine fine scale patterns of haplotypic variation with additional sites examined from across much of the eastern Midwest states (i.e., Illinois, Indiana, Michigan, Ohio, and Wisconsin), Pennsylvania, and Ontario, Canada to begin to resolve larger geographic patterns of haplotype radiation following the Wisconsinan glaciation. I also heavily focused sampling efforts in the Beaver Archipelago of northeastern Lake Michigan as the evolutionary history of taxa on these islands is a point of some controversy (Hatt et al., 1948) and it may act as a point of secondary contact for two lineages. Samples were obtained from numerous sources as frozen muscle tissue or tail tips from live specimens

subsequently released at the point of capture, or previously extracted samples (see Table 2.1 for sources).

DNA extraction and sequencing—For most individuals, I used tail tips to obtain total genomic DNA with the DNeasy® Tissue Kit (Qiagen). The entire ~1020 bases of ND2 were PCR-amplified using the forward primer L4437b (5'-CAG CTA AAA AAG CTA TCG GGC CCA TAC C-3'; Kumazawa et al., 1996), which lies in the tRNA-Met upstream of ND2, and the reverse primer Sn-ND2r (5'-GGC TTT GAA GGC TMC TAG TTT-3'; R. Lawson, pers. comm.), which lies in the tRNA-Trp downstream of ND2. Polymerase chain reactions (PCR) were conducted in 25-µL volumes with 1.0 µL DNA, 1X ExTaq PCR buffer (PanVera / TaKaRa), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µg/µL bovine serum albumin, 0.1 mM each primer, and 1.25 units of ExTaq polymerase (Panvera / TaKaRa). Amplification conditions involved 30 cycles each consisting of 1 min of denaturing at 94ºC, 1 min of primer annealing at 55ºC, and 1.5 min of extension at 72ºC. A negative control was included for all PCR reactions. PCR products were purified prior to sequencing using ExoSAP-IT™ (USB Corporation).

Sequencing reactions were carried out using the internal primers H5382 (5'-GTG TGG GCR ATT CAT GA-3') and L5238 (5'-ACM TGA CAA AAA ATY GC-3') (de Queiroz et al., 2002) and Big Dye® Terminator v3.1 Cycle Sequencing kits (Applied Biosystems), and read on an automated sequencer (Applied Biosystems 3100, University of Tennessee Molecular Biology Resource Facility). Sequences were edited using the program Sequencher 3.1.1 (Gene Codes Corporation, Ann Arbor, MI). Alignments were

performed initially using Clustal X (Thompson et al., 1997) and subsequently manually refined. In addition to the ND2 sequences generated for this study, 36 sequences were obtained from GenBank (Table 2.1).

Data analyses—Network analysis to estimate a gene genealogy was carried out using TCS 1.13 (Clement et al., 2000) which implements the Templeton et al. (1992) statistical parsimony procedure. The haplotype network was then converted into a series of nested clades (Templeton, 1998) for interpretation and for use in statistical analyses of geographic vs. genetic variation (see below). Ambiguous connections (loops or reticulations) in the haplotype network were resolved using approaches from coalescent theory (see Crandall, 1994). In the case of DNA sequence data, this resolution generally involves a comparison of the probabilities of whether a haplotype arose via mutation from either a high- or low- frequency haplotype. Coalescent theory would suggest that, based on these probabilities, the new haplotype arose from the higher-frequency haplotype.

Statistical analyses of geographic/genealogical associations were conducted using GeoDis ver. 2.0 (Posada et al., 2000). All statistical analyses in GeoDis were performed using 10,000 (Monte Carlo) replications. Results obtained from GeoDis were then interpreted using the revised inference key of Templeton (1998, 2004; revised version dated 14 July 2004 available at http://darwin.uvigo.es/download/geodisKey_14Jul04.pdf).

The population genetic structure elucidated by the network analysis was further assessed and supported by performing analysis of molecular variance (AMOVA). Φ statistics (analogous to the F-statistics of Wright, 1965), were calculated using ARLEQUIN ver. 2.000 (Excoffier et al., 1992; Schneider et al., 2000). Two sets of analyses were performed by grouping individuals. These groups represent the highest level of genetic apportionment. The first analysis grouped individuals into clades based on the nested clade analysis (see below), while the second analysis divided individuals into 7 geographic groups (Ohio, Indiana, Illinois, Wisconsin, lower peninsula of Michigan, upper peninsula of Michigan, and the Beaver Archipelago of Lake Michigan). Michigan was split into three separate geographic groups, as none of the three are currently connected to each other via any landmass. The group apportionment of variation with respect to all haplotypes is described by Φ_{CT}, while Φ_{SC} describes the apportionment within populations of the defined groups, and Φ_{ST} refers to the variation in a single population relative to all haplotypes. The AMOVA assumes that groupings represent populations and that the populations are in drift-migration equilibrium, conditions that may be unrealistic for combined population groupings. Levels of significance of the Φ statistics were determined through 1000 permutation replicates.

In addition to employing a gene genealogy approach, I performed phylogenetic analyses of the ND2 data under the criterion of maximum likelihood (ML) as implemented in PAUP* (Swofford, 2002). Gene genealogical approaches are usually used for intraspecific analyses because they allow ancestral haplotypes to exist at internal nodes, which more accurately reflects haplotype relationships. Genealogical approaches

such as statistical parsimony implemented in TCS (Clement et al., 2000), however, may be unable to connect all sequences into a single network if divergence between sequences is large. Therefore, I used ML analysis to complement the genealogical analysis, assess relationships among major clades, and root the tree. For the ML analysis, I used the hierarchical likelihood ratio test from Modeltest (Posada and Crandall, 1998) to determine the appropriate ML model for the analysis. The model I used was the HKY + G. ML analyses were conducted with 100 random sequence addition heuristic search replicates with tree-bisection-reconnection (TBR) branch swapping. Bootstrap analysis was employed to assess internal support for the inferred phylogeny using 100 bootstrap replicates with simple taxon addition heuristic searches and TBR branch swapping.

In addition to the sequences produced for this study I included in the ML analysis sequences of *T. sirtalis* from western N. America reported by Alfaro and Arnold (2001) (n = 1), de Queiroz et al. (2002) (n = 1), and Janzen et al. (2002) (n = 32) to assess the relationship among Midwest/eastern and western N. American *T. sirtalis* populations. To root the ML tree I included sequences of *T. elegans* (Janzen et al., 2002; GenBank Accession No. AY136238), *T. proximus* (Alfaro and Arnold, 2001; de Queiroz et al., 2002; GenBank Accession No. AF383847, AF420163) and *T. sauritus* (de Queiroz et al., 2002; GenBank no. AF420179). These taxa were chosen based on broad-scale phylogenetic analyses of *Thamnophis* phylogeny (Alfaro and Arnold, 2001; de Queiroz et al., 2002) that show *T. proximus* and *T. sauritus* as sister to *T. sirtalis*, with *T. elegans* representing a more distant outgroup.

RESULTS

Sequence variation—I obtained complete ND2 sequences for 148 *T. sirtalis* individuals from 37 populations. 37 unique haplotypes were detected in these 148 sequences. Of the 1101 bp used in analyses, 72 were variable, and 56 were phylogenetically informative. With up to 6.5 % divergence between sequences, the populations used in this study express more intraspecific mtDNA sequence divergence than many other species of gartersnake (2.5 % in the Mexican gartersnake (*T. eques*), 5.5 % in the terrestrial gartersnake (*T. elegans*); de Queiroz and Lawson, 1994) including previous estimates for *T. sirtalis* (2.5 %; Janzen et al., 2002). No stop codons or indels were detected in any of the sequences, indicating the functionality of the ND2 gene. Using the program DnaSP (Rozas and Rozas, 1999), I found that 19 of the 72 variable sites represented nonsynonymous substitutions, which may be under selection (Zink, 2005), and thus may confound the geneaological and phylogenetic analyses. However, only 9 of these were phylogenetically informative sites (of 56 total phylogenetically informative sites) and of those only 2 were distributed in populations that were geographically isolated from each other. Removing these 2 sites from the analyses resulted in no difference in the structure of either the nested cladogram or the maximum likelihood tree; therefore, they were kept in the data set for all analyses, as it is possible that they resulted from geographical isolation rather than selection (Zink, 2005). If selection is acting on these sites, it appears to be negligibly influencing the phylogeographic hypotheses.

Nested clade analyses—Gene genealogy reconstruction resulted in two independent networks that could not be connected with 95% confidence levels by the statistical parsimony approach implemented in TCS (Templeton et al., 1987). The smaller network, which includes 6 haplotypes from localities in Ohio, Illinois, Pennsylvania, southern Michigan, and Ontario, Canada, is far removed from the remaining network (over 25 mutational steps) and mostly includes a small number of sequences from poorly sampled locations from the outskirts of the area of interest for this study. As a result, it and the haplotypes within it were not used in the statistical analyses of geographic/genealogical associations or the AMOVA analyses. These haplotypes are, however, utilized in the maximum likelihood analysis to help understand the more broad scale implications of my data. The larger network includes 30 haplotypes from localities throughout the entire sampling range, except Ontario. Each of the 37 haplotypes from both networks were assigned a number, 1 to 37, and the localities they represent are detailed in the Table 2.1. The nested cladogram representing the second network (Fig. 2.2), as well as the maximum likelihood tree also utilize these numbers (Fig. 2.3).

Nesting procedures resulted in three levels of nested clades (Fig. 2.2). At the highest level of nesting, three clades were recognized (3.1, 3.2, 3.3). The first and largest (3.1, 15 haplotypes, 87 individuals with 38 from the Beaver Archipelago) is split into three main clades (2.1, 2.2, 2.3). Clade 2.1 is the most restricted, including only individuals from the Beaver Archipelago; clade 2.2 is comprised of individuals from Wisconsin and the Beaver Archipelago, and clade 2.3, which is basal to clades 2.1 and 2.2, is the most widespread including individuals from the upper peninsula of Michigan,

Wisconsin, the Apostle Islands of Lake Superior, Indiana, Illinois, and Ohio. The second largest level 3 clade (3.2, 10 haplotypes, 28 individuals with 9 from the Beaver Archipelago) is also split into three smaller clades (2.4, 2.5, 2.6). Clade 2.6 is the most restricted, represented by only one individual from Indiana, clade 2.5 includes individuals from the Beaver Archipelago and the lower peninsula of Michigan, and clade 2.4, which is basal to clades 2.5 and 2.6, is the most widespread with individuals from Indiana, the Beaver Archipelago, and the lower peninsula of Michigan. The third and smallest level 3 clade (3.3, 5 haplotypes, 5 individuals) is further broken down into two main clades (2.7, 2.8) both of which include sequences from Illinois individuals only.

The haplotype network illustrates that tip clades (n = 20) are more widespread geographically than ancestral (internal) haplotypes (n = 11), an expectation of range expansion, and this is supported by the nested distance analysis of the dispersion of the clades. The analysis suggests that four nested groups in the overall nested cladogram show geographic distributions that are significantly different from random expectations (Table 2.2). I infer from these patterns of genetic variation that past fragmentation, followed by both contiguous range expansion and long distance colonization has occurred throughout the Midwest (Table 2.3). In addition, patterns of isolation by distance, suggesting reduced gene flow, occur between the Beaver Archipelago and mainland populations (Table 2.3). In particular, clade 1.5 (with individuals from the upper peninsula of Michigan, Wisconsin, and the Apostle Islands) and clade 2.4 (with individuals from Indiana, the Beaver Archipelago, and the lower peninsula of Michigan) both exhibit patterns of genetic differentiation that indicate past fragmentation followed

by range expansion. Clade 2.3 (upper peninsula of Michigan, Wisconsin, Apostle Islands, Indiana, Illinois, Ohio) shows evidence of long-distance colonization. Genetic variation in clade 3.1, which includes the majority of sequences from the Beaver Archipelago populations (as well as Illinois, Wisconsin, Indiana, and Ohio), is characterized by restricted gene flow with isolation by distance.

Analyses of molecular variance—The AMOVA conducted with the nested clade clusters revealed significant genetic structuring across all hierarchical levels (all $P < 0.0001$). Overall, 70.53 % of the variation was a result of differences among the three clusters (i.e., clades 3.1-3.3) ($\Phi_{CT} = 0.71$). 13.51 % of the total variation resulted from differences among populations within these groups ($\Phi_{SC} = 0.46$) and 15.95 % was due to variation within populations ($\Phi_{ST} = 0.84$). When individuals were grouped into the seven geographic regions defined in the methods, significant genetic structuring existed across all hierarchical levels (all $P < 0.05$), but only 16.23 % of the variation was a result of differences among the groupings ($\Phi_{CT} = 0.16$). The remainder of the total variation resulted from differences among populations of these groups (42.81 %, $\Phi_{SC} = 0.51$) and within populations (40.95 %, $\Phi_{ST} = 0.59$).

Phylogenetic analyses—To reduce the computational time, maximum likelihood analysis was performed on the 37 unique haplotypes rather than on all 148 individual sequences (Fig. 2.3). In addition, as noted above, all sequences used by Janzen et al. (2002) were also included in this analysis, as well as 1 sequence each from Alfaro and Arnold (2001) and de Quieroz et al. (2002). Note that the very short branch lengths of

the maximum likelihood tree (Fig. 2.3) indicate very little divergence between most haplotypes. In addition, while the results of the phylogenetic analysis relating to the sequences are topologically concordant with the network analysis, important differences are highlighted in interpretation between phylogenetic and genealogical approaches. Rather than forming distinct clades (as with the network analysis), the haplotypes form two grades. The first grade represents the more distantly related network of haplotypes that was detected in the nested analysis but that was not used in the AMOVA analysis or the statistical analyses of geographic/genealogical associations. The second grade represents the larger network (Fig. 2.2) that includes the remainder of the sequences. This grade also includes all of the western *T. sirtalis* sequences from previous studies (Alfaro and Arnold, 2001; de Queiroz et al., 2002; Janzen et al., 2002) and indicates that the haplotypes found in the larger network are actually more closely related to the western haplotypes than they are to haplotypes found in the smaller network. Thus, nested clade analysis defines clades based on dissection of existing and inferred haplotypic variation into groups for further analysis of geographic vs. genetic variation without respect to monophyly vs. paraphyly. This allows observed haplotypes to occupy internal nodes (i.e., ancestral haplotypes). Traditional phylogenetic analysis, on the other hand, infers relationships under the assumption that all haplotypes occupy terminal positions, and does not allow existing haplotypes to occupy internal node positions.

The phylogeny inferred from maximum likelihood analysis (Fig. 2.3) depicts an initial divergence in the sample between two well-supported haplotype groups corresponding to a more eastern grade vs. clades 3.1, 3.2, and 3.3 of the network analysis

(Fig. 2.2). Geographically this indicates a primary divergence between a primarily eastern/southern clade (Ontario, Pennsylvania, Ohio, lower peninsula of Michigan, and one Illinois population) vs. a primarily northern/western clade (Illinois, Indiana, Wisconsin, Michigan lower and upper peninsula, and Beaver and Apostle Islands). Within this latter group, one of the clades delimited by nested clade analysis is monophyletic (3.1), one is unresolved, but its monophyly is not directly contradicted (3.3), and one is paraphyletic to the other two (3.2). Geographically, the paraphyletic grade of 3.2 includes individuals from the north-central portion of the range (northern Indiana, lower peninsula of Michigan, and the Beaver Islands) with the southernmost population (Indiana, haplotype 13) occupying the basalmost position. Clade 3.3 consists entirely of individuals from Illinois, primarily from the southwestern portion of the state. Finally, clade 3.1 is resolved as monophyletic group although not with strong bootstrap support (< 50 %). Geographically this clade is principally confined to the western portion of the sample range with individuals from Illinois, Wisconsin, the upper peninsula of Michigan, the Beaver and Apostle Islands. One population from northwestern Ohio (OH2) is also included in this clade. In addition, two haplotypes from this more western distributed group appear to be the sister group for all of the western haplotypes. The distribution of the western haplotypes is concordant with the findings of Janzen et al. (2002) with only very minor differences (e.g., a sequence from an Oregon *T. sirtalis*, AY136317, is more closely related to several California *T. sirtalis* sequences than to other Oregon sequences, as originally indicated by Janzen et al. (2002)). The sequence from the Alfaro and Arnold (2001) study (AF383838), which represents a Humboldt County, California *T. sirtalis*, falls out with the Janzen et al. (2002) Humboldt

County *T. sirtalis* sequence (AY136213). The sequence produced for the de Queiroz et al. (2002) study (AF420195) was from Santa Clara County, California, which was not sampled by Janzen et al. (2002); however, it is most similar to *T. sirtalis* sequences from Sonoma County, California, which is in the same vicinity as Santa Clara County.

DISCUSSION

General phylogeographic patterns—The goal of this study was to reconstruct the phylogeographic patterns and to evaluate the role of Pleistocene glaciations in the Great Lakes region of North America on the evolutionary history of the common gartersnake, *T. sirtalis*, especially in Michigan. The data collected contribute to a growing body of evidence suggesting that Pleistocene events played an important role in the differentiation of North American vertebrate populations (e.g., Hays and Harrison, 1992; Byun et al., 1997; Klicka and Zink, 1997; Arbogast, 1999; Burbrink et al., 2000; Conroy and Cook, 2000; Demboski and Cook, 2001; Austin et al., 2002; Brant and Orti, 2003; Zamudio and Savage, 2003). The majority of the results presented herein are in agreement with earlier hypotheses related to the recolonization of the Great Lakes region by reptiles and amphibians, but at least one earlier hypothesis is rejected, with an alternate hypothesis suggested, and detailed data on patterns of recolonization not available in the past are also provided.

Both the nested clade analysis and a standard phylogenetic analysis resulted in similar tree topologies, with strong support for two distinct lineages. Nested clade analysis also suggests that one of these lineages is split into three main clades, some of

which are supported by the phylogenetic analysis. AMOVA results strongly support the genetic distinctiveness of these three clades, which better explain the genetic variation displayed by the data than grouping snakes into their respective states. From these analyses, two important patterns emerge. First, Great Lakes populations recolonized areas that were covered by the glacier via two routes: 1) from Indiana along the eastern coast of Lake Michigan into the lower peninsula of Michigan, and 2) from Illinois and Indiana into Wisconsin and the upper peninsula of Michigan along the western coast of Lake Michigan. Second, the Beaver Archipelago in northeastern Lake Michigan is an area of secondary contact for the two lineages that diverged around either side of Lake Michigan. In addition, although not explicitly examined here, there is some evidence that the more southern and eastern states I sampled may have acted as refugia for *T. sirtalis* during the glaciation and that Ontario, Canada was recolonized by Ohio and Michigan populations, presumably via the gap between Lake Huron and Lake Erie. More sampling along the east coast and states south of Illinois, Indiana, and Ohio are needed to more fully support such hypotheses.

In general, however, the data support hypotheses concerning the recolonization of the Great Lakes region following the Wisconsinan glaciation (Fig. 2.4), especially the recolonization of Michigan. Primary reptile and amphibian invaders of the region are believed to have stayed close to the margins of the retreating ice sheet and quickly recolonized the region via two routes located to the east and west of Lake Michigan. The data agree with past hypotheses that the upper peninsula of Michigan was colonized via Wisconsin and a route west of Lake Michigan, while the lower peninsula of Michigan

was recolonized by animals from Indiana and Ohio. In addition, I found an enigmatic group of haplotypes that occur only in Illinois populations. Suggestions for the origin of this group are discussed below. I rejected a long-standing hypothesis that the Beaver Archipelago in northeastern Lake Michigan was recolonized solely by lower peninsula populations. The data indicate that the Beaver Archipelago acts as a secondary contact point between the east and west coast Lake Michigan lineages, although whether the contact was natural dispersion or human assisted remains unknown. Interestingly, 81 % of the Beaver Archipelago snakes I sampled are found in the western group of haplotypes. Most hypotheses regarding the recolonization of the Beaver Archipelago suggest that island populations most likely originated from the lower peninsula of Michigan. If any animals colonized from the west or from the upper peninsula of Michigan, their contribution to the genetic make-up of the island populations was believed to be minimal at best. The results suggest the opposite. The Beaver Archipelago is also characterized by seven unique haplotypes not found on the mainland (haplotypes 8, 9, 14, 15, 34, 35, and 36) indicating that genetic divergence and evolution may be ongoing on these islands. The two areas that colonized the islands (i.e., the LP and the UP) are also characterized by unique haplotypes, but not as many (2 for the LP and 3 for the UP).

In addition to testing hypotheses regarding the paths animals took when recolonizing the Great Lakes region, the data also allow me to infer specific patterns of recolonization, as one of the advantages of using nested clade analysis is to discriminate statistically among various biological explanations for significant associations between

geography and genetic data (Templeton et al., 1987). Knowles and Maddison (2002) have criticized the methods used by Templeton et al. (1987) to examine such associations, citing the frequent failure of nested clade analysis to recover the true history of simulated data sets. In response, Templeton (2004) modified the nested clade analysis inference tree, resulting in improved performance with simulated data. Further validation comes from analysis of 42 molecular data sets for which strong *a prior* phylogeographic expectations could be inferred (Templeton, 2004).

There are three major biological factors that may cause spatial or temporal associations of haplotype variation: 1) restricted gene flow, 2) past fragmentation, and 3) range expansion (Templeton et al., 1995). My analyses indicate that all three of these factors are or have been at work among my populations, but that recurrent gene flow is also common, which may explain the short branch lengths of the maximum likelihood tree. Restricted gene flow (due to isolation by distance) led to the nonrandom geographic/genealogical associations between the Beaver Archipelago and the surrounding mainland, especially Wisconsin and the upper peninsula of Michigan. Given that the islands are currently separated from the mainland by ca. 25-30 km of water, restricted gene flow between the populations found on these islands and the mainland are to be expected. Past fragmentation followed by range expansion is seen throughout the data set.

Phylogeography of Great Lakes region taxa—In general, the data support past hypotheses regarding recolonization of the Great Lakes region following the retreat of the

Wisconsinan glacier, and provide the most extensive sampling of any taxon in this region. However, others have sampled within this region on a smaller scale. Rye (2000) provides evidence from Canadian gartersnake population allozyme and mtDNA cytochrome *b* data that support the hypothesis for multiple fronts of post-Pleistocene colonization within the Great Lakes region. Samples from an east-west transect reveal two genetic lineages that meet north of Lake Superior. A western lineage occurs in western Ontario and Manitoba and an eastern lineage occurs in central and eastern Ontario. The location of contact between lineages is consistent with two fronts of colonization diverging around the Great Lakes and meeting secondarily. Zamudio and Savage (2003) found that spotted salamander (*Ambystoma maculatum*, Ambystomatidae) haplotypes from individuals collected in Indiana were basal to two separate Midwest lineages. One of these lineages extends up the west coast of Lake Michigan into the upper peninsula of Michigan, while the other extends from the southernmost counties of the lower peninsula of Michigan and gave rise to southern Ontario, Canada populations. While these results mirror mine, salamander populations in the central and northern portions of the lower peninsula of Michigan, the Beaver Archipelago, and Ohio were not sampled. Therefore, the complete evolutionary history of Ohio and Michigan populations, and the origin of the Beaver Archipelago populations, were not elucidated. Analysis of mtDNA from the spring peeper, *Pseudacris crucifer* (Anura: Hylidae), also suggests multiple fronts of colonization within the Great Lakes region (Austin et al., 2002). Different lineages occur east and west of Lake Michigan, consistent with the hypothesis that the lake has functioned as a barrier to gene flow. However, routes of colonization differ from those hypothesized here. The southern Great Lakes region was

apparently colonized from unglaciated areas to the south whereas the northern Great Lakes region was colonized by a lineage originating east of the Appalachian Mountains that expanded counterclockwise around the Great Lakes into Minnesota and northern Wisconsin (Fig. 6 in Austin et al., 2002). During the Xerothermic period (4-6,000 ybp), contact between lineages was prevented by the prairie peninsula, which extended eastward across N Indiana, NW Ohio, and SW Ontario. Once this barrier disappeared, secondary contact occurred in SW Ontario. Even though Holman (1992) considers both *T. sirtalis* and *P. crucifer* primary invaders, it is possible that they recolonized the Midwest differently given that differences in behavior, ecology, and physiology exist between them. More sampling of *T. sirtalis* populations on the east coast of the United States and Canada are needed to further understand these distinct recolonization patterns. For mammals, Brant and Orti (2003) recently examined the postglacial recolonization of the eastern United States by the Northern short-tailed shrew (*Blarina brevicauda*, Soricidae), and while they did not extensively sample in the Great Lake region, they did designate a phylogroup of east-central haplotypes that includes populations from Indiana and Wisconsin, indicating a possible west coast of Lake Michigan migration route. Several other studies have been conducted in the general vicinity of the Great Lakes region, but they do not focus on the recolonization of that area specifically (e.g., Fuerst and Austin, 2004; Starkey et al., 2003) and are not as detailed as those discussed above. In addition, I have found no other studies on any taxa that have specifically examined the molecular phylogeography of Beaver Archipelago populations, so the data on this unique system cannot be compared to any other study.

The Beaver Archipelago was traditionally thought to have been colonized solely by lower peninsula of Michigan populations. This hypothesis was based on two main facts: 1) in the last 10,000 years, the islands of the Beaver Archipelago were connected to the lower peninsula of Michigan by a land bridge, and 2) at least one species currently found on the islands occurs in the lower peninsula but not in the upper peninsula of Michigan and several species that are rare in the upper peninsula of Michigan are common on the islands and the lower peninsula. However, while the islands were connected to the lower peninsula, they were only separated from the upper peninsula by the Mackinac River, which may have been only 1.6 km wide in some areas. The common gartersnake is an excellent swimmer and often can be found in logs; therefore, it could have easily crossed the river via swimming or rafting. In addition, Native Americans and European settlers often traveled from the upper peninsula to the islands carrying with them supplies that gartersnakes and other small animals may have had access to (e.g., straw bales for livestock). As a result, animals may have migrated to the islands from the upper peninsula as stowaways on human vessels at any time in the last 14,000 years (Tanner, 1986). The data indicate that the islands have been colonized by populations from both the lower and upper peninsula of Michigan and that the islands act as a secondary contact point for the east and west coast Lake Michigan lineages. Therefore, any future hypotheses regarding the origin and divergence of Beaver Archipelago taxa should take into account the underlying genetics of the populations.

The third distinct clade detected in the analyses is found only in Illinois. Based on my phylogenetic analysis this group appears to be an offshoot of the clade that is

found along the west coast of Lake Michigan; therefore, it may be a more recently derived lineage arising from that group (e.g., *T. s. semifasciatus*), or it may be a precursor to that group. In terms of the former hypothesis, the range of *T. s. semifasciatus*, the Chicago gartersnake, overlaps with that of *T. s. sirtalis*, which was the subspecies of focus for this study, in northeastern Illinois. It is possible that snakes that were identified as *T. s. sirtalis* were actually *T. s. semifasciatus*, as the two are very similar morphologically (Rossman et al., 1996). However, only one county (Cook County, IL 9 from Fig. 2.1) in this third clade is found within the known range of *T. s. semifasciatus*, which leads me to believe some other phenomenon may be responsible for the third clade; however, it may also be that the range of *T. s. semifasciatus* is expanding southward or interbreeding between the two subspecies may be producing a hybrid that is found throughout Illinois. A second more likely explanation requires further sampling of the states south and west of Illinois, as gartersnake populations from states not sampled (e.g., Iowa, Missouri, Arkansas), may be ancestral to lineages found in the Great Lakes region and my enigmatic Illinois group may be related to them. For example, Austin et al. (2002) indicated that southern Illinois spring peeper populations may have been colonized by populations from Missouri, Kansas, and Arkansas, and this may also be the case for *T. sirtalis.*

Implications for the rangewide phylogeography of Thamnophis sirtalis—By combining the ND2 sequences produced by three other studies (Alfaro and Arnold, 2001; de Queiroz et al., 2002; Janzen et al., 2002), especially those from the Janzen et al. (2002) study, I provide the most complete *T. sirtalis* phylogeny published to date. This

phylogeny reveals several important patterns in the evolutionary history of *T. sirtalis*. First, subspecies classifications are not supported by the underlying genetics of the populations sampled. While I sampled only one subspecies (*T. s. sirtalis*), Janzen sampled four and all five appear to be distinguished more based on the location they were collected from than on morphological and behavioral differences. In addition, within the subspecies I examined, more differences exist between the three clades detected by the nested analysis (clades 3.1, 3.2, & 3.3) and an eastern/southern grade than between all of the sequences from the Janzen et al. (2002) study or between clades 3.1, 3.2, and 3.3 and the western haplotypes combined.

There are some similarities between my data and the western data set that may explain the patterns we see. In particular, the maximum likelihood tree is poorly resolved and indicates low genetic divergences for both my haplotypes and the western haplotypes. Janzen et al. (2002) attribute this to mainly be the result of historical forces. Since most of the locations they sampled would have either been under ice or inhospitable to *T. sirtalis* less than 10,000 years ago, west coast *T. sirtalis* are believed to have recolonized from glacial refugia in only the last 10,000 years. While restricted to glacial refugia, the fixation of respective mtDNA variants occurred before *T. sirtalis* recolonized following the glaciation. The bulk of my sampling locations were also not populated by *T. sirtalis* until relatively recently (i.e., in the last 10,000 years) due to the last glaciation, which indicate a similar scenario. It is possible that certain variants had a selective advantage under such conditions, which may explain the similarities we see between clades 3.1, 3.2, and 3.3, and the western haplotypes. Several nonsynonymous

substitutions were detected in among my sequences. Another possibility is that the western haplotypes and clades 3.1, 3.2, and 3.3 share a more recent common ancestor with each other than with the eastern/southern grade. The enigmatic southern Illinois clade (clade 3.3) may provide evidence of such ancestry. If both the Pacific coast and Midwest were recolonized by populations from states such as Utah, Colorado, Kansas, Missouri, Arizona, New Mexico, Oklahoma, and Arkansas this may be the case. More sampling both in those states, as well as along the east coast and southeast is needed to more fully understand, what appears to be, a complex evolutionary history. Unfortunately, modern *T. sirtalis* are currently absent or rare in Utah, Colorado, New Mexico, and Arizona due to current climatic conditions and habitat availability, so this analysis may remain incomplete.

***Conclusions*—**I have provided the most conclusive data in support of the recolonization pathways hypothesized for the vertebrates of the Great Lakes region following the Wisconsinan glaciation. I present novel data on the colonization of the Beaver Archipelago in northeastern Lake Michigan rejecting previous hypotheses in this regard. In addition, my data indicate that *T. sirtalis* populations found in the Great Lakes region and states directly to the east of them exhibit a greater level of sequence divergence (up to 6.5 %) than most species of gartersnakes studied to date. A greater level of sequence divergence (up to 7.7 %) has been detected in the terrestrial gartersnake, *Thamnophis elegans*, (Bronikowski and Arnold, 2001), but three subspecies were sampled in that study, while I intentionally focused only on the most widespread subspecies of *T. sirtalis*, *T. s. sirtalis*. However, when sampling four subspecies of *T.*

sirtalis from the west coast of North America, Janzen et al. (2002) found only up to 2.5 % sequence divergence. While anecdotal evidence on Great Lakes region gartersnake populations suggests there is not nearly the level of morphological variation as seen between populations on the west coast of the United States, preliminary behavioral and morphological data (Burghardt and Schwartz, 1999; King unpubl. data) suggest geographic differences that may mirror the clades distinguished by the nested clade analyses (i.e., snakes from the west coast of Lake Michigan display behavior and scalation patterns that differ significantly from snakes from the east coast of Lake Michigan). More detailed studies of geographic variation in the behavior and morphology of the Great Lakes region *T. sirtalis* populations are in progress and such studies conducted with other taxa are strongly encouraged (Arbogast and Kenagy, 2001).

I also note that the amount of sequence divergence detected in my study (6.5 %) exceeds the amount typically seen in gartersnakes (2.5 – 5.5 %; de Queiroz and Lawson, 1994; Janzen et al., 2002). In fact, although working with a different species, Burbrink et al. (2000) based their decision to classify two groups of ratsnakes (*Elaphe obsoleta,* Colubridae) as separate species based on only 2.83 – 4.37 % sequence divergence. The focus of my study was not to question the taxonomy of gartersnakes in North America, but given the amount of variation I detected, researchers should consider my data along with additional molecular and phenotypic data when focusing on the taxonomy of this species in the future. Given the high level of intraspecific sequence variation I detected, I strongly encourage researchers examining higher level phylogenetic relationships to sample more than one specimen/sequence per species. For example, the majority of the

species examined in the two most recent phylogenetic studies on natricine snakes (Alfaro and Arnold, 2001; de Queiroz et al., 2002) relied on sequence data from single individuals. In addition to the amount of variation I detected in *T. sirtalis*, sequence data from the plains gartersnake (*T. radix*) and Butler's gartersnake (*T. butleri*) indicate that the two share many haplotypes (Burghardt and Placyk, unpubl. data) further indicating the need to sample more than one individual per species in interspecific molecular phylogenetic studies.

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APPENDIX II

TABLES AND FIGURES

Table 2.1. Collection data for common gartersnakes, *Thamnophis sirtalis*, included in this study. For each population sampled (alphanumeric locality codes refer to those in Fig.1) I list collection locality (state, county, and approximate coordinates), sample sizes (n), the unique mtDNA haplotype numbers present, the nested clade analysis level 3 grouping (see Fig. 2; all those marked NCA for this category are found in the eastern grade, see Fig. 3), and the source of the specimen. Tissue sample providers included John S. Placyk, Jr. (JP), Gordon M. Burghardt (GMB), Richard B. King (RK), Gary S. Casper (GC), Jace W. Robinson (JR), Ronald K. Gratz (RG), Briar J. Howes (BH), Erik R. Wild (ER), Alan Resetar (AR), Paul T. Andreadis (PA), John Marshall (JM), Scott R. Ballard (SB), and Harlan D. Walley (HW). One sequence was acquired from GenBank (GB).

Locality	State/province	County	Coordinates	n	Haplotype no.	NCA group	Source
BA (Beaver Island 1)	Michigan (MI)	Charlevoix	46°03'N, 85º58'W	10	7, 9, 28, 34	3.1, 3.2	JP
BA (Beaver Island 2)	MI	Charlevoix	46º04'N, 85°59'W	11	14, 15, 28, 34	3.1, 3.2	JP
BA (Garden Island)	MI	Charlevoix	46°19'N, 85°49'W	11	7, 9, 28, 34, 36	3.1, 3.2	JP
BA (High Island)	MI	Charlevoix	46°13'N, 86°05'W	12	7, 28, 34, 35	3.1, 3.2	JP
BA (Squaw Island)	MI	Charlevoix	46°23'N, 85°59'W	1	8	3.2	JP
BA (Trout Island)	MI	Charlevoix	46°17'N, 86°09'W	2	34	3.1	JP
LP1	MI	Antrim	45°03'N, 84°57'W	9	7, 12	3.2	JP
LP2	MI	Saginaw	43°19'N, 84°02'W	1	7	3.2	GMB
LP3	MI	Wayne	42°15'N, 83°17'W	3	1, 2	N/A	GMB
LP4	MI	Barry	42°36'N, 85°18'W	1	16	3.2	AR
LP5	MI	Monroe	41°55'N, 83°30'W	1	1	N/A	GMB
UP1	MI	Marquette	46°49'N, 87°58'W	4	23	3.1	JP
UP2	MI	Houghton	47°11'N, 88°57'W	2	25	3.1	RG
UP3	MI	Alger	47°09'N, 86°28'W	5	23, 24	3.1	GC

Table 2.1. continued.

Locality	State/province	County	Coordinates	n	Haplotype no.	NCA group	Source
AI(Apostle Islands)	Wisconsin (WI)	Bayfield	46°95'N, 90°63'W	1	26	3.1	GMB
WI1	WI	Bayfield	46°37'N, 91°10'W	9	28, 31, 32, 33	3.1	ER/GC
WI2	WI	Marinette	45°20'N, 88°00'W	7	28, 30	3.1	GC
WI3	WI	Crawford	43°13'N, 90°55'W	3	22, 28	3.1	HW
WI4	WI	Portage	44°28'N, 89°30'W	1	28	3.1	ER
WI5	WI	Waukesha	43°00'N, 88°14'W	3	22, 29	3.1	GMB
PA	Pennsylvania (PA)	Venango	41°23'N, 79°45'W	1	1	N/A	GMB
IN	Indiana (IN)	Porter	41°37'N, 87°04'W	9	7, 10, 11, 13, 22	3.1, 3.2	RK
IL1	Illinois (IL)	Carroll	42°05'N, 90°08'W	1	22	3.1	GB
IL2	IL	Dekalb	41°58'N, 88°41'W	8	1	N/A	RK
IL3	IL	Perry	38°05'N, 89°22'W	2	22	3.1	AR/SB
IL4	IL	Richland	38°42'N, 88°05'W	1	17	3.3	AR
IL5	IL	La Salle	41°20'N, 88°52'W	2	22	3.1	JR
IL6	IL	Monroe	38°16'N, 90°10'W	2	21, 22	3.1, 3.3	SB
IL7	IL	Union	37°27'N, 89°15'W	1	20	3.3	SB
IL8	IL	Alexander	37°11'N, 89°20'W	1	19	3.3	SB
IL9	IL	Cook	41°53'N, 87°39'W	1	18	3.3	AR
OH1	Ohio (OH)	Licking	40°04'N, 82°25'W	2	5, 6	N/A	PA
OH2	OH	Ottawa	41°30'N, 82°56'W	8	22, 27	3.1	RK
OH3	OH	Williams	41°40'N, 84°33'W	6	1, 4	N/A	RK/JM
OH4	OH	Wyandot	40°51'N, 83°17'W	2	1	N/A	RK
ONT	Ontario	Addington	45°00'N, 77°17'W	4	2, 3	N/A	BH

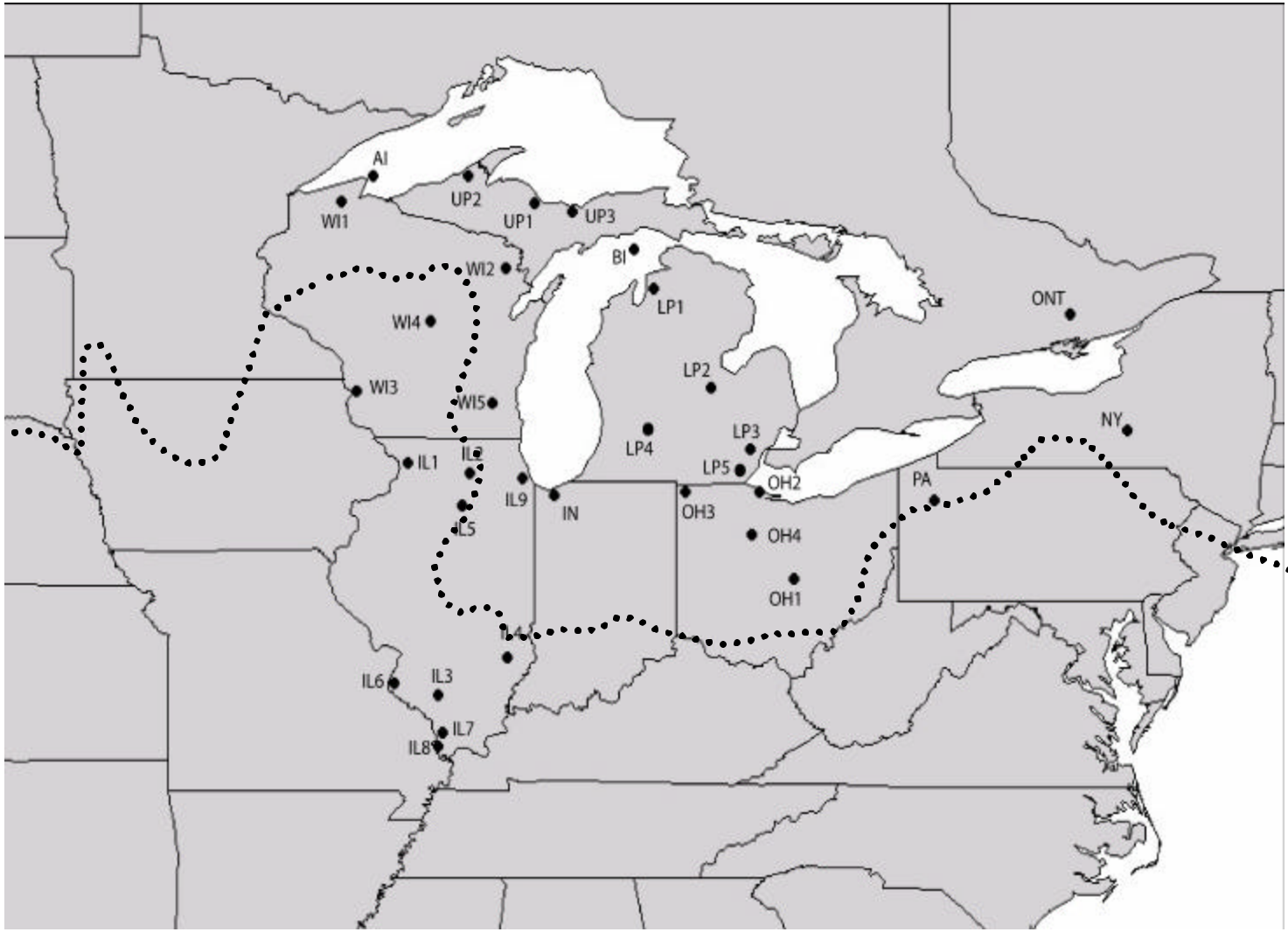
Table 2.2. Results of the nested geographic distance analysis for common gartersnake, *Thamnophis sirtalis*, populations from the Great Lakes region. For each nested clade with significant geographical associations I report the levels of clade/haplotype dispersion (D_C), displacement from clades at higher nesting levels (D_N), tip-interior contrasts (I-T), and probabilities for larger (<=) and smaller (>=) than expected distance values based on comparisons with the null hypothesis of no geographical association. Significant probabilities ($P < 0.05$) are indicated by an asterisk.

	Position	D_C	$P<=$	$P>=$	D_N	$P<=$	$P>=$
Clade 1-5							
Haplotype 23	interior	58.943	0.016*	0.995	62.056	0.011*	1.000
Haplotype 26	tip	0.000	1.000	1.000	252.039	1.000	0.086
Haplotype 25	tip	0.000	0.256	1.000	81.523	0.505	0.509
Haplotype 24	tip	0.000	1.000	1.000	109.775	0.916	0.509
I-T clades	--	58.943	0.667	0.334	-69.159	0.043*	0.958
Clade 2-3							
Clade 1-6	interior	264.174	0.000*	1.000	319.303	0.085	0.915
Clade 1-5	tip	77.543	0.000*	1.000	445.253	0.938	0.062
I-T clades	--	186.632	0.995	0.005*	-125.951	0.069	0.931
Clade 2-4							
Clade 1-10	interior	186.516	0.396	0.616	189.393	0.304	0.708
Clade 1-7	tip	239.842	0.972	0.038*	247.581	0.786	0.224
Clade 1-8	tip	0.000	1.000	1.000	343.853	1.000	0.295
Clade 1-9	tip	0.000	1.000	1.000	74.382	0.385	1.000
I-T clades	--	42.611	0.045*	0.956	-42.802	0.234	0.767
Clade 3-1							
Clade 2-1	tip	14.000	0.000*	1.000	260.425	0.058	0.942
Clade 2-2	interior	170.086	0.005*	0.995	205.125	0.000*	1.000
Clade 2-3	interior	362.473	0.892	0.108	386.568	0.982	0.018*
I-T clades	--	258.914	1.000	0.000*	41.679	0.770	0.230

Table 2.3. Demographic inferences from nested geographical distance analysis for the Great Lakes region common gartersnake, *Thamnophis sirtalis*, clades (after Templeton, 2004).

Clade	Inference chain	Inferred pattern
Clade 1-5	1-2-11-12-13-Yes	past fragmentation followed by range expansion
Clade 2-3	1-2-3-5-15-No	long-distance colonization
Clade 2-4	1-2-11-12-13-Yes	past fragmentation followed by range expansion
Clade 3-1	1-2-3-4-No	restricted gene flow with isolation by distance

Fig. 2.1. Collection localities for common gartersnake, *Thamnophis sirtalis*, populations sampled for this study. Exact localities for all collection sites are listed Table 3, and are identified by alphanumeric characters. The dotted line represents the maximum southern extent of Wisconsinan ice sheets (modified from Holman, 1992).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WI1
UP1
UP3
BI
WI2
LP1
ONT
WI4
LP2
WI3
WI5
NY
LP3
LP4
IL1
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OH2
PA
IL9
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OH1
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IL6
IL3
IL7
IL8

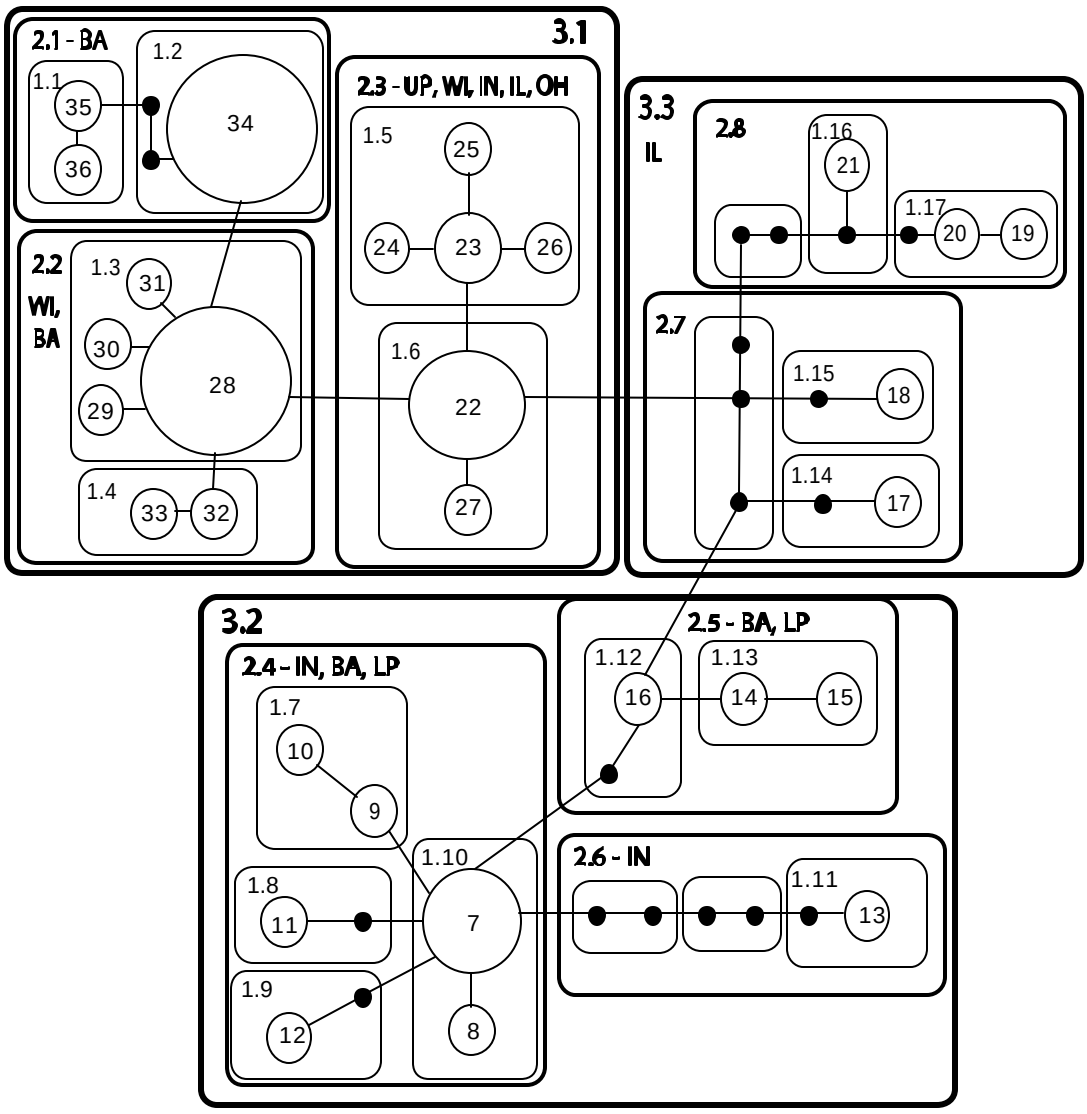
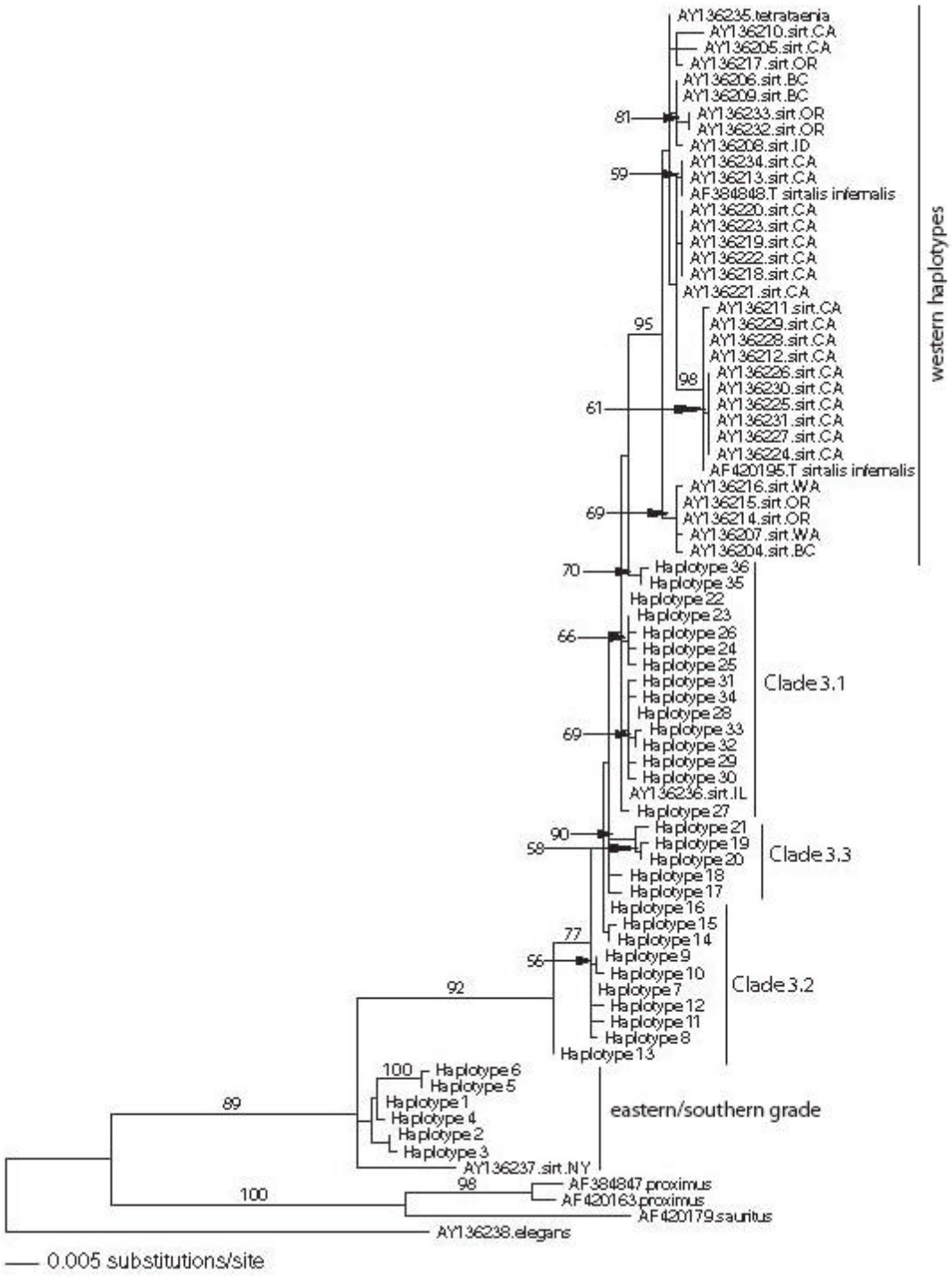


Fig. 2.2. Nested arrangement of common gartersnake, *Thamnophis siratlis*, ND2 haplotype network following the procedure given by Templeton et al. (1987, 1992). Haplotypes are labeled as in Table 1. Solid lines connect haplotypes with a single step. Missing intermediates are indicated by closed circles. Letters indicate the collection origin of haplotypes in each clade. Points of origin include the Beaver Archipelago (BA), the lower peninsula of Michigan (LP), the upper peninsula of Michigan (UP), Wisconsin (WI), Indiana (IN), Ohio (OH), and Illinois (IL).

Fig. 2.3. Maximum likelihood tree recovered from 37 unique common gartersnake, *Thamnophis sirtalis*, ND2 haplotypes. Numbers above or to the left of branches are bootstrap values (>50%, 100 random sequence addition heuristic search replicates with tree-bisection-reconnection (TBR) branch swapping) for those nodes recovered in the maximum likelihood analysis. Wording to the right of the tree indicates either 1) which of the three highest level nested clade analysis clusters particular haplotypes belong to, 2) haplotypes from a smaller more distant network detected by my nested analysis (i.e., the eastern/southern grade, and 3) western haplotypes from previous studies.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AY136210.sirt.CA
AY136205.sirt.CA
AY136217.sirt.OR
AY136206.sirt.BC
AY136209.sirt.BC
81
AY136233.sirt.OR
AY136232.sirt.OR
AY136208.sirt.ID
59
AY136234.sirt.CA
AY136213.sirt.CA
AF384848.T sirtalis infernalis
AY136220.sirt.CA
AY136223.sirt.CA
AY136219.sirt.CA
AY136222.sirt.CA
AY136218.sirt.CA
AY136221.sirt.CA
95
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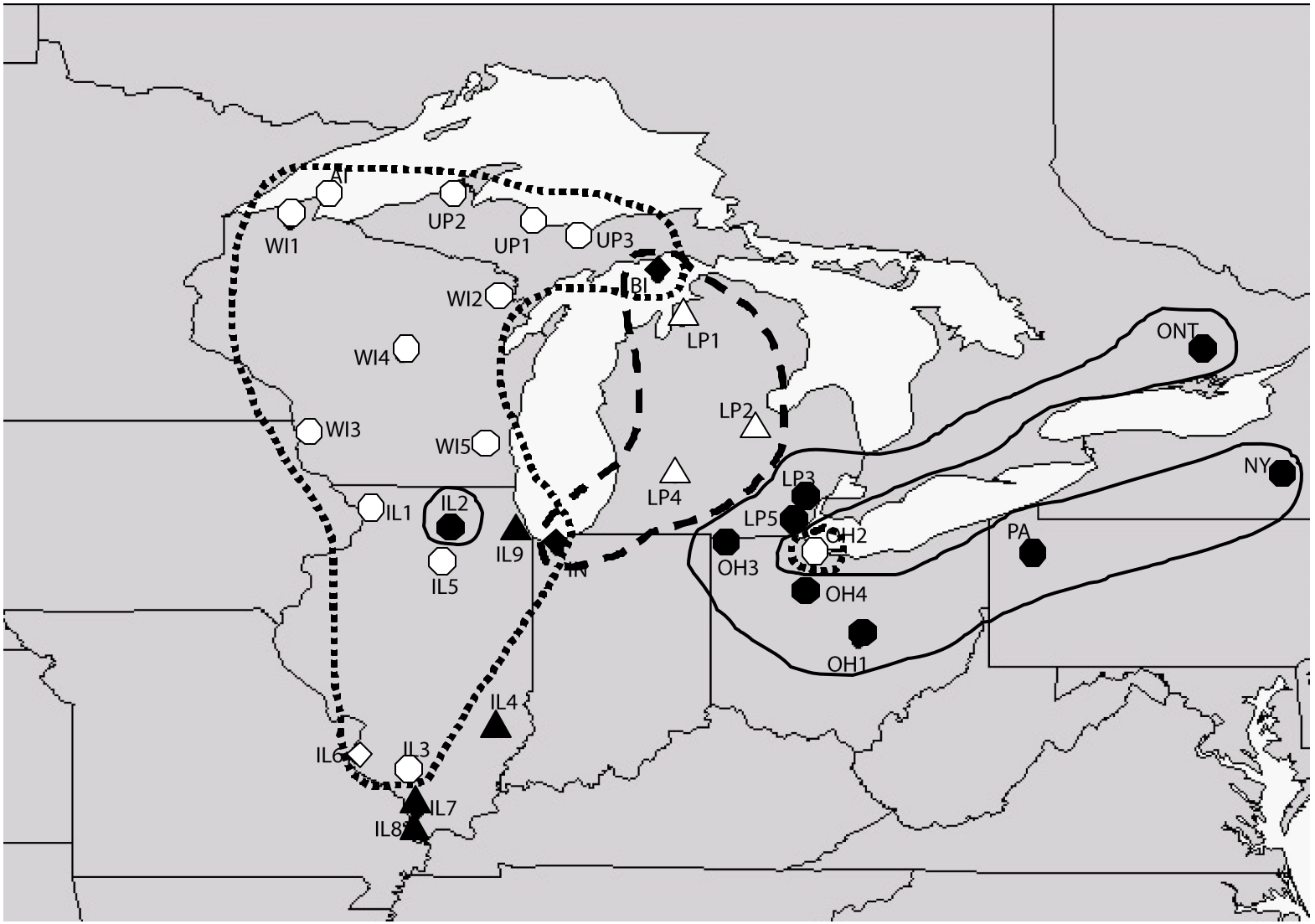
AY136228.sirt.CA
AY136212.sirt.CA
98
AY136226.sirt.CA
AY136230.sirt.CA
61
AY136225.sirt.CA
AY136231.sirt.CA
AY136227.sirt.CA
AY136224.sirt.CA
AF420195.T sirtalis infernalis
AY136216.sirt.WA
69
AY136215.sirt.OR
AY136214.sirt.OR
AY136207.sirt.WA
AY136204.sirt.BC
western haplotypes
70
Haplotype 36
Haplotype 35
Haplotype 22
Haplotype 23
66
Haplotype 26
Haplotype 24
Haplotype 25
Haplotype 31
Haplotype 34
Haplotype 28
69
Haplotype 33
Haplotype 32
Haplotype 29
Haplotype 30
AY136236.sirt.IL
Haplotype 27
Clade 3.1
90
Haplotype 21
58
Haplotype 19
Haplotype 20
Haplotype 18
Haplotype 17
Clade 3.3
Haplotype 16
Haplotype 15
77
Haplotype 14
56
Haplotype 9
Haplotype 10
92
Haplotype 7
Haplotype 12
Haplotype 11
Haplotype 8
Haplotype 13
Clade 3.2
100
Haplotype 6
Haplotype 5
89
Haplotype 1
Haplotype 4
Haplotype 2
Haplotype 3
AY136237.sirt.NY
eastern/southern grade
98
AF384847.proximus
100
AF420163.proximus
AF420179.sauritus
AY136238.elegans
0.005 substitutions/site

Fig. 2.4. Map showing the current distribution of common gartersnake, *Thamnophis sirtalis*, mtDNA clades (as inferred from nested clade analysis) following glacial retreat. Symbols represent the geographic locations of the identified clades: open circles = 3.1; open triangles = 3.2; closed triangles = 3.3; closed circles = eastern/southern grade; open diamonds = 3.1 & 3.3; closed diamonds = 3.1 & 3.2). Current distribution of clade 3.1 is represented by a dotted line, current distribution of clade 3.2 is represented by a dashed line, and current distribution of the eastern/southern grade is represented by a solid line.


UP2
UP1
UP3
WI1
WI2
WI4
WI3
WI5
LP1
LP2
LP4
LP3
LP5
ONT
NY
PA
IL1
IL2
IL5
IL9
IN
OH2
OH3
OH4
OH1
IL4
IL6
IL3
IL7
IL8

Part III

GEOGRAPHIC VARIATION IN THE ANTIPREDATOR BEHAVIOR AND VENTRAL SCALE COUNTS OF INSULAR POPULATIONS OF THE COMMON GARTERSNAKE, *THAMNOPHIS SIRTALIS*

INTRODUCTION

Most vertebrates inhabiting islands are believed to be characterized by fearlessness and tame behavior as a result of fewer predators than exist on comparable mainlands (e.g., Pimm, 1987; Atkinson, 1989; Case et al., 1992); however, this common belief may be misleading (McLaughling and Roughgarden, 1989; Alcover and McMinn, 1994). In addition, this trend of fearlessness and tameness in island species has traditionally been based on studies of endemic island species (e.g., Stone et al., 1994), rather than species that occur both on the mainland and in insular habitats with only a few recent intraspecific examples being documented. Insular spiny-tailed iguanas (*Ctenosaura hemilopha*, Iguanidae), for example, are believed to have modified their behavior in response to a decline in predation risk by basking at greater distances from their refuges and taking flight at shorter distances than *C. hemilopha* from mainland populations (Blazquez et al., 1997). Similarly, insular tiger snake (*Notechis scutatus occidentalis*, Elapidae) populations are more placid than mainland populations as the result of phenotypic plasticity (Bonnet et al., 2005). However, despite the support these studies provide for the hypothesis that island animals are less reactive than mainland animals, caution should still be taken before applying the island tameness hypothesis broadly, as a variety of factors (both intrinsic and extrinsic) need to be taken into account when examining such geographic variation in behavior. For example, Scharf (1973) observed that gartersnakes, *Thamnophis sirtalis* (Colubridae), found on South Manitou Island in northern Lake Michigan are seemingly more "aggressive" than mainland *T. sirtalis* which occur with more predators, and even at the interspecific level, after 3-4 millions years, Galapagos finches (Geospizinae) retain alarm responses to snakes, owls,

and hawks on islands from which these predators are missing (Curio, 1964, 1966, 1969, 1976).

My research was designed to be a detailed examination of intraspecific geographic variation in the antipredator behavior of insular populations, incorporating traditionally neglected data (e.g., morphological data, geological history, underlying genetics), and attempting to elucidate the forces that drive and maintain such variation. Specifically, I examined the antipredator behavior of several insular and mainland populations of the common gartersnake (*Thamnophis sirtalis*) using both neonate snakes naïve to environmental influences and wild-caught adult snakes in experiments designed to examine the influence of genetic and environmental influences on behavioral variation. Experiments were also conducted to determine the role that phenotypic plasticity may play in creating behavioral variation. In addition, I surveyed variation in a morphological character (ventral scale number) associated with antipredator behavior (see below for details), as variation in such characters may correlate with and possibly restrict variation in behavioral characters (e.g., Arnold and Bennett, 1988). Behavioral divergence is also believed to occur in a "different way" than divergence in other aspects of phenotype (including morphological traits) resulting from the same selection pressures (e.g., Mayr, 1960; Hailman, 1982; Huey and Bennett, 1987; Futuyma and Moreno, 1988); however, few studies incorporate data on the two together. Snakes prove to be ideal test subjects for examinations of behavioral variation for many reasons.

Snakes, in general, are excellent candidates for studying the role of genetics and environment on behavior, as they have highly precocial young that can be tested when naïve to external stimuli that elicit behavioral responses (e.g., Burghardt, 1993). In addition, many snakes have large litter/clutch sizes, which provide an opportunity to split litters/clutches among environmental treatments to study genotype-by-environment interactions and common family environment (including maternal) effects (e.g., Brodie and Garland, 1993; Burghardt and Schwartz, 1999; King et al., 2001; Badyaev et al., 2002; King, 2002). Gartersnakes (*Thamnophis* spp.) of the subfamily Natricinae are good model species for studying many aspects of behavior, physiology, reproduction, ontogeny, genetics, and life-history (e.g., Seigel et al., 1987; Seigel and Collins, 1993; Rossman et al., 1996). Population differentiation is well known in morphology (color, pattern, size, scalation), life-history traits, and food habits with some species exhibiting geographic variation in phenotypic traits as both neonates and adults making *Thamnophis* an ideal genus in which to study the divergence of phenotypic traits.

Thamnophis sirtalis is the most wide-ranging snake in North America (Rossman et al., 1996) being found throughout the Midwest region of the United States and elsewhere, and it is considered the most common snake species in the Great Lakes region (Harding, 1996). This, in part, is why I centered my study in the Beaver Archipelago of Michigan and the mainland surrounding it. The Beaver Archipelago is located in northeastern Lake Michigan and is ca. 25-30 km from both the lower and upper peninsula of Michigan (Fig. 3.1; all figures for Part III are located in Appendix III). It is the largest group of islands in Lake Michigan consisting of one main island (Beaver, 15130.0

hectares (ha)), three moderately large islands (Garden, High, and Hog, 1023.9 to 1989.0 ha), and six smaller islands (Hat, Pismire, Shoe, Squaw, Trout, and Whiskey, 1.0 to 52.3 ha). This archipelago is ideal for studies on geographic variation in phenotypic traits, as species abundance and diversity varies from island to island and between the islands and the surrounding mainland roughly following island biogeography theory (Bowen and Gillingham, 2004) as do all of the archipelagos found in the Great Lakes region that have been studied to date (Hecnar et al., 2002). In addition, the archipelago has a well-documented geological history (Hough, 1958), both the lower and upper peninsula of Michigan and all but the smallest islands (i.e., Hat, Pismire, and Shoe) support *T. sirtalis* populations (Hatt et al., 1948; Placyk and Gillingham, 2002), the origin and underlying genetics of the island populations were recently revealed (Placyk, 2006; Placyk et al., in prep), mainland populations from which island snakes are thought to have originated from vary in antipredator behavior (Burghardt and Schwartz, 1999), gartersnake predators and prey vary from island to island (Placyk and Burghardt, 2005), and the frequency of gartersnakes wounded by failed predatory attacks varies among islands and between the islands and the mainland (Placyk and Burghardt, 2005).

Variation in antipredator behavior was chosen, not only for the reasons listed above, but also because, in gartersnakes, Herzog and Schwartz (1990) have indicated that this type of behavior has heritabilities in the range of 0.40-0.60 suggesting that rapid evolutionary change is capable. In fact, Magurran (1999) has indicated that, at least with guppies (Poeciliidae), differences in antipredator behaviors resulting from evolutionary change were detectable after only 16 years (Magurran et al., 1995), while Endler (1980)

found that in only 2 years, guppies showed significant differences in morphological traits related to predator pressures. In terms of the morphological character I chose to include in my data set, while many morphological characters may change as a result of varying environmental conditions throughout the life of an organism confusing distinctions between evolutionary processes and plasticity, I chose a discrete morphological character that is known to vary with different predator selection pressures, but not with environmental differences (i.e., number of ventral scales). Ventral scales are attached internally to muscles, which are very important in locomotion; therefore, animals with an increased number of ventral scales are able to flee faster than animals with fewer ventral scales. In gartersnakes, differences in the number of ventral scales is associated with varying predation pressures with even minor variations having detectable impacts on the ability of a snake to flee from a predator (e.g., Arnold and Bennett, 1988; Lindell et al., 1993; Forsman et al., 1994; Kelley et al., 1997) making the character ideal for studies of variation in antipredator-related traits. In addition, Burghardt and Schwartz (1999) found significant differences in the number of ventral scales between Michigan and Wisconsin *T. sirtalis* populations indicating that such differences may also exist in the Beaver Archipelago system which is also found in the Midwest and may have been colonized by populations genetically similar to both Wisconsin and mainland Michigan populations (Placyk, 2006; Placyk et al., under rev.).

The overall goals of this work are to determine (1) if variation in antipredator behavior and ventral scale count exists between my populations, (2) if variation in ventral scale count and any behavioral characters correspond with each other, (3) the influence of

microevolution and phenotypic plasticity on any such variation, and (4) the role of historical/geological processes on any phenotypic variation.

GENERAL METHODS

Gartersnakes were collected from field sites in both the lower (LP) and upper peninsula (UP) of Michigan and from the three largest islands located in the Beaver Archipelago (i.e., Beaver Island (BI), High Island (HI), and Garden Island (GI)) (Fig. 3.1) that are known to vary in predator pressures and support substantial snake populations (Placyk and Burghardt, 2005). Given the size of Beaver Island and this work's focus on island phenomenon, it was possible to sample two Beaver Island sites nearly 1 km apart (i.e., Miller's Marsh (MM) and Sawmill (SM)) that vary in predator pressures (Placyk and Burghardt, 2005). Following capture, snakes were placed, by site, into 61 X 32 X 33 cm aquaria to eliminate breeding and disease transmission between different populations. Aquaria were kept between 20 and 25 ° C on a 16:8 Light:Dark cycle, to simulate northern Michigan summer conditions, with fresh water and shelter available at all times. Snakes were permitted to acclimate for 48 h after which behavioral testing was conducted. Since *T. sirtalis* is diurnal, all behavioral tests were conducted during photophase. Following behavioral testing, all animals had their ventral scales counted (the number of scales from the first scale above the anal plate to the neck), and nonpregnant females and males were scale-clipped for future identification (Brown and Parker, 1976), had tissue samples taken for genetic analyses and were released to their respective capture locations. I also recorded if an animal was missing part of its tail or had other types of wounds or scars that may have been caused by a failed predatory

attack, as such data may correspond with differences in antipredator behavior and variation in this type of data has been documented in this system (Placyk and Burghardt, 2005). Some pregnant females (determined via palpating for developing embryos) were held until parturition after which time they were released to their respective capture locations. Neonates were kept for additional testing and were maintained in 15 X 30 X 9 cm cages with shelter, offered water ad libitum, and fed chopped worms (*Lumbricus* spp.) three times per week. Following neonate behavioral testing, ventral scales were counted and animals were released to the site of their mother's capture, maintained for other experiments, or transferred to other research facilities. Any adult snakes held more than 72 hours and all neonates were submitted to a health screening before being released.

For behavioral experiments, adult snakes were typically tested at the Central Michigan University Biological Station on Beaver Island if they were caught in the lower peninsula or on any of the islands or at Northern Michigan University if they were caught in the upper peninsula of Michigan. All neonate testing took place at the University of Tennessee. Only snakes that appeared outwardly healthy and, for neonates, were eating on a regular basis were tested.

Specific statistical analyses conducted with each data set are detailed below; however, for data sets that did not fit parametric assumptions, Mann-Whitney U tests and Kruskal-Wallis ANOVAs and Friedman's ANOVAs followed by Kruskal-Wallis multiple-comparison Z-value tests and Tukey-Kramer multiple-comparison tests,

respectively, were used accordingly. Alpha was set at 0.05 for all analyses. Chi-squared tests were performed using JMP 5.1.2 (SAS Institute Inc.). NCSS (Hintze, 2001) was used for all ANOVAs, t-tests, and nonparametric tests.

EXPERIMENT I: RESPONSES TO A SIMULATED PREDATORY ATTACK

Geographic variation in behavior may be congenital or develop over time due to experience or maturational changes (see review in Foster and Endler, 1999a). In fact, congenital variation may still undergo ontogenetic shifts due to experience or maturation (see review in Foster and Endler, 1999a). For Experiments I and II, both neonates and adults from all sites were tested to determine if geographic variation in antipredator behavior exists in my system and if such differences are present during both infancy and adulthood. To accomplish this, a standardized antipredator behavioral test (Herzog and Burghardt, 1986; Mori et al., 1996) and an ophiophagous snake antipredator response test (see Experiment II) were used.

Methods—For standardized antipredator behavioral tests (modified by Mori et al., 1996), test arenas consisted of 51 cm X 26.5 cm X 30.5 cm aquaria lined with a corrugated cardboard cage liner and sterilized with Alconox®, a clinical, concentrated, anionic detergent powder. Trials began with snakes being left undisturbed for 30 sec after which time the experimenter's finger was slowly brought within 2 cm of the snakes snout and held stationary for 60 sec (nonmoving stimulus session). If the snake crawled away during the test, it was followed, with the extended finger in front of the snake. The

snake was then given a 30 sec undisturbed period followed by the researcher, again, extending the forefinger to within 1 to 2 cm of the snake's snout, but this time moving it back and forth at the rate of ca. three to four oscillations per sec throughout the 60 sec period (moving stimulus session). Finally, after another 30 sec undisturbed period, the snake was gently tapped or had its body touched (head to tail) ca. once every sec throughout the 60 sec period (tapping stimulus session). Behavioral measures such as fleeing, anal gland discharge (musking), defecating, urinating, body flattening, striking, biting, head hiding, and tail wagging were recorded using counters for each time they occurred for each trial (Table 3.1; tables 3.1 – 3.3 are located in Appendix III). Adults were tested within 1 to 2 days after capture, and neonates were always tested 1 to 3 days after birth. All behavioral tests for this experiment were carried out by John S. Placyk, Jr.

Statistical analyses—Significant differences in fleeing due to a site effect were evaluated using one-way ANOVAs followed by Tukey-Kramer multiple-comparison tests. Since all other observed behaviors occurred infrequently, chi-squared tests of independence were used to determine if the frequency of the behavior was independent of the site. If a test indicated that site and the behavior were not independent of each other, the model was partitioned and Fisher exact probability tests were used to determine pairwise differences with *P*-values adjusted for multiple comparisons using Holm's method (Holm, 1979; Aickin and Gensler, 1996). Two-sample t-tests and ANOVAs (for fleeing) and chi-squared tests of independence (for all other behaviors) were also used to determine if differences in the behavior of adult snakes existed due to sex or reproductive state (male, nonpregnant female, pregnant female) and snakes exhibiting wounds from

failed predatory attacks vs. snakes that did not exhibit such wounds. Sex differences were examined in neonates as well, with litter effects on behavior also being examined and controlled for whenever possible by nesting litter within each site. Neonates were always tested 1 to 3 days after birth. A simple linear regression was used to determine if the number of times a neonate fled from a simulated predator was related to the number of times that dams fled from a simulated predator. This analysis could not be performed for any other behavior due to the infrequency of all other behaviors.

Results—For the adults, a significant site effect was detected for 8 of the 9 possible different responses that could have been exhibited ($P < 0.05$). In general, adults from mainland sites (LP and UP) and Beaver Island (BI) displayed a greater diversity of antipredatory responses (6-9 different types of responses) than adults from either Garden Island (GI) or High Island (HI) (2-5 different types of responses). In addition, of the 9 different responses that were observed, adults from mainland sites and the two sites on BI (MM and SM) tended to display them each significantly more than adults from GI and HI (Table 3.2). As for specific site differences, the number of times adult snakes tried to flee from the simulated predator differed significantly among sites ($H_{5,301} = 188.84$, $P < 0.0001$; Fig. 3.2a) with snakes from the LP, UP, and BI fleeing from the simulated predator significantly more than snakes from GI and HI ($P < 0.05$; Fig. 3.2a). Adult snakes from both mainland Michigan sites bit the experimenter significantly more than snakes from most of the islands (Table 3.2) and struck at the experimenter significantly more than animals from MM and from GI and HI ($P < 0.05$; Table 3.2). Adult snakes from BI wagged their tails more than snakes from GI or HI ($P < 0.05$; Table 3.2). Adults

from the UP urinated more than animals from HI ($P < 0.05$; Table 3.2). Adults from the two mainland sites defecated more than animals from HI, and adults from the UP defecated more than animals from GI ($P < 0.05$; Table 3.2). Adults from the UP discharged anal gland secretions (musked) more than adult animals from any most other sites, and MM adults musked more than snakes from HI ($P < 0.05$; Table 3.2). Finally, LP adults flattened more than MM adults ($P < 0.05$; Table 3.2)

In addition to site differences, sex and reproductive state resulted in significant differences for a variety of the adult snake responses. The presence of wounds from failed predatory attacks, however, did not result in significant differences for any of the observed antipredator responses ($P > 0.05$). As for general sex differences, adult females (17.64 $\pm$ 1.12) fled from the simulated predator more than adult males (14.26 $\pm$ 1.27) ($Z_1 = -2.13$, $P = 0.03$), while adult males (11 %) tended to wag their tails more than adult females (5 %) ($X^2 = 3.98$, $G^2 = 3.87$, df = 1, $P < 0.05$). In regard to reproductive state, pregnant females struck more (9%) ($X^2 = 4.57$, $G^2 = 3.80$, df = 1, $P < 0.05$), bit more (5 %) ($X^2 = 6.57$, $G^2 = 5.86$, df = 1, $P < 0.05$) and flattened their bodies more (5 %) ($X^2 = 6.57$, $G^2 = 5.86$, df = 1, $P < 0.05$) than nonpregnant females (2 % struck, none bit or flattened their bodies) and flattened their bodies more than reproductive males (none flattened their bodies) ($X^2 = 5.52$, $G^2 = 5.33$, df = 1, $P < 0.05$). In addition, adult males bit more (3.4 %) than nonpregnant females ($X^2 = 4.84$, $G^2 = 6.35$, df = 1, $P < 0.05$). Despite these differences, sex ratios between the six sites did not differ significantly ($X^2 = 7.28$, $G^2 = 7.41$, df = 5, $P > 0.05$) and although the ratio of pregnant females to nonpregnant females ($X^2 = 20.16$, $G^2 = 20.11$, df = 4, $P < 0.001$) differed among sites,

only one pairwise difference was found with more pregnant females being taken from the LP (70 %) than from MM (13 %) ($P < 0.05$). This may have some influence on other relationships between these two sites, but broader island/mainland trends may not be as affected.

Unlike with the adults, neonates almost always exhibited all 9 antipredator behaviors regardless of where their mothers were collected. Neonates also appeared to be more reactive than adults, displaying many of these behaviors at greater frequencies (Table 3.3). However, significant differences due to a site effect were less pronounced than with the adults, with some of the behavioral differences that were found exhibiting different patterns. For example, while, on average, neonates from the UP, LP, and MM fled more than neonates from SM, HI, and GI, after taking litter into account, no significant site difference was detected ($F_{5,294} = 1.24$, $P = 0.31$; Fig. 3.2b). A simple linear regression, however, indicated that there is a significant positive linear relationship between the number of times a dam fled from a simulated predator and the number of times her offspring fled from a simulated predator ($r^2 = 0.06$; $P < 0.001$; Fig. 3.3), but this relationship only explains 6 % of the variation in the neonate fleeing data. In addition to differences in the number of times neonates fled from the simulated predator, MM neonates musked significantly more than LP neonates ($P < 0.05$; Table 3.3) and UP, HI, and SM neonates flattened their bodies more than LP neonates ($P < 0.05$, Table 3.3). No significant differences in the frequency of strikes, tail wagging, urinating, head hiding, or biting were found between sites ($P > 0.05$). As with the adults, a sex effect was detected for the neonates, but it also was not as pronounced. Specifically, neonate

males (4 %) tended to defecate more than neonate females (0.5 %) ($X^2 = 4.16$, $G^2 = 4.33$, df = 1, $P < 0.05$). In addition, a litter effect was detected for neonate fleeing ($F_{35,336} = 5.53$, $P < 0.0001$) but this effect was controlled for by nesting litters within sites. Possible litter effects for the remaining behaviors could not be assessed, as the frequency of those behaviors, once classified based on litters, occurred less than 5 times for more than 90 % of the cells of the chi-squared table.

Discussion—Adult snakes from both mainland sites and both BI sites tended to exhibit antipredator behaviors more frequently than snakes from GI or HI. Specifically, adult snakes from both mainland sites and both BI sites, fled more than adult snakes from HI and GI, and various combinations of adult snakes from mainland sites and BI sites struck more, tail-wagged more, defecated more, and musked more than adult snakes from GI and HI (Table 3.2). Mainland adults were observed biting significantly more and LP adults flattened significantly more than adults from all of the island sites. These data suggest that the pattern displayed by adults in this regard corresponds with the richness of predators at each site. Snakes at both mainland sites occur with more predators than any of the island snakes, and snakes from BI occur with more predators than snakes on either GI or HI (Placyk and Burghardt, 2005). While no behavioral differences could be directly attributed to the presence of wounds derived from failed predatory attacks, any such significant effect would only indicate that adult animals may have learned to respond to predators differently following an attack, whereas, the differences I saw may be due either to a strong genetic component, environmental influences that have occurred over the lifetime of individuals from each population, or a combination of the two. To

further understand the possible influence of genetic components on antipredator behavior in these populations, I tested neonate snakes under the same conditions as I tested adults. Since neonates were naïve to the occurrence of predatory attacks, they would have had no experience by which to shape their response to a simulated attack.

As with the adults, site differences were detected, and while neonates born to dams from the two mainland sites and the MM appear to flee more than neonates from HI or GI indicating possible evolutionary adaptive changes, these differences were not significant. However, a simple linear regression did indicate that the more a dam fled the more her offspring fled indicating some genetic connection between neonate and adult fleeing behavior. Few other similarities, however, were found between neonate responses and adult responses. For example, MM neonates musked more than LP neonates, while neonates from the UP, GI, MM, and HI defecated more than LP neonates and along with SM neonates flattened their bodies more than LP neonates. That adult patterns and neonate patterns vary may indicate several things. First, predator pressures that influence neonates may differ from those that influence adults. Generally, smaller animals tend to have more predators than larger animals, and this may explain why, in general, neonates responded to a simulated predator more than adults. However, this does not explain the significant site differences I detected. One explanation for these site differences may be due to the underlying genetics of these populations (e.g., Mori et al., 1996; Mori and Burghardt, 2000). Burghardt and Schwartz (1999) found neonates born to mothers from Wisconsin were more reactive than neonates born to LP mothers. Since UP animals are derived from WI populations (Placyk, 2006; Placyk et al., in prep), the

difference I saw between UP and LP populations may be the result of the underlying genetics of these populations. My data also indicate that neonates from MM, SM, and HI also appear to be more reactive than the LP neonates in some regards (musking, body flattening), where more gartersnake predators occur. Placyk (2006) also indicates that most individuals sampled (80.9 %) from the Beaver archipelago are derived from UP populations rather than LP populations. However, since fleeing is the most common antipredator behavior exhibited by gartersnakes, in general, it makes sense that this behavior may have been one of the first to undergo adaptive evolutionary change as indicated by the qualitative differences between MM and GI and HI neonates. Since many of the behaviors exhibited by adult animals correspond with predator richness, but not with neonate behaviors, the experiment (see Experiment III below), in which I attempted to examine the plasticity of such behaviors was useful in determining the role of environmental influences on adult behavior.

In addition to site effects, both adults and neonates exhibited some behavioral differences due to a sex effect, and adults also varied as a result of reproductive state (nonpregnant female, pregnant female, male). In particular, adult females fled more, while males wagged their tails more and neonate males defecated more than neonate females. I am unsure if these differences are spurious results or if there is some ecological or evolutionary explanation for them. King (2002) also found that a sex effect influenced the display of antipredator behavior in *T. sirtalis* with females from his study striking more than males, but he also was unsure of the natural correlate(s) of such differences. It is interesting to note that in a recent study in which I examined the

frequency of tail stubs in these same populations that I found males tended to be characterized by a higher frequency of tail stubs than females (Placyk and Burghardt, 2005). Given these new behavioral data, one can make the connection between males wagging their tails more in response to predatory attacks and as a result suffering more tail loss than females. In terms of differences due to reproductive state, pregnant females struck more, bit more, and flattened more than nonpregnant females, pregnant females flattened more than adult males and adult males bit more than nonpregnant females. These differences may be the result of variation in circulating sex hormone levels. Recent work suggests that progesterone levels as well as testosterone levels influence "aggressive" behavior in reptiles (Weiss and Moore, 2004) and differences in these levels may be the cause of the differences in behavior I observed, but hormonal assays of pregnant vs. nonpregnant females vs. males are required to further support such a hypothesis. Neonates also displayed a litter effect, which King (2002) suggests is a major contributor to gartersnake behavior, but this effect did not influence site effects.

EXPERIMENT II: ANTIPREDATOR RESPONSES TO OPHIOPHAGOUS SNAKES

Introduction—Antipredator responses to ophiophagous snakes are common among snakes that they may potentially prey upon (e.g., Weldon and Burghardt, 1979; Weldon, 1982; Gutzke et al., 1993). While such responses have been found to result from both congenital factors and experience (e.g., Weldon and Burghardt, 1979; Weldon, 1982), few have examined neonates and adults from the same populations. In addition, based on mtDNA sequence data (Placyk, 2006; Placyk et al., under rev.), the island populations examined in my study are derived from two source populations, one that

occurs with ophiophagous snakes (LP populations) and one that does not (UP populations). As a result, the island populations provide a unique system in which to examine the factors that may produce differences in response to ophiophagous snakes and that may be driving and maintaining such differences. To test hypotheses in this regard, I developed a new ophiophage antipredator test and surveyed both adults and neonates from both mainland and island sites.

Methods—For the ophiophage antipredator test, gartersnake behavior was observed under three conditions: (1) in a clean test arena, (2) in the presence of ophiophage chemical cues, and (3) in the presence of nonophiophage chemical cues. Eastern milksnakes (*Lampropeltis t. triangulum*) were used to provide ophiophagous snake chemical cues, as they both elicit ophiophage antipredator responses (Weldon and Burghardt, 1979) and they occur over most of the Great Lakes Region, so adult snakes from this area may have experience with them. Northern ring-necked snakes (*Diadophis punctatus edwardsii*, Colubridae) provided nonophiophagous snake chemical cues, as throughout most of the Great Lakes region, they prey almost exclusively on salamanders (Harding, 1996) and are frequently found sharing cover objects with gartersnakes, so gartersnakes should find them rather inoffensive (Placyk, pers. obs.). Milksnakes were maintained on a diet of various small snakes and lab-bred mice, whereas, the ring-necked snakes were maintained on a diet of eastern red-backed salamanders (*Plethodon cinereus*, Plethodontidae), their prey of choice in northern Michigan.

Trials for the ophiophagous antipredator testing were conducted in 51 cm X 26.5 cm X 30.5 cm aquaria that previously housed either an ophiophagous snake or a nonophiophagous snake or that had been sterilized. The ophiophages and the nonophiophages were permitted 24 hours to deposit chemical cues. Trials began with a 30 sec undisturbed period followed by a 2 min period during which mobility duration and the frequency of tongue-flicks were recorded. Tongue-flicking is an appropriate measure of antipredator behavior in gartersnakes, as Weldon (1982) found that *Thamnophis* emit significantly greater numbers of tongue-flicks in the presence of ophiophagous snake chemical cues than with nonophiophagous snake chemical cues. Adults were tested 1 to 2 days after capture and neonates were always tested within 1-5 days following birth. All behavioral tests for this experiment were carried out by John S. Placyk, Jr.

Statistical analyses—Differences in either the number of tongue-flicks or mobility duration within a treatment (control, ringneck, milksnake) due to a site effect were detected using one-way ANOVAs, while differences across treatments were detected using repeated-measures ANOVAs. Significant pairwise differences were detected with Tukey-Kramer multiple comparison tests. For adults, two-sample t-tests and ANOVAs were used to test for effects of sex, reproductive state, or the presence of wounds from failed predatory attacks for every combination of treatment and behavior. Sex effects were examined for neonates and any litter effect was also tested and controlled for each treatment and behavior combination using a nested one-way ANOVA.

Results—When adult snakes were observed in test arenas that were sanitized (control) or that previously housed either an ophiophagus snake (milksnake) or a nonophiophagus snake (ringneck snake) both mobility duration ($F_{2,536} = 198.61$, $P < 0.001$; Fig 3.4a) and frequency of tongue-flicks ($F_{2,536} = 166.84$, $P < 0.001$; Fig 3.5a) significantly differed among treatments. In general, adults tongue-flicked and were more active in the presence of milksnake chemical cues than when in the presence of ringneck chemical cues ($P < 0.05$) or when in the control test arena ($P < 0.05$). Adult snakes also tongue-flicked more in the presence of ringneck chemical cues than when tested in the control treatment ($P < 0.05$), but this difference did not exist for mobility duration ($P > 0.05$). In addition to these general responses, site differences, both within and across treatments, in mobility duration (Fig. 3.4a) and tongue-flicking (Fig. 3.5a) were also detected.

Specifically, adult MM and SM animals differed in the amount of time they spent in motion between the control and ringneck treatments ($P < 0.05$; Fig 3.4a). A site effect was also detected for tongue-flicking with adult UP animals not differing in the amount of tongue-flicking they did between the three treatments and adult LP animals not tongue-flicking more in the ringneck condition than in the control treatment ($P > 0.05$; Fig 3.5a). Adult animals from HI and LP tongue-flicked significantly more in the presence of milksnake chemical cues than adults from MM, SM, GI, or the UP ($P < 0.05$; Fig. 3.5a), and HI adults tongue-flicked more than MM adults under the control treatment ($P < 0.05$; Fig 3.5a). In terms of mobility duration ($F_{10,536} = 2.50$, $P < 0.01$; Fig 3.4a), LP, GI, and HI adults moved more than MM adults under the milksnake treatment, SM

adults moved more than MM and HI adults under the ringneck treatment, and GI and HI adults moved more than MM adults under the control treatment ($P < 0.05$). Within treatments, differences due to sex, reproductive state, or the presence of wounds from failed predatory attacks did not influence site effects for either adult tongue-flicking or mobility duration ($P > 0.05$).

Neonates were also observed differing in their amount of mobility ($F_{2,180} = 110.35$, $P < 0.001$; Fig 3.4b) and frequency of tongue-flicking ($F_{2,180} = 109.04$, $P < 0.001$; Fig 3.5b) as a result of the treatments they were tested under. As with adults, neonates, in general, moved and tongue-flicked more in the presence of milksnake chemical cues than when observed in either a control treatment or in the presence of nonophiophagous snake chemical cues ($P < 0.05$) and this held for neonates from all sites except those from HI, which did not vary in mobility duration between any of the treatments ($P > 0.05$; Fig. 3.4b). In addition, only GI neonates tongue-flicked more in the presence of nonophiophagus chemical cues than when tested under the control treatment ($P < 0.05$) and only UP neonates moved more under the ringneck treatment than in the control treatment ($P < 0.05$; Fig. 3.4b). Differences in mobility duration ($F_{10,180} = 2.12$, $P < 0.05$; Fig 3.4b) and tongue-flicking ($F_{10,180} = 2.25$, $P < 0.05$; Fig 3.5b) among sites within each treatment also varied significantly for neonates. Specifically, LP and GI neonates tongue-flicked more than MM neonates, LP neonates tongue-flicked more than UP neonates in the milksnake condition ($P < 0.05$; Fig. 3.5b), and UP neonates tongue-flicked less than GI neonates in the ringneck condition ($P < 0.05$; Fig. 3.5b). As for mobility duration, LP neonates spent more time moving than UP and HI neonates in the

milksnake condition, GI neonates spent more time moving than HI and MM neonates in the ringneck condition, and UP neonates spent more time moving than GI and LP neonates in the control condition ($P < 0.05$). As with the adults, sex differences for each site within treatments were not detected ($P > 0.05$). However, when nesting litter within site, a significant litter effect was detected for neonate mobility duration ($F_{15,90} = 1.91$, $P = 0.03$), but this effect was controlled for by nesting it within site. In addition, no treatment by litter effect was detected ($F_{30,180} = 0.76$, $P = 0.82$). No litter effect was detected for neonate tongue-flicking ($F_{15,90} = 1.00$, $P = 0.47$).

Discussion—When testing the response of snakes to the chemical cues of ophiophagous snakes (milksnakes) versus nonophiophagus snakes (ringnecks), both neonates and adults from most collection sites showed the same trend. Snakes tongue-flicked and moved more in the presence of milksnake chemical cues than either in the presence of ringneck chemical cues or in a sanitized "odorless" control treatment. While adult snakes from the UP did not tongue-flick more in the milksnake condition than in the other two conditions, they did move more in the milksnake condition and neonates born to UP mothers moved and tongue-flicked more in the milksnake condition than in the other two conditions, despite rarely, if ever, occurring with milksnakes (Harding, 1996). This may indicate that the behavior is present at birth and may be lost if not utilized on a regular basis, but since mobility duration of adults is still higher in the milksnake condition than in the other two conditions, there could also be a maturation effect. I did observe, however, that ophiophage antipredator behavior is maintained in neonates of UP populations. This may indicate that 1) the cost of maintaining such a behavior may be

less than the benefit, 2) UP populations have not been separated from other populations that still occur with milksnakes long enough to have lost the behavior, or 3) gene flow between populations that occur with milksnakes and those that do not is maintaining the behavior. In addition, the fact that neonates, in general, responded similarly to adults in this test is not surprising, as there is an obvious advantage to expressing such behaviors given that the first encounter with an ophiophagous snake could be the last, especially for smaller individuals. In addition, this data corresponds with past research indicating naïve neonate gartersnakes, as well as naïve neonates of other species of snakes (e.g., Cooper et al., 2000) respond to ophiophagous snakes (Weldon and Burghardt, 1979) even when they no longer occur with them. In addition, some antipredator behaviors of snakes that may be ineffective may persist in some populations (e.g., Mori and Burghardt, 2000). For example, Japanese natricine snakes, *Rhabdophis tigrinus* (Colubridae), which are believed to sequester toxins from their toad prey in special nuchal glands on their necks (e.g., Azuma et al., 1986), still display behaviors (e.g., neck arching, neck butting), albeit at lower frequencies, even when the primary source of the toxins in those nuchal glands (i.e., toads) are absent from their diets (Mori and Burghardt, 2000). In fact, innate behavioral responses to predators are known from a wide variety of animal taxa, not only from reptiles (see review in Coss, 1999).

However, despite differences between treatments, the fact that UP adults did tongue-flick less than adults from any other site indicates that some "relaxation" of the behavior may be occurring (e.g., Coss, 1999). The difference in response to ophiophages between adult and neonate UP snakes may indicate that 1) there may be an maturational

shift in the behavior, or 2) some environmental influence is shaping the responses of the adults. The experiment I designed to examine the plasticity of gartersnake responses to ophiophagous chemical cues suggests that latter (see Experiment IV below).

Other site differences indicate that LP and HI animals were more reactive than other populations and GI animals moved less than HI animals, but since all of the populations I sampled encounter milksnakes, except UP animals, it is unclear what these differences may indicate. My data also indicate that adults react differently to ringneck chemical cues than when tested in the control treatment, but that neonates do not exhibit this difference. In addition, some site differences were detected for neonates with MM animals moving less in the presence of ringneck chemical cues than GI babies and with MM babies moving less than LP babies in the presence of milksnake cues. The significance of these additional differences is not known.

EXPERIMENT III: PLASTICITY OF RESPONSES TO A SIMULATED PREDATORY ATTACK

Introduction—If differences in antipredator behavior exist between neonates and adults from the same populations, one possible mechanism that may be driving such change is phenotypic plasticity (Via, 1994; Via et al., 1995). As with the experiments designed to examine geographic variation in antipredator behavior, the influence of phenotypic plasticity on antipredator behavior was tested in two similar fashions. Both Experiment III and IV utilized lab-born neonate *T. sirtalis* born to wild caught pregnant females from each of the various sites to determine what effect if any experience has on both antipredator behavior in general and response to ophiophagous snakes, respectively.

For both experiments (described below), neonates were reared under conditions that I thought would lead either to a heightened development of antipredator behavior or a relaxation of antipredator behavior. Experiment III utilized the standardized antipredator behavioral test as described above to examine the role of phenotypic plasticity on antipredator behavior in general.

Methods—One hundred and eighty eight neonates were evenly split and randomly assigned, in terms of sex, site (LP, SM, MM, GI, and HI), and litter (19 different litters total), to one of three groups. Since more LP neonates were available than neonates from any other site and since sex ratios of litters tended to be female biased (although not significantly; Placyk, 2006), all three groups were LP neonate and female biased. UP neonates were not included in this experiment, as this experiment was carried out in 2001 when no pregnant UP females were captured. Individuals from one group were handled only when their cage required cleaning, one group was gently handled (e.g., held in hand and prevented from falling) for 60 sec at a time on a weekly basis, and one group was roughly handled (e.g., held in a shaking hand) for 60 sec at a time on a weekly basis. After 42 days, all three groups were tested again using the standardized antipredator behavioral test. The 42 day treatment period began 10-12 days after birth, after Experiment I and behavioral testing not related to this study had been carried out. At the end of the 42 week treatment period, several snakes from each treatment group either died or escaped (5 from control group, 7 from handled group, and 9 from shaken group) (Table A.1; tables A.1 – A.22 are located in the Appendix). Treatments were carried out by Michael Boling, an undergraduate assistant, and all behavioral tests were

carried out by John S. Placyk, Jr.

Statistical analyses—Data collected following the 42 day treatment protocol were examined using one-way ANOVAs and chi-square tests of independence to detect differences in behavior due to the treatment group (not handled, handled gently, handled roughly) the animal was assigned to. Tukey-Kramer multiple-comparison tests and Fisher exact probability tests were then used to detect significant pairwise differences. Differences due to sex were examined using two-sample *t*-tests and chi-squared tests of independence, while site and litter effects were detected using nested one-way ANOVAs and chi-squared tests of independence.

Results—Before the 42 day treatment period began, neonates that were placed in the three different treatment groups (control, handled, shaken) did not differ across treatment groups in the number of times they fled ($F_{2,137} = 0.29$, $P = 0.75$; Fig. 3.6) from a simulated predatory attack or in the frequency of strikes ($X^2 = 1.23$, $G^2 = 1.23$, df = 2, $P > 0.05$) and bites ($X^2 = 1.14$, $G^2 = 1.15$, df = 2, $P > 0.05$). At the end of the 42 week treatment period, differences due to the treatment were detected for fleeing ($F_{2,123} = 134.75$, $P < 0.001$; Fig. 3.6), striking ($F_{2,123} = 6.87$, $P = 0.001$; Fig. 3.7) and flattening ($X^2 = 6.94$, $G^2 = 7.17$, $df = 2$, $P < 0.05$). Specifically, animals that were roughly handled fled more, struck more, and flattened their bodies more (42 % compared to 20.4 and 22.6 % for control and handled animals, respectively) than animals that were held or not handled ($P < 0.05$). No differences in biting, tail-wagging, musking, urinating, or defecating were detected ($P > 0.05$).

Differences within treatment groups between the two testing periods were also detected. Specifically, animals in the control group (t_1 = 2.96, $P < 0.01$; Fig. 3.6) and in the group that was gently handled (t_1 =2.64, $P = 0.01$; Fig. 3.6) fled less at the end of their treatments, while animals from the roughly handled group fled more at the end of their treatment (t_1 = -18.07, $P < 0.0001$; Fig 3.6). In addition, all three treatment groups bit more at the end of the treatment period than at the beginning ($P < 0.05$) and the two groups that were handled struck more at the end of their treatments than before the treatments were initiated (52.8 % vs. 20.8 % for the group that was gently handled, 90.2 % vs. 21.6 % for the group that was handled roughly; $P < 0.05$; Fig. 3.7), while the control group did not vary in the frequency of strikes they exhibited between the two testing periods (41.8 % vs. 29.1 %; $P > 0.05$).

No sex effect was detected for either fleeing ($F_{1,123}$ = 2.23, $P = 0.14$) or striking ($F_{1,123}$ = 0.70, $P = 0.41$). In addition, no site effect was detected for fleeing ($F_{4,123}$ = 1.28, $P = 0.30$) or for striking ($F_{4,123}$ = 0.35, $P = 0.84$). When controlling for a litter effect by nesting litters in each site, both fleeing ($F_{14,123}$ = 1.91, $P = 0.03$) and striking ($F_{14,123}$ = 1.79, $P = 0.05$) exhibited significant litter effects.

Discussion—At the end of the treatment period, snakes in the group that experienced simulated predatory attacks on a regular basis fled more and struck more than animals from the other two treatment groups. In addition, snakes from all three treatment groups bit more, snakes that were gently handled struck more, and snakes from

both the control group and the group that were gently handled fled less at the end of the treatment period. These results indicate two things. First, environmental influences appear to effect the antipredator behavior exhibited by adult snakes, which may be one reason I saw differences between antipredator responses displayed by adults and neonates. Second, maturational shifts in behavior, as, perhaps, evidenced by the increase in biting across all treatment groups, may also account for differences in adult and neonate behavior. In fact, ontogenetic (maturational) shifts of this type are well known for gartersnakes even within only the first few months of life (e.g., Herzog et al., 1992).

Herzog (1990) carried out a similar study with neonate gartersnakes from Wisconsin and found that early experience with simulated predatory attacks and the resulting increase in antipredator behavior were relatively long-lasting, further supporting my data and assertions that the antipredator behavior of the adults from my study may have resulted from phenotypic plasticity. In fact, changes due to ontogenetic (maturational) shifts are believed to cease entirely 40-60 days after birth (Herzog, 1990; Herzog et al., 1992).

EXPERIMENT IV: PLASTICITY OF RESPONSES TO OPHIOPHAGOUS SNAKES

Introduction—As with Experiment III, Experiment IV was designed to examine the influence of phenotypic plasticity on antipredator behavior. Specifically, Experiment IV focused on responses to ophiophagous snakes. While responses to ophiophagous snakes appear to be maintained at birth in populations of snakes that no longer occur with

such predators, there is some confusion as to how such responses may change over time (e.g., Weldon and Burghardt, 1979; Weldon, 1982; Gutzke et al., 1993). Some studies indicate that the behavior is maintained throughout the life of potential snake prey and change very little, while other studies indicate that regular exposure to ophiophagous snakes is required to maintain such responses (e.g., Weldon and Burghardt, 1979; Weldon, 1982; Gutzke et al., 1993). When including this experiment with the results from Experiment II, I provide one of the more complete examinations of response to ophiophagus snakes by a potential prey species.

Methods—Sixty neonate snakes were randomly assigned in respect to site (LP, MM, SM, GI, HI), litter, and sex to one of two treatment groups. UP animals were not included in this experiment, as it was carried out in 2002 when no pregnant UP females were collected. Eight to 10 days following the initial testing described in Experiment II and other behavioral testing, one group of 30 was exposed to ophiophagous snake chemical cues on a weekly basis and the other group of 30 was not (modified from Herzog, 1990). After 42 days, the two groups were tested again using the ophiophage antipredator test used in Experiment II. Four snakes from the exposed group and seven snakes from the not exposed group died or escaped before the end of the 42 day treatment period (Table A.2). Exposure to ophiophagous snake chemical cues entailed placing snakes from the exposed treatment group into a 15 X 30 X 9 cm holding cage (not their home cage) for 15 min, once a week, with a corrugated cardboard substrate that had been placed in a holding tank with one of two milksnakes, *L. t. triangulum*, for a minimum of 12 hours. Snakes from the unexposed treatment group were placed in a similar holding

cage with a corrugated cardboard substrate that had not previously been used as substrate for another animal. All treatments and behavioral tests for this experiment were carried out by John S. Placyk, Jr.

Statistical analyses—Differences in responses both within and between groups across the two testing periods were compared using repeated-measures ANOVAs with group and testing period as independent factors. Post hoc tests consisted of Tukey-Kramer tests.

Results—When compared to their responses before the 42 day treatment period, neonates in the treatment group that were not exposed to milksnake chemical cues showed a significant decrease in the number of tongue-flicks exhibited ($Z_1 = 3.92$, $P < 0.0001$; Fig. 3.8a) and in the time spent moving ($Z_1 = 3.88$, $P < 0.001$; Fig. 3.8b) when testing in the presence of milksnake chemical cues after the treatment period. No other differences were detected when comparing the number of tongue-flicks and the time spent moving for any other condition (control, ringneck, milksnake) for either treatment group (i.e., 1) those exposed to milksnake chemical cues, 2) those not exposed to milksnake chemical cues) ($P > 0.05$). However, despite the difference in behavior following treatment, the treatment group that was not exposed to milksnake chemical cues continued to tongue-flick ($F_{2,69} = 4.81$; $P = 0.01$; Fig. 3.8a) and move more ($F_{2,69} = 4.84$; $P = 0.01$; Fig. 3.8b) when tested in the presence of milksnake chemical cues than when tested in either in the control condition or in the presence of ringneck chemical cues ($P < 0.05$).

Discussion—Specifically, I found that, after the 42 day treatment period, while neonates that were not exposed to milksnake chemical cues still responded more strongly to milksnake cues than to nonophiophagus cues or in the control treatment, the number and frequency of tongue-flicks and mobility duration significantly decreased, supporting the hypothesis that adult responses to ophiophagous snakes are shaped by environmental influences. However, that differences still exist between conditions indicates there may be some benefit to maintaining the behavior. The results of my plasticity experiment do not completely correspond with those of Gutzke et al. (1993), who found that, in crotaline snakes, if snakes are not periodically confronted by ophiophagous snakes they stop responding. My snakes continued to respond to ophiophagous snakes, but responses were less intense. It is not clear how long the snakes from the Gutzke et al. (1993) study were not permitted to come in contact with ophiophagous snakes, so it is possible that if my treatment period were extended, snakes from my populations may have stopped responding to ophiophagous snakes altogether. In addition crotaline snakes (Viperidae) are from an entirely different family of snakes than gartersnakes (Colubridae), which may also influence differences between the two data sets.

EXAMINING VARIATION IN VENTRAL SCALE COUNTS

Introduction—Number of ventral scales in snakes is associated with the speed at which a snake can escape from a potential predator and is fixed at birth (e.g., Arnold and Bennett, 1988). Therefore, any differences seen in adults are most likely the result of evolutionary change. If such differences correspond with the colonization patterns of the

sites studied, historical processes and/or phylogenetic constraints may be implicated ("the ghost of selection past"). If differences correspond with differences in current predation pressures, current natural selection may be implicated. If differences do not correspond with either colonization patterns or current predation pressures, genetic drift or founder effects may be at play. In addition, by testing both neonates and adults, I could tell if the differences that were present at birth differed from adults, which may indicate that selection may be actively playing a role on the number of ventral scale counts in my system.

Methods—Ventral scales of adult snakes were counted directly after capture and ventral scales of neonate snakes were counted after all behavioral testing was completed. Since neonates are relatively small, both myself and an assistant counted ventral scales for each neonate. When our counts did not match, we both recounted the scales to correct for any counting errors. Live adults and neonates were restrained by hand and ventral scales were counted with the aid of a pen, with which a mark was placed at every 25^{th} scale in case myself or my assistant lost count. Ventral scale counts of dead animals were similarly conducted, although much less restraint was required. To determine if ventral scale counts (VSCs) for adult snakes differed based on site, a one-way ANOVA was used. A nested ANOVA was used when comparing neonate VSCs with litters nested within each site and site considered as the independent factor. King et al. (2001), using maternal half-sib analyses, found that number of ventral scales may be influenced by maternal effects. As a result, I also held mother's VSC as a covariate in the neonate VSC analysis.

Results—For both adults ($F_{5,500}$ = 7.08, P < 0.0001; Fig. 3.9a) and neonates ($F_{5,451}$ = 18.43, P < 0.0001; Fig. 3.9b) the number of ventral scales significantly differed among sites. While a nested ANOVA indicated that litter did not influence the observed site differences ($F_{35,451}$ = 1.09; P = 0.34), there was a significant effect of maternal VSC ($F_{1,451}$ = 22.21; P < 0.0001; Fig. 3.10). Specifically, the more ventral scales a mother snake has, the more ventral scales her offspring tend to have; however, this relationship is relatively weak (r^2 = 0.08; P < 0.0001).

In terms of pairwise differences, patterns of significant differences were almost identical for adults and neonates (Fig. 3.9). Both adults and neonates from the UP, MM, and HI have significantly more ventral scales than animals from the LP or GI (P < 0.05). Adults from the UP, MM, and HI also have significantly more ventral scales than adults from the SM. SM neonates have significantly fewer ventral scales than neonates from the UP and HI. Adult snakes from MM are also characterized by having significantly fewer ventral scales than those from HI (P < 0.05).

Discussion—In general, both neonates and adults varied in the number of ventral scales based on what site either they or their mother came from. Both adult and neonate snakes from the UP and HI have significantly more ventral scales than animals from the LP, GI, or SM. MM adult animals have more ventral scales than LP, SM, and GI adults, but less than HI adults. MM neonates have more ventral scales than LP and GI neonates. Overall, these site differences do not appear to correlate with predator richness; however,

selection may be acting on some of the island populations, as MM adults and neonates have more ventral scales than GI neonates and adults and occur with more predators. This may indicate either that MM snakes with more ventral scales or GI snakes with less ventral scales may have been selected for, indicating either an adaptation to deal with increased predator pressures or a relaxation of predation pressure. Regardless, the majority of the observed site differences do not correspond with predator pressures, and it is my hypothesis that these differences are more likely the result of the underlying genetics of the populations I sampled. Based on genetic data UP snakes are more closely related to Wisconsin *T. sirtalis* populations than to LP populations (Placyk, 2006; Placyk et al., under rev.) and previous work indicates that Wisconsin populations tend to have a greater number of ventral scales than LP populations (Burghardt and Schwartz, 1999). Therefore, that UP snakes have a greater number of ventral scales than LP snakes, despite occurring with fewer predators, may result from the underlying genetics of UP populations rather than current selection pressures. In terms of the islands, genetic analyses indicate that the Beaver Archipelago was colonized by both UP and LP *T. sirtalis* populations (Placyk, 2006; Placyk et al., under rev.). As a result, to find that snakes from two of the island sites (HI and MM) do not differ from UP snakes, while snakes from the other two sites (GI and SM) do not differ from LP snakes, is not all that unusual. In fact, GI is closer to the LP than it is to the UP, while HI is closer to the UP than to the LP indicating that gene flow is more likely between these pairs than vice versa, which supports my data. That snakes from MM and SM populations differ from each other, despite both being on Beaver Island, may indicate that the populations that founded the two originated from the UP and SM, respectively, and that they have

changed little over the last 10,000 years. In addition, my specific values closely match early data collected in the Great Lakes region. Specifically, Schwartz (1989) found that neonates from a Wisconsin population averaged 154.6 ventral scales, whereas neonates from a population from the lower peninsula of Michigan averaged 150.1 ventral scales. My averages for both neonates and adults from MM, HI, and UP are between 151.03 and 153, whereas adults and neonates from SM, GI, and LP average between 148.59 and 149.81. While these differences seem miniscule, even minor differences in the number of ventral scales could have a significant influence on locomotion (e.g., Arnold and Bennett, 1988). Given my data and what it known about the molecular genetics of these populations, it is likely that ventral scale count site differences represent the underlying genetics of the populations and that current selection pressures may have played only a small role in shaping the differences that are seen.

MULTIPLE DISCRIMINANT ANALYSES: COMBINING BEHAVIORAL AND VENTRAL SCALE COUNT DATA AND TESTING HYPOTHESES REGARDING OBSERVED VARIATION

Introduction—Multiple discriminant function analyses (MDA) were used allowing me to 1) combine behavioral and ventral scale count data in the same test and 2) test hypotheses regarding why the patterns of variation that I observed might exist. Such analyses also allowed me to assess the relative importance of the various phenotypic characters in distinguishing between snakes from the different sites and to test whether or not I could predict what site a snake may have originated from based on those characters.

In total, I ran five MDAs for each age class (i.e., adults and neonates) with each including all behavioral data collected from the standardized antipredator tests (See Table 3.1) and ventral scale counts.

Methods—The first MDA was used to determine how well snakes could be correctly classified to their sites of origin (i.e., LP, UP, MM, SM, HI, GI). The second combined snakes from the LP and UP to form one mainland group with snakes from MM, SM, HI, and GI forming an island group. Both the third and fourth MDA examined the role that the recent geological history of the islands may play. Specifically, the third model combined snakes from the MM, SM, and HI into one group while all other sites remained as single units. This model was based on the idea that Beaver Island and HI remained above water, while GI became submerged ca. 7,800 to 6,200 years before present (ybp) (Hough, 1958; Kapp et al., 1969; Dietrich, 1988; Petty et al., 1996). The fourth model is based on the idea that the islands were primarily recolonized by populations from the LP when the LP was connected to the archipelago via a land bridge ca. 8,900 to 7,800 ybp (Hough, 1958; Kapp et al., 1969; Dietrich, 1988). As a result, for this model, island and LP data were combined and UP data was held as a separate group. The fifth MDA grouped island and UP data and held LP data separate based on recent mtDNA analyses that indicate that the island populations are more closely related to UP populations than to LP populations (Placyk, 2006; Placyk et al., under rev.)

If any overall MDA was significant, the contribution of each phenotypic character to the discriminant function(s) for each model and the ability of each discriminant model to correctly predict and classify which site a snake originated were reported. SPSS (SPSS Inc.) was used for MDAs.

Results—All ten discriminant models (5 for adults, 5 for neonates; Tables A.3-A.22) were highly significant with the majority having *P*-values below 0.001; however, these models varied significantly in their ability to correctly classify individuals. For adults, when snakes were split into groups based on their site of origin (Wilks' Lambda = 0.27; X^2 = 387.56; df = 50; $P < 0.001$; Table A.3), 50.8 % were correctly classified (Table A.4), while the island/mainland model (Wilks' Lambda = 0.77; X^2 = 77.98; df = 10; $P < 0.001$; Table A.5) correctly classified 89.9 % (Table A.6). The model used to examine the role high lake levels may have played when several islands were submerged (Wilks' Lambda = 0.47; X^2 = 224.64; df = 30; $P < 0.001$; Table A.7) correctly classified 65.7 % of individuals (Table A.8), while the model used to test the influence of the islands being connected to the LP (Wilks' Lambda = 0.72; X^2 = 100.22; df = 10; $P < 0.001$; Table A.9) correctly classified 98.4 % of individuals (Table A.10). Finally, the model in which the ability of the underlying genetics of the populations were used to correctly classify individuals (Wilks' Lambda = 0.82; X^2 = 58.83; df = 10; $P < 0.001$; Table A.11), a hit ratio of 88.3 % was observed (Table A.12). While the ability of these models to correctly classify individuals varies, in all five the majority of variation was usually explained by three behaviors (i.e., fleeing, biting, and striking).

As for neonates, the discriminant model that held all six sites separate (Wilks' Lambda = 0.63; X^2 = 150.33; df = 50; $P < 0.001$; Table A.13) was able to correctly classify 41.4 % of individuals (Table A.14), while the island/mainland model (Wilks' Lambda = 0.90; X^2 = 33.42; df = 10; $P < 0.001$; Table A.15) correctly classified 63.1 % (Table A.16). The two models based on the recent geological history of the islands correctly classified 47 % when MM, SM, and HI were combined as one unit (Wilks' Lambda = 0.70; X^2 = 117.95; df = 30; $P < 0.001$; Tables A.17 and A.18) and 74.1 % when LP was combined with the islands (Wilks' Lambda = 0.92; X^2 = 27.88; df = 10; $P < 0.01$; Table A.19 and A.20). The model based on the underlying genetics of the populations (Wilks' Lambda = 0.83; X^2 = 61.39; df = 10; $P < 0.001$; Table A.21) correctly classified 65.5 % of all individuals (Table A.22). For the majority of these models, ventral scale count contributed to the majority of the variation in the data; however, in the model which held all six sites as separate units and the model in which MM, SM, and HI sites were combined, fleeing helped explain 28.8 % and 23.6 % of the variation, respectively.

The detection of multicollinearity in multivariate data sets is typically detected in two fashions: 1) by running *t*-tests to determine if variables in the model are significantly different from each other or not, and 2) examining correlation coefficients (*r*) to see if a strong correlation exists between any pairs of the variables. In terms of what correlation coefficient value should be used as a cut-off has been debated, with the most conservative value being $\pm$ 0.5 and the least conservative being $\pm$ 0.99. (e.g., Johnson, 1998; Hintze, 2001). For my analyses, I used use an *r* of $\pm$ 0.90 as the cut-off value, as it is commonly

used by multivariate statisticians (e.g., Johnson, 1998). Given this, and the fact that sample sizes were large (n = 307 for adults; n = 336 for neonates) (Hintze, 2001), multicollinearity was not detected for either the adult or neonate data sets, with all but one correlation coefficient being above 0.50 (neonate biting vs. neonate striking).

Discussion—The discriminant analyses allowed me to 1) combine VSC data and behavioral data, 2) determine which characters contribute most to distinguishing between individuals from different groups for the two different age classes, and 3) test hypotheses regarding why phenotypic variation in antipredator-related traits may exist in my system. For all models, most variation in the adult data sets resulted from three behavioral measures (i.e., fleeing, striking, and biting); however, when I ran the same models with the VSC data removed, the models were less significant and less able to correctly classify individuals indicating a benefit to combining morphological and behavioral data. The majority of the variation in the neonate data was better explained by ventral scale counts. If the antipredator behavior measures recorded in my tests are strongly influenced by environmental factors or maturation rather than genetic factors, differences in those measures may become more pronounced the longer an animal lives. This may explain why adults could be distinguished based on the behavioral measures, but the neonates could not. However, ventral scale number, which appears to have a maternal component and is fixed a birth, allows for the distinction of the neonates, as any differences in behavior in naïve neonates are not strong enough directly after birth. However, the number of ventral scale counts may give a better indication of the underlying genetics of my populations, while the antipredator behaviors may be a better indicate of the role of

environment on the populations. By constructing different models that rearranged individuals from the six sites into different groups to test different hypotheses regarding variation in this system, some support for differences between the neonate and adult data sets as discussed above can be seen.

The two adult discriminant models that were best able to classify individuals included 1) the model that tested the hypothesis that island animals are more like LP animals than like UP animals based on geological data that indicate the Beaver Archipelago was connected to the LP via a land bridge and 2) the model that divided snakes into either an island and mainland group. In addition, the model based on the data that indicate the genetic composition is more closely related to UP populations was the third best model. Similarly, neonates were best classified by the latter two models with the island/mainland model following close behind. These results indicate several things. To begin, there appears to be some genetic component to the antipredator-related traits used in the models that is detectable in both adults and neonates. This may be either the result of historical processes (based the islands being colonized by both the UP and LP) and of recent processes (current gene flow between the islands and the UP and LP). In addition, that both adult and neonate snakes from the different populations could be accurately classified based on if they were from either the mainland or islands suggests that some evolutionary genetic change, possibly due to different selection pressures experienced by island populations, has occurred in island populations. Analyses of mtDNA sequences (Placyk, 2006; Placyk et al., under rev.) also suggest this with 7 (18.9 %) haplotypes (out of 37 found in populations from across the Midwest) being unique to

island populations. In addition, the island/mainland models provide some evidence for the presence of plasticity in this system, as the adult model was able to correctly classify 89.9 % of individuals while the neonate model only correctly classified 63.1 %.

GENERAL DISCUSSION

Several Michigan populations of the common gartersnake (*T. sirtalis*) show geographic variation in both antipredator behavior and number of ventral scales and the variation that was detected between the two types of traits does not correspond with each other, at least not for adult snakes. In addition, it appears as though both evolutionary processes and phenotypic plasticity play a role in shaping behavioral variation, but since the morphological character I measured was discrete, I can only tell that it has been influenced by genetic processes. Finally, historical processes and the molecular phylogeography of the populations appear to play a major role in both the variation in behavior and morphology that I detected.

The data indicate that the differences in antipredator behavior exhibited by my populations are most likely the result of both environmental and genetic influences. While site differences in adult antipredator behavior indicate that populations appear to have adapted to variation in environmental influences, by experimenting with neonates, I found that such differences are most likely also the result of phenotypic plasticity, which is further confirmed by the plasticity experiments I conducted. Without examining the genetics of these behaviors via neonatal analyses, the differences exhibited by the adults may have been attributed solely to microevolutionary behavioral divergence. Therefore,

it is essential that experiments similar to those I carried out be conducted in other studies on behavioral variation when possible. In addition, without knowing the molecular phylogeography of my populations, I would have been unable to interpret the variation in behavior seen at the neonate level. While phylogenetic relationships have been used to explain differences in behavior across taxa at the interspecific and higher order levels (e.g., Martins, 1996), few have used them at the intraspecific level (Foster, 1988, 1994). Finally, many have suggested that behavioral divergence occurs more quickly than morphological divergence, the majority of my data indicate that neither of the traits have undergone significant divergence or evolutionary change in my populations providing little if any support for such a hypothesis.

Why might we not see much evolutionary change in the Beaver Archipelago *T. sirtalis* populations? To begin, since my populations have only been separated from each other, geographically, for a maximum of 18,000 years, it is possible that insufficient time has passed for such divergence to occur; however, recent work with insular tiger snake populations indicate that morphological and behavioral divergence may have occurred in less than 100 years in that system (Aubret et al., in press). It is also possible that gene flow among my populations may be preventing genetic changes that may lead to behavioral and morphological divergence, as preliminary analyses using microsatellite markers suggest that gene flow between these populations occurs on a regular basis (Placyk, unpub. data). However, recent work in this area indicates that phenotypic divergence may still be possible despite high levels of gene flow (Jordan et al., 2005). This may be due to the strength of selection pressures acting on a population. Given that

T. sirtalis is considered a generalist species that can adapt to a wide variety of habitats and predator/prey regimes, the selection pressures needed to drive evolutionary change in phenotypic traits may need to be relatively strong in this species before evolutionary change is seen. The combination of weak selection pressures, the time since my populations colonized the islands, and the apparent gene flow between island and mainland populations may be why I can attribute few differences to genetic change at this point.

In terms of where my results fit into the current literature on behavioral differences in insular systems, I found no general island vs. mainland trend. Only the standardized antipredator testing indicated any type of island/mainland differences that would suggest that island populations are less reactive or more "tame" than mainland populations. However, such differences were only seen for adults from GI and HI. The traditional view that island populations or species become less fearful on islands is based on the idea that there are generally less predators in island systems. This idea is supported by island biogeography theory and appears to apply to GI and HI populations. However, individuals from both BI sites tended not to differ much from individuals from mainland sites. Given that BI has roughly the same number of species of predators as UP and LP sites, this is not surprising; however, the general idea that fewer predator species occur on islands needs to be taken into account in future studies. GI and HI populations do occur with fewer predators and therefore fit the traditional view that there tend to be fewer predators on islands which may translate into relaxation of predator pressures that may lead to phenotypic changes.

To summarize, my study is one of few on geographic variation in behavior, in which the underlying genetics of the populations under study were taken into account by both testing neonates naïve to environmental influences and by utilizing molecular phylogeographic data. In addition, the predator composition at my sites has been documented and a morphological character has also been examined making this one of the most complete studies on geographic variation in antibehavior to date. Second, this study provides much needed data on the antipredator behavior of island fauna at the intraspecific level, as only a few empirical studies of population-level antipredator behavior variation in insular systems exist (e.g., Blazquez et al., 1997; Bonnet et al., 2005). In addition, much of the information that does exist and that often forms the basis of hypotheses generated for intraspecific level studies are often based on the more readily available studies done at the interspecific level. My data may provide a better framework from which to base future hypotheses and studies on behavioral changes in island systems at the population level.

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APPENDIX III

TABLES AND FIGURES

Table 3.1. Antipredator behaviors exhibited by common gartersnakes, *Thamnophis sirtalis*, during standardized antipredator testing (modified from Mori et al., 1996).

Antipredator Behavior	Description
Flee	The snake rapidly crawls away from the stimulus object. The snake may move around inside the arena or attempt to climb up the wall of the arena.
Anal gland discharge (musking)	The snake expels products of the cloacal glands.
Defecation	The snake expels feces from the cloaca.
Urination	The snake expels uric acid from the cloaca.
Strike	The snake, with either closed or open mouth, rapidly orients the head toward the stimulus object or straightens the anterior body to strike the stimulus. Actual contact with the stimulus object may or may not occur, but the snake does not hold on to the stimulus with its jaws.
Bite	The snake strikes the stimulus and holds on to it with its jaws.
Head hiding	The snake hides its head under part of its body.
Tail wagging	The snake slowly or rapidly vibrates its tail.
Flatten	The snake dorsoventrally flattens part of its body or its head.

Table 3.2. Frequency of antipredator behaviors (except fleeing) exhibited during standardized antipredator testing by adult common gartersnakes, *Thamnophis sirtalis.* Snakes used for testing were collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). *P*-values calculated from chi-squared tests of independence. Significant site differences for each behavior are indicated by different letters. Significant pairwise differences detected by Fisher exact probability tests (determined by *P*-values calculated using the Holm method).

	Site							
Behavior	LP	UP	MM	SM	GI	HI	X^2	*P*
Bite	0.15^{a}	0.33^{a}	0^{b}	0.02^{ab}	0^{b}	0^{b}	44.60	**< 0.0001**
Strike	0.23^{a}	0.33^{a}	0.01^{b}	0.09^{ab}	0^{b}	0^{b}	43.39	**< 0.0001**
Tail-wag	0.12^{ab}	0^{ab}	0.12^{a}	0.14^{a}	0^{b}	0^{ab}	17.24	**< 0.001**
Head -hide	0.12	0	0.11	0.09	0.01	0.02	9.25	0.10
Urinate	0.04^{ab}	0.33^{a}	0.12^{ab}	0.14^{ab}	0.03^{ab}	0^{b}	15.01	**0.01**
Defecate	0.08^{ac}	0.33^{a}	0.07^{a}	0.12^{a}	0.01^{bc}	0^{b}	13.54	**0.02**
Musk	0.08^{a}	0.67^{b}	0.15^{bc}	0.05^{ac}	0.03^{ac}	0^{ac}	28.89	**< 0.0001**
Flatten	0.08^{a}	0^{ab}	0^{b}	0^{ab}	0^{ab}	0^{ab}	21.76	**< 0.001**
n =	26	3	113	43	68	54		

Table 3.3. Frequency of antipredator behaviors (except fleeing) exhibited during standardized antipredator testing by lab-born neonate common gartersnakes, *Thamnophis sirtalis*. Neonates used for testing were born to mothers from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). *P*-values calculated from chi-squared tests of independence. Significant site differences for each behavior are indicated by different letters. Significant pairwise differences detected by Fisher exact probability tests (determined by *P*-values calculated using the Holm method).

	Site							
Behavior	LP	UP	MM	SM	GI	HI	X^2	*P*
Bite	0.10	0.10	0.08	0.19	0.18	0.06	5.14	0.40
Strike	0.17	0.24	0.22	0.06	0.28	0.19	5.19	0.39
Tail-wag	0.54	0.57	0.51	0.56	0.58	0.75	3.50	0.62
Head -hide	0.15	0.10	0.11	0.13	0.08	0.06	2.62	0.76
Urinate	0.34	0.33	0.34	0.31	0.26	0.44	2.06	0.84
Defecate	0.0	0.05	0.03	0.0	0.06	0.06	9.56	0.09
Musk	0.51^{a}	0.67^{ab}	0.72^{b}	0.69^{ab}	0.56^{ab}	0.75^{ab}	13.12	**0.02**
Flatten	0.0^{a}	0.10^{b}	0.05^{ab}	0.06^{b}	0.04^{ab}	0.13^{b}	13.43	**0.02**
# of litters =	12	2	11	4	8	4		
n =	154	21	79	16	50	16		

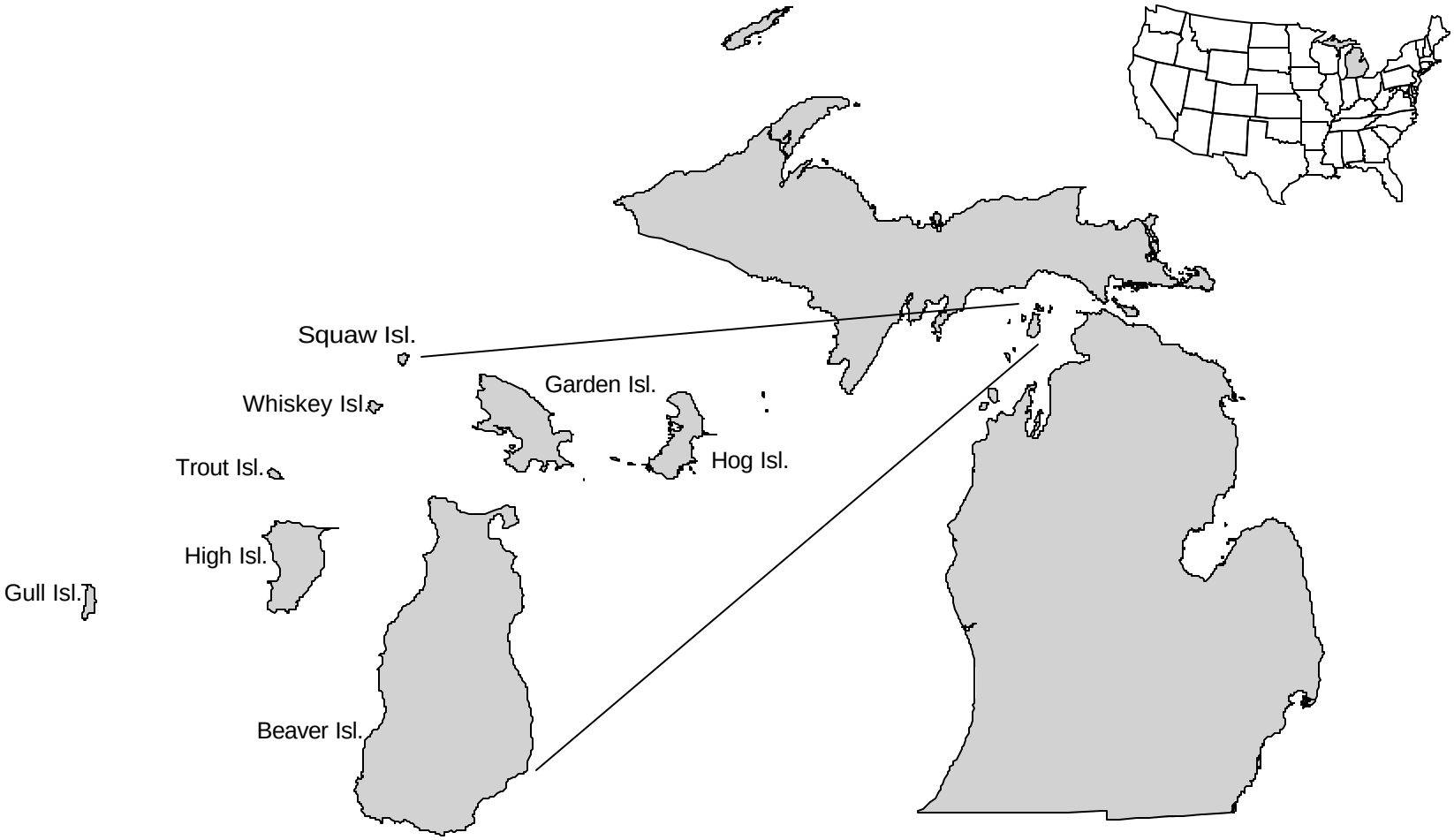


Fig. 3.1. Collection localities for common gartersnake, *Thamnophis sirtalis*, populations sampled for this study (modified from Hansknecht, 2003).

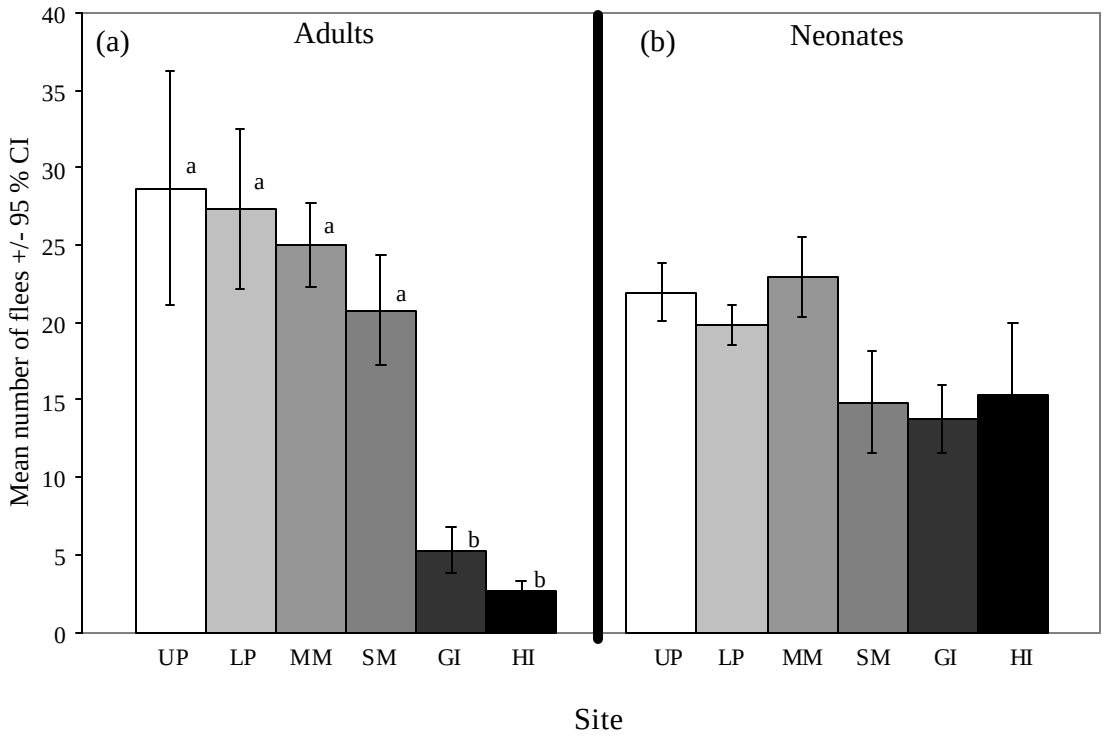


Fig. 3.2. Mean number of times (± 95% CI) adult (a) and neonate (b) common gartersnakes, *Thamnophis sirtalis*, from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI) and High Island (HI) fled from a simulated predator during standardized antipredator testing. Different letters indicate significantly different means ($P < 0.05$) within each age class. Litter means, not individual means, are shown for neonates. Sample sizes are listed in Tables 1 and 2.

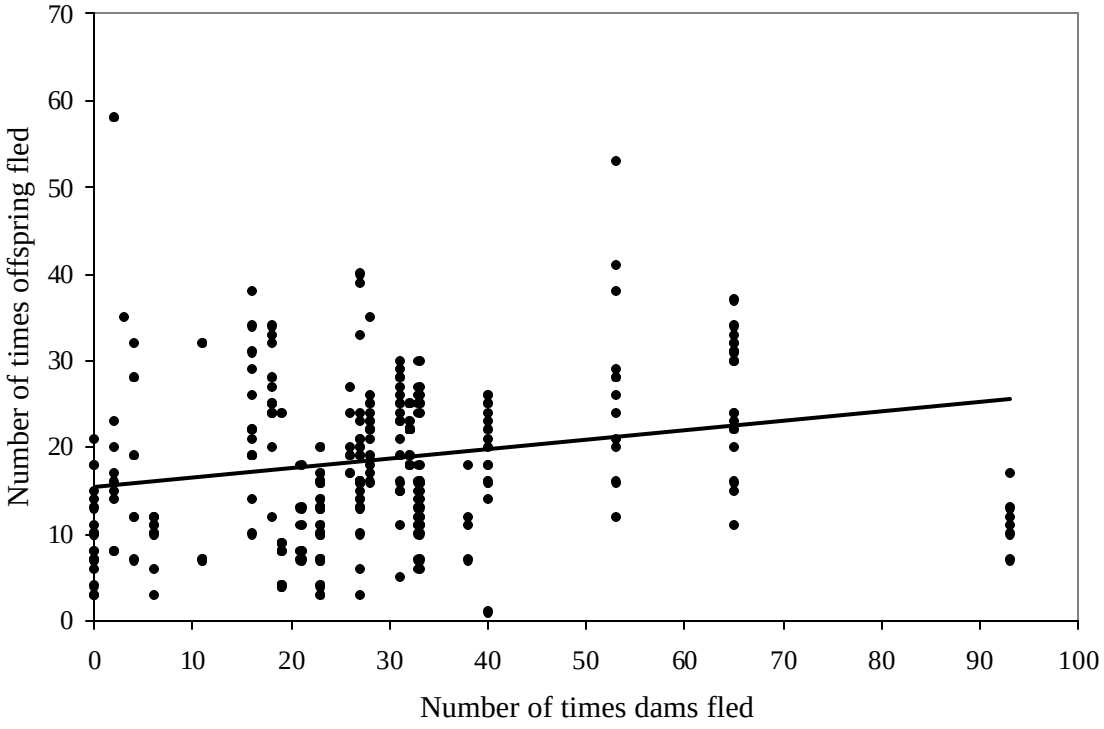


Fig. 3.3. Relationship between the number of times common gartersnake, *Thamnophis sirtalis,* dams fled from a simulated predator and the number of times their offspring fled from a simulated predator (neonate flee = 15.34 + 0.11 dam flee; $r^2 = 0.06$; $P < 0.001$).

Fig. 3.4. Mean mobility duration (sec) (± 95 % CI) exhibited by adult (n = 274) (a) and neonate (n = 117) (b) common gartersnakes, *Thamnophis sirtalis*, when tested in either a sanitized test arena or in the presence of chemical cues from either ophiophagous eastern milksnakes, *Lampropeltis t. triangulum*, or nonophiophagous northern ring-necked snakes, *Diadophis punctatus edwardsii*. Different letters indicate significantly different means ($P < 0.05$) within each treatment within each age class. Litter means, not individual means, are shown for neonates.

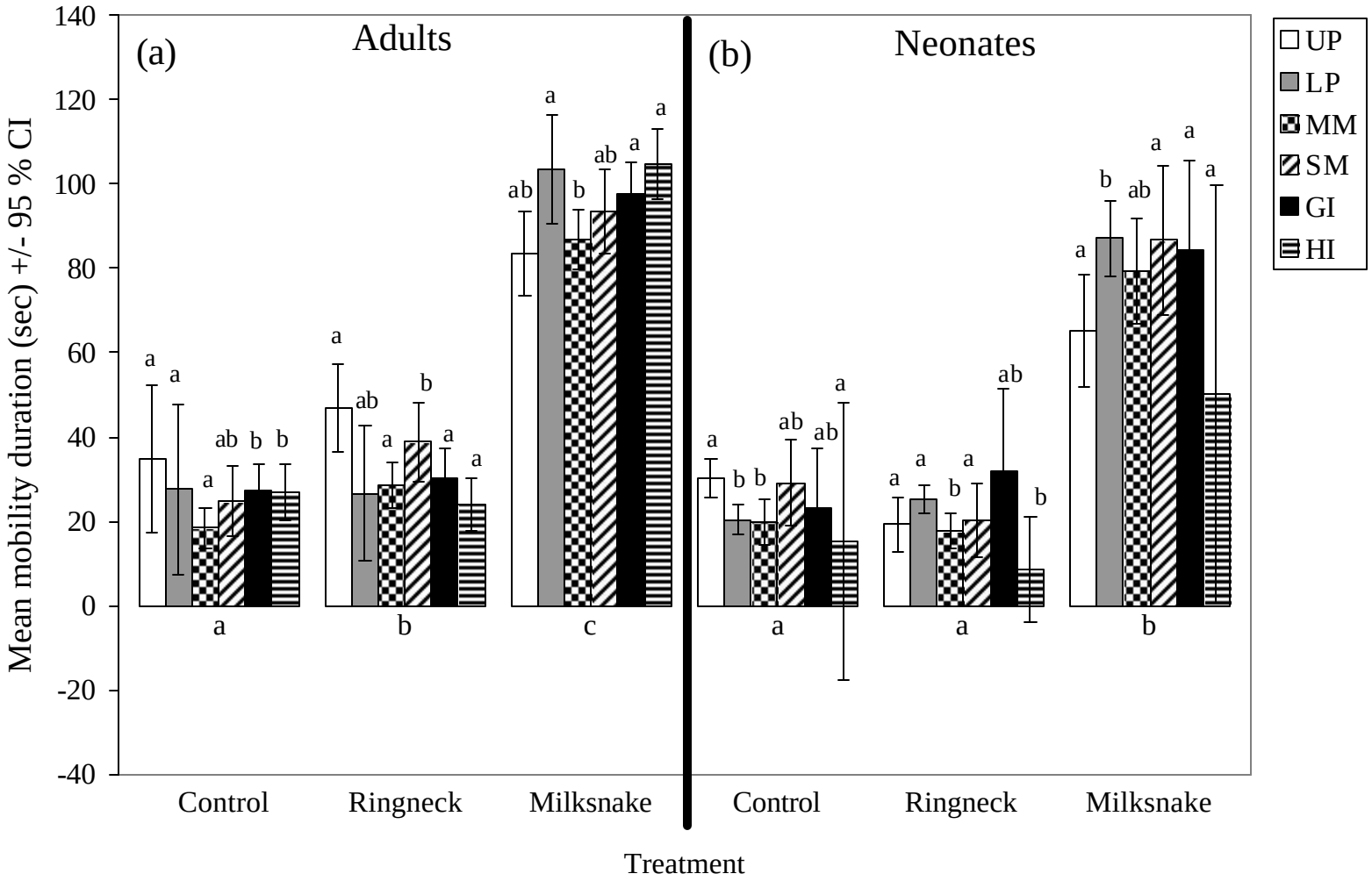
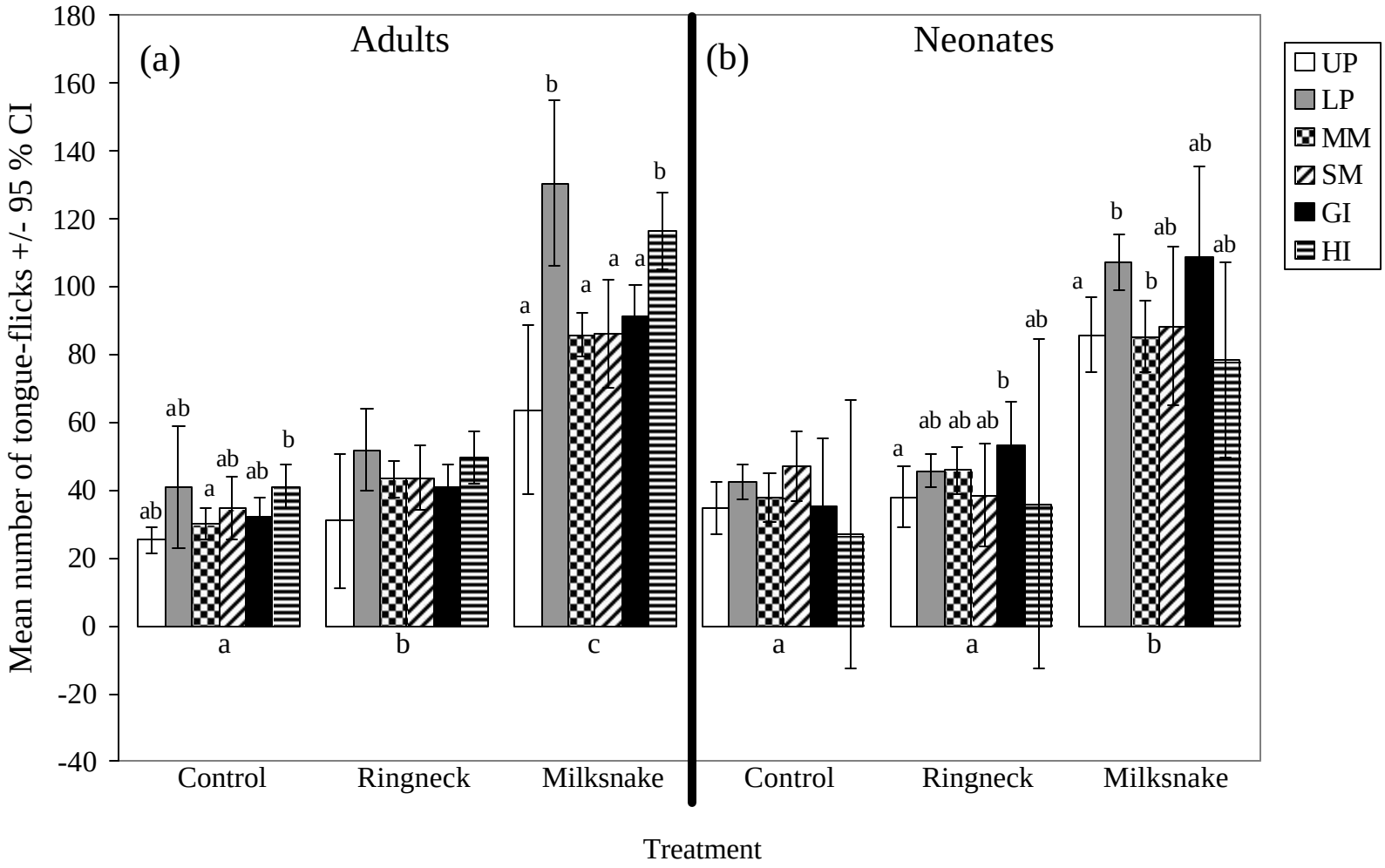

(a)
Adults
(b)
Neonates
UP
LP
MM
SM
GI
HI
Mean mobility duration (sec) +/- 95 % CI
140
120
100
80
60
40
20
0
-20
-40
Control
Ringneck
Milksnake
Control
Ringneck
Milksnake
Treatment

Fig. 3.5. Mean number of tongue-flicks (± 95 % CI) exhibited by adult (n = 274) (a) and neonate (n = 117) (b) common gartersnakes, *Thamnophis sirtalis*, when tested in either a sanitized test arena or in the presence of chemical cues from either ophiophagous eastern milksnakes, *Lampropeltis t. triangulum*, or nonophiophagous northern ring-necked snakes, *Diadophis punctatus edwardsii*. Different letters indicate significantly different means ($P < 0.05$) within each treatment within each age class. Litter means, not individual means, are shown for neonates.


(a)
Adults
(b)
Neonates
UP
LP
MM
SM
GI
HI
Mean number of tongue-flicks +/- 95 % CI
180
160
140
120
100
80
60
40
20
0
-20
-40
Control
Ringneck
Milksnake
Control
Ringneck
Milksnake
Treatment

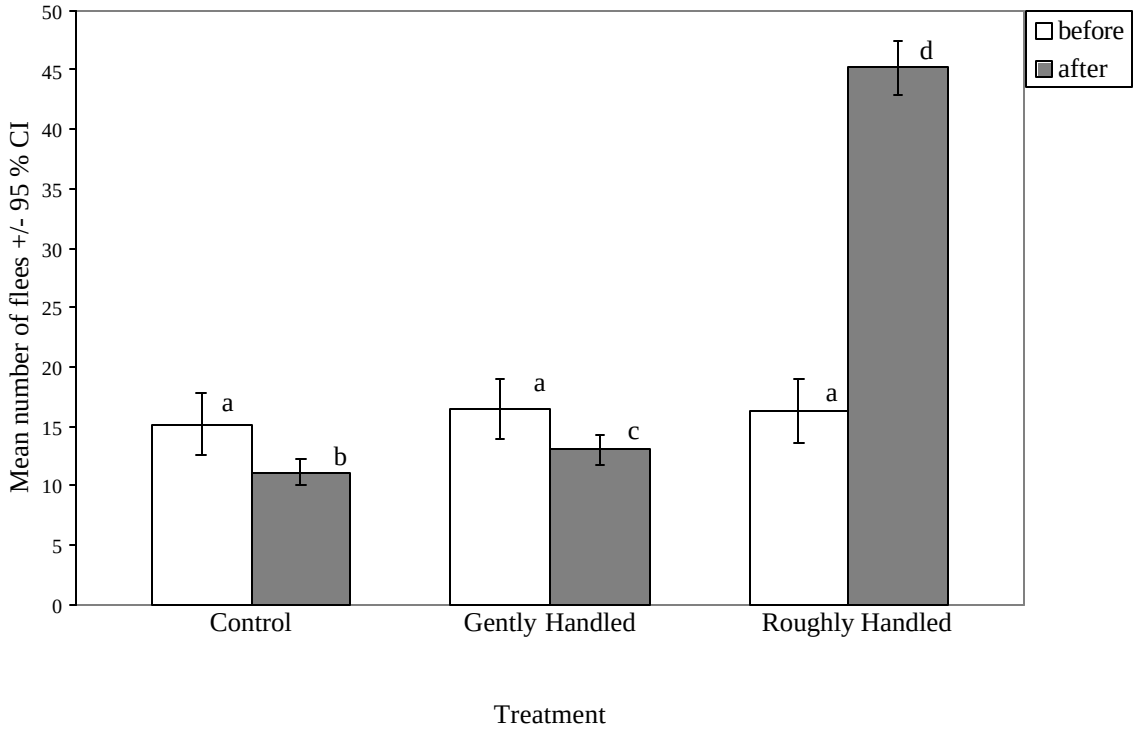


Fig. 3.6. Mean number of times (± 95 % CI) neonate common gartersnakes, *Thamnophis sirtalis*, fled from a simulated predator during standardized antipredator testing before and after treatments (not handled/control (n = 55), handled gently (n = 53), handled roughly (n = 51)) designed to examine plasticity of antipredator behavior. Different letters indicate significantly different means ($P < 0.05$).

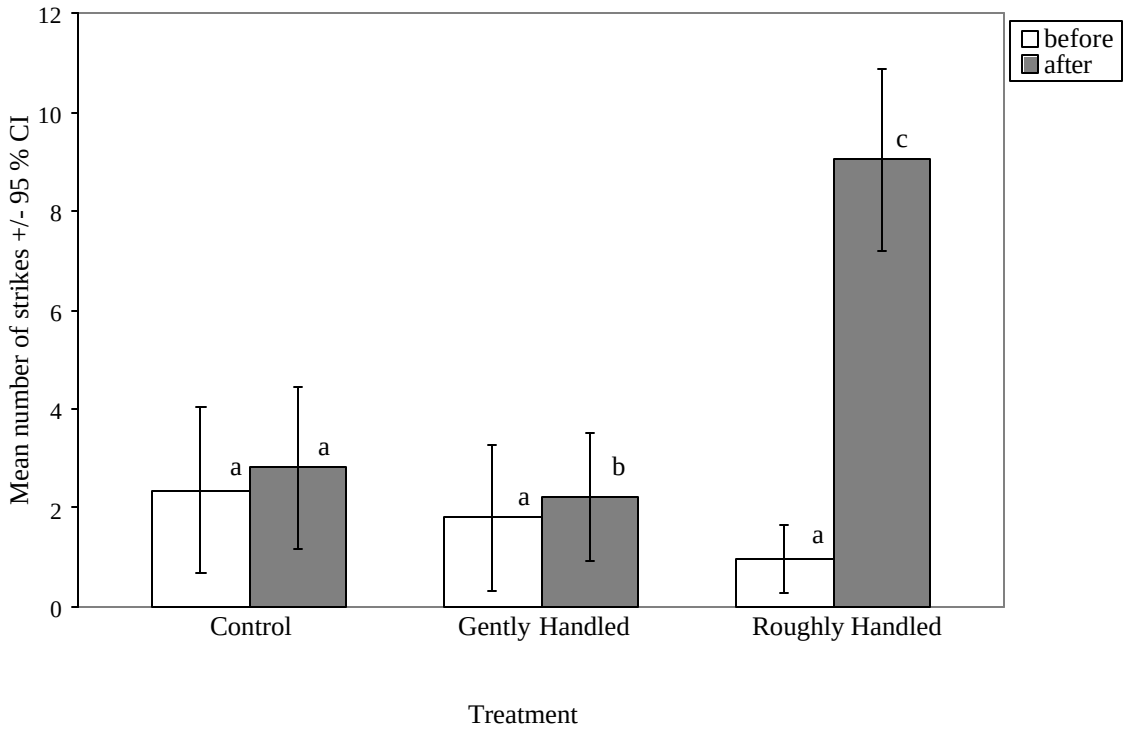
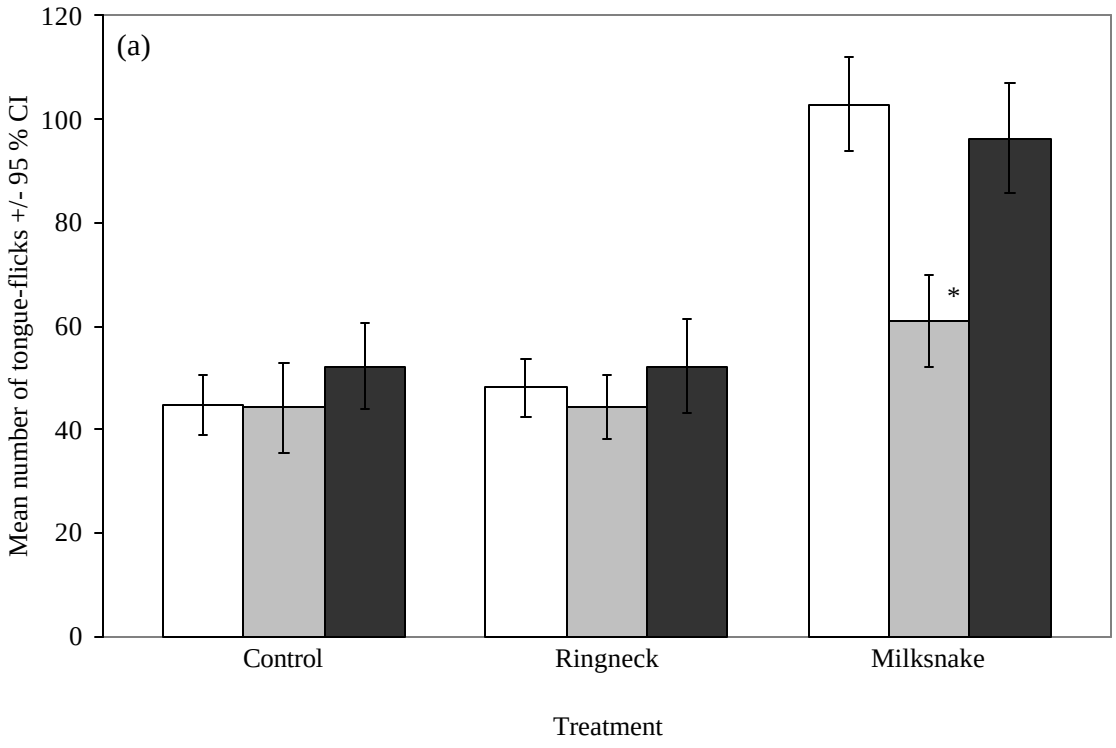
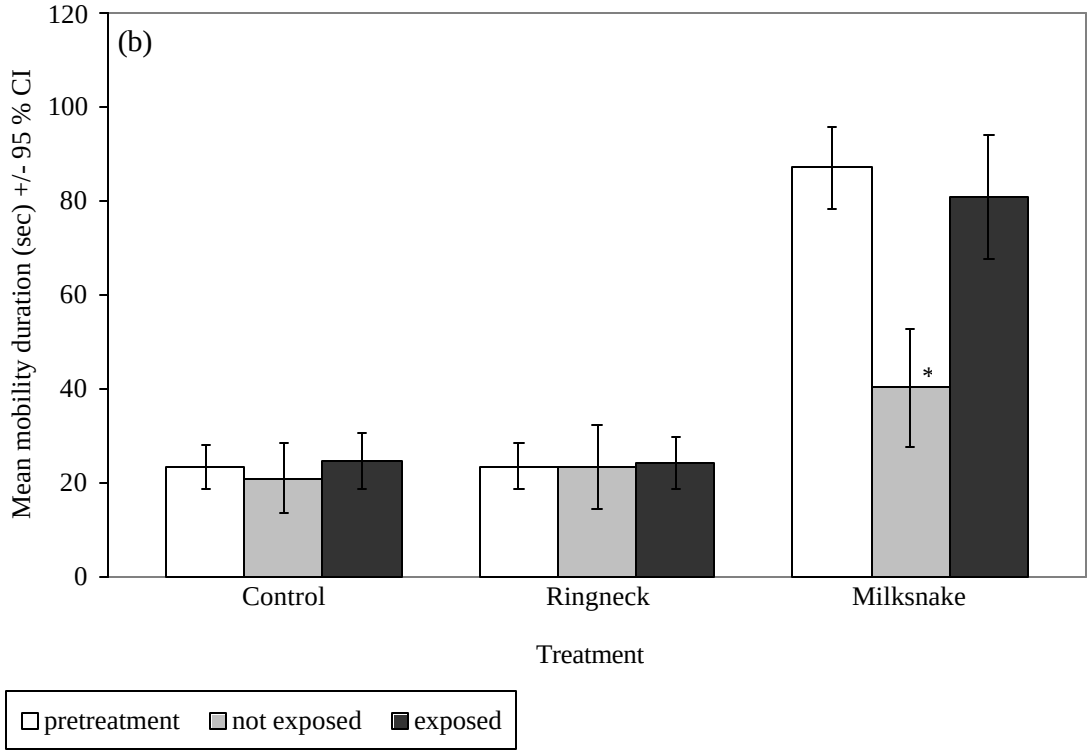


Fig. 3.7. Mean number of times (± 95 % CI) neonate common gartersnakes, *Thamnophis sirtalis*, struck at a simulated predator during standardized antipredator testing before and after treatments (not handled/control (n = 55), handled gently (n = 53), handled roughly (n = 51)) designed to examine plasticity of antipredator behavior. Different letters indicate significantly different means ($P < 0.05$).

Fig. 3.8. Mean number of tongue-flicks (± 95 % CI) (a) and mean mobility duration (sec) (± 95 % CI) (b) exhibited by neonate common gartersnakes, *Thamnophis sirtalis*, before and after treatments (not exposed to milksnake chemical cues (n = 23), exposed to milksnake chemical cues (n = 26)) designed to examine plasticity of response to ophiophagous eastern milksnakes, *Lampropeltis t. triangulum*. Neonates not exposed to *L. t. triangulum* chemical cues during the treatment period showed a significant decrease in both number of tongue-flicks and mobility duration (sec) as indicated by asterisks (*) ($P < 0.05$).


(a)
Mean number of tongue-flicks +/- 95 % CI
120
100
80
60
40
20
0
*
Control
Ringneck
Milksnake
Treatment


(b)
Mean mobility duration (sec) +/- 95 % CI
120
100
80
60
40
20
0
*
Control
Ringneck
Milksnake
Treatment
pretreatment
not exposed
exposed

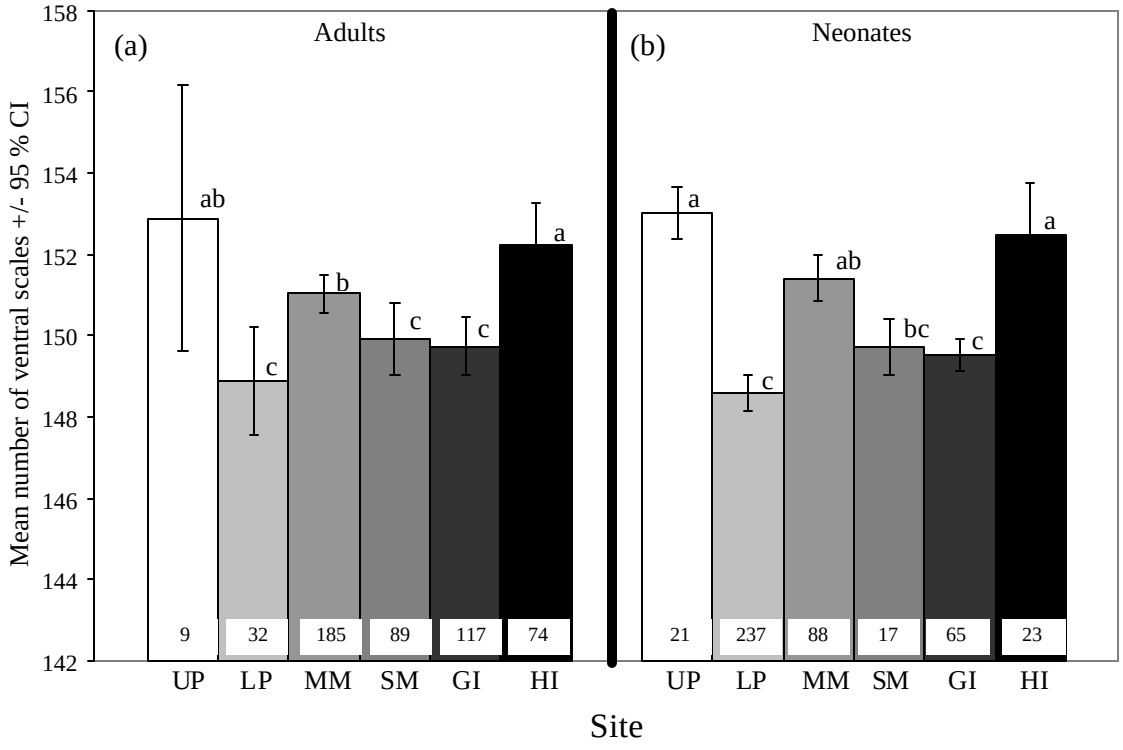


Fig. 3.9. Mean number of ventral scales (± 95 % CI) for adult (a) and neonate (b) common gartersnakes, *Thamnophis sirtalis*, from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI) and High Island (HI). Numbers at the bottom of each bar indicate sample sizes for each site and age class. Different letters indicate significantly different means ($P < 0.05$) within each age class. Litter means, not individual means, are shown for neonates.

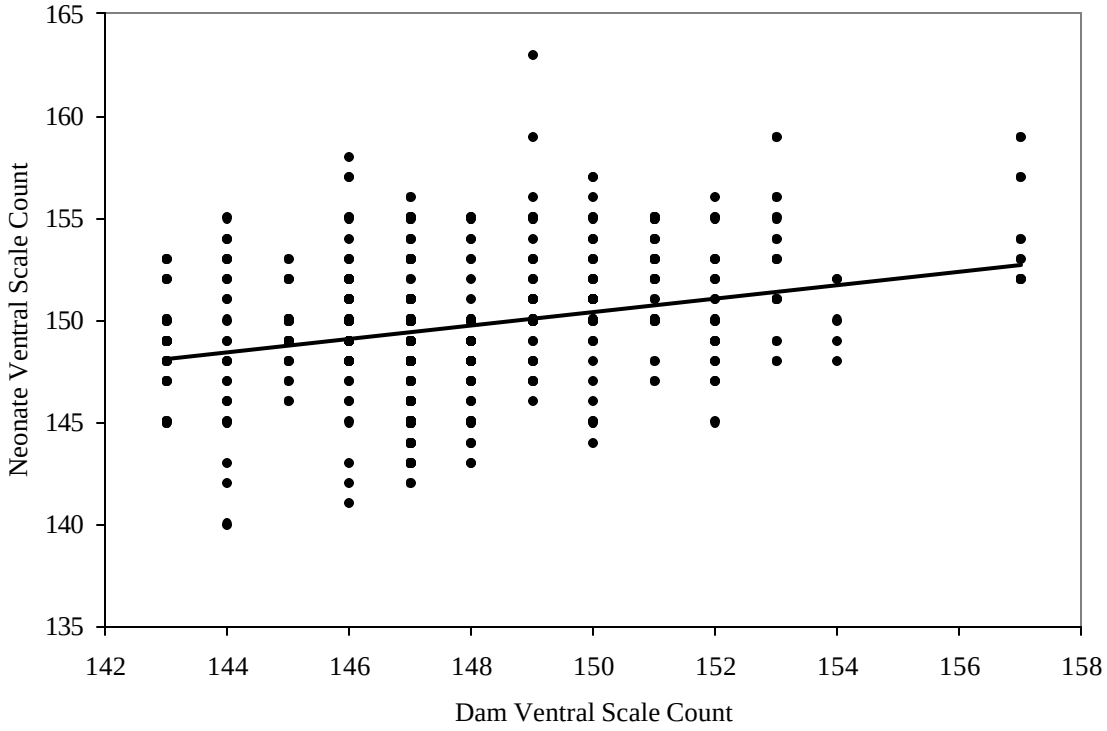


Fig. 3.10. Relationship between number of dam ventral scales and number of neonate ventral scales (neonate ventral scales = 101.29 + 0.33 dam ventral scales; $r^2 = 0.08$; $P < 0.0001$).

Part IV

GEOGRAPHIC VARIATION IN THE HEAD MORPHOLOGY AND FORAGING BEHAVIOR OF INSULAR POPULATIONS OF THE COMMON GARTERSNAKE, *THAMNOPHIS SIRTALIS*

INTRODUCTION

A wide variety of taxa exhibit significantly different morphological characters when found on islands as compared to their mainland conspecifics and congeners (e.g., Case, 1978, 1982; Heaney, 1978; Schwaner, 1985; Plummer, 1987; Shine, 1987; Schwaner and Sarre, 1988; Case and Schwaner, 1993; Aubret et al., 2004a, 2004b). These differences may be attributed to a founder effect or as the result of significantly different biotic and abiotic factors between island and mainland ecosystems resulting in different selection pressures. If selection pressures vary, the resulting differences in morphological characters could represent evolutionary change (e.g., Berry et al., 1987; Miller et al., 2000), which, in island systems, has been seen within several thousand years (Heaney, 1978) and in some genera (e.g., *Mus*, Muridae) in as little as seventy years (Berry, 1964; Berry and Jakobson, 1975). However, adaptive evolutionary change would depend not only on the strength of selection but on the amount of gene flow between populations. While gene flow is usually restricted, if not absent, in many island systems, even minimal amounts may counter selection (Vucetich and Waite, 2000), especially in small populations. As a result, many of the observed differences in ecologically important phenotypic characters that may have developed between island and mainland populations may be the result of phenotypic plasticity rather than evolutionary change (Via, 1994). Therefore, studies of variation in morphology between island and mainland populations should take into account and test for genetic (resulting from either historical processes or more recent selection), as well as environmental influences on such variation to fully understand the cause and maintenance of such variation. In addition, it would be ideal to know how selection pressures acting on the trait(s) in question differ between

populations and if morphological differences correspond with differences in other phenotypic traits (e.g., behavior), as covariation in traits is common (Arnold and Bennett, 1988) and sometimes act as evolutionary constraints (Bell, 2004). My study incorporates data on differences in selection pressures and attempts to elucidate the role that historical, recent evolutionary change, and phenotypic plasticity play in shaping differences in morphology between island and mainland populations. I also examined behaviors that one would expect to respond to the same selection pressures as the morphological characters I examined. To complete my study, I choose an archipelago with a well-documented geological history, the Beaver Archipelago in northeastern Lake Michigan.

The Beaver Archipelago consists of one main island (i.e., Beaver, the only one inhabited by people), three moderately large islands (i.e., Garden, High, and Hog), and six smaller islands (i.e., Hat, Pismire, Shoe, Squaw, Trout, and Whiskey) situated in northeastern Lake Michigan (Fig. 4.1; all figures for Part IV are located in Appendix IV). The islands are separated from the lower and upper peninsula of Michigan by approximately 30 and 25 km of open water, respectively. The recent geological history of the archipelago is marked by drastic lake level fluctuations due, in part, to complex interactions between the retreating Laurentide Ice Sheet, differential isotatic rebound, and regional climate change (Petty et al., 1996). Specifically, 13,000 to 8,900 years before present (ybp), directly following the last glaciation of the Holocene, the largest and latest proglacial lake (i.e., Lake Algonquin) occupied the three upper Great Lake basins (Larsen, 1987). During this time, the lake level around the archipelago stood at ca. 250 m with only the higher elevation islands (i.e., Beaver and High Island) remaining emerged

(Kapp et al., 1969). Starting ca. 8,900 to 7,800 ybp, lake levels dropped allowing the archipelago to be connected with the lower peninsula of Michigan and separated from the upper peninsula by the Mackinac River, which may have only been 1.6 km wide in some areas (Hough, 1958; Kapp et al., 1969; Dietrich, 1988). This low lake level persisted until ca. 7,800 to 6,200 ybp when a drastic increase in the lake level, once again, caused all but the higher elevation islands to become submerged (Hough, 1958; Kapp et al., 1969; Dietrich, 1988; Petty et al., 1996). Recently (i.e., 4,500 ybp to present day), islands of low elevation have reemerged (Hough, 1958; Kapp et al., 1969; Dietrich, 1988). As a result of these lake level fluctuations, evolutionary hypotheses as to the amount of divergence present in island populations can be developed in relation to the recent colonization, extinction, and recolonization events that may have occurred throughout the Beaver Archipelago. Since many species of plants and animals exist throughout the archipelago, I attempted to focus on a species that 1) exists on the majority of the islands and the surrounding mainland, 2) colonized the islands early on, so that any evolutionary change has had longer to work, 3) is experiencing variation in selection pressures, and 4) would be good for examining differences due to both genetic and environmental factors. One species that fits all of these criteria is the common gartersnake (*Thamnophis sirtalis*).

Snakes, in general, are excellent candidates for studying the role of genetics and environment on phenotypic traits, as many have large litter/clutch sizes, which provide an opportunity to split litters/clutches among environmental treatments to study genotype-by-environment interactions and common family environment (including maternal) effects (Brodie and Garland, 1993; King et al., 2001). In addition, in terms of studying

genetic and environmental influences on behavior, snakes have highly precocial young that can be tested when naïve to the external stimuli that elicit behavioral responses (e.g., Burghardt, 1993).

With prey generalist *Thamnophis* species, such as *T. sirtalis*, population differentiation is well known in morphology (color, pattern, size, scalation: see Rossman et al., 1996; Fitch, 1980), habitats, and food habits and geographic variation in phenotypic and life-history traits is exhibited in neonates as well as in adults (review in Burghardt and Schwartz, 1999; Brodie and Brodie, 1991; Bronikowski, 2000; Bronikowski and Arnold, 1999; Gregory and Nelson, 1991), making it an ideal genus in which to study the divergence of phenotypic characters. Several natricines reside in the Beaver Archipelago, but I choose to focus on the common gartersnake (*T. sirtalis*) for several reasons.

First, *T. sirtalis* is found on most of the islands of the Beaver Archipelago (Hatt et al., 1948; Placyk and Gillingham, 2002), as well as on the surrounding mainland. In fact, Harding (1997) lists *T. sirtalis* as being the most familiar and frequently encountered snake in the Great Lakes region. In addition, fossil evidence suggests that *T. sirtalis* have most likely existed in the region since the late Pleistocene (Holman, 2000) and were one of the primary vertebrate invaders of both the mainland surrounding the Beaver Archipelago and the islands themselves. Therefore, this taxon has had the opportunity to evolve independently throughout the entire Beaver Archipelago for at least the last 4,500 years. *Thamnophis sirtalis* prey upon and are preyed upon by a wide variety of animals

(Harding, 1997), and historical documents indicate that prey availability and predator pressures for *T. sirtalis* vary between and among the islands of the Beaver Archipelago and the surrounding mainland (Hatt et al., 1948). Therefore, traits related to foraging and antipredator related traits may vary. Finally, data on the phylogeography of Midwest *T. sirtalis* populations and the origin of Beaver Archipelago populations have recently become available (Placyk, 2006; Placyk et al., under rev.), which can be used along with the geological data on the archipelago to better understand the historical processes that may account for current variation in phenotypic traits.

Since most morphological differences that have been documented between island and mainland populations are usually related to differences in prey availability, and since prey availability is known to differ between the Beaver Archipelago and surrounding mainland, I choose to focus on traits related to foraging for this study. In particular, I was interested in head morphology and foraging behavior.

Snakes are often described as "gape-limited" predators (e.g., Arnold, 1993; Greene, 1997), because the type of prey they can take is usually dictated by characteristics of their heads, in particular, jaw characteristics. As a result, snake head morphology may reflect local adaptation to local prey resources (e.g., Forsman, 1991, 1996; Forsman and Shine, 1997). Given this, I focused on head morphology, as it is likely to vary in my system, especially given that there are differences in prey availability for *T. sirtalis* both between the islands of the Beaver Archipelago and between the archipelago and the mainland. In fact, Grudzien et al. (1992) found head shape

differences between *T. sirtalis* in this system, but their analyses were incomplete in that they did not fully explore how this variation is driven and maintained (See discussion for specifics). My work not only documents differences in head morphology between populations of the Beaver Archipelago and surrounding mainland, but it elucidates the roles historical processes, recent evolutionary change, and phenotypic plasticity play in driving and maintaining such variation. I also conducted new prey availability surveys for all of my populations and used recent phylogeographic information related to the origin of those populations and information on the geological history of the Beaver Archipelago. In addition, I conducted prey preference behavioral tests to determine if the morphological difference I observed correspond with behavioral differences and to disseminate any other relationships between the two types of traits.

The main goals of this work are to (1) update *T. sirtalis* prey availability surveys for the Beaver Archipelago and mainland Michigan, (2) determine if geographic variation in head morphology and foraging behavior exist, (3) determine if such variation corresponds to each other, and (4) determine the role of phenotypic plasticity and genetics may play in causing any such variation.

MATERIALS AND METHODS

Gartersnakes were collected from field sites in both the lower and upper peninsula of Michigan and from several islands located in the Beaver Archipelago (Fig. 4.1). Given the size of Beaver Island and this work's focus on island phenomenon, it was possible to sample two Beaver Island sites (Miller's Marsh (MM) and Sawmill (SM)) that

varied in prey availability (Table 4.1; tables 4.1 – 4.4 are located in Appendix IV). Upon initial capture, prey being utilized by each population was determined from palpation, regurgitated stomach contents (Carpenter, 1952), and observations of snakes subduing prey. Prey collected via regurgitation were fixed in 10 % formalin and preserved in 95 % ethyl alcohol for future positive identification to the nearest taxonomic group. In addition, prey availability was also determined by collecting, preserving, and positively identifying aquatic and terrestrial prey from each site. Data on available prey were used to update and verify historical accounts of the various *T. sirtalis* prey species in this system (Table 4.1).

Adult snakes used in this study were placed, by site, into 61 X 32 X 33 cm aquaria to eliminate breeding and disease transmission between different populations and permitted to acclimate for 48 h. Aquaria were kept between 20 and 25 ° C on a 16:8 Light:Dark cycle, to simulate northern Michigan summer conditions, with fresh water and shelter available at all times. After the acclimation period, adult snakes were used in behavioral trials conducted as part of a broader study on geographic variation in phenotypic traits. Following behavioral testing, snakes had their snout-vent-length (SVL) measured and had various head morphology measurements taken (Table 4.2). These particular morphological measurements were chosen, because they tend to vary within species of natricines and many of them are subject to individual, sexual, and geographic variation (e.g., Rossman et al., 1996; King, 1997). The various head measurements were taken to the nearest 0.01 mm with a pair of Mitutoyo® digital calipers; whereas, SVL was recorded to the nearest 0.1 cm using a standard meter stick. Following collection of

morphological measurements, males and nonpregnant females were then scale-clipped for future identification (Brown and Parker, 1976), had tissue samples taken for future genetic analyses, and were released to their respective capture locations.

Pregnant females (determined via palpating for developing embryos) were held until parturition after which time they were released to their respective capture locations. Morphological measurements were taken for neonates directly after birth. Neonates were then kept for behavioral testing and for head morphology plasticity experiments (see below). Neonates were maintained in 15 X 30 X 9 cm cages with shelter, offered water ad libitum, and fed chopped worms (*Lumbricus* spp.) three times per week (unless involved in an experiment that required manipulation of the food being offered). Following neonate behavioral testing and experimenting, animals were released to the site of their mother's capture, maintained for other experiments, or transferred to other research facilities. Any adult snakes held more than 72 h and all neonates were submitted to a health screening before being released.

Analyses of morphological measures—To determine if the entire suite of morphological measurements recorded differed based on site or sex, factorial MANCOVAs, with SVL as the covariate, were used. If a MANCOVA was significant, factorial ANCOVAs, with SVL as the covariate, were used to detect site and sex differences for each specific measurement. For both MANCOVAs and ANCOVAs SVL acted as a covariate, because it is assumed to relate to age or growth, with sex and site as independent variables (e.g., How et al., 1996a, 1996b). In addition, since SVL increases

in a curvilinear fashion with age, SVL data was log-transformed prior to analysis. As a result of log-transforming the data, a Least Square Means post hoc test was employed to determine any pairwise differences. Interactions between the independent variables were also examined for statistical significance. Morphological data taken from neonates were also analyzed with MANCOVAs and ANCOVAs, but in addition to having sex and site as independent factors, litter was nested within site, as King et al. (2001), using maternal half-sib analyses, found morphology may be less influenced by heritability and more reliant on maternal effects.

Five multiple discriminant function analyses (MDA) were also performed for both adults and neonates allowing me to test several hypotheses regarding variation in head morphology. The first analysis estimated the ability to assign individuals to their population of origin (UP, LP, MM, SM, GI, or HI). The second MDA was used to determine whether individuals could be discriminated between island and mainland. The third and fourth MDAs were used to determine whether the morphological variation had a pattern of differentiation similar to that predicted from the recent geological history of the Beaver Archipelago. Specifically, for the first geological history analysis, MM, SM, and HI were combined as one group, and GI, LP, and UP were each held as separate groups. These groupings are based on the idea that Beaver Island and HI remained above water ca. 7,800 to 6,200 cal yr BP when rising lake levels caused GI to become submerged. For the second geological history analysis, LP and all the island sites were grouped together and UP was held as a second group. These groupings are based on the fact that ca. 8,900 to 7,800 cal yr BP the archipelago was attached to the lower peninsula.

The fourth MDA was used to assess recent molecular phylogenetic data (Placyk, 2006; Placyk et al., under rev.) which indicate that Beaver Archipelago populations are genetically more similar to UP populations than to LP populations. Therefore, I grouped UP with all island populations and held LP as a separate group. If any of the overall MDAs were significant, the contribution of each morphological character to the discriminant function(s) for each model and the ability of each discriminant model to correctly predict and classify which site a snake originated were reported. SPSS (SPSS Inc.) was used for my MDA.

Head morphology plasticity experiment—To test for phenotypic plasticity in the morphological measurements examined above in response to prey type or size, 90 newborn *T. sirtalis* were raised on two feeding regimes: (1) half were fed one large nightcrawler (*Lumbricus terrestris*) piece twice a week and (2) half were fed two small leafworms (*L. rubellus*) or leafworm pieces twice a week. Male and female siblings from the different sites and litters were assigned to treatments at random in a factorial design. Thus, this design allowed for tests of the effect of prey size on morphological measurements while controlling for differences between the sexes and among site and litters. The two types of earthworms used in this experiment are regularly taken as prey in the diet of Michigan *T. sirtalis*. Queral-Regil and King (1998), using similar treatments, found that neonate watersnakes, *Nerodia s. sipedon* (Colubridae), offered large prey increased in body size and jaw length more than snakes offered small prey suggesting that these two morphological characters respond plastically to the size of prey. Therefore, these particular treatments will allow me to determine the influence prey size

may have on the adult head morphology of *T. sirtalis.* To account for the difference in the amount of prey taken, the number and size of prey items was selected so that all snakes were offered approximately the same mass of food at each feeding. The morphological head measurements that were recorded included: HL, HW, JL, IOD, and ED. Morphological measurements were taken 48 h prior to the onset of the experiment and again 10, 25, and 35 weeks during which the two different feeding regimes were maintained. Statistical analyses consisted of independent *t*-tests for each head measurement recorded for each time period (e.g., 10, 25, or 35 weeks).

Behavioral testing of neonates—Using data from my prey availability survey, I was able to further explore the prey preferences of animals from each site utilizing a standardized chemical extract test (Burghardt, 1969). While diet data collected from adults give some idea of prey that are being taken at each site, standardized chemical extract tests utilize neonates that are ingestively-naïve to better understand the underlying genetic basis for prey preferences and to eliminate the role environmental stimuli may be playing in adult preferences. Specifically, 9-10 days after birth, neonates were exposed to one of 6 cotton swabs soaked in either distilled water for a control or one of 5 prey surface wash (Burghardt, 1969) stimuli including earthworm (*L. terrestris*), green frog (*Rana clamitans*, Anura: Ranidae), American toad (*Bufo americanus*, Anura: Bufonidae), fathead minnow (*Pimephales promelus*, Cyprinidae), and eastern red-backed salamander (*Plethodon cinereus*, Plethodontidae). Chemosensory tests were conducted 9 or 10 days after birth with neonates not having any previous encounter with prey. Each snake was tested twice with each of the six different stimuli. Each stimulus was presented for 30

sec in a systematically balanced order followed by the presentation of the stimuli in reversed order to control for order effects. A period of at least 20 min occurred between each test. Behavioral measures included the number of tongue-flicks directed towards the cotton swab, the total number of tongue-flicks, latency to attack, and the number of attacks. If a swab was bitten the trial ended at the time the swab was bitten. All behavioral tests for this experiment were carried out by John S. Placyk, Jr.

From the tongue-flick and latency to attack data, the tongue-flick attack score (TFAS) was calculated for each individual as per Cooper and Burghardt (1990). This measure incorporates both tongue-flicks and latency to attack into a single index. TFAS(R) for repeated measures was calculated by adding the greatest number of tongue-flicks for any stimulus given by each individual in any trial without an attack to the latency component. The equation is as follows:

$$TFAS(R)_I = TF_{max(i)} + (TL - latency_i)$$

Where $TF_{max(i)}$ is the maximum of tongue-flicks emitted by individual *i* in any trial, TL is trial length in sec in the absence of an attack, and $latency_i$ is the latency of attack by an individual *i*. The TFAS(R) was natural log transformed (TFAS +1) in order to reduce the variance and heteroscadicity.

Repeated measures ANOVAs were used to test for any possible order effects. Repeated measures ANOVAs with sex and stimulus as independent variables were used to test for differences across the six stimuli. One-way factorial ANOVAs with sex and

site as independent variables and litter nested within site were used to test for differences among sites within each stimulus. Tukey tests were employed as the multiple comparison procedure. To determine if frequency of attacks varied among sites or stimuli or if sites varied within each stimulus, chi-squared tests of independence were used. If a test indicated that two variables were not independent of each other, the respective model was partitioned and Fisher exact probability tests were used to determine pairwise differences with *P*-values adjusted for multiple comparisons using Holm's method (Holm, 1979; Aickin and Gensler, 1996). Alpha was set at 0.05.

RESULTS

Adult head morphology—MANCOVAs indicate that SVL (Wilks' Lambda = 0.23; $F_{5,338} = 225.82$; $P < 0.0001$) and the site (Wilks' Lambda = 0.73; $F_{5,1257.12} = 4.39$; $P < 0.0001$) an adult snake was collected from had a significant influence on overall head morphology. In addition, while the sex (Wilks' Lambda = 0.98; $F_{5,338} = 1.64$; $P = 0.15$) of adult snakes did not have a significant influence on overall head morphology, a significant sex by site interaction was detected (Wilks' Lambda = 0.0.87; $F_{5,1257.12} = 1.93$; $P = 0.004$). To examine site differences for specific adult head measurements, univariate ANCOVAS were employed. While SVL has a significant influence for all of the head measures examined (each measure increases in size with SVL), my univariate analyses indicate that differences in individual measures do not always correspond with the suite of measures included in my multivariate analyses. For example, most of the individual measures are significantly influenced by a sex effect.

For interocular distance (IOD), the influence of both SVL ($F_{1,355}$ = 560..48; P < 0.0001) and site ($F_{5,355}$ = 6.00; P < 0.0001) were significant, with island adults having larger IODs than mainland adults (Fig. 4.2), but there was neither a sex ($F_{1,355}$ = 0.00; P = 0.96) or interaction effect ($F_{5,355}$ = 0.67; P = 0.65). For eye diameter (ED), there was both a SVL ($F_{1,355}$ = 300.64; P < 0.0001) and a site ($F_{5,355}$ = 6.75; P < 0.0001) effect, but no sex effect ($F_{1,355}$ = 0.14; P = 0.71) ; however, a significant site by sex interaction ($F_{5,355}$ = 3.38; P > 0.01) was detected (Fig. 4.3a). For head length (HL) a significant SVL ($F_{1,355}$ = 841.54; P < 0.0001) and site ($F_{5,355}$ = 7.00; P < 0.0001) effect were detected, but no sex ($F_{1,355}$ = 1.77; P = 0.18) or interaction ($F_{5,355}$ = 1.69; P = 0.14) effects were initially detected. Upon removing the interaction term from the model, however, a sex effect ($F_{1,355}$ = 4.76; P = 0.03) was revealed for HL (Fig. 4.3b) with adult females, in general, having larger HLs than adult males. For both ED and HL, island adults appear to be characterized by larger measures, but only adults from the SM show a significant difference, having significantly larger EDs and HLs than adults from any other site (Fig. 4.3).

For head width (HW), after removing the nonsignificant interaction term ($F_{5,355}$ = 1.30; P = 0.26) from the model, significant SVL ($F_{1,355}$ = 183.44; P < 0.0001), site ($F_{5,355}$ = 2.75; P = 0.02) (both present before removing the interaction), and sex ($F_{1,355}$ = 10.78; P = 0.001) effects were detected. Here, LP adults have larger HWs than three of the island sites (HI, GI, and SM), but none of the island sites differ from UP snakes (Fig. 4.4a). For jaw length (JL), significant SVL ($F_{1,355}$ = 674.66; P < 0.0001), site ($F_{5,355}$ = 10.68; P < 0.0001), sex ($F_{1,355}$ = 4.14; P = 0.04) and interaction ($F_{5,355}$ = 2.24; P = 0.05)

effects were detected. In terms of site differences, LP and SM adults have larger JLs than HI, GI, MM, and UP adults (Fig. 4.4b). As with HL, the sex effect for JL and HW both show a general trend of adult females having larger measures than adult males (Fig. 4.4). As for the site X sex interactions for both ED and JL, while, in general, both measures tend to be larger for adult females than for adult males, at some sites, this relationship is significantly reversed (Figs. 4.3a and 4.4b).

To better understand the site by sex interactions I detected for some of the head measurements and to make more powerful assessments of site differences within each sex, I also conducted ANCOVAs for adult males only and adult females only. For IOD, while adult males show no significant difference among sites ($F_{5,140} = 0.93$; $P = 0.46$), adult females appear to account for the island vs. mainland differences I saw in my full model with adult females from the islands all being represented by larger IODs than adult females from the mainland sites ($F_{5,213} = 5.55$; $P < 0.001$). In addition, UP adult females have significantly larger IODs than LP females ($P < 0.05$), which was not detected in the model that pooled the sexes.

For both ED ($F_{5,140} = 5.79$; $P < 0.001$) and HL ($F_{5,140} = 5.24$; $P < 0.001$) , adult males show the same trend that the pooled data show with SM snakes have greater measures than snakes from any other site ($P < 0.05$); however, the adult female data is more involved. For ED ($F_{5,213} = 4.50$; $P = 0.001$), Beaver Island (SM & MM) adult females have the greatest EDs , HI and GI EDs fall in between, LP adult female EDs are the smallest, and UP EDs do not differ from any other site (Fig. 4.3a). For HL ($F_{5,213} =$

3.84; $P = 0.002$; Fig. 4.3b), there appears to be an island vs. mainland trend with island adult females, in general, having greater HLs than mainland adult females, however, this difference is not seen between GI and UP adult females.

For HW, unlike with the pooled data, no differences were found among sites for either adult males ($F_{5,140} = 1.84$; $P = 0.11$) or adult females ($F_{5,213} = 2.12$; $P = 0.07$). For JL, adult male ($F_{5,140} = 7.01$; $P < 0.001$; Fig. 4.4b) and adult female ($F_{5,213} = 5.17$; $P < 0.001$; Fig 4.4b) differences roughly adhere to differences seen with the pooled data with a few exceptions. Specifically, UP adult females have smaller JLs than adult females from any other site ($P < 0.05$), while the JL of LP adult females do not differ from the JLs of HI, GI, or MM adult females ($P > 0.05$). For adult males, the only difference from the pooled data differences is that the JL of GI adult males does not differ from the JL of LP adult males ($P > 0.05$).

Multiple discriminant function analyses of adult head morphology—All five discriminant models for adult head morphology were significant ($P < 0.05$). The model in which adults were grouped based on which site they originated from correctly classified 27.9 % of individuals (Wilks' Lambda = 0.71; $X^2 = 118.0$; df = 25; $P < 0.001$; Tables A.23 and A.24; tables A.23 – A.42 are located in the Appendix), whereas the second model in which sites were grouped based on if they were either an island or a mainland site correctly classified 75.8 % of individuals (Wilks' Lambda = 0.87; $X^2 = 48.21$; df = 5; $P < 0.001$; Tables A.25 and A.26). As for the two models used to test the influence of the geological history of the islands, the one in which SM, MM, and HI were

grouped together correctly classified 44.5 % of individuals (Wilks' Lambda = 0.81; X^2 = 75.44; df = 15; $P < 0.001$; Tables A.27 and A.28), while the model in which island adults were grouped with LP adults, resulted in 77.2 % of individuals being correctly classified (Wilks' Lambda = 0.97; X^2 = 12.57; df = 5; $P = 0.03$; Tables A.29 and A.30). Finally, the model based on recent molecular phylogenetic data, which indicates the island populations are more genetically similar to UP populations, resulted in the correct classification of 76.9 % of individuals (Wilks' Lambda = 0.88; X^2 = 45.93; df = 5; $P <$ 0.001; Tables A.31 and A.32). For all but the second geological history model (e.g., UP vs. all other sites), JL accounted for the majority of variation. For the second geological history model, HL accounted for the majority of the variation.

The detection of multicollinearity in multivariate data sets is typically detected in two fashions: 1) by running *t*-tests to determine if variables in the model are significantly different from each other or not, and 2) examining correlation coefficients (*r*) to see if a strong correlation exists between any pairs of the variables. In terms of what correlation coefficient value should be used as a cut-off has been debated, with the most conservative value being $\pm$ 0.5 and the least conservative being $\pm$ 0.99. (e.g., Johnson, 1998; Hintze, 2001). For my analyses, I used use an *r* of 0.90 as my cut-off value, as it is commonly used by multivariate statisticians (e.g., Johnson, 1998). Given this, and the fact that the sample size was large (n = 355) (Hintze, 2001), multicollinearity was not detected for this data set, with all but one correlation coefficient being below 0.90 (JL vs. HL); however, a *t*-test confirms that these two variables are significantly different from each other (t = -14.13; df =708; $P < 0.0001$).

Neonate head morphology—MANCOVAs indicate that SVL (Wilks' Lambda = 0.81; $F_{5,374} = 17.31$; $P < 0.0001$), site (Wilks' Lambda = 0.79; $F_{25,1390.85} = 3.73$; $P < 0.0001$), sex (Wilks' Lambda = 0.91; $F_{5,374} = 7.09$; $P < 0.0001$), and litter (Wilks' Lambda = 0.13; $F_{185,1861.67} = 5.05$; $P < 0.0001$) have significant effects on the overall head morphology of neonates. No interaction between site and sex was detected (Wilks' Lambda = 0.93; $F_{25,1390.85} = 1.14$; $P = 0.29$). To examine site differences for specific head measurements, univariate ANCOVAS with litters nested within sites were employed. SVL, as with the adults, significantly increases with all of the head measures (except one, see below), my univariate analyses indicate that differences in individual measures do not always correspond with differences in the suite of measures included in my multivariate analyses.

Site differences were not detected for any of the neonate head measures ($P > 0.05$); however, significant sex, litter, and SVL effects ($P < 0.05$) were detected for all measures, except ED, which was not influenced by SVL. However, given that ED is extremely small in neonate *T. sirtalis* (sometimes less than 2 mm), differences corresponding with SVL may have been too small to detect with the digital calipers used for measurements. When conducting nested ANCOVAs with only neonate males or only neonate females, site differences remained nonexistent for all head measurements ($P > 0.05$). In terms of differences due to sex, the JL ($F_{1,378} = 29.52$; $P < 0.001$), HL ($F_{1,383} = 17.92$; $P < 0.001$), HW ($F_{1,383} = 16.61$; $P < 0.001$), IOD ($F_{1,383} = 20.57$; $P < 0.001$), and

ED ($F_{1,383}$ = 3.74; P = 0.05) of neonate females were significantly larger than those of neonate males (Figs. 4.5 and 4.6). Only JL had a significant site by sex interaction, which was brought about by the lack of significant difference between neonate males and neonate females from both the SM and UP site, respectively (Fig. 4.5).

Multiple discriminant function analyses of neonate head morphology—As with the adult head morphology data set, all five discriminant models for neonate head morphology were significant ($P < 0.05$). The model in which neonates were grouped based on which site they originated from correctly classified 34.1 % of individuals (Wilks' Lambda = 0.70; X^2 = 148.73; df = 25; $P < 0.001$; Tables A.33 and A.34), whereas the second model in which sites were grouped based on if they were either an island or a mainland site correctly classified 65.4 % of individuals (Wilks' Lambda = 0.85; X^2 = 68.86; df = 5; $P < 0.001$; Tables A.35 and A.36). As for the two models used to test the influence of the geological history of the islands, the one in which SM, MM, and HI were grouped together correctly classified 41.4 % of individuals (Wilks' Lambda = 0.77; X^2 = 108.43; df = 15; $P < 0.001$; Tables A.37 and A.38), while the model in which island neonates were grouped with LP neonates, resulted in 74.1 % of individuals being correctly classified (Wilks' Lambda = 0.93; X^2 = 31.31; df = 5; $P < 0.001$; Tables A.39 and A.40). Finally, the model based on recent molecular phylogenetic data, which indicates the island populations are more genetically similar to UP populations, resulted in the correct classification of 62.6 % of individuals (Wilks' Lambda = 0.90; X^2 = 45.35; df = 5; $P < 0.001$; Tables A.41 and A.42). For all neonate head morphology MDAs, IOD accounted for the majority of variation.

As with the adult data set, multicollinearity was not detected, as the sample size was large (n = 428) and each of the five head measures (i.e., JL, HL, HW, IOD, ED) showed *r* values less than 0.75 with the other head measures.

Plasticity of head morphology—After 10 weeks no significant differences in JL, HL, HW, IOD, or ED were detected between neonates fed leafworms vs. neonates fed nightcrawlers (Table 4.3; Fig. 4.7); however, the *P*-value for HW is marginally significant with neonates being fed nightcrawlers having slightly larger HWs than neonates being fed leafworms. At both 25 and 35 weeks differences in JL, HL, HW, IOD, and ED were detected with the neonates on the nightcrawler diet showing significantly larger head measurements than the neonates on the leafworm diet (Table 4.3; Fig. 4.7).

Prey chemical preferences: Tongue-flick attack scores—While the position (i.e., 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, or 7^{th}) of a stimulus in the testing order significantly influenced the TFAS ($F_{25,3977} = 1.69$, $P = 0.03$) as has been documented in the past (e.g., Schwartz, 1989), TFAS did not differ depending on if the stimuli were presented in forward or reverse order ($F_{5,3977} = 0.87$, $P = 0.51$). Given the lack of an order effect and the fact that the position of a stimulus in my presentations was balanced throughout, the average TFAS for each stimulus was calculated and used in prey chemical preference analyses as has been done in many similar studies (e.g., Schwartz, 1989; Lyman-Henley, 1991; Kirby, 2005).

A repeated measures ANOVA indicated that stimulus had a significant effect on TFASs ($F_{5,1989} = 56.73$, $P < 0.0001$, Fig. 4.8). Specifically, TFASs for the control stimulus were significantly lower than TFASs exhibited for any other stimuli ($P < 0.05$). The greatest TFASs were calculated for the worm and toad stimuli, which did not differ from each other significantly ($P < 0.05$). The salamander stimulus resulted in the third highest TFASs, which differed from toad TFASs ($P < 0.05$), but not from worm TFASs ($P > 0.05$). The frog and fish stimuli resulted in the lowest TFASs, which differed significantly from every other stimuli ($P < 0.05$) except each other ($P > 0.05$).

A significant site by stimuli interaction ($F_{25,1989} = 3.36$, $P < 0.0001$) was also detected indicating site differences in response to each stimuli. Specifically, significant differences were detected for both the frog ($F_{5,293} = 2.95$, $P = 0.02$) and salamander ($F_{4,274} = 2.78$, $P = 0.03$) stimuli, but not for the control ($F_{5,293} = .992$, $P = 0.43$), worm ($F_{5,293} = 1.17$, $P = 0.34$), toad ($F_{5,291} = 1.02$, $P = 0.41$), or fish ($F_{5,293} = 1.71$, $P = 0.15$) stimuli (Fig. 4.8). HI neonates exhibited significantly lower TFASs for the salamander stimulus than neonates from any other site ($P < 0.05$), while responses to the frog stimulus were less clear cut (Fig. 4.8). UP neonates exhibited the lowest TFAS for the frog stimuli, but did not differ from HI neonates ($P > 0.05$), which exhibited the second lowest TFAS for that stimuli (Fig. 4.8). Neonates from Beaver Island (SM & MM), GI, and the LP exhibited the highest TFASs for the frog stimuli with all differing from the UP ($P < 0.05$) and all but GI differing from HI ($P > 0.05$, Fig 4.8). In addition, a litter

effect was detected for all stimuli ($P < 0.05$), but no sex effect ($P > 0.05$) or sex X site interaction effect ($P > 0.05$) were detected.

Prey chemical preferences: Attacks—The frequency of attacks was related to site ($G^2 = 38.22$, df = 5, $P < 0.001$) with UP neonates attacking the cotton swab significantly less often than neonates from any other site ($P < 0.05$; Table 4.4). Attack frequency was also related to stimulus ($G^2 = 330.37$, df = 5, $P < 0.0001$; Table 4.4) with the frequency of attacks observed for each stimulus differing from the frequency of attacks observed for every other stimuli with one exception (i.e., attack frequency did not differ between the frog and fish stimuli). Attacks were most common with the toad stimuli (attacks witnessed in 38.5 % of trials with the toad stimuli) followed by the worm (23.3 %), salamander (12.7 %), fish (3.9 %), frog (3.0 %) and control (0 %) stimuli. In addition, site differences within each stimulus were also detected for the worm ($G^2 = 14.30$, df = 5, $P = 0.01$) and toad ($G^2 = 30.27$, df = 5, $P < 0.0001$) stimuli but not for the control (no attacks observed for any sites), frog ($G^2 = 3.53$, df = 5, $P = 0.62$), fish ($G^2 = 5.83$, df = 5, $P = 0.0.32$), or salamander ($G^2 = 6.80$, df = 5, $P = 0.15$) stimuli (Table 4.4). With the worm stimuli, after adjusting for multiple testing using the Holm method, no site differences could be detected. As for the toad stimuli, the UP neonates attacked less then neonates from any other site ($P < 0.05$) except those from GI.

DISCUSSION

The head morphology of both adult and neonate gartersnakes varied depending on the site they originated from, their sex, their length, and for neonates, their litter. For

both age classes, head characteristics increased in size with increasing snout-vent length and female head characteristics were almost always larger than corresponding male head characteristics. However, while discriminant functions indicate that variation in head morphology is explained similarly for both neonates and adults, observed site differences for specific head characters do not correspond between the two age classes. This may be due to different environmental influences experienced by snakes from each site as they grew to adulthood, as my plasticity experiment provides evidence that the head characteristics I measured were significantly influenced by the size of prey taken by neonate *T. sirtalis* in only a matter of months. Regardless, both neonates and adults could be classified to their respective sites, to some degree, possibly indicating that both genetics and environment may play a role in shaping the head morphology of these particular populations. In terms of the chemical prey preference testing, site differences were minimal and most do not clearly correspond with differences in adult or neonate head morphology, possibly due to the generalist nature of *T. sirtalis*.

Head morphology: Site differences—Multivariate analyses of both neonate and adult head morphology indicate that the suite of characters I observed distinguished snakes from different sites; however, this did not always hold when univariate analyses were employed. For example, for neonates, while both a MANCOVA and an MDA indicate significant site differences and the ability to classify snakes to their sites of origin, respectively, univariate analyses revealed no site differences for any of the individual head characters. In addition, when univariate analyses did detect site differences, the differences they detected were not always easily explained (e.g., adult

SM animals have larger eye diameters and head lengths than adults from any other site). MDAs, on the other hand, give us some idea as to what might be causing the variation I observed.

Interestingly, the percentage of variation in head morphology explained by the different MDAs showed the same trends for both neonates and adults in terms of how much variation was explained by each model. Specifically, the MDAs in which I grouped sites based on which islands remained above water ca. 7,800 to 6,200 cal yr BP, explained only 44.5 % and 41.4 % amount of variation in adults and neonates, respectively. The MDAs in which I held each site separate explained the least amount of variation for both adult (27.9 %) and neonates (34.1 %). The MDAs that explained the most variation for both neonates and adults, were the models that 1) divided the sites into either an island or mainland group, 2) were based on the genetic make-up of the island populations by grouping the UP with the islands while holding the LP as a separate group, and 3) were based on geological data that indicate the LP was connected to the archipelago ca. 8,900 to 7,800 ybp and lumped all island sites with the LP while holding the UP as a separate group. It was the latter model for both neonates and adults that correctly classified snakes correctly the best (ca. 75 % correctly classified for both neonates and adults). The significance of these last three models indicates several important issues concerning the current head morphologies seen in island snakes.

Specifically, these models indicate that both the underlying genetics and environmental factors may be influencing head morphology variation in my system.

Both the model related to the colonization of the islands from the LP and the model used to test the significance of the genetic make-up of the island populations indicate that genetic factors appear to have the greatest influence on the current head morphology seen in my system. This may be the result of historical processes or the result of current levels of gene flow between the Beaver Archipelago and the surrounding mainland. However, the model that splits the sites into island and mainland groups indicates that environmental differences between the islands and mainland may also drive morphological differences. Since this model is able to correctly classify over 75 % of adults and over 60 % of neonates there is some indication that the differences between islands and the mainland have a genetic component. However, since the adult model was somewhat better at classifying individuals than the neonate model, there is also some indication that plasticity may play a role.

In fact, my plasticity experiment clearly shows the influence of prey size on head morphology. While this laboratory experiment indicated that increases in head character lengths was directly related to a diet consisting of larger prey, transferring these results to my observations of wild adult head characteristics is not easily done, as a variety of prey and prey sizes are available and univariate analyses provided little explanation of multivariate site differences. There is no clear indication from my prey surveys that larger prey are available at specific sites, but prey richness does vary. The complication of taking into account the variety of prey taken by individual snakes in each population and the influence such a diet would have on head morphology would be a daunting task.

When adult male and adult female data was pooled, the only clear univariate result is for interocular distance, which is larger in island populations than in mainland populations. Spreading the eyes farther apart on the head may be an adaptation found in populations with higher predation pressures (e.g., Kardong, 2006), as it allows the animal to survey more of its environment; however, predator surveys (Placyk and Burghardt, 2005) indicate fewer predators on the islands when compared to the mainland. Another possibility is that larger IODs indicate broader heads, but I saw no such island/mainland trend in head width, so this option is discounted. The differences in IOD I saw may simply be the result of genetic drift or a founder effect and requires more testing at this point. When analyzing the various head measurements for adult females and adult males separately, HL also showed a mainland/island split with most island females having larger HLs than mainland females. Longer heads may be an adaptation related to foraging, but given that prey availability varies among the islands any advantage longer heads convey to island populations is unclear. The observed differences in HL may most likely be the result of either a founder effect or genetic drift. Other, less explainable differences were also seen for individual head measurements.

Differences in head width and jaw length were detected with the most obvious result being that LP adults are, in general, characterized by longer jaws and wider heads than adults from many other sites. This may correspond with the greater array of prey available at the LP site. At the same time, that the UP adults did not vary as dramatically from island adults in terms of HW and JL may indicate a genetic component to these two traits which would support genetic data indicating that island snakes are more closely

related to UP snakes than to LP snakes. Other univariate differences in adult head morphology included larger eye diameters and head lengths in Sawmill snakes when compared to snakes from every other site and I have no ready explanation for this. As for additional differences in individual sexes, the EDs of adult females are smaller in LP snakes than Beaver Island and GI adult females, but do not differ from HI adult females, while UP adult females do not differ from any other site. Given the variety of differences in head morphology observed between my 6 sites it is obvious that much needs to be taken into account when trying to explain these differences. My results indicate that genetics (both as a result of historical and current processes) and phenotypic plasticity may both play important roles in head morphology differentiation.

While others have noted significant differences in head morphology between island and mainland populations of snakes and between populations in general, none of these past studies are as detailed as mine. To begin, Grudzien et al. (1992), who also studied the head morphology of Beaver Archipelago gartersnake populations, did not examine differences due to sex or SVL, did not sample snakes from the UP, and did not observe neonates from any sites. Their results indicated that variation in head morphology in this system was most likely solely due to natural selection, as they determined the majority of the differences they detected were the result of animals being either from the islands or from the LP. While they did take into account which islands were recently submerged and found little concordance with their head morphology data, as I did, they did not take into account the underlying genetics of the populations or the fact that the islands were connected to the LP in the recent past. As a result, they quickly

dismissed the role of historical processes and proposed that all the differences they detected were genetically based and due to differences in selection pressures between the islands and the LP. They made no attempt to take into account the role of phenotypic plasticity. Krause et al. (2003) also worked in this system but only sampled from two sites on Beaver Island. The differences in head morphology they found between sites were mainly due to sex, but some were also attributed to plasticity as a result of diet differences. While Krause et al. (2003) took into account sex and SVL, the underlying genetics of their populations were not known, neonates were not examined (although these data can be found is his dissertation (Krause, 2000)), and surrounding island and mainland populations were not sampled.

In addition to work in the Beaver Archipelago and with gartersnakes, two other species of snakes (the adder, *Vipera berus*, Viperidae, and tiger snakes, *Notechis scutatus*, Elapidae) have also been widely used for studies of head morphology, but these systems and bodies of work also differ from mine (Forsman, 1991, 1996; Forsman and Shine, 1997; Aubret et al., 2004a). To begin *V. berus* and *N. scutatus* are both more specialized in terms of the prey they take than *T. sirtalis.* As a result, hypotheses generated with these species may not apply to a more generalist species like *T. sirtalis.* In addition, the studies conducted with these two species are incomplete and sometimes contradictory even for the same species. For example, Forsman (1991) found that relative head length of *V. berus* was smallest on the mainland and increased on the islands with increasing body size of its main prey and suggested that stabilizing selection for head size is acting in each population. However, in a later study, directional selection

is indicated (Forsman and Shine, 1997). Regardless of the mechanism maintaining the differences, the possibility of phenotypic plasticity influencing the differences in head shape is discounted (e.g., Forsman, 1996). However, besides running experiments that indicate that growth rate does not influence difference in head morphology, plasticity of head morphology is never directly tested. The studies conducted with tiger snakes are more complete with the most recent indicating that head size differences in tiger snakes were due to both genetics and plasticity; however, only one island was examined and historical processes that may influence differences have not been thoroughly evaluated (Aubret et al., 2004a). In addition to the work on tiger snakes and adders, smaller projects have been carried out, but all are incomplete in terms of fully explaining differences in head size (e.g., King, 1997; Bonnet et al., 2001). Not to say that my work is by any means complete, it does 1) take into account historical processes (e.g., phylogeography of the populations in question), 2) include detailed prey availability surveys, and 3) attempt to examine behaviors that may be associated with prey availability differences.

Head morphology: Sex differences—The majority of the head characters I observed for both neonates and adults indicate that females have larger head dimensions than males, even when holding SVL as a covariate. This is not unexpected, as other studies with both neonate (e.g., King et al., 1999; Krause 2000) and adult (e.g., Kind et al., 1999; Krause et al., 2003) gartersnakes have shown similar trends. The physiological reason for this trend is that testosterone hinders growth in male gartersnakes (Crews et al., 1985; Shine and Crews, 1988; King et al., 2004) with these differences being present

even at birth (King et al., 1999), which was also observed for my study. Adaptive scenarios can also be hypothesized for these differences. For example, females may need to take more prey of varying sizes to meet the energy requirements of developing neonates. Large heads facilitate the capture of a wider variety of prey and possibly the intake of more prey overall. Resource partitioning between males and females may also be a possibility. Regardless, adaptive mechanisms for sex differences in gartersnake head morphology were not examined here and require further study. Interestingly though, the only adult head measurement in which I did not detect a sex difference was adult IOD, which corresponds with Krause et al. (2003); however, my data indicates that this difference is present at birth.

Prey chemical preferences—Site differences in prey chemical preferences were less marked and do not appear to correspond with differences in head morphology. In general, neonates did not vary in their responses to the various stimuli based on sex, which is understandable, but there was a litter effect for all stimuli. For the most part, the various stimuli elicited similar responses from snakes from all sites with TFASs and attacks being highest for worm and toad stimuli, followed by moderate responses to frog, fish, and salamander stimuli, and very low or no responses towards the control. These trends fit with the prey available at my different sites, as worms are the most abundant prey source for all and baby toads/toadlets are readily available at the time baby snakes would be born. Salamanders and frogs are also available at most sites, but are less common and fish, while a staple of some gartersnake populations, are not common or available at most of my sites.

In terms of specific site differences, the frog and salamander stimuli elicited differences in TFAS with HI snakes exhibiting lower TFASs for the salamander stimulus than snakes from any other site, and with UP snakes exhibiting lower TFASs for the frog stimulus than most other sites. These differences may correspond with UP and HI prey availability. Until recently, the ubiquitous eastern red-backed salamander (*P. cinereus*), was not documented as occurring on HI (Placyk and Gillingham, 2002; Placyk et al., 2002). Surveys for this study detected one individual somewhat removed from the collection site of my snakes indicating that *P. cinereus* is present on the island, but that it may be relatively rare. In addition, green frogs (*R. clamitans*) occur and compete with mink frogs (*R. septentrionalis*) and may be more abundant and common than *R. clamitans* in the UP, but do not occur at any of my other sites (Placyk, pers. obs.). In terms of site differences for attacks, UP neonates attacked the toad stimuli less than snakes from most of the other sites, but an examination of overall responses from neonates from each site indicate that UP neonates were less responsive in general. Since the responses I measured were congenital, my results may provide support for the geological hypothesis that suggests the islands were primarily colonized by LP populations and as a result both island and LP animals are more responsive in general than UP snakes.

That differences in response to various prey chemical stimuli did not vary greatly, however, is no surprise. *Thamnophis sirtalis* is a generalist predator and may have innate tendencies to respond to a variety of prey despite whether it may actually occur with the

prey in question or not. The cost of maintaining the ability to detect different prey may not be costly enough for the behaviors to be removed from the populations or gene flow from surrounding populations may reinforce preferences for prey that some populations may no longer occur with. In fact, even the worm and leech specialist Butler's gartersnake, *T. butleri*, maintains the ability to detect and ingest prey (i.e., fish, amphibians) not included in its typical diet possibly, as the result of phylogenetic inertia from its presumed plains gartersnake, *T. radix*, ancestor, a prey generalist (e.g., Burghardt, 1967; Halloy and Burghardt, 1990; Lyman-Henley and Burghardt, 1995). However, geographic variation in response to prey in *Thamnophis* is known to vary over greater distances (e.g., Burghardt, 1970; Arnold, 1977, 1992). Of the few site differences I did see, there is some evidence that responses to certain prey may vary depending on the occurrence of such prey, but overall, such differences were lacking. If there is little cost to maintaining the ability to respond to prey not normally taken, the benefit of detecting such prey on rare occasion may outweigh it, as the *T. sirtalis* active season in Michigan is relatively short (late April thru September) and any and all prey may have great value. Aubret et al. (in press) in their study of island populations of tiger snakes (*N. scutatus*) arrived at similar results finding that island populations exhibited responses to prey not consumed naturally, but that occurred with surrounding mainland populations. In addition, their results also indicate that the foraging behaviors they observed most likely resulted from some combination of genetic change and plasticity.

Summary—Differences in both *T. sirtalis* head morphology and prey preference vary among Beaver Archipelago island populations and between island populations and

mainland populations. While these differences do not appear to correspond with each other, both provide evidence that underlying genetics and differences in selection pressures influence both behavioral and morphological traits related to feeding. In addition, my work demonstrates that plasticity may play a role in shaping head morphology in my system, while past work already suggests the same for foraging and feeding behaviors (e.g., Burghardt and Krause, 1999). In addition, since I controlled for the amount of food taken by neonates in my plasticity experiment, according to Schuett et al. (2005), this is the first study to unequivocally demonstrate that head morphology responds plastically to increased mechanical stimuli imposed by larger prey.

Overall, my study corresponds best with the recent work of Aubret et al. (2004a) who also demonstrated that head morphology results from a combination of complex interactions between evolutionary change and plasticity. However, my work is more complete, also examining differences due to sex and SVL, sampling several islands and surrounding mainland sites, including data from neonates, and including data on related behavioral characteristics. While this study provides the most detailed work on head morphology in snakes to date, additional research is still needed. Can the influence of genetics and plasticity be teased apart to fully understand the role prey intake throughout the life of an organism may play on shaping morphological characters? What role might ontogenetic shifts in morphology or behavior play in this system? What might be the significance of IOD in terms of both site differences and in terms of not differing between the sexes?

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APPENDIX IV

TABLES AND FIGURES

Table 4.1. Descriptions of morphological measurements taken from common gartersnakes, *Thamnophis sirtalis.*

Morphological Measurment	Description
Snout-vent length (SVL)	Distance between the tip of the rostral scale and the posteriormost point of the anal plate
Head length (HL)	Distance between the posteriormost point of the parietal scales and the tip of the rostral scale
Head width (HW)	Measured at the hinges of the jaws
Jaw length (JL)	Distance between the posterior edge of the posteriormost labial scale and the tip of the rostral scale
Interocular distance (IOD)	Distance between the outermost edges of the supraocular scales, above the eyes
Eye diameter (ED)	Diameter of the eye

Table 4.2. Prey available to common gartersnake, *Thamnophis sirtalis*, populations in the lower peninsula (LP), upper peninsula (UP), and Beaver Archipelago of Michigan (Miller's Marsh (MM), Sawmill (SM), Garden Island (GI), High Island (HI)). The presence of species at each site as documented in the past (Hatt et al., 1948; Phillps et al., 1965; Harding, 1996; Placyk and Gillingham, 2002) was verified for this study with the presence of a species at each site being marked by an **X**. The presence of an asterisk (*) indicates that a species was detected at a particular site for the first time as a result of this study (Placyk et al., 2002).

	Site					
Prey	LP	UP	MM	SM	GI	HI
Earthworms	**X**	**X**	**X**	**X**	**X**	**X**
Small pond fish (e.g., Cyprinidae)	**X**	**X**	**X**			**X**
Green frog (*Rana clamitans*)	**X**	**X**	**X**		**X**	
Mink frog (*Rana septentrionalis*)		**X**				
Northern leopard frog (*Rana pipiens*)	**X**	**X**	**X**			
American bullfrog (*Rana catesbeiana*)	**X**	**X**	**X**			
Gray treefrog (*Hyla versicolor*)	**X**	**X**	**X**			
Spring peeper (*Pseudacris crucifer*)	**X**	**X**	**X**			
American toad (*Bufo americanus*)	**X**	**X**	**X**	**X**	**X**	**X**
Eastern newt (*Notophthalmus viridescens*)	**X**	**X**	**X**		**X**	**X**
Eastern red-backed salamander (*Plethodon cinereus*)	**X**	**X**	**X**	**X**	**X**	**X***

Table 4.3. Results of independent *t*-tests used to compare head measurements of neonate common gartersnakes, *Thamnophis sirtalis*, that had been maintained either on a leafworm or a nightcrawler diet at 10, 25, and 35 weeks. Significant *P*-values are in bold print.

	10 weeks			25 weeks			35 weeks		
Head Measurement	*t*	df	*P*	*t*	df	*P*	*t*	df	*P*
Jaw length (JL)	1.29	77	0.20	4.30	64	**<0.001**	4.89	57	**<0.001**
Head length (HL)	-0.21	77	0.83	3.49	64	**0.001**	4.65	57	**<0.001**
Head width (HW)	1.86	77	0.07	5.65	64	**<0.001**	4.94	57	**<0.001**
Interocular distance (IOD)	0.34	77	0.73	3.30	64	**0.002**	3.22	57	**<0.01**
Eye diameter (ED)	1.18	77	0.24	2.59	64	**0.01**	4.07	57	**<0.001**

Table 4.4. Frequency of attacks exhibited for six stimuli during chemosensory testing of lab-born neonate common gartersnakes *Thamnophis sirtalis* from the lower (LP) and upper peninsula (UP) of Michigan, Miller's March (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). *P*-values calculated from chi-squared tests of independence. Significant differences ($P < 0.05$) are indicated by different letters. Overall site and stimuli differences are noted by letters following the name of each site or stimuli. Significant site differences with each stimulus are noted by letters following the frequency of attacks observed for each site at each stimulus. Differences among stimuli are also indicated by letters following each stimulus. Significant pairwise differences detected by Fisher exact probability tests (determined by *P*-values calculated using the Holm method).

	Site							
Stimuli	LP[a]	UP[b]	MM[a]	SM[a]	GI[a]	HI[a]	G^2	P
Control[a]	0.0	0.0	0.0	0.0	0.0	0.0	n/a	n/a
Worm[b]	0.25	0.0	0.29	0.25	0.20	0.13	14.30	**0.01**
Frog[c]	0.03	0.0	0.04	0.0	0.04	0.0	3.53	0.62
Toad[d]	0.46[a]	0.0[b]	0.36[a]	0.63[a]	0.28[ab]	0.40[a]	30.27	**< 0.01**
Fish[c]	0.04	0.0	0.03	0.13	0.06	0.0	5.83	0.32
Salamander[e]	0.19	0.0	0.10	0.06	0.12	0.0	6.80	0.15
Total	0.16[a]	0.0[b]	0.14[a]	0.18[a]	0.12[a]	0.09[a]	38.22	**< 0.01**
litter =	12	2	11	4	8	4		
n =	155	21	78	16	50	15		

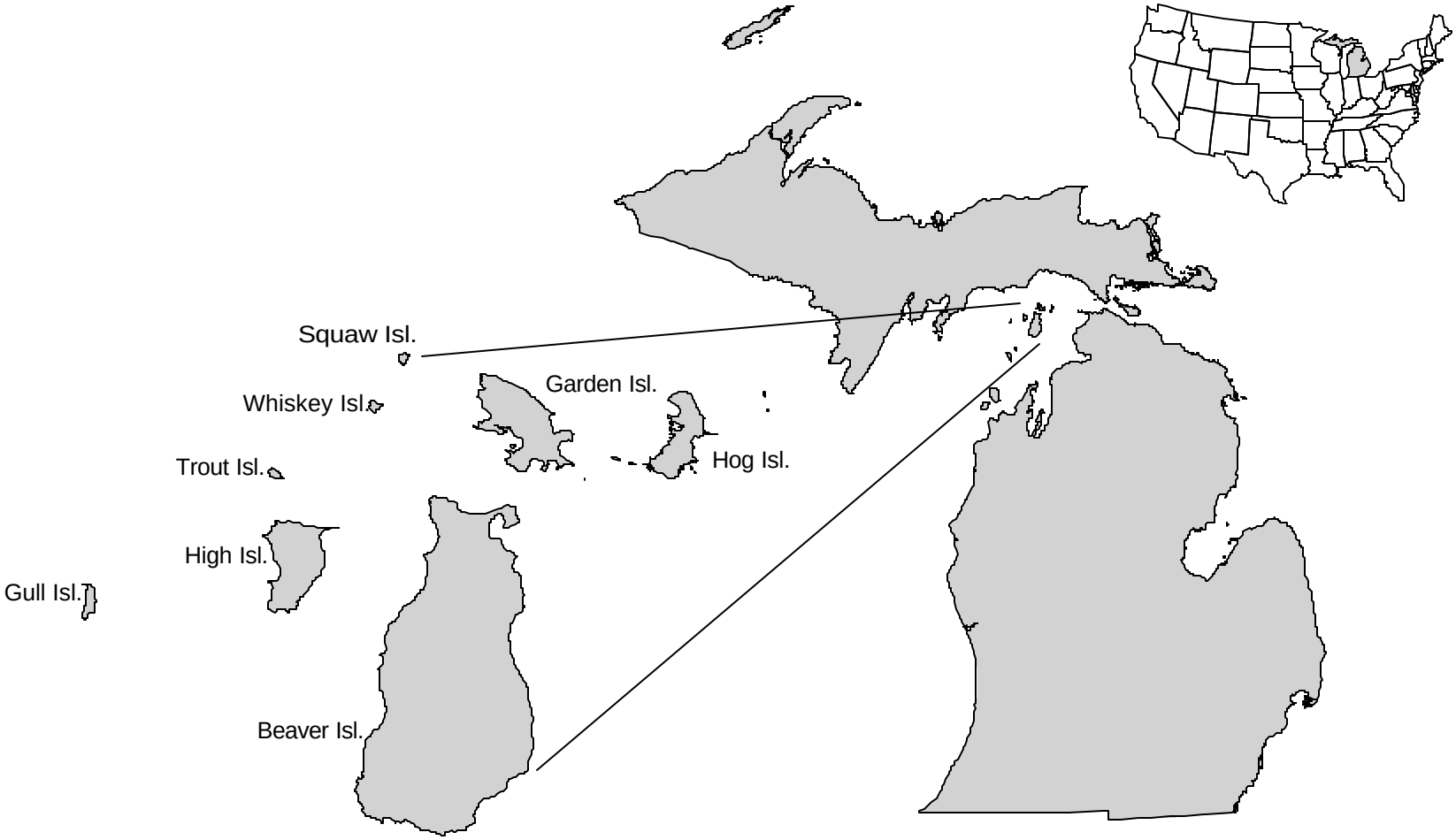


Fig. 4.1. Collection localities for common gartersnake, *Thamnophis sirtalis*, populations sampled for this study (modified from Hansknecht, 2003).

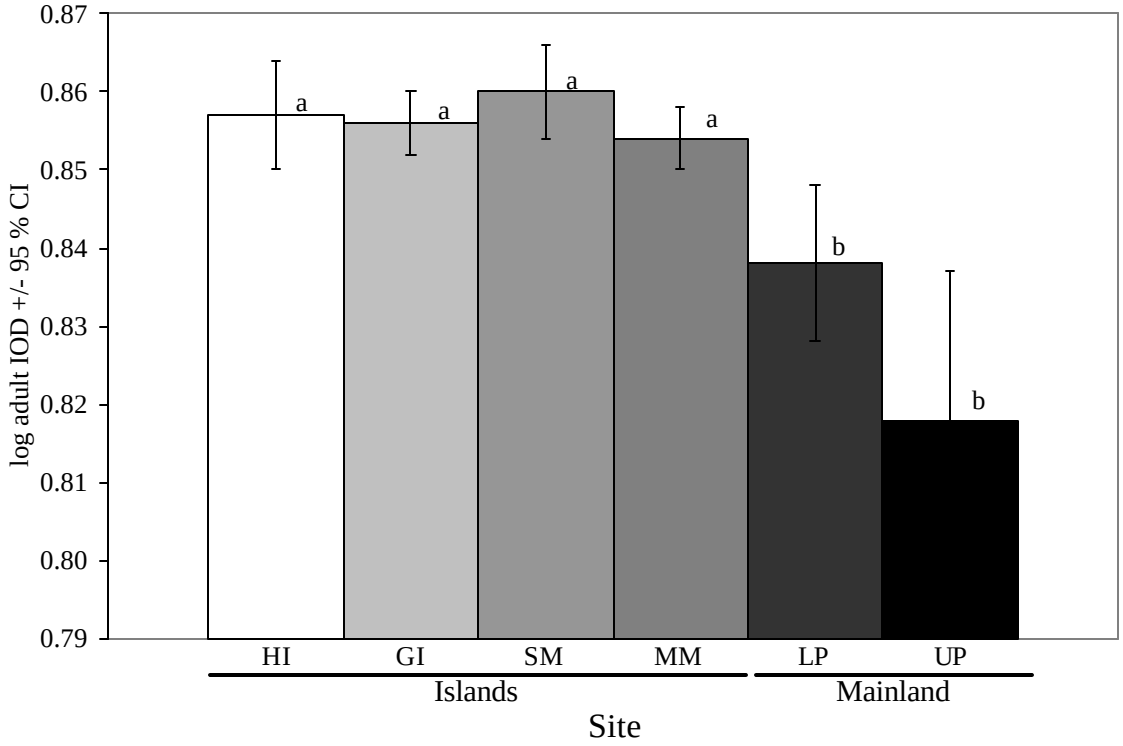
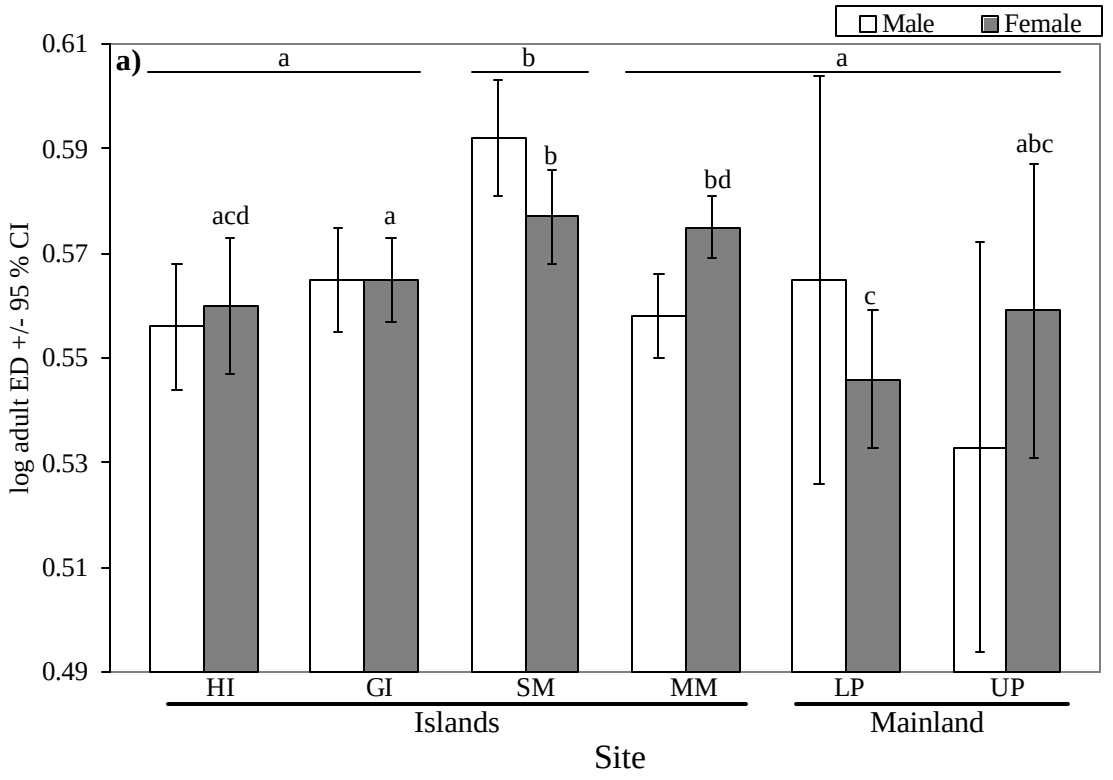


Fig. 4.2. Mean interocular distance (IOD) (± 95 % CI) of adult male and female common gartersnakes, *Thamnophis sirtalis* collected from the lower (LP) (n = 22) and upper peninsula (UP) (n = 6) of Michigan, Miller's Marsh (MM) (n = 126) and Sawmill (SM) (n = 69) on Beaver Island, Garden Island (GI) (n = 94) and High Island (HI) (n = 38). Measurements were taken in cm and then log-transformed. Different letters indicate significantly different means ($P < 0.05$).

Fig. 4.3. Mean eye diameter (ED) (± 95 % CI) (a) and head length (HL) (± 95 % CI) (b) of adult male and female common gartersnakes, *Thamnophis sirtalis*, collected from the lower (LP) (n = 22) and upper peninsula (UP) (n = 6) of Michigan, Miller's Marsh (MM) (n = 126) and Sawmill (SM) (n = 69) on Beaver Island, Garden Island (GI) (n = 94) and High Island (HI) (n = 38). Measurements were taken in cm and then log-transformed. Different letters above lines indicate significantly different means ($P < 0.05$) among sites for males and females combined; different letters directly above bars indicate significantly different means ($P < 0.05$) among sites for females only.


Male
Female
a)
a
b
a
0.61
0.59
0.57
0.55
0.53
0.51
0.49
log adult ED +/- 95 % CI
acd
a
b
bd
c
abc
HI
GI
SM
MM
LP
UP
Islands
Mainland
Site

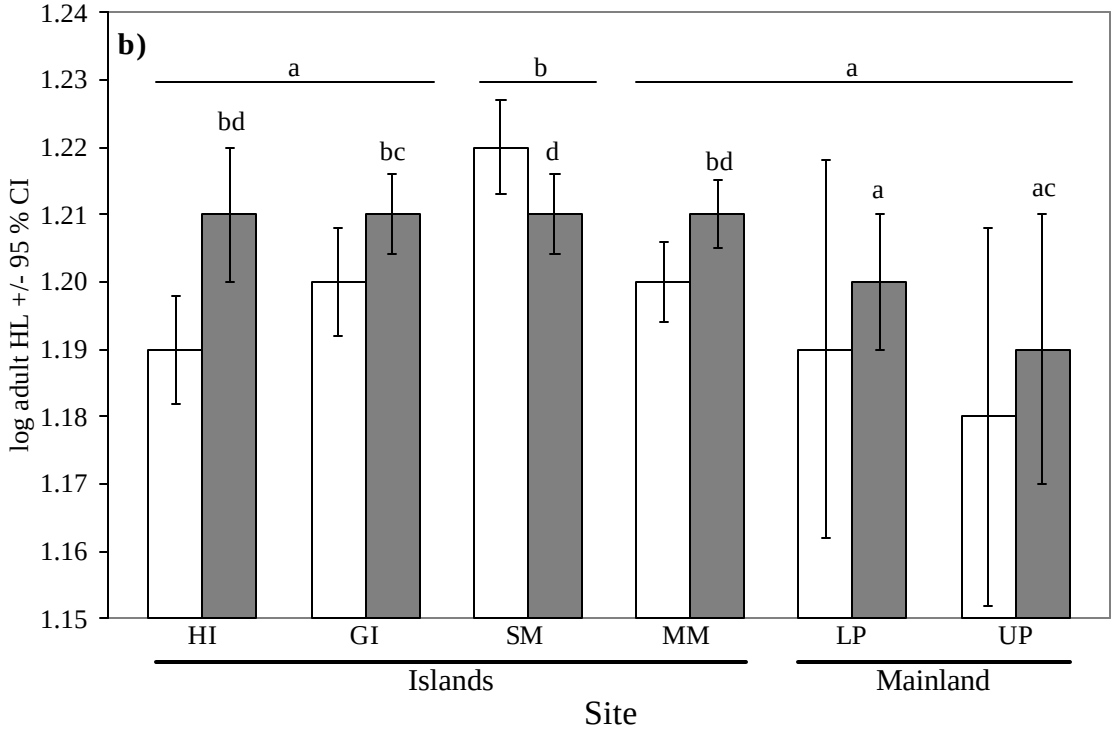
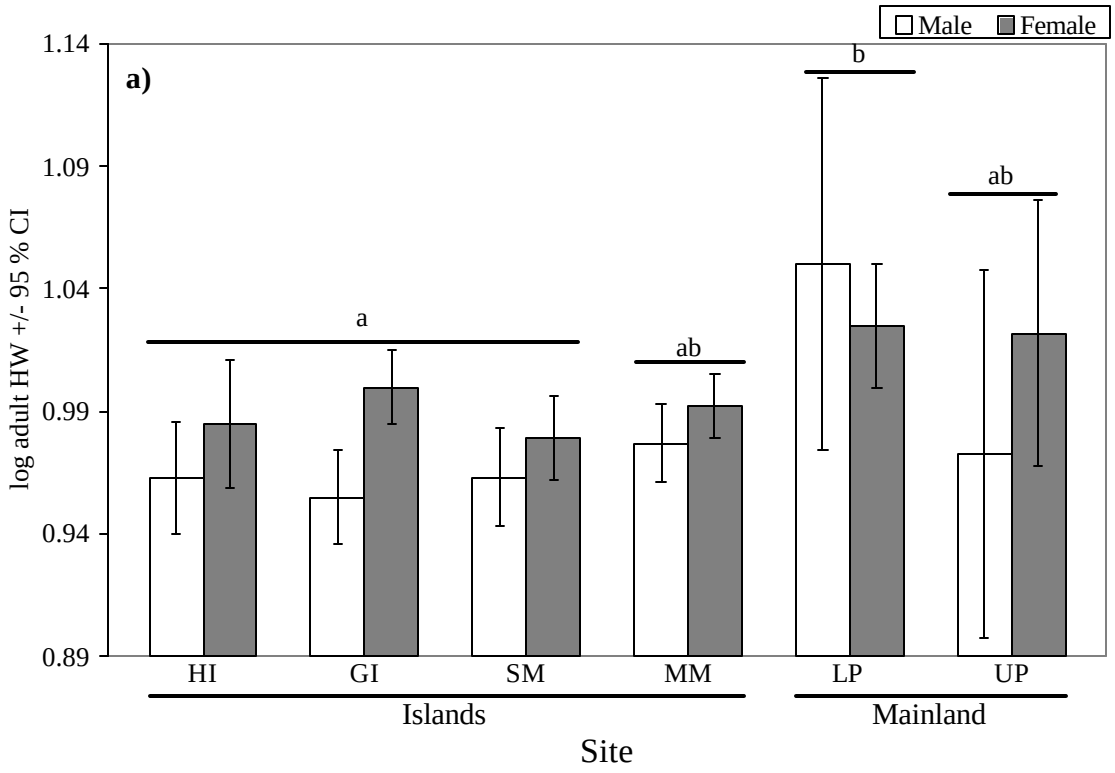
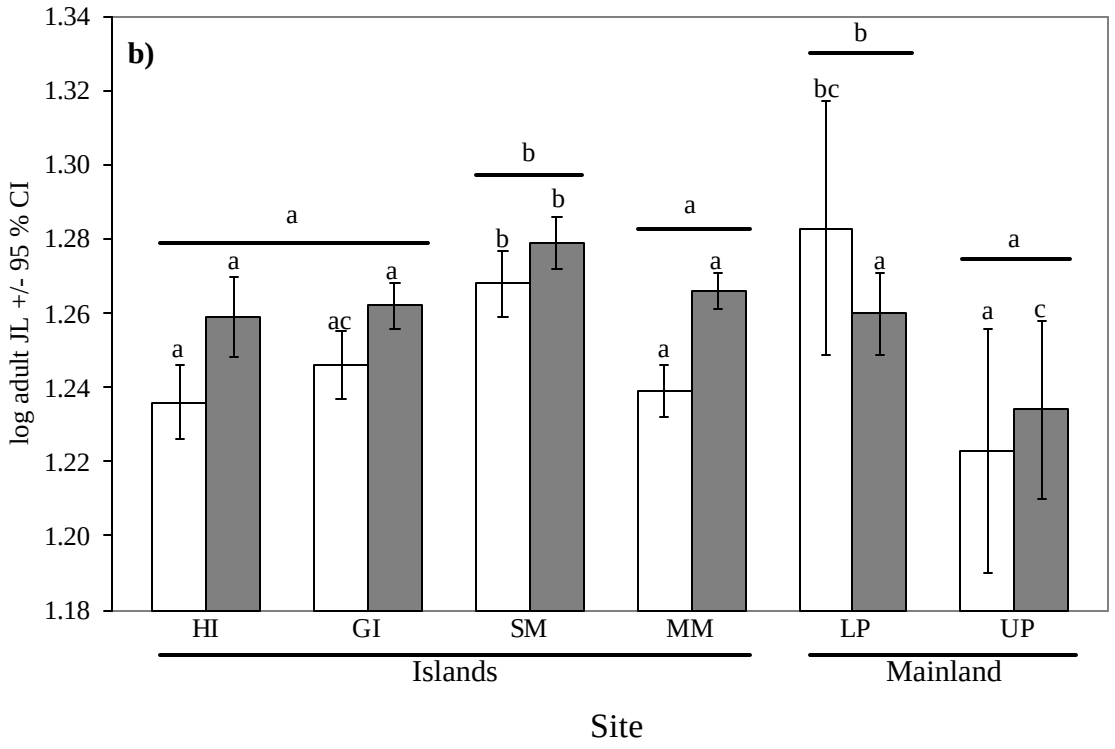

b)
a
b
a
1.24
1.23
1.22
1.21
1.20
1.19
1.18
1.17
1.16
1.15
log adult HL +/- 95 % CI
bd
bc
d
bd
a
ac
HI
GI
SM
MM
LP
UP
Islands
Mainland
Site

Fig. 4.4. Mean head width (HW) (± 95 % CI) (a) and jaw length (JL) (± 95 % CI) (b) of adult male and female common gartersnakes, *Thamnophis sirtalis*, collected from the lower (LP) (n = 22) and upper peninsula (UP) (n = 6) of Michigan, Miller's Marsh (MM) (n = 126) and Sawmill (SM) (n = 69) on Beaver Island, Garden Island (GI) (n = 94) and High Island (HI) (n = 38). Measurements were taken in cm and then log-transformed. Different letters above lines indicate significantly different means ($P < 0.05$) among sites for males and females combined; different letters directly above bars (Fig. 4b only) indicate significantly different means ($P < 0.05$) among sites for females only and males only.


Male
Female
a)
log adult HW +/- 95 % CI
1.14
1.09
1.04
0.99
0.94
0.89
a
ab
b
ab
HI
GI
SM
MM
LP
UP
Islands
Mainland
Site


b)
log adult JL +/- 95 % CI
1.34
1.32
1.30
1.28
1.26
1.24
1.22
1.20
1.18
a
b
a
b
b
bc
a
a
a
ac
a
b
a
a
a
c
HI
GI
SM
MM
LP
UP
Islands
Mainland
Site

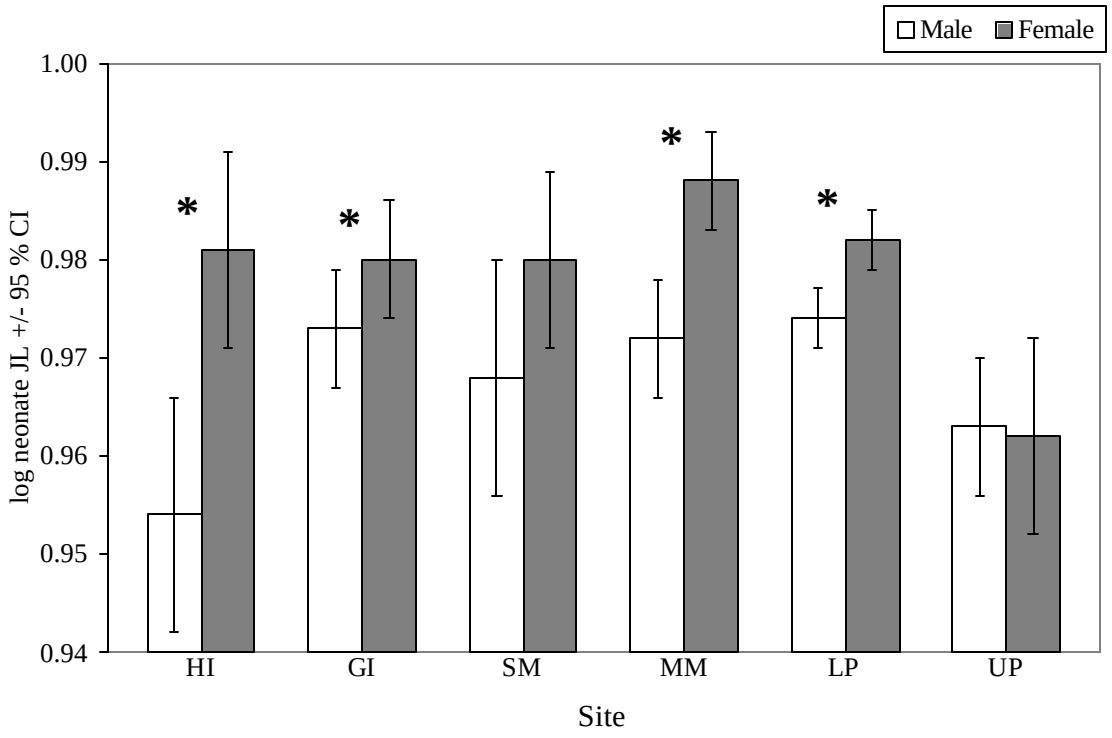
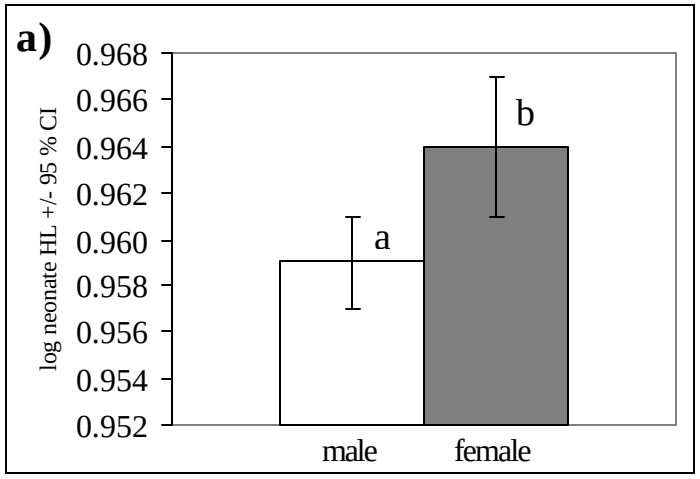
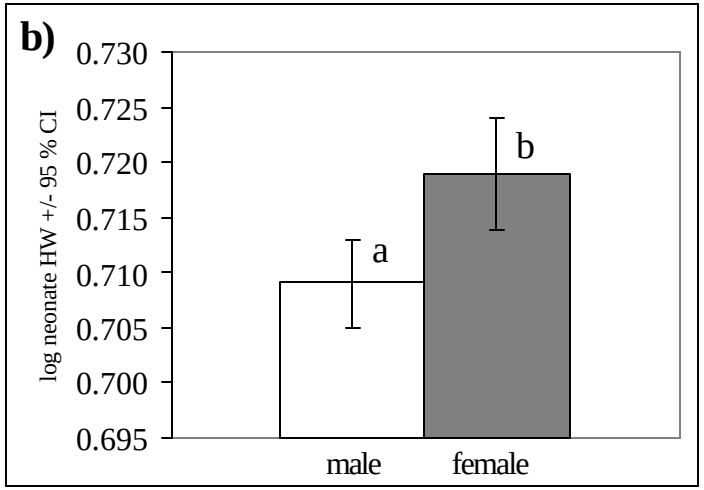
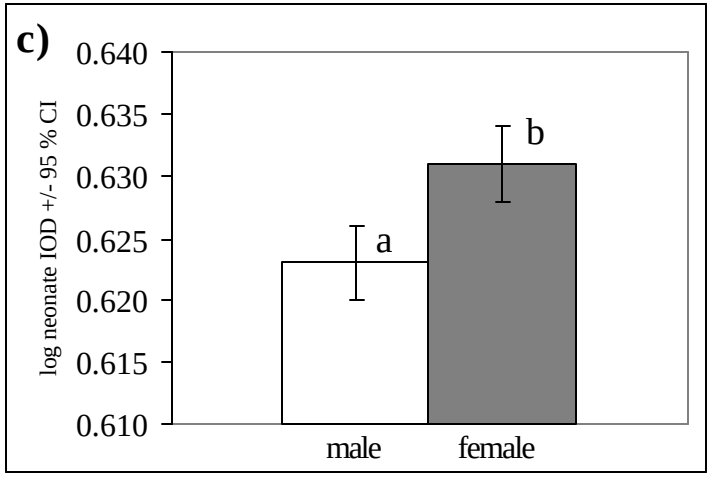


Fig. 4.5. Mean jaw length (JL) (± 95 % CI) of neonate male and female common gartersnakes, *Thamnophis sirtalis*, born to mothers collected from the lower (LP) (n = 231) and upper peninsula (UP) (n = 20) of Michigan, Miller's Marsh (MM) (n = 78) and Sawmill (SM) (n = 17) on Beaver Island, Garden Island (GI) (n = 64) and High Island (HI) (n = 18). Measurements were taken in cm and then log-transformed. A significant site effect was not detected, but a significant site X sex interaction exists. Asterisks (*) indicate the sites in which males significantly differ from the females ($P < 0.05$). Litter means, not individual means, are shown.

Fig. 4.6. Mean head length (HL) (± 95 % CI) (a), head width (HW) (± 95 % CI) (b), interocular distance (IOD) (± 95 % CI) (c), and eye diameter (ED) (± 95 % CI) (d) of neonate male and female common gartersnakes, *Thamnophis sirtalis*, born to mothers collected from 6 Michigan field sites. Since site effects were not detected, data from the 6 sites were pooled for each measurement. Measurements were taken in cm and then log-transformed. Different letters indicate significantly different means ($P < 0.05$).


a)
log neonate HL +/- 95 % CI
0.968
0.966
0.964
0.962
0.960
0.958
0.956
0.954
0.952
a
b
male
female


b)
log neonate HW +/- 95 % CI
0.730
0.725
0.720
0.715
0.710
0.705
0.700
0.695
a
b
male
female


c)
log neonate IOD +/- 95 % CI
0.640
0.635
0.630
0.625
0.620
0.615
0.610
a
b
male
female

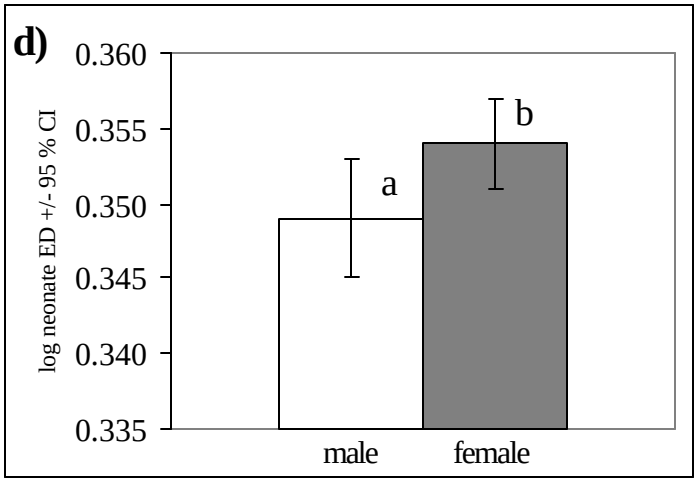

d)
log neonate ED +/- 95 % CI
0.360
0.355
0.350
0.345
0.340
0.335
a
b
male
female

Fig. 4.7. Mean jaw length (JL) (± 95 % CI) (a), head length (HL) (± 95 % CI) (b), head width (HW) (± 95 % CI) (c), interocular distance (IOD) (± 95 % CI) (d), and eye diameter (ED) (± 95 % CI) (e) of neonate common gartersnakes, *Thamnophis sirtalis*, from groups offered either leafworms (LW) or nightcrawlers (NC) at 10 weeks (LF, n = 44; NC, n = 35), 25 weeks (LF, n = 37; NC, n = 29) and 35 weeks (LF, n = 31; NC, n = 28). Asterisks (*) indicate significant differences between the two groups at any given time period ($P < 0.05$).

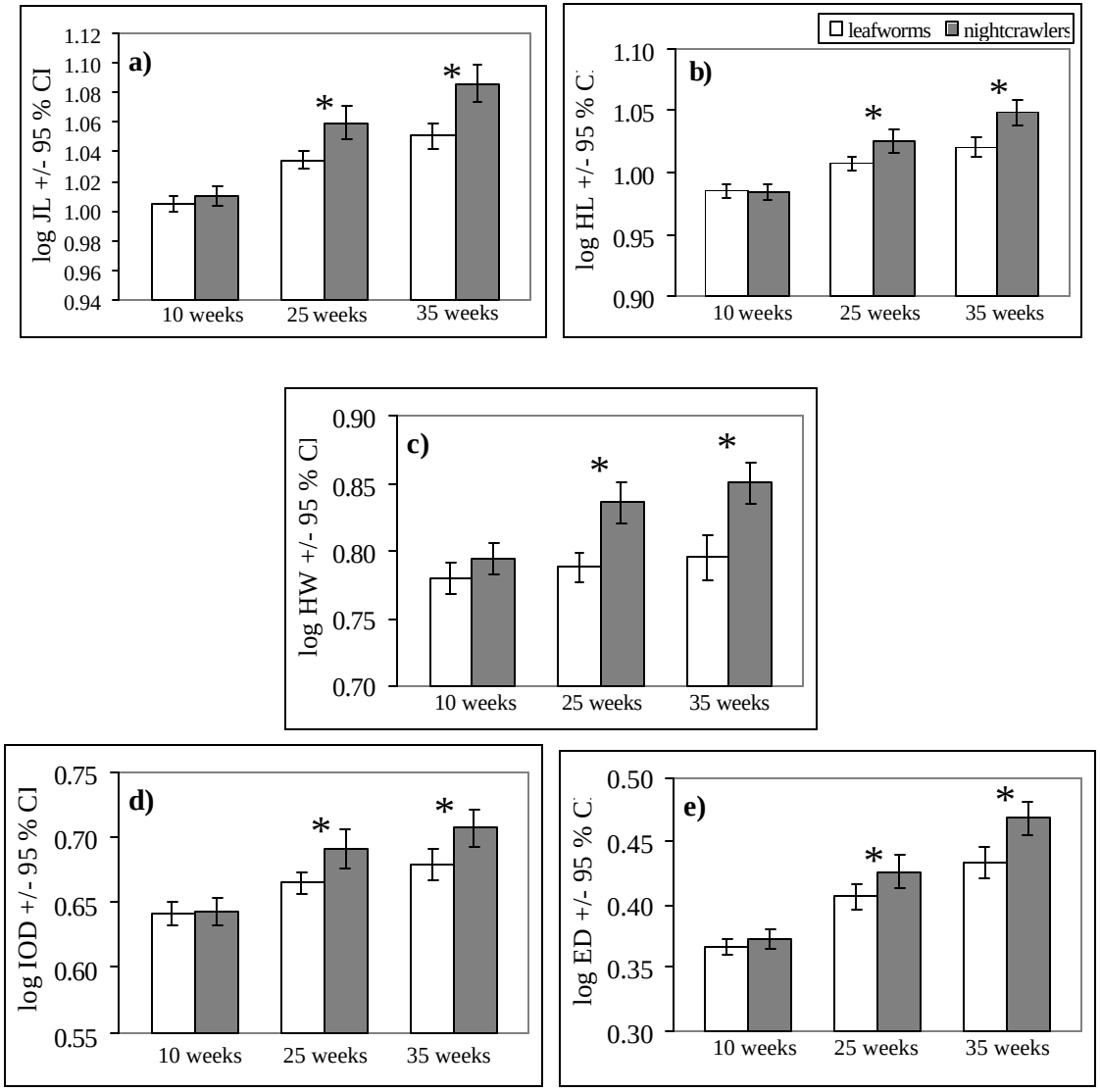
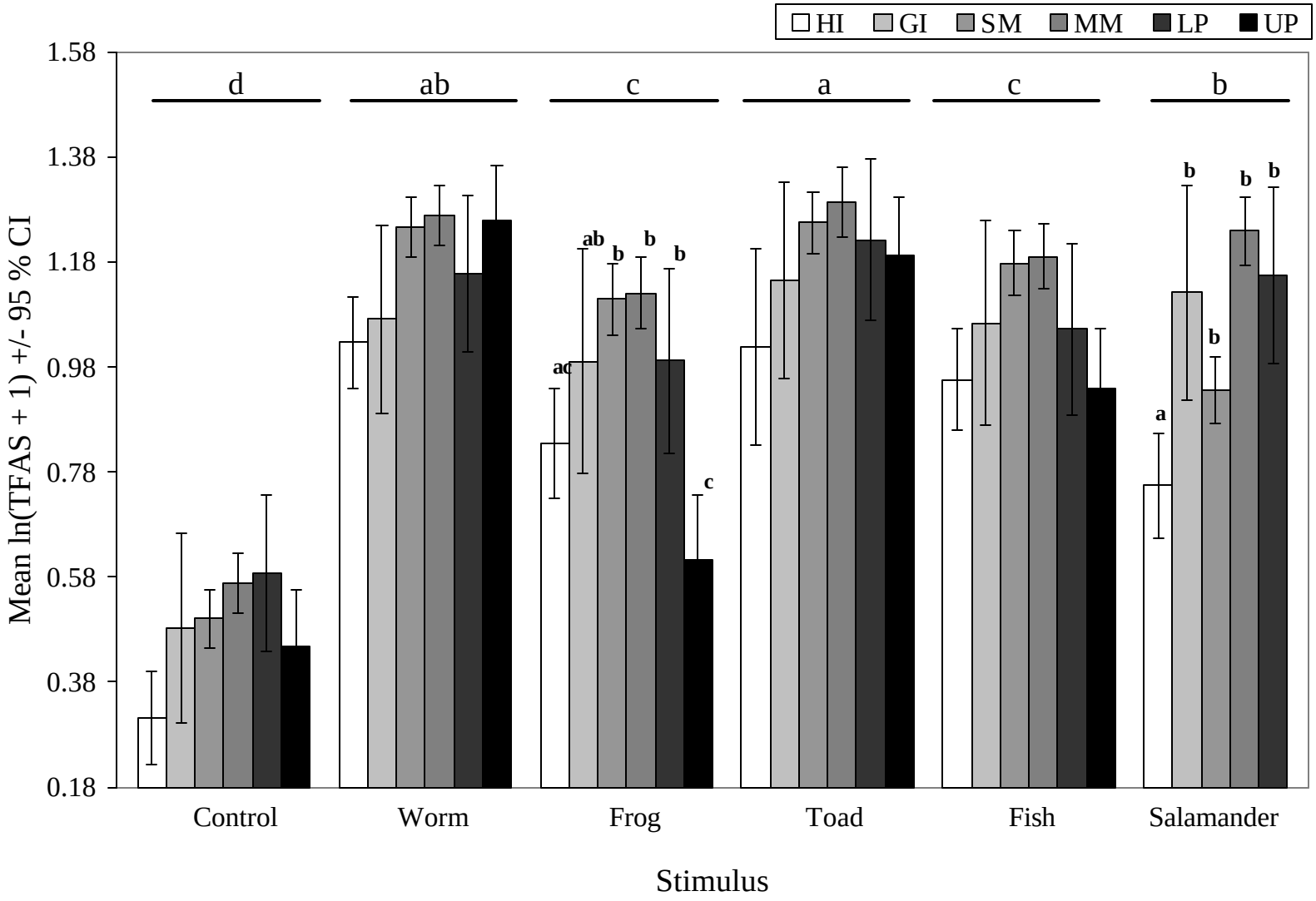

a)
log JL +/- 95 % CI
1.12
1.10
1.08
1.06
1.04
1.02
1.00
0.98
0.96
0.94
10 weeks
25 weeks
35 weeks
*
*
b)
leafworms
nightcrawlers
log HL +/- 95 % C
1.10
1.05
1.00
0.95
0.90
10 weeks
25 weeks
35 weeks
*
*
c)
log HW +/- 95 % CI
0.90
0.85
0.80
0.75
0.70
10 weeks
25 weeks
35 weeks
*
*
d)
log IOD +/- 95 % CI
0.75
0.70
0.65
0.60
0.55
10 weeks
25 weeks
35 weeks
*
*
e)
log ED +/- 95 % C
0.50
0.45
0.40
0.35
0.30
10 weeks
25 weeks
35 weeks
*
*

Fig. 4.8. Mean ln(tongue-flick attack score (TFAS) +1) (± 95 % CI) for neonate common gartersnakes, *Thamnophis sirtalis*, born to dams collected from the lower (LP) (litters = 12; n = 155) and upper peninsula (UP) (litters = 2; n = 21) of Michigan, Miller's Marsh (MM) (litters = 11; n = 78) and Sawmill (SM) (litters = 4; n = 16) on Beaver Island, Garden Island (GI) (litters = 8; n = 50) and High Island (HI) (litters = 4; n = 15). Different letters indicate significantly different means among and within stimuli ($P < 0.05$). Litter means, not individual means, are shown.


HI
GI
SM
MM
LP
UP
1.58
1.38
1.18
0.98
0.78
0.58
0.38
0.18
Mean ln(TFAS + 1) +/- 95 % CI
d
ab
c
a
c
b
ac
ab
b
b
b
c
a
b
b
b
b
Control
Worm
Frog
Toad
Fish
Salamander
Stimulus

Part V

VARIATION IN ADULT SIZE AND LIFE-HISTORY TRAITS OF INSULAR POPULATIONS OF THE COMMON GARTERSNAKE, *THAMNOPHIS SIRTALIS*

INTRODUCTION

Geographic variation in phenotypic traits takes many forms, and particularly important for population biology is variation in life-history traits. Life-history traits are generally related to adaptations of an organism that may both result from, and more or less directly, influence demographic patterns, especially survival and fecundity (Ricklefs, 1990). Stearns (1992) considers 1) size at birth, 2) patterns of growth, 3) age at maturity, 4) size at maturity, 5) number, size, and sex of offspring, 6) age and size-specific reproductive investment, 7) age and size-specific mortality schedules, and 8) length of life as the principle life-history traits. The importance of variation in life-history traits stems from their close relationship to fitness. For example, fecundity and mortality (e.g., length of life) are components of fitness, while growth patterns and age at maturity are directly related to fitness. While theoretical and empirical interest in the study of variation in life-history traits is common, in only a few cases, have researchers been able to explain, and even more rarely predict, how different environments affect life-history traits (e.g., Roff, 1992). In general, we lack a comprehensive theory that applies to a wide range of organisms and environments. This is largely due to a shortage of experimental data that would allow tests of existing theory, as well as provide a basis for developing alternative theories (Dunham et al., 1989; Niewiarowski, 1994, 2001; Zera and Harshman, 2001). The work provided herein should provide useful empirical data on life-history trait variation and I will explore the possible influence of both genetics and environment on such variation. To conduct such a study naturalistically, a system in which such variation is expected to exist is needed.

Island systems, in particular, are ideal for studying variation in phenotypic traits. The utility of using such systems stems from the general trend that the biota in such systems tend to experience fewer, simpler, or stronger selective pressures when compared to their mainland counterparts (Whittaker, 1998; Schluter, 2001). Specifically, predator composition, prey availability, habitats, and various abiotic factors often vary among mainland and island populations. This variation in selection pressures often results in microevolutionary and plastic adaptive responses (e.g., Aubret et al., 2004); however, relaxation of such pressures may also lead to the retention of traits that no longer have a current utility (e.g., Coss, 1999; Aubret et al., in press). In addition to the influence of environmental differences, populations of insular systems are often characterized by unique genetic attributes. To begin, such populations are often the result of founder events, which may result in a decrease in genetic and possibly phenotypic diversity starting from the colonization of the population (MacArthur and Wilson, 1967; Johnson et al., 2000). Secondly, gene flow between island and mainland populations may be absent or reduced, and since island populations may be considerably smaller than mainland populations, random genetic drift may also greatly influence their genetic and phenotypic attributes (Grant, 1998). As a result of these various environmental and genetic factors, one can appreciate the value of island systems for studies of variation in phenotypic traits. In addition, since variation in life-history traits may be influenced by both environmental (Roff, 1992; Stearns, 1992) and genetic factors (Mousseau and Roff, 1987), they provide the perfect setting for studies of life-history trait variation. As a result, I chose to focus my work on such a system, specifically, the Beaver Archipelago of Lake Michigan.

The Beaver Archipelago consists of one main island (i.e., Beaver, the only one inhabited by people), three moderately large islands (i.e., Garden, High, and Hog), and six smaller islands (i.e., Hat, Pismire, Shoe, Squaw, Trout, and Whiskey) situated in northeastern Lake Michigan (Fig. 5.1; all tables and figures for Part V are located in Appendix V). The islands are separated from the lower and upper peninsula of Michigan by approximately 30 and 25 km of open water, respectively. The recent geological history of the archipelago is marked by drastic lake level fluctuations due, in part, to complex interactions between the retreating Laurentide Ice Sheet, differential isotatic rebound, and regional climate change (Hough, 1958; Petty et al., 1996). As a result, hypotheses as to the variation in life-history traits in island populations can be developed in relation to the recent colonization, extinction, and recolonization events that may have occurred throughout the Beaver Archipelago. In addition, the floral and faunal composition of the islands vary within the archipelago and between the islands and the mainland, roughly adhering to island biogeography theory (i.e., with more species found on the mainland and larger islands than on the smaller islands) (MacArthur and Wilson, 1967; Bowen and Gillingham, 2004). As a result, available habitats, prey availability, and predator pressures vary substantially in this system. While the earliest islands may have been colonized is 11,000 years before present, recent work in other more distant systems indicates this may not be an issue. For example, in less than 10,000 years, tiger snake (*Notechis* sp., Elapidae) populations off the coast of Australia have seen independent and rapid evolution of significant shifts in adult size resulting from similar and strong selective pressures (Keogh et al., 2005). In addition, work with other genera (e.g., *Mus*, Muridae) found on islands indicates that evolutionary change in phenotypic

characters is possible in as little as seventy years (Berry, 1964; Berry and Jakobson, 1975). Therefore, given the environmental differences, the potential genetic attributes of island populations, in general, and the age of this system, the Beaver Archipelago is an ideal location in which to examine geographic variation in phenotypic traits, including life-history traits.

For animals, adult body size is one of the morphological traits related to life-history that tends to change most readily on islands, and it is a well-known phenomenon that islands can support populations of gigantic and dwarf forms that are similar to their mainland conspecifics in most aspects except adult body size (e.g., Case, 1978; Boback, 2003). In general, the reduced competition in depauparate insular communities enables small species to increase in size and take advantage of resources normally consumed by larger members of the communities, while resource limitation causes larger species to become dwarfed. Shifts in body size are also often the result of relaxation of predation and random genetic drift. In terms of insular mammals, specifically, Heaney (1978) believes that the four main factors that influence body size are (1) predation, (2) competition, (3) food limitation, and (4) physiological efficiency. Regardless of the cause, differences in body size between island and mainland populations are the most commonly documented island phenomenon, but variation in other types of life-history traits also often occur. To begin, any of Stearns (1992) eight principal life-history traits that have some genetic component, including size at maturity, may vary between island and mainland populations due to the unique genetic attributes and history of island populations. In addition, any of these traits may be influenced by environmental

differences that may also vary. While much work has been devoted to describing and explaining differences in body size between island and mainland populations (e.g., Case, 1978; Heaney, 1978; Boback, 2003), the literature is lacking in any major focus on variation in other types of life-history traits in island systems; however, there is evidence of such variation.

For example, insular Japanese keelback snakes, *Amphiesma vibakari* (Colubridae), have smaller clutch sizes than mainland *A. vibakari* (Hasegawa, 1990). Adult island *Anolis* lizards (Polychrotidae) have considerably greater survivorship and significantly greater sexual dimorphism ratios than mainland *Anolis* (Andrews, 1979) resulting, in part, from differences in predator pressures (Lister and Aguayo, 1992). Black tiger snakes, *Notechis ater niger* (Elapidae), on offshore islands in South Australia exhibit marked differences in sexual dimorphism and sex ratio from mainland populations (Schwaner, 1985). Island populations of European grass snakes, *Natrix natrix* (Colubridae), exhibit a greatly reduced degree of sexual body size dimorphism compared to nearby mainland populations due to modified growth patterns resulting from the lack of large prey items on islands (Madsen and Shine, 1993). Therefore, not only size, but several of Stearns (1992) principal life-history traits vary between mainland and island populations. In this study, I will focus on describing and attempting to explain variation in several life-history traits or life-history-related traits to 1) provide much needed empirical data on such variation, and 2) to test traditional hypotheses related to life-history trait variation. To do so, I enlisted the help of a model species for my study, the common gartersnake, *Thamnophis sirtalis*, Colubridae.

Gartersnakes (*Thamnophis* spp.) of the subfamily Natricinae, especially the thamnophiine, have been model species for studying many aspects of behavior, physiology, reproduction, ontogeny, genetics, and life-history (e.g., Seigel et al., 1987a; Seigel and Collins, 1993; Rossman et al., 1996). Population differentiation is well known in life-history traits, morphology (color, pattern, size, scalation), and behavior (antipredator, food habits) with some species exhibiting geographic variation in phenotypic traits as both neonates and adults (Burghardt, 1967, 1969, 1970; Arnold 1980, 1981a, 1981b, 1981c; Arnold and Bennett, 1988; Ford and Seigel, 1989; Arnold, 1992; Gregory and Larsen, 1993; Larsen et al., 1993; Rossman et al., 1996; Burghardt and Schwartz, 1999). *Thamnophis sirtalis*, specifically, is the most wide-ranging snake in North America (Rossman et al., 1996) and wide-ranging species of animals, in general, often show geographic variation in demographic characteristics, including reproductive traits (e.g., Hemelaar, 1988; Ritke, 1990; Leslie, 1990). Specifically, *T. sirtalis* is found throughout the Midwest region of the United States and is considered the most common species of snake in the Great Lakes region (Harding, 1996), where the Beaver Archipelago is located. In addition, *T. sirtalis* vary in clutch size, offspring size and mass, and reproductive frequency throughout their range, and at least, variation in clutch size is known to result from changes in prey availability (Rossman et al., 1996). Gartersnakes are easily maintained in a laboratory setting with pregnant females giving birth to live young in captivity. As a result, data on litters and individual neonates can easily be collected. In addition, regarding the Beaver Archipelago populations

specifically, the origin and underlying genetics of the island populations were recently revealed (Placyk, 2006; Placyk et al., in prep) and gartersnake predators (Placyk and Burghardt, 2005) and prey (Placyk, 2006; Placyk and Burghardt, in prep) vary from island to island. Therefore, variation in life-history traits can easily be corresponded with the geological history of the islands, the phylogeography of gartersnakes in the United States Midwest, and with the variable environmental factors experienced by each population in question. Having this kind and amount of data on hand will allow me to hypothesize as to the nature of any life-history trait variation I detect much easier than those who have studied such variation without access to such data.

For this study, I focus on some of Stearns's principle life-history traits including (1) size at birth and (2) number, size, and sex ratio of offspring. I was also interested in examining differences in size at maturity, but since *T. sirtalis* is characterized by indeterminate growth focused instead on adult size. In addition, to examine the general condition of both adults and neonates, which usually indicates the health of an organism, and as a result, may act as an indicator of environmental quality (e.g., prey availability), I also calculated a condition index for use in comparisons (Rivas, 2000). Since differences in the morphological characters of individuals (both neonates and adults) may vary as the result of sex (Shine and Crews, 1988; Shine, 1990; Houston and Shine, 1993; Shine, 1994) and since sex effects may vary from site to site (e.g., Schwaner, 1985; Schwaner and Sarre, 1988; Shine and Crews, 1988; King, 1997; Bonnet et al., 2002b; Krause et al. 2003), sexual dimorphism was also examined both within and among populations.

As mentioned above, differences in adult size have been extensively studied in insular systems and in snakes, in particular, have been the focus of several reviews (e.g., Case, 1978; Boback, 2003; Boback and Guyer, 2003) and long-term studies (e.g., Schwaner, 1985; Shine, 1987; Keogh et al. 2005). However, the majority of the empirical data and theory derived from this work has been based on predators with more specialized diets, which may be more heavily influenced by differences in prey availability (the driving force in island snake size differences (Boback, 2003)), then on prey generalists (c.f., King, 1989) that may be able to easily shift to different prey on islands with little or no effect. As a result, general trends of island dwarfism and gigantism displayed by many of the insular snake species studied to date may not apply to generalist species despite experiencing genetic and environmental differences similar to those experienced by specialists. In fact, Boback (2003) notes that species for which size may not change between islands and mainlands are most likely underrepresented in the published literature and data on such species is needed to more fully understand body size change in insular populations. Since *T. sirtalis* is a generalist predator eating earthworms, leeches, fish, amphibians, other reptiles, small mammals and birds, and even roadkill (e.g., Rossman et al., 1996), variation in prey availability may have little effect on their adult body size. This study will be one of the first to test the hypothesis that body size variation in insular populations may depend on the ecology of the organism in question and that general trends may not apply to large taxonomic groupings.

Few have specifically focused on the other life-history traits I examined for this study (e.g., size at birth & number, size, and sex ratio of offspring), in island systems.

However, general studies that have focused on size and number of offspring indicate that population differences in these traits result from genetic and environmental influences. For example, in the checkered gartersnake, *T. marcianus*, number of offspring and clutch/litter mass are significantly affected by prey availability, but relative clutch mass and offspring size are fixed (Ford and Seigel, 1989). In the common viper or adder, *Vipera berus*, Viperidae, offspring size varies as a function of prey availability (Andren and Nilson, 1983). Although, differences in prey availability may not influence the size of a generalist predator, the availability and quality of prey may still effect neonate characteristics (Smith and Fretwell, 1974), therefore, I expect such characteristics are more likely to vary in my system than adult characteristics. In fact, such differences are commonly seen in insular populations of snakes (Schwaner, 1985; Shine, 1987; Schwaner and Sarre, 1988; Hasegawa, 1990) and are usually the result of differences in prey availability; however, in addition to relying on snakes that have more specialized diets, most of the work done in this area has focused on 'capital' breeders (Drent and Daan, 1980), which rely on stored energy as their primary energy source for reproduction. *Thamnophis sirtalis*, is an 'income breeder' relying more on recently acquired energy for reproduction (e.g., Whittier and Crews, 1990). Therefore, the differences observed in many of the insular populations of snakes studied to date may not apply to snakes that are classified as 'income breeders'. Therefore, my work not only provides novel data on a generalist predator, but also novel data on an 'income breeder'. As a result, not only does this work provide much needed data on life-history trait variation in general, but it also provides novel data on a generalist predator that is an 'income breeder'.

Overall, the objectives of this study are to (1) determine if variation in life-history traits exists between the populations of the Beaver Archipelago and the surrounding mainland (2) determine if any such variation corresponds with current abiotic and biotic influences, and (3) determine the role the underlying genetics of these populations may play in determining such variation.

MATERIALS AND METHODS

General methods—Gartersnakes were collected from field sites in both the lower (LP) and upper peninsula (UP) of Michigan and from two sites on Beaver Island (MM & SM), Garden Island (GI), and High Island (HI) of the Beaver Archipelago (Fig. 5.1) during the summers of 2001 and 2002. Since UP animals proved elusive throughout the study, additional snakes from this area were collected in 2004. Following capture, snakes were placed, by site, into 61 X 32 X 33 cm aquaria to eliminate breeding and disease transmission between different populations and permitted to acclimate for 48 h. Aquaria were kept between 20 and 25° C on a 16:8 Light:Dark cycle, to simulate northern Michigan summer conditions, with fresh water and shelter available at all times. After the acclimation period, snakes were used in behavioral trials conducted as part of a broader study on geographic variation in phenotypic traits. Following behavioral testing, snakes had their snout-vent length (SVL) and tail length measured and were weighed. Mass was determined to the nearest 0.1 g using an Ohaus® triple beam balance. SVL and TL were recorded to the nearest 0.1 cm using a standard meter stick. Following collection of morphological measurements, snakes were then scale-clipped for future

identification (Brown and Parker, 1976), had tissue samples taken for genetic analyses, and were released to their respective capture locations. Some pregnant females (determined via palpating for developing embryos) were held until parturition after which time they were released to their respective capture locations. Morphological measurements (SVL, mass) were taken for neonates directly after birth. In addition, number of neonates per litter, total mass of each litter, and ratio of stillborns per litter were also recorded. Following neonate behavioral testing and experimenting associated with other studies, animals were released to the site of their mother's capture, maintained for other experiments, or transferred to other research facilities. Any adult snakes held more than 72 h and all neonates were submitted to a health screening before being released.

Statistical analyses—Condition indices (CI) for all animals were calculated by taking the cubic root of an animal's mass (g) and dividing that value by its length (mm) (Andrews, 1982; Rivas, 2000). This particular type of condition index applies a 'condition with isometry assumption', which allows for easier interpretation than other condition indices that are typically calculated (Rivas, 2000). For analysis of adult morphological characters, only males with a SVL over 350.0 mm and females with a SVL over 400.0 mm were used, as smaller animals may not be considered adults (King, 1989; Krause, 2000; Krause et al., 2003). Adult SVL and CIs were compared between sites via ANOVAs, while adult mass was compared via an ANCOVA with SVL as a covariate. Sex and reproductive state (male, nonpregnant female, pregnant female) were also added to adult ANOVAs, separately. For models in which sex was added as an

independent variable, interactions between sex and site were used to detect differences in relative sexual size dimorphism among sites. Litter mass, litter size, and stillborn ratio were compared across populations using ANCOVAs with maternal SVL as a covariate, as female size, in both gartersnakes (e.g., Rossman et al., 1996) and reptiles in general (e.g., Pough et al., 2003), often corresponds with characteristics of her offspring. Differences in both adult and neonate sex ratios within and among sites were determined using chi-squared tests. Significant chi-squared tests were partitioned to determine significant pairwise differences with *P*-values being adjusted for multiple testing using the Holm method (e.g., Aickin and Gensler, 1996). Neonate length, neonate mass, and condition indices were also compared in this fashion, but litter was nested within each site. In addition, the effect of sex on neonate length and neonate mass was also examined via two-sample t-tests. If SVL covaried with any dependent variable, simple linear regressions were performed to determine the degree of monotone association between the two variables. For all analyses involving ANOVAs, the year an animal was collected or born was included to examine possible temporal effects in life-history trait variation. In addition, any possible interactions between all independent variables were examined whenever an ANOVA was employed. Data were natural log transformed to normalize the data and to linearize variate-covariate relationships. Alpha was set at 0.05 for all analyses.

RESULTS

Adult characteristics—The SVL of an animal had a significant effect on its mass ($F_{1,357} = 848.09$; $P < 0.0001$) with longer snakes weighing more than shorter snakes ($r^2 =$

0.83; $P < 0.0001$). This effect was controlled for by adding SVL as a covariate in the analysis of mass. Both SVL and mass of adult snakes varied as a result of collection site ($F_{5,357} = 9.85$; $P < 0.0001$ for SVL; $F_{5,357} = 18.58$; $P < 0.0001$ for mass), sex ($F_{1,357} = 70.76$; $P < 0.0001$ for SVL; $F_{1,357} = 12.38$; $P < 0.0001$ for mass), and reproductive state ($F_{2,357} = 42.14$; $P < 0.0001$ for SVL; $F_{2,357} = 17.47$; $P < 0.0001$ for mass) (Figs. 5.2 and 5.3), but neither SVL ($F_{2,357} = 1.49$; $P = 0.23$) or mass ($F_{2,357} = 1.78$; $P = 0.17$) varied depending on the year snakes were collected. Before determining site differences, sex and reproductive state effects need to be determined. In general, females were longer ($F_{1,357} = 70.76$; $P < 0.0001$) and heavier ($F_{1,357} = 12.38$; $P < 0.0001$) than males, and pregnant females were longer ($F_{2,357} = 42.14$; $P < 0.0001$) and heavier ($F_{2,357} = 17.47$; $P < 0.0001$) than both males and nonpregnant females. That females are larger than males in this system was also detected via the Index of Sexual Size Dimorphism (ISSD) (Gibbons and Lovich, 1990; Shine, 1993), which is calculated by dividing the mean SVL of the larger sex by the smaller sex, and subtracting this ratio from one. The resulting overall index for the populations from this study was -0.19, which reflects a female biased size dimorphism. However, differences in sex ratios among sites and differences in the ratio of pregnant females to nonpregnant females may confound site differences. To begin, male to female ratios are do not significantly differ from a 1:1 ratio at UP, SM, and HI sites ($P > 0.05$); however, GI, MM, and LP are female heavy populations ($P < 0.05$) In addition, chi-squared tests of independence indicate that there are significant site differences in both sex ratios ($X^2 = 14.94$, $G^2 = 16.78$; df = 5; $P < 0.05$) and the ratio of pregnant females to nonpregnant females to males ($X^2 = 57.97$, $G^2 = 50.39$; df = 10; $P < 0.0001$). When partitioning those tests, I found that more females were collected from

the LP site than from MM, SM, HI ($P < 0.05$; Table 5.1). The proportion of females collected from the UP did not differ from any other sites. In terms of reproductive state, more LP females were pregnant than females from HI and more GI females were pregnant than females from MM ($P < 0.05$; Table 5.1). Thus, any examination of site differences in this system needs to take into account sex and reproductive state.

After taking sex and reproductive state into account, no significant differences in adult SVL were detected among sites ($P > 0.05$; Fig. 5.2). In terms of both mass and condition indices (CIs), after taking SVL into account, SM males are heavier and have a greater CI than MM males, nonpregnant females from SM and GI are heavier and have greater CIs than nonpregnant HI and MM females, and pregnant females from LP and GI are heavier and have greater CIs than pregnant females from MM ($P < 0.05$; Fig. 5.3; Note: Since site differences for mass and condition are identical and since the condition index includes mass in its calculations, Fig. 5.3 only shows CI results). In addition, CI varied depending on reproductive state ($F_{2,355} = 19.09$; $P < 0.001$) with pregnant females being in better condition than nonpregnant females and both pregnant and nonpregnant females being in better condition than males ($P < 0.05$) for all six sites. As with SVL and mass, no significant difference in CI existed from year to year ($F_{2,355} = 1.26$; $P = 0.29$)

In addition, to site, sex, and reproductive state differences, significant interactions were detected for SVL between site and sex ($F_{5,357} = 2.63$; $P = 0.02$) and between site and reproductive state ($F_{10,357} = 1.91$; $P = 0.04$), and for mass between site and sex ($F_{5,357} = 5.12$; $P < 0.001$) and site and reproductive state ($F_{10,357} = 4.04$; $P < 0.0001$) indicating

that differences in SVL and mass between the sexes varies between sites. Specifically, LP females tend to be substantially longer than LP males and UP females tend to be closer to the same size as UP males when compared to sex differences for island sites (Fig. 5.2). In terms of mass (Fig. 5.3), GI females weigh more than GI males when compared to other site sex differences, while once again UP males and females tend to be similar in size. The interaction between sex and site for SVL is also apparent when the site ISSDs are calculated. While the ISSD for snakes from HI (-0.13), GI (-0.19), SM (-0.15), MM (-0.18) do not differ much from each other, substantial differences appear to exist between both the islands and the mainland and between the two mainland sites with the LP exhibiting an ISSD of -0.49 and the UP exhibiting an ISSD of -0.08. These values indicate that LP females are much larger than LP males and that UP males and females are close to the same size when compared to the other sites sampled in this study.

Litter characteristics—After taking maternal SVL into account, the total number of neonates per litter significantly differed among sites ($F_{5,43} = 16.27$; $P < 0.0001$; Fig. 5.4) with LP mother's giving birth to more babies than any of the island or UP mothers ($P < 0.05$). When testing for the influence of the mother's SVL on the number of neonates per litter, no significant effect was found ($F_{1,43} = 3.81$; $P = 0.06$); however, this *P*-value is marginal, and a regression analysis supports a significant positive relationship ($r^2 = 0.26$; $P < 0.001$; Fig. 5.5a) between the number of babies per litter and the size of the mother. In terms of total litter mass (g), significant site differences were also found ($F_{5,43} = 6.40$; $P = < 0.001$; Fig. 5.6). Specifically, the total mass of litters born to mothers from HI, GI, and SM was significantly less than those born to LP mothers ($P < 0.05$).

While MM litters did not differ significantly in mass from the LP litters, when pooling MM and SM data into a Beaver Island group, Beaver Island litters weighed significantly less than LP litters ($P < 0.05$). As with the total number of babies per litter, total litter mass increases as the mother's SVL increases ($r^2 = 0.21$; $P < 0.01$; Fig. 5.5b); however, again, the *P*-value from the ANOVA is only marginally significant ($F_{1,43} = 3.46$; $P = 0.07$). The number of stillborns per litter was not influenced by site ($F_{5,43} = 0.65$; $P = 0.67$) or maternal SVL ($F_{1,43} = 0.11$; $P = 0.74$). In addition, the year a litter was born did not influence the number of neonates per litter, the total litter mass, or the stillborn ratio ($P > 0.05$).

Differences in neonate sex ratios were detected with litters from the LP (0.56), MM (0.56), SM (0.65), GI (0.54), and HI (0.57) being female "heavy" and litters from the UP being male "heavy" with only 0.33 females. However, chi-squared tests indicate that none of these proportions deviate significantly from 0.5 ($P > 0.05$). In addition, no site differences in sex ratios were detected ($X^2 = 4.82$, $G^2 = 4.85$, df = 5, $P = 0.44$).

Neonate characteristics—In addition to litter differences, site specific morphological differences in neonates also existed. For example, after taking maternal SVL into account, the SVL ($F_{5,451} = 94.21$; $P < 0.0001$; Fig. 5.7) and mass ($F_{5,451} = 126.95$; $P < 0.0001$; Fig. 5.8) of neonates differed significantly both among sites and among litters within sites ($F_{36,451} = 11.45$; $P < 0.0001$ for SVL, Fig. 7; $F_{36,451} = 13.77$; $P < 0.0001$ for mass, Fig. 5.8). The sex of individual neonates, however, did not influence differences in either SVL ($t = -0.61$; df = 449; $P = 0.54$) or mass ($t = -0.80$; df = 449; $P =$

.42). Further, neonate SVL is not effected by maternal SVL ($F_{1,451} = 2.31$; $P = 0.13$); however, a significant negative relationship between the two variables is indicated by a simple linear regression ($r^2 = 0.06$; $P < 0.0001$; Fig. 5.9a). Neonate mass is under the influence of the mother's SVL ($F_{1,451} = 8.38$; $P = 0.004$) decreasing as maternal SVL increases ($r^2 = 0.05$; $P < 0.001$; Fig. 5.9b). As for site differences, in general, island neonates are longer and weigh more than mainland neonates. Specifically, the SVL and mass of neonates from the LP are significantly less than island neonates ($P < 0.05$), and UP animals are shorter than Beaver Island animals and weigh less than animals from HI and Beaver Island ($P < 0.05$). UP animals tend to be longer and weigh more than LP animals, but this was significant only for mass. As for differences between the islands, MM animals tend to be longer than animals from any other island site and weigh more than GI animals. In terms of condition ($F_{5,451} = 5.73$; $P < 0.0001$; Fig. 5.10), LP (0.77) and MM (0.77) neonates have significantly lower CIs than HI (0.81) and GI (0.79) neonates ($P < 0.05$). In addition to site differences on neonate morphology, neonates varied in SVL due to the year they were born ($F_{4,430} = 60.81$; $P < 0.0001$) with 2002 neonates averaging larger SVLs than 2001 neonates (Fig. 5.7). CIs also differed from year to year ($F_{2,408} = 17.96$; $P < 0.0001$; Fig. 5.10) with 2002 neonates having a significantly lower average CI (0.75) than 2001 neonates (0.8) While litter differences were detected for neonate SVL ($F_{36,451} = 11.45$; $P < 0.0001$), neonate mass ($F_{36,451} = 13.78$; $P < 0.0001$), and neonate CIs ($F_{37,451} = 11.29$; $P < 0001$), these were taken into account via nesting the litter variable within the site variable; therefore, the site differences discussed above exist regardless of any litter effect (Figs. 5.7, 5.8, and 5.10).

DISCUSSION

Variation in adult, litter, and neonate life-history characteristics is evident in the Beaver Archipelago and surrounding mainland populations. To begin, adult females are heavier and longer than adult males and pregnant females are, in general, the heaviest and longest of all of the snakes collected. In addition, significant differences in this sexual dimorphism among sites were also detected. In terms of site differences, taking sex and reproductive state into account eliminates most, but some differences remain. In terms of neonate and litter differences mainland animals tend to have large litter sizes with smaller babies, while island populations tend to have small litter sizes with larger babies. Maternal effects on litter and neonate characteristics were also detected, but do not appear to greatly influence site differences. In addition, temporal differences in both neonate SVL and neonate condition occur for all sites.

Variation in adult characteristics: Differences in adult size—While initially there appeared to be a trend of adult animals being longer and heavier on the mainland and shorter and lighter on the islands, which would have fit Case's (1978) hypothesis that snakes, in general, become smaller on islands, when taking into account, the sex and reproductive state of the animals sampled from each site, most of these differences disappeared. In the end, SM males are heavier than MM males, SM and GI nonpregnant females are heavier than HI and MM nonpregnant females, and pregnant females from LP and GI are heavier than pregnant females from MM. No significant SVL differences exist. The lack of many size differences in this system is more easily explained than the differences that were detected. To begin, despite having colonized the islands of the

Beaver Archipelago about 10,000 years ago, preliminary microsatellite analyses indicate that gene flow between island and mainland *T. sirtalis* populations is common (Placyk, unpubl. data) possibly canceling out any genetic influence on size shifts. In terms of environmental differences, while prey availability (the main driving force in most insular snake population size shifts) varies considerably among sites (Placyk, pers. obs.), *T. sirtalis* is a generalist predator and may easily adapt to such differences with little morphological effect. In terms of the site differences that were detected, logical explanations are not evident, as MM animals have a greater abundance and diversity of prey available to them (Krause et al., 2003) than GI and SM snakes with many other environmental and probably genetic differences being canceled out, especially between MM and SM populations which are found less than 1 km apart. Interestingly, Krause et al. (2003) found that MM females were longer and heavier than females from a Beaver Island population with poorer resource availability, but Krause's second Beaver Island site (i.e., McCafferty Farm site) was also more than 2 km away from the MM site indicating much less gene flow and travel between the two sites. One reason for the lack of differences detected in my data may be that the age structures of these populations differ, but size differences that vary between the sexes among sites do not easily fit this hypothesis. Boback (2003) indicated that while there are general trends of dwarfism and gigantism on islands that many snake populations may not vary in such systems; however, he indicated if that was the case, then such studies have not been published. Given my data, it appears as though this island system may be one in which insular populations do not vary significantly from their mainland conspecifics in terms of body

size, despite being geologically separated for an extended period of time and experiencing variation in selection pressures.

In terms of differences due to sex and reproductive state, females, in general tended to be longer and heavier than males regardless of their reproductive state, which is common in gartersnakes (e.g., Shine and Mason, 2001; Krause, 2000). When taking reproductive state into account, pregnant females were longer and heavier than both nonpregnant females and males. There may be several reasons for these trends. First, Crews et al. (1985) have indicated that the small size of male gartersnakes was mainly due to the effect of testosterone. Second, the larger many female reptiles grow, the more offspring they are able to produce (e.g., Ford and Seigel, 1989), thus there may be an evolutionary advantage in terms of reproductive fitness for females to growth larger than males; however, evolutionary pressures may also select for smaller males. For example, in some species of snakes, including northern populations of *T. sirtalis*, animals breed in large aggregations (e.g., Shine and Mason, 2001). Often times males use the size of a conspecific, along with several other cues (Shine and Mason, 2001; Shine et al., 2003a, 2003b), to discriminate females from other males (Rivas and Burghardt, 2001; Shine and Mason, 2001). As a result, it may be advantageous for males to be smaller in such systems so that other males do not disrupt their locomotion and courtship behavior or even copulate with them (Pfrender et al., 2001). Such systems may also select for larger females as well, since the bigger a female, the more likely the male will be able to identify her and mate with her. In terms of size differences between pregnant and nonpregnant females, pregnant females may be larger than nonpregnant females, because

there may be a critical threshold that must be reached in terms of body reserves that must be met before females will become sexually receptive (Aubret et al., 2002). In addition, the ability to regain mass after giving birth has been linked to female survival (Bonnet et al., 2002), and since larger animals are probably able to eat a greater variety of prey, this may also play a role in this system.

In addition to a general trend of females being larger than males, differences in size between males and females also varied among sites. Specifically, LP females tend to be much larger than LP males, while UP females tend to be close to the size of UP males. Island animals fit in between these two extremes. Madsen and Shine (1993) found a reduced degree of sexual size dimorphism in island populations of snakes and attributed the difference to prey availability. This may explain why LP females and UP males and females are all quite large, but it does not explain why LP males are relatively small. Another possibility is that age structures vary between my populations. Usually, the age of a snake is determined by its length, while I found no significant differences in SVL between my sites, it is still possible that the age structure varies, as once an animal with indeterminate growth reaches a certain size their growth rate decreases steadily. Sexual selection may also play a role, as larger body size may enhance the mating success of male gartersnakes, probably because they can displace their rivals' tails from the vicinity of the female's cloaca prior to coitus (Shine et al., 2000; Shine et al., 2001; Shine et al., 2004). Since LP populations are found at much lower densities than island populations, selection for larger LP males may be weaker than on the islands.

Variation in adult characteristics: Differences in adult sex ratios and reproductive state ratios—There are several possible reasons why more females are represented in my data than males. First, while not significant, data on the sex ratios of litters produced by individuals from my populations indicate that there may be a female bias at birth. Second, males may suffer from higher predator pressures than females. Evidence from the populations in this system suggests that males may be less likely to survive predatory attacks than females (Placyk and Burghardt, 2005). This is believed to be the case because many snake predators often damage the tails of snakes and since the reproductive organs of males are found in their tails, damage to their tails may result in damage that may be fatal. In addition, since males are smaller than females, they may 1) have more predators than females, and 2) may not be able to fend off predators as easily as females. For example, milksnakes, *Lampropeltis triangulum*, Colubridae, which are found at many of the sites (Placyk and Burghardt, 2005), may only be able to restrain and swallow smaller male snakes.

Finally, males may exhibit behaviors that would make their capture more difficult. Males of many species often disperse greater distances to find females than females do to find males. Often females move very little when searching for mates. As a result, male *T. sirtalis* may have been in transit more than females and as a result, were not sampled as easily or conversely, suffer higher predation due to this mobility. Shine et al. (2000) also found that males were more likely to flee from collectors than females. In addition, since large, pregnant females need to thermoregulate more precisely then males, they may have been easier to catch because 1) they may be found out in the open more,

and 2) because the weight associated with their pregnancy may slow them down. In fact, Shine (2003b) and Brown and Shine (2004) confirmed that pregnancy significantly impairs maternal locomotion in both lizards and snakes and this has also been demonstrated in a wide variety of invertebrates (Shaffer and Formanowicz, 1996; Isaacs and Byrne, 1998; Gu and Danthanarayana, 2000) and vertebrates (Shine, 1980; Seigel et al., 1987b; Cooper et al., 1990; Lee et al., 1996; McLean and Speakman, 2000; Veasey et al., 2000, 2001). In addition, both gravid and non-gravid adult females of tiger snakes have reduced muscle mass when compared to males (Schwaner and Sarre, 1988). In addition, males may not be as easy to catch or they may have been inadvertently selected against in collection procedures. Since males are smaller, they may be less noticeable when fleeing from a potential collector. Males may also be harder to grasp and fit into hiding spots more easily than larger snakes, and as indicated above, they may have more muscle mass devoted to locomotion than females. In fact, differences in sex ratios observed in tiger snakes were attributed to differences in catchability (Schwaner, 1985) and behavioral differences (Shine, 1987; Schwaner and Sarre, 1988). While Bonnet et al. (2002) indicated that sex didn't influence catchability directly, they suggested that size was the reason more males were sampled than females in their study of *Notechis scutatus*, Elapidae, a species in which males are generally larger than females.

Still, why would there be differences in adult sex ratios among sites? Sex ratios do not vary from site to site at birth. It is more likely that these site differences are the result of variation in various environmental factors. For example, predator pressures appear to be greater at the mainland sites than at the island sites (Placyk and Burghardt,

2005) and the sample of adults from the LP had the greatest frequency of females of all the populations sampled. Similarly, Carpenter (1952) estimated female biased sex ratios in *T. sirtalis* populations (80 % females for 1949) in his seminal study on the ecology of three species of gartersnakes in the lower peninsula of Michigan, so my results do not appear to differ much from Michigan *T. sirtalis* population sex ratios from over 50 years ago. Carpenter (1952) indicated that females appeared to be more active in June and July (two prime collection times for my study), which may offer yet another reason for such bias in the observed sex ratios. In addition, mainland populations are less dense than island populations. As a result, more males may have been sampled on the islands, because they were more easily found than mainland males.

Neonate and litter characteristics: Optimal litter and neonate sizes—Animals from the LP produced more, smaller offspring while island animals produced fewer, larger offspring. These observations fit life-history theory that predicts that litter size and neonate size should be negatively correlated (e.g., Smith and Fretwell, 1974). However, while Smith and Fretwell (1974) also suggest that animals producing fewer, larger babies have a greater fitness than those producing more, smaller babies, the model does not discriminate between specific environmental and genetic differences that may lead to variation in litter and neonate characteristics. When environmental factors, particularly (e.g., prey availability, density of competitors) and other neonate characteristics (e.g., condition), are taken into account, the fitness of babies from populations of the same species that vary in litter and neonate characteristics may be the same. However, if populations vary due to genetic constraints, these differences may not confer the same

level of fitness. We can begin to understand the fitness of the neonates in my system by examining differences in the condition indices I calculated. While LP neonates have one of the lowest CIs of all of the populations sampled, supporting the idea that smaller neonates may not be as fit as larger neonates, a simple linear regression indicates no relationship between neonate CI and litter size ($r^2 = 0.002$; $P = 0.3$; Fig. 5.11), possibly indicating larger offspring may be no better than smaller offspring and that producing more equally fit babies may actually be a better strategy. Therefore, my system provides conflicting information on the influence of neonate size on fitness.

What, however, is responsible for site differences in litter and neonate size? Stearns (1992) synthesized much of the work in this area and found that when food is readily available, population densities are low, and temperature regimes are optimal, more offspring are produced, but that the opposite occurs when food availability is low, population densities are high, and temperature regimes are not optimal. In addition, differences in predator pressures also strongly influence reproductive energy allocation (e.g., Reznick, 1982; Reznick et al., 1990). While these proximate influences play a large role in much of the reproductive energy allocation variation that is observed, the genetics of such allocation also plays a part. In fact, Mousseau and Roff (1987) found that there is considerable genetic variation for clutch-size, litter size, and fecundity. Therefore, many factors may be acting on the variation in my system.

Between the Beaver Archipelago and the surrounding mainland, differences in most of the factors mentioned above are common. For example, the richness of prey

available on the islands is less than that found on the mainland (Placyk and Burghardt, in prep.), the population density of the island populations is much higher than those for the mainland populations (Placyk, pers. obs.), and the temperature regimes vary, with the islands experiencing cooler summers and colder winters than the LP and with the islands freezing before and thawing later than mainland sites (Phillips and McCulloch, 1972). In terms of genetics, Placyk et al. (under rev.) recently discovered that the islands were colonized by both LP and UP populations with more island haplotypes being shared with UP populations than with LP populations.

Given this information on environmental differences, one can hypothesize that the island populations reside under harsher environmental conditions than the LP populations and that given their underlying genetics, may be more similar to UP populations than LP populations for traits with strong genetic components. As a result of the environmental differences, one would expect to see more energy allocated per offspring on the islands than on the mainland, which is the general trend seen here. However, UP litter and neonate characteristics do not differ as considerably from the island populations as the LP does. This may be due to the genetics of my populations or to environmental factors. In terms of molecular genetics, as mentioned above, mtDNA haplotypes found on the islands represent a greater genetic contribution from the UP than from the LP (Placyk, 2006; Placyk et al., under rev.). In addition, UP populations were founded by populations that followed a recolonization route along the west coast of Lake Michigan (Placyk, 2006; Placyk et al., under rev.). Both Fitch (1985) and Gregory and Larsen (1993) indicate that there are longitudinal differences in reproductive characteristics of *T.*

sirtalis with an east-west trend in which the farther east you travel the bigger the litters and the smaller the babies produced by *T. sirtalis*. In terms of environmental differences, both summer and winter temperatures that UP populations experience are lower than those experienced by the LP populations (Phillips and McCulloch, 1972) creating harsher conditions for UP *T. sirtalis* populations.

Neonate and litter characteristics: Maternal influences—Maternal body size has often been positively correlated with litter size in snakes, and, although less commonly documented, neonate size can show either positive or negative correlations with maternal size (Shine, 2003). My data indicate that there are significant positive relationships between maternal size and number of neonates per litter and total litter mass, and a significant negative relationship between maternal size and neonate size. Specifically, maternal size accounts for 26 % of the variation in litter size and 21 % of the variation in total litter mass (21 %), but only 6 % of the variation in neonate SVL and 5 % of variation in neonate mass. Gregory and Larsen (1993) also found a significant positive relationship between maternal size and litter size, but found that no such relationship existed for neonate SVL, which my analysis indicates is influenced very little by maternal size. Krause (2000), who also studied *T. sirtalis* populations from Beaver Island, found a positive relationship between maternal SVL and litter size, which corresponds with my work, but found positive linear relationships between maternal SVL and both neonate SVL and neonate mass, which contradicts my results. However, unlike my results, none of the relationships Krause (2000) detected were statistically significant. Regardless of the significance, there may be several reasons for such differences being found in the

same system. First, Krause (2000) sampled 286 neonates from 33 litters from only 2 sites (both on Beaver Island), while I sampled 451 neonates from 43 litters from 6 sites. Therefore, his work gives us more of an idea about how maternal SVL may be influencing neonate and litter characteristics from one island, but not its overall influence on multiple populations in the same system. For example, the full size range of snakes in the entire system is not represented by populations from one island. My populations show a range of 320 to 700 mm for maternal SVL. If I were to only consider Beaver Island populations (SM & MM), the range would be 320 to 580 mm. In addition, since Krause (2000) sampled his sites in different years than I sampled mine, environmental differences could have been dramatically different. Regardless of the differences between my study and Krause's (2000), both of us found that a good amount of variation in litter size was accounted for by maternal size (25.9 % for litter size in Krause's study (2000)). Bonnet et al. (2000) suggest that larger body size has a more consistent effect on ecological attributes than on reproductive output, which may account for the remainder of the variation not explained by my results.

In addition to the effect of maternal SVL on litter and neonate characteristics, significant litter effects were also found indicating possible other maternal effects. Regardless, maternal SVL and litter effects were taken into account in all analyses possible and none appear to alter the detected site effects. Therefore, while some variation in litter and neonate characteristics appear to be controlled by maternal influences, either environmental or other genetic factors are maintaining the site differences I observed.

Neonate and litter characteristics: Temporal variation—In addition to the differences noted above, temporal variation in both neonate SVL and neonate CIs were also detected. Overall, neonates were longer with smaller CIs in 2002 with neonates being born in 2001 being shorter with larger CIs. Since, *T. sirtalis* are considered 'income breeders', in which reproduction is fuelled by recently acquired energy (Drent and Daan, 1980) or food intake immediately before ovulation, the influence of environmental factors of the year they are attempting to produce offspring will have a major effect on the phenotypes of those offspring. Since 2002 had a much hotter and drier active season than 2001, the primary prey of *T. sirtalis* in northern Michigan (i.e., earthworms, amphibians, and fish) were less common, as all are associated with wet conditions. This decrease in prey availability between 2001 and 2002 may have resulted in pregnant mothers obtaining less energy to allocate to her offspring resulting in lower condition indices for her neonates. Temporal fluctuations in reproductive output and the amount of energy allocated to offspring in a given year are common (e.g., Murphy, 1968; Schaffer, 1974; McGinely et al., 1987; Madsen and Shine, 1999; Shine and Downes, 1999; Bonnet et al., 2001; Lourdais et al., 2003) resulting primarily from environmental factors, especially, due to fluctuations in prey availability (Andren, 1982; Andren and Nilson, 1983; Madsen and Shine, 1999; Shine and Downes, 1999) so to see it in my system is to be expected. It is interesting to note, though, that neither total litter mass or the number of neonates varied from year to year. This may further indicate that for this system, those factors may have a strong genetic component. Other studies with *Thamnophis* indicate that as food intake increases litter size increases (Ford and Seigel,

1989) indicating that *T. sirtalis* not only exhibits population level differences in litter number, but also genus level differences.

Conclusions—Variation in adult, litter, and neonate life-history traits appear to be common in my system. This work provides more empirical evidence, from which broader hypotheses may be proposed and life-history models may be tested and constructed. In addition, my data provides some evidence that both genetic and environmental factors need to be taken into account when examining intraspecific variation in life-history traits. More detailed studies focusing specifically on these factors are needed to further support my hypotheses. In particular, a long-term study in which animals from these various sites are reared and bred under controlled laboratory settings, where predator pressures are absent, food is readily available, and temperature regimes are constant would be the first step and would not be difficult to carry out given time. Such a long-term study would allow a better understanding of the proximate and ultimate influences on variation in life-history traits and would further support and strengthen current and future hypotheses and models. In addition to providing much needed data on life-history trait variation in general, this study also provides data on a generalist snake species that is an 'income' breeder. The data indicate that the idea that snakes either become giants or dwarfs on islands may not apply to generalist species, and more studies using generalist predators are needed to confirm this. In addition, temporal variation in prey availability and other environmental conditions appear to directly influence the condition of neonates but not litter characteristics in this 'income' breeder.

As for the broader implications of this study, much has been hypothesized about changes in body size on islands and optimal litter and neonate characteristics. This work contradicts previous data on body size changes in island systems but supports parental investment theory. Specifically, snakes, in general, have either become dwarfed or gigantic on island (e.g., Case, 1978; Boback, 2003), usually due to predator pressures and resource availability. While both predator and prey richness vary between the sites examined for this study, no significant differences in adult size were detected. This may be due to the fact that I took into account both sex and reproductive state, while most past studies have not and because *T. sirtalis* is a generalist species that may readily adapt to variation in environmental characteristics. Most past studies on body size change in snakes have focused on snakes that have more specialized diets. In addition, studies in which no significant size differences between island and mainland populations of snakes exist may not be published as often due to the nonsignificance of the results.

In terms of parental investment theory, my work appears to support hypotheses that are typically made in this area. Snakes from populations under harsher conditions appear to allocate more energy per offspring than snakes from populations under less harsh conditions. As a result, island snakes produce fewer, bigger babies while lower peninsula snakes produce more, smaller babies. Mothers from the upper peninsula are poorly represented in my sample, but appear to fit the island trend of having smaller litters with bigger babies. This may be due to the harsher conditions, especially in terms of temperature, or it may indicate more underlying genetic influences of the litter and neonate characteristics that they appear to share with the island populations.

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APPENDIX V

TABLES AND FIGURES

Table 5.1. Number and frequency of male, total female, and pregnant female adult common gartersnakes, *Thamnophis sirtalis*, from the upper (UP) and lower (LP) peninsulas of Michigan and from High Island (HI), Garden Island (GI) and the Sawmill (SM) and Miller's Marsh (MM) sites on Beaver Island of the Beaver Archipelago. Different letters in each column represent significant site differences ($P < 0.05$) for that specific column as detected by partitioning X^2 tests of independence.

Collection Site	Males		Total females		Pregnant females	
	Count	Frequency	Count	Frequency	Count	Frequency
HI	21	0.55	17	0.45[a]	5	0.13[a]
GI	33	0.35	61	0.65[ab]	25	0.27[ab]
SM	31	0.45	38	0.54[a]	4	0.06[abc]
MM	52	0.41	74	0.58[a]	18	0.14[c]
LP	2	0.09	20	0.91[b]	16	0.73[bc]
UP	2	0.25	6	0.75[ab]	2	0.25[abc]

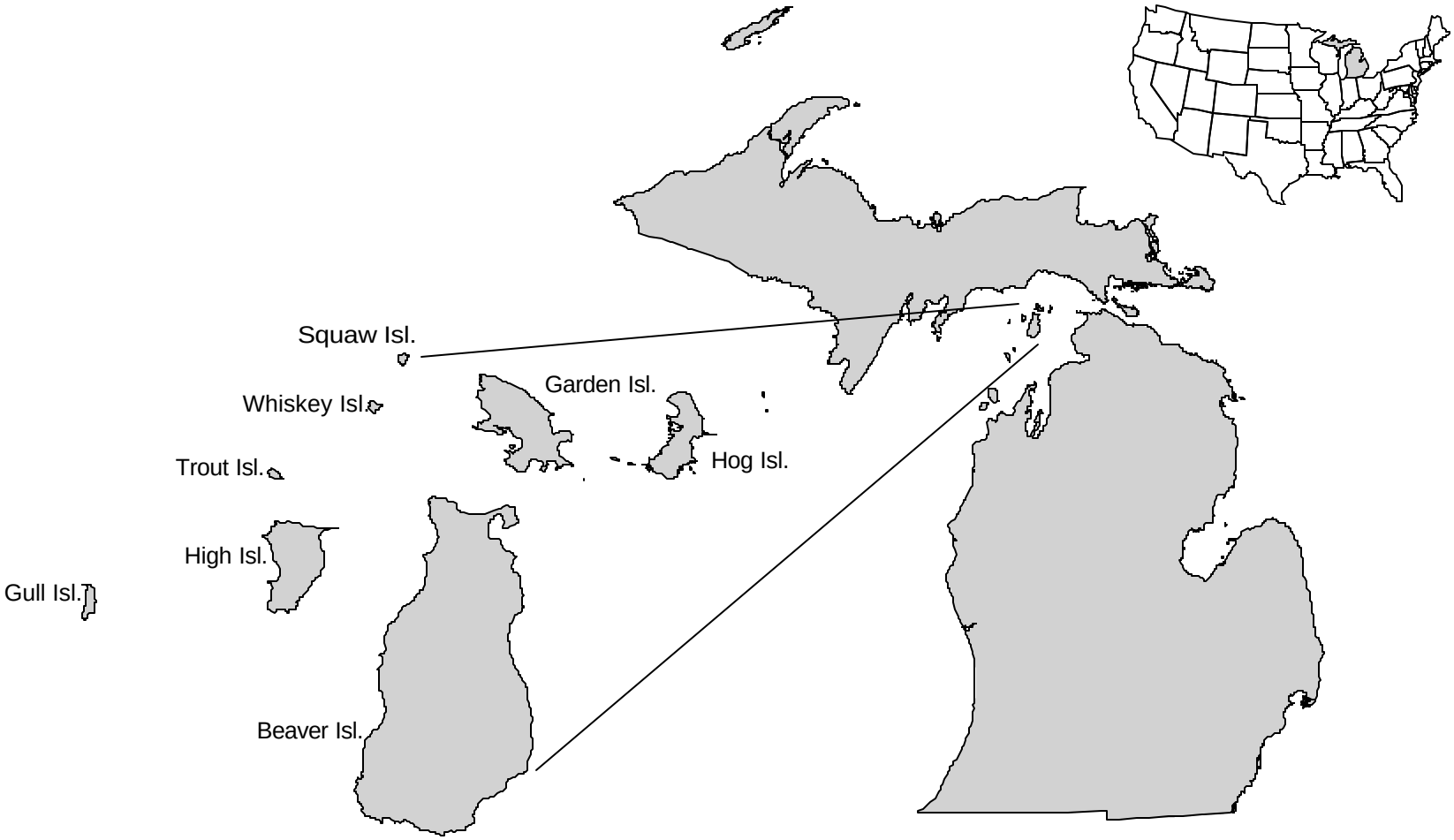


Fig. 5.1. Collection localities for common gartersnake, *Thamnophis sirtalis*, populations sampled for this study (modified from Hansknecht, 2003).

Fig. 5.2. Mean snout-vent length (SVL) (± 95 % CI) (mm) of adult male (a), nonpregnant female (b), and pregnant female (c) common gartersnakes, *Thamnophis sirtalis*. Snakes were collected from the lower (LP, n = 22) and upper (UP, n = 7) peninsula of Michigan and High Island (HI, n = 38), Garden Island (GI, n = 94), and two sites on Beaver Island (SM, n = 69; MM, n = 126) of the Beaver Archipelago in northeastern Lake Michigan. Values at the bottom of each bar indicate sample sizes (n) for each reproductive state for each site. For all sites, males are significantly shorter than both nonpregnant and pregnant females and nonpregnant females are significantly shorter than pregnant females ($P < 0.05$) as detected by Tukey-Kramer Multiple-Comparison Tests. No site differences in SVL were detected.

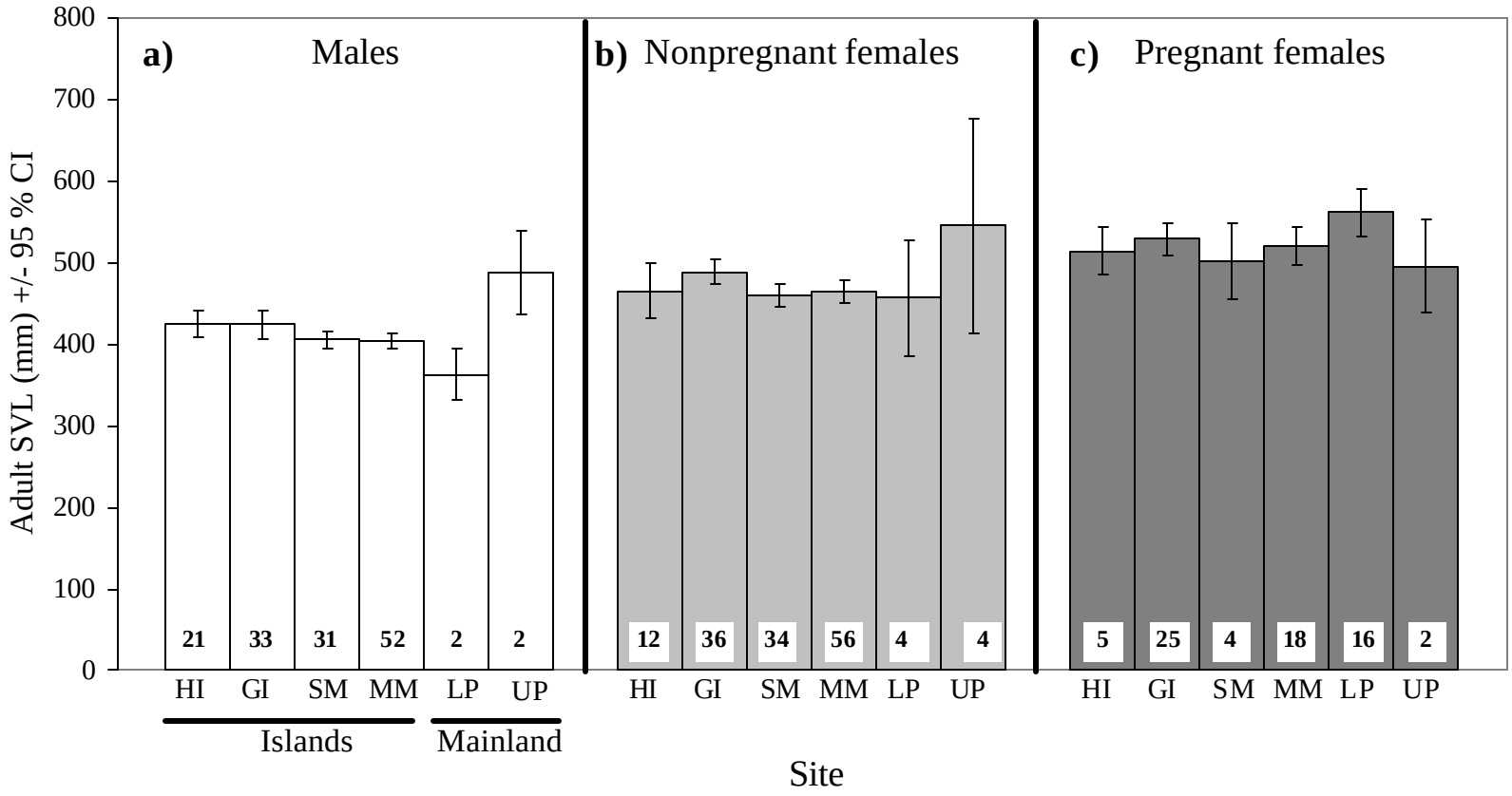

a) Males
b) Nonpregnant females
c) Pregnant females
Adult SVL (mm) +/- 95 % CI
800
700
600
500
400
300
200
100
0
21
33
31
52
2
2
12
36
34
56
4
4
5
25
4
18
16
2
HI
GI
SM
MM
LP
UP
Islands
Mainland
Site

Fig. 5.3. Mean condition index (CI) (± 95 % CI) of adult male (a), nonpregnant female (b), and pregnant female (c) common gartersnakes, *Thamnophis sirtalis*. Snakes were collected from the lower (LP, n = 22) and upper (UP, n = 7) peninsula of Michigan and High Island (HI, n = 38), Garden Island (GI, n = 94), and two sites on Beaver Island (SM, n = 69; MM, n = 126) of the Beaver Archipelago in northeastern Lake Michigan. Values at the bottom of each bar indicate sample size (n) for each reproductive state for each site. For all sites, males are significantly lighter than both nonpregnant and pregnant females and nonpregnant females are significantly lighter than pregnant females ($P < 0.05$). Significant site differences within each reproductive state are indicated with different letters ($P < 0.05$). All significant differences detected by Tukey-Kramer Multiple-Comparison Tests.

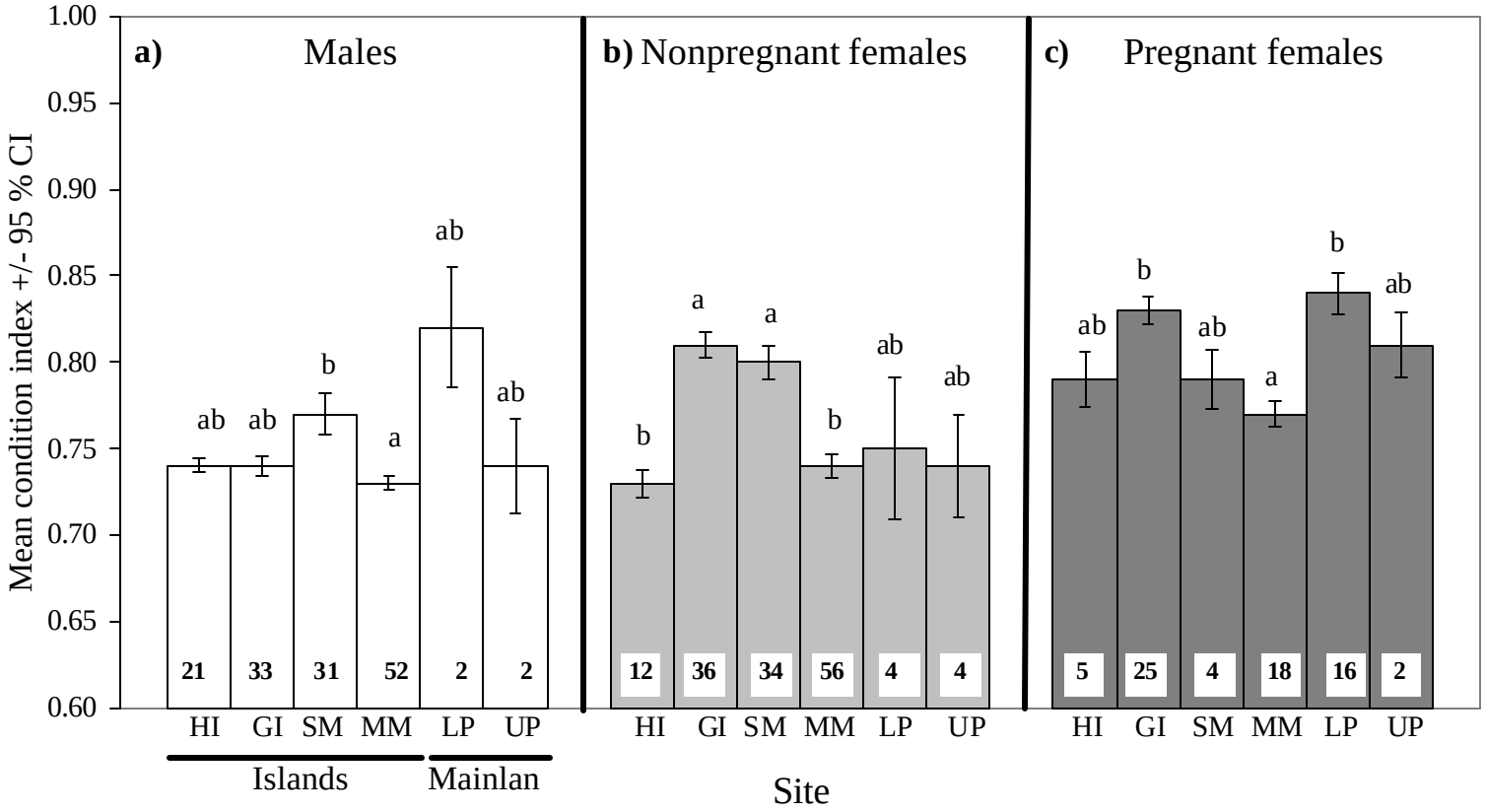
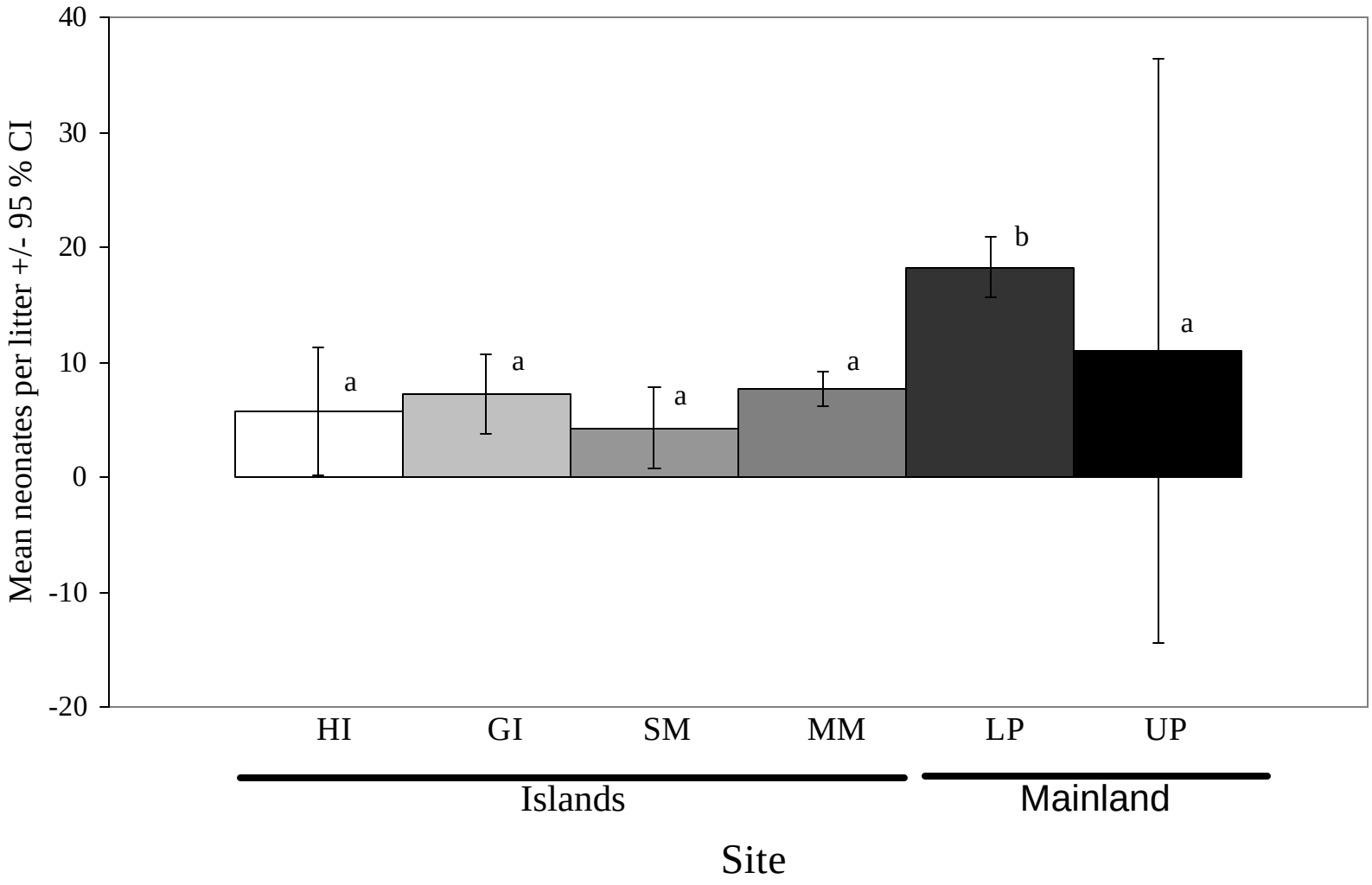

a) Males
b) Nonpregnant females
c) Pregnant females
Mean condition index +/- 95 % CI
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
ab ab b a ab ab
21 33 31 52 2 2
b a a b ab ab
12 36 34 56 4 4
ab b ab a b ab
5 25 4 18 16 2
HI GI SM MM LP UP
Islands
Mainlan
Site

Fig. 5.4. Mean number (± 95 % CI) of neonates born per litter to common gartersnakes, *Thamnophis sirtalis*. Litters were born to mothers collected from the lower (LP, n = 13) and upper (UP, n = 2) peninsula of Michigan and High Island (HI, n = 4), Garden Island (GI, n = 9), and two sites on Beaver Island (SM, n = 4; MM, n = 11) of the Beaver Archipelago in northeastern Lake Michigan. Different letters indicate significantly different means ($P < 0.05$) as detected by Tukey-Kramer Multiple-Comparison Tests.


40
30
20
10
0
-10
-20
Mean neonates per litter +/- 95 % CI
a
a
a
a
b
a
HI
GI
SM
MM
LP
UP
Islands
Mainland
Site

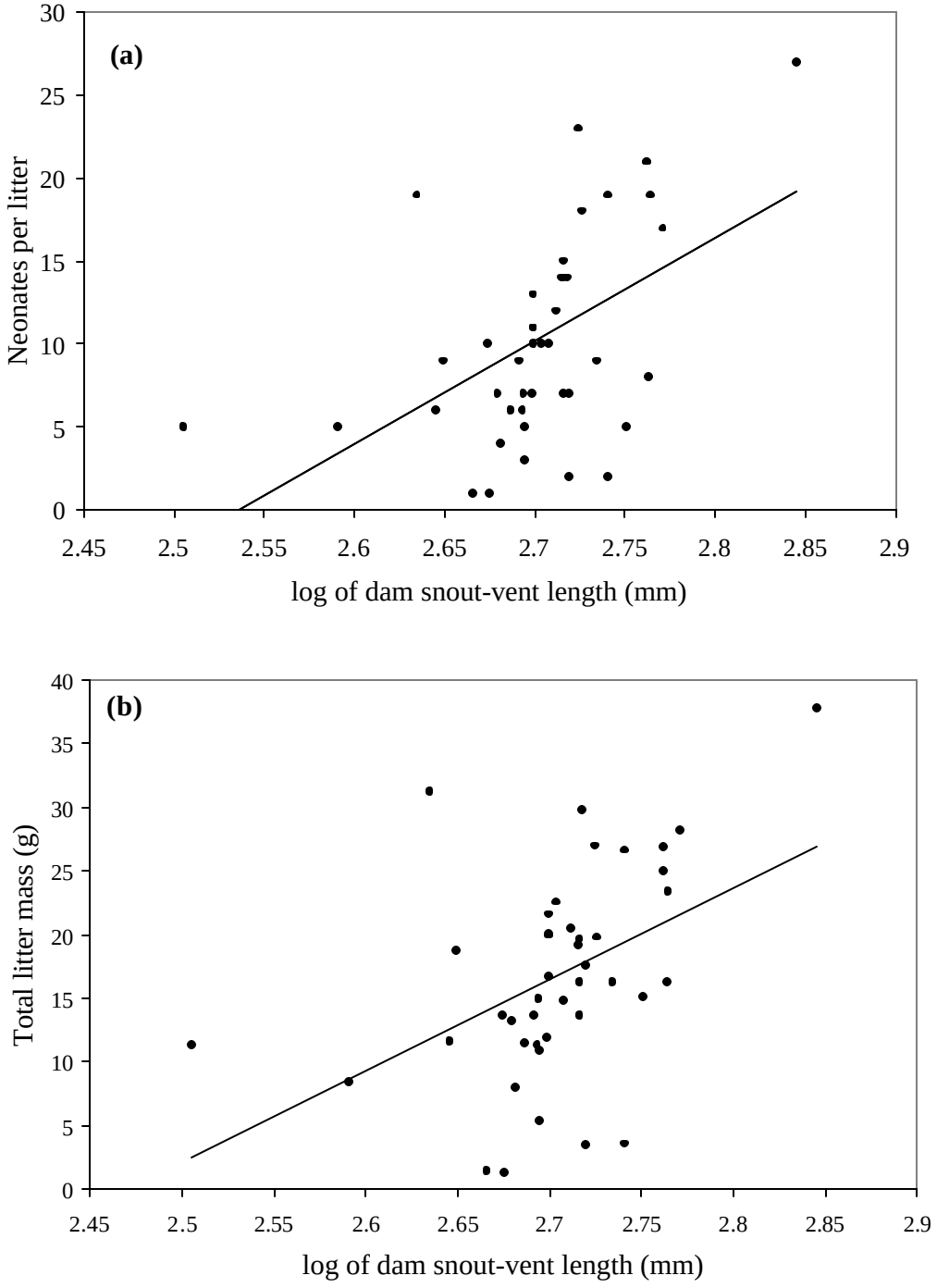


Fig. 5.5. Relationship between the log of dam snout-vent length (mm) and both number of neonates per litter (number of neonates = -156.39 + 61.70 log dam SVL; $r^2 = 0.26$; $P < 0.001$) (a) and total litter mass (g) (litter mass = -176.19 + 71.36 log dam SVL; $r^2 = 0.21$; $P < 0.01$) (b).

Fig. 5.6. Mean total litter mass (g) (± 95 % CI) for litters born to common gartersnakes, *Thamnophis sirtalis.* Litters were born to dams collected from the lower (LP, n = 13) and upper (UP, n = 2) peninsula of Michigan and High Island (HI, n = 4), Garden Island (GI, n = 9), and two sites on Beaver Island (SM, n = 4; MM, n = 11) of the Beaver Archipelago in northeastern Lake Michigan. 95 % confidence intervals are also plotted. Different letters indicate significantly different means ($P < 0.05$) as detected by Tukey-Kramer Multiple-Comparison Tests.

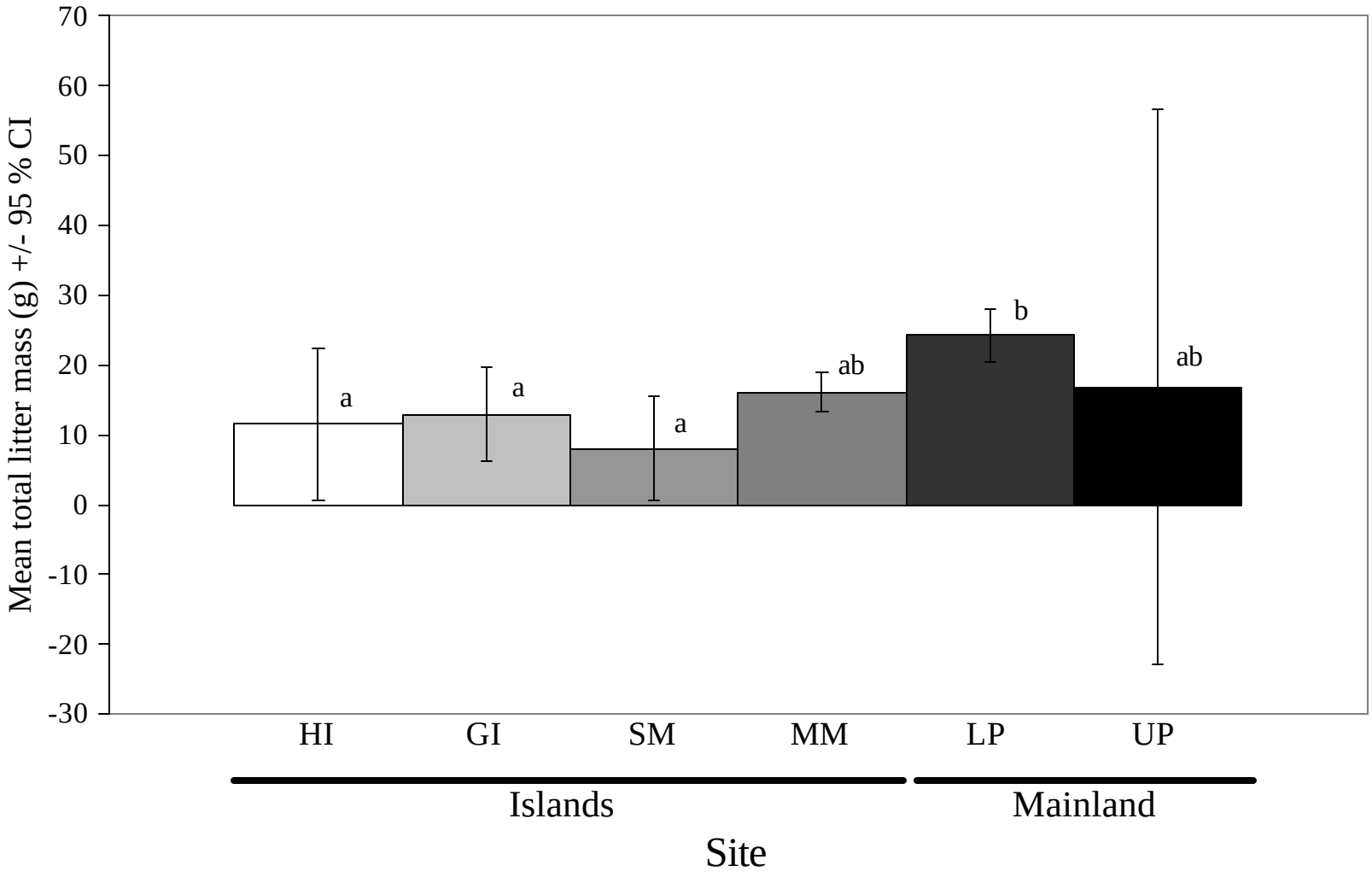

70
60
50
40
30
20
10
0
-10
-20
-30
Mean total litter mass (g) +/- 95 % CI
a
a
a
ab
b
ab
HI
GI
SM
MM
LP
UP
Islands
Mainland
Site

Fig. 5.7. Mean snout-vent length (SVL) (mm) (± 95 % CI) of neonates born to common gartersnakes, *Thamnophis sirtalis.* Neonates were born in litters to dams from the lower (LP, n = 237) and upper (UP, n = 21) peninsula of Michigan and High Island (HI, n = 23), Garden Island (GI, n = 65), and two sites on Beaver Island (SM, n = 17; MM, n = 88) of the Beaver Archipelago in northeastern Lake Michigan collected in 2001, 2002 & 2004. Bars nested within sites represent different litters. For HI, GI, SM, MM, and LP, mean neonate SVL was significantly longer in 2002 than in 2001 ($P < 0.05$). Significant site differences are indicated by different letters ($P < 0.05$). All significant differences detected by Tukey-Kramer Multiple-Comparison Tests. There was no interaction between the year a neonate was born and site ($P < 0.05$).

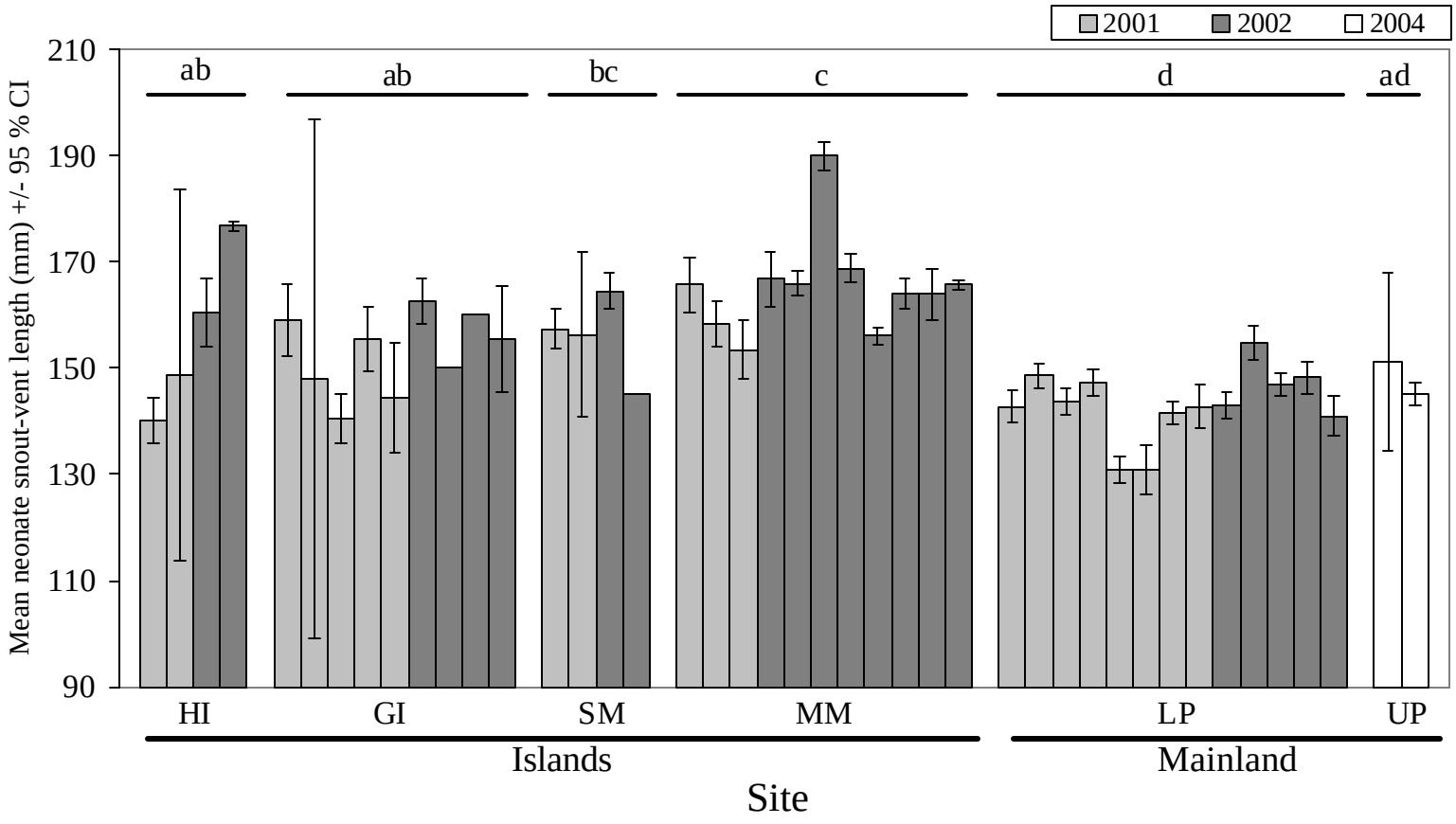
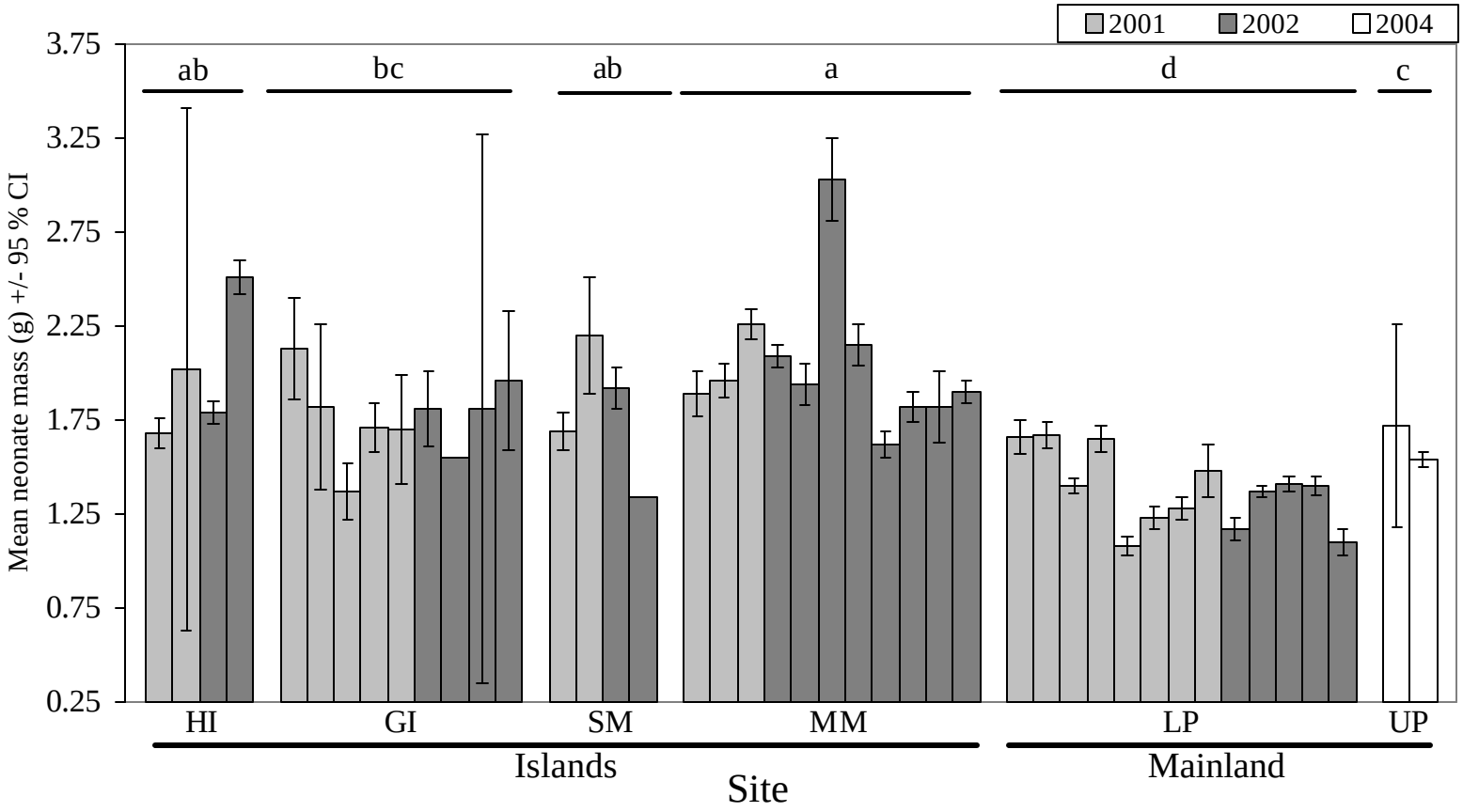

2001
2002
2004
Mean neonate snout-vent length (mm) +/- 95 % CI
210
190
170
150
130
110
90
ab
ab
bc
c
d
ad
HI
GI
SM
MM
LP
UP
Islands
Mainland
Site

Fig. 5.8. Mean mass (g) (± 95 % CI) of neonates born to common gartersnakes, *Thamnophis sirtalis.* Neonates were born in litters to dams from the lower (LP, n = 237) and upper (UP, n = 21) peninsula of Michigan and High Island (HI, n = 23), Garden Island (GI, n = 65), and two sites on Beaver Island (SM, n = 17; MM, n = 88) of the Beaver Archipelago in northeastern Lake Michigan. Bars nested within sites represent different litters. The year a neonate was born did not influence its mass ($P < 0.05$). Significant site differences are indicated by different letters ($P < 0.05$). All significant differences detected by Tukey-Kramer Multiple-Comparison Tests.


2001
2002
2004
3.75
3.25
2.75
2.25
1.75
1.25
0.75
0.25
Mean neonate mass (g) +/- 95 % CI
ab
bc
ab
a
d
c
HI
GI
SM
MM
LP
UP
Islands
Mainland
Site

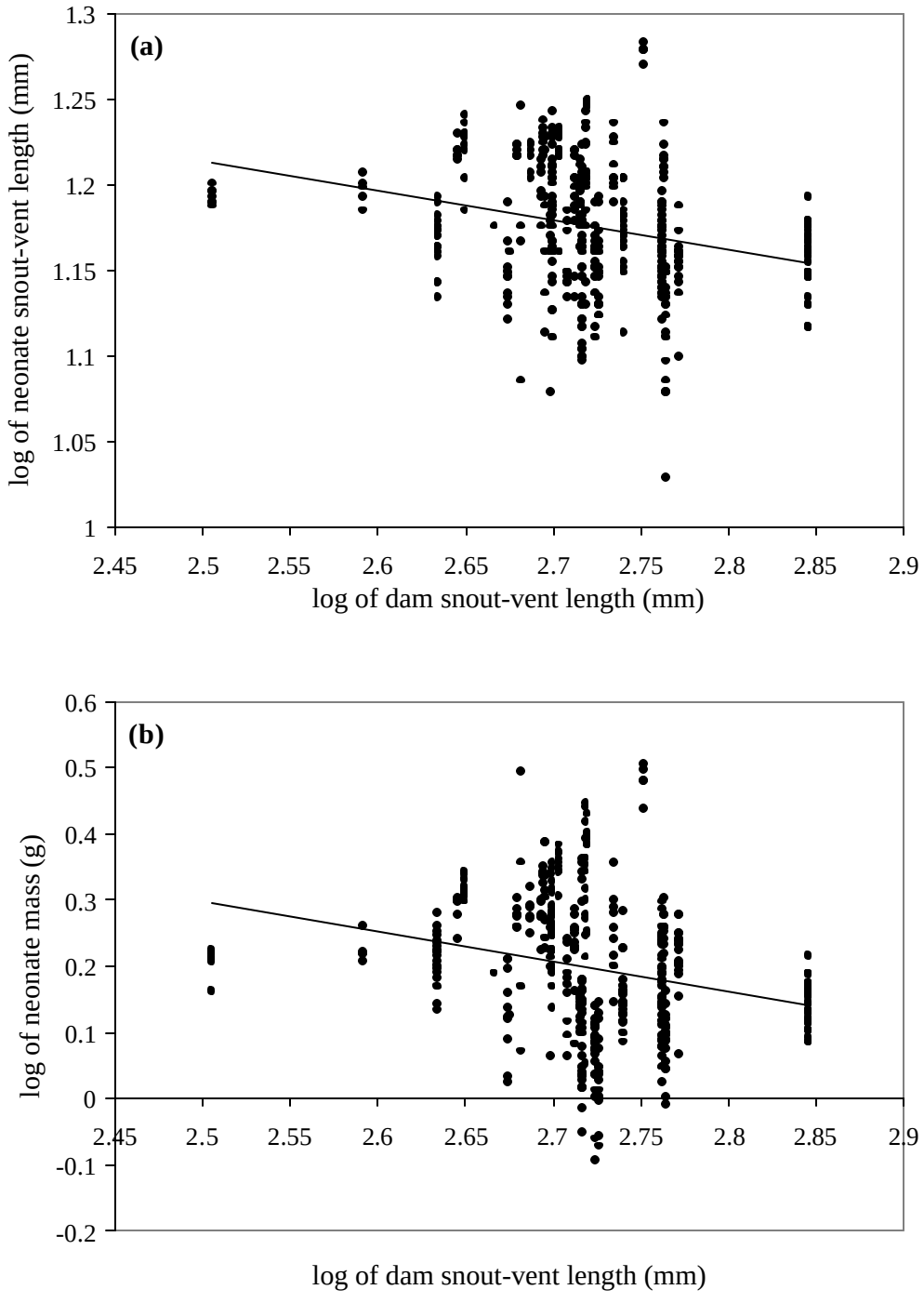
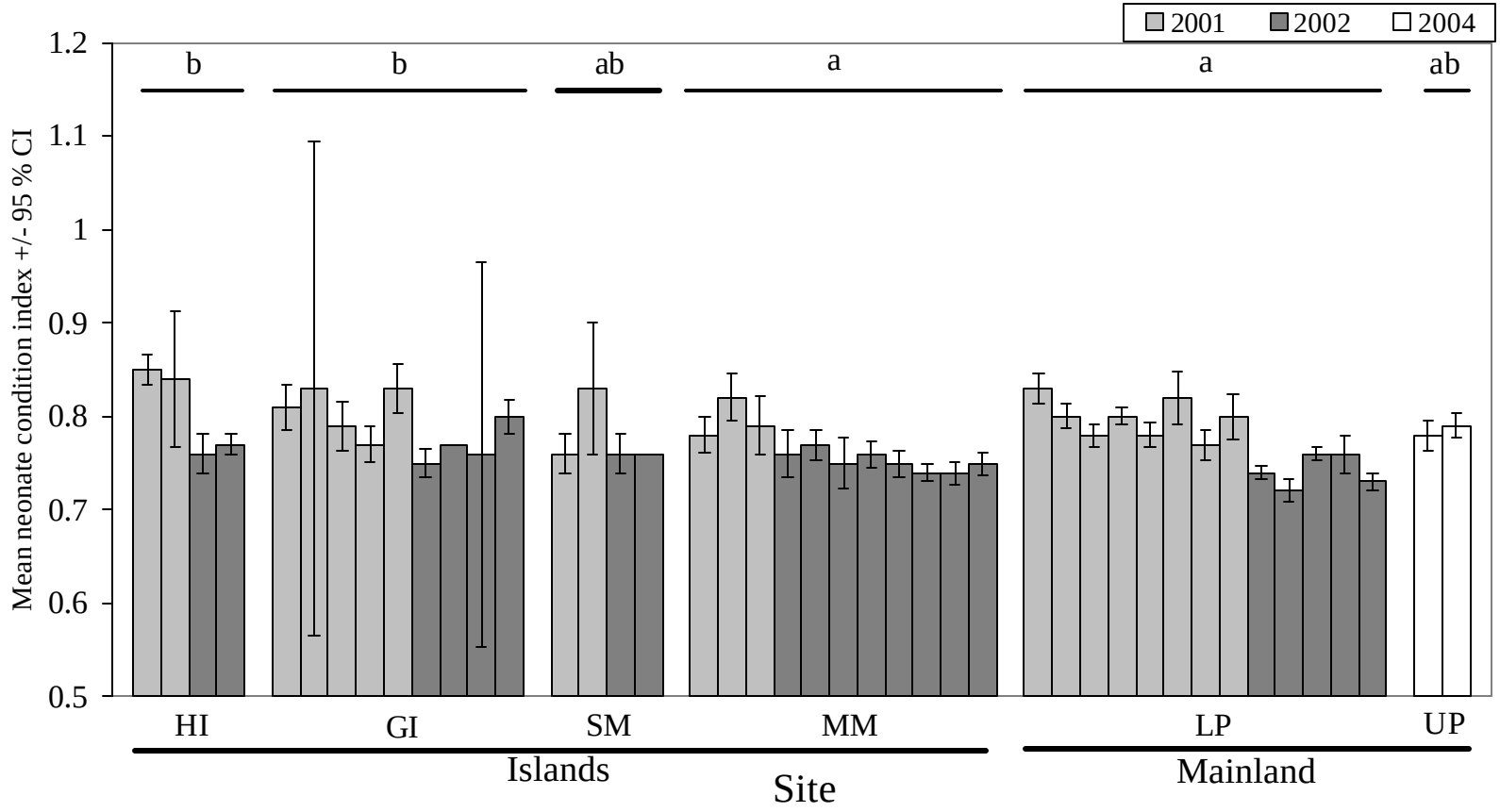


Fig. 5.9. Relationship between log of dam snout-vent length (mm) and both log of neonate snout-vent length (mm) (log neonate SVL = 1.64 – 0.17 log dam SVL; $r^2 = 0.06$; $P < 0.0001$) (a) and log of neonate mass (g) (log neonate mass = 1.43 – 0.45 log dam SVL; $r^2 = 0.05$; $P < 0.0001$) (b).

Fig. 5.10. Mean condition index (CI) (± 95 % CI) for neonate common gartersnakes, *Thamnophis sirtalis.* Neonates were born in litters to dams from the lower (LP) and upper (UP) peninsula of Michigan and High Island (HI), Garden Island (GI), and two sites on Beaver Island (SM; MM) of the Beaver Archipelago in northeastern Lake Michigan. Bars nested within sites represent different litters. For HI, GI, SM, MM, and LP, mean neonate CI was significantly lower in 2002 than in 2001 ($P < 0.05$). Significant site differences are indicated by different letters ($P < 0.05$). All significant differences detected by Tukey-Kramer Multiple-Comparison Tests. There was no interaction between the year a neonate was born and site ($P < 0.05$).


2001
2002
2004
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
Mean neonate condition index +/- 95 % CI
b
b
ab
a
a
ab
HI
GI
SM
MM
LP
UP
Islands
Mainland
Site

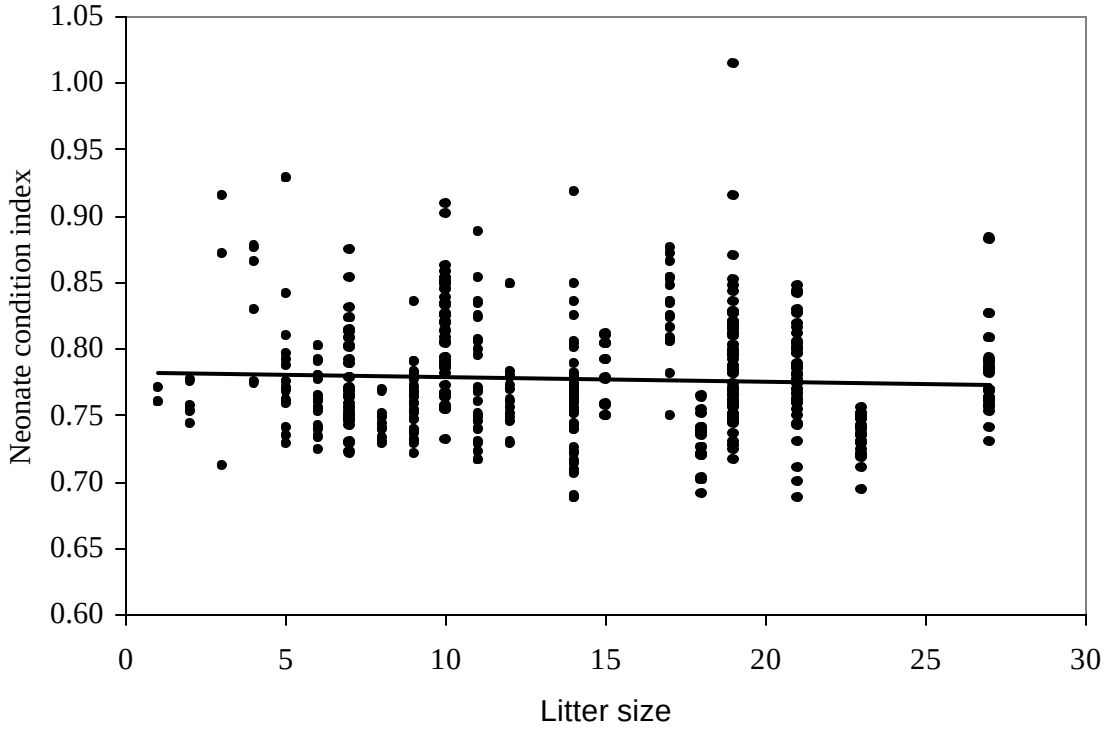


Fig. 5.11. Relationship between number of neonates per litter and neonate condition index (neonate condition index = 0.78 – 0.0003 litter size; $r^2 = 0.002$; $P = 0.30$).

Part VI

General Conclusions and Future Directions

The initial goal of my dissertation was to examine geographic variation in behavior, morphology, and life-history traits of gartersnakes (*Thamnophis sirtalis*) from the Beaver Archipelago of northeastern Lake Michigan. Both the Beaver Archipelago and *T. sirtalis* proved to be ideal for this work. The Beaver Archipelago, for example, has a well-documented geological history, allowing me to incorporate this history into discriminant analyses conducted with the antipredator- and foraging- related trait parts of this dissertation, appears to adhere to island biogeography theory, as the number of species (including *T. sirtalis* predators and prey) varies from island to island, in part, due to the size of each island, and was relatively easily accessible. *Thamnophis sirtalis* were abundant at most sites and easily maintained and observed in a laboratory setting. In addition, neonate *T. sirtalis* provided invaluable information regarding the genetics of the various traits I observed and also fared well under laboratory conditions. The importance of this work includes but is not limited to: (1) revealing the origin of Beaver Archipelago populations, (2) providing support for hypotheses regarding the recolonization of the Great Lakes region following the last glaciation, (3) documenting geographic variation in a wide variety of phenotypic traits for the same species in the same system, (4) providing updated species accounts for the Beaver Archipelago and surrounding mainland, (5) determining the role that historical processes, evolution, and phenotypic plasticity play in driving and maintaining variation in the Beaver Archipelago, (6) providing a detailed study (including data on geology, molecular genetics, morphology, behavior, and life-history traits) on geographic variation in phenotypic traits, and (7) providing data that may help workers understand the beginnings of phenotypic divergence in island systems.

The work completed for Part 2 of my dissertation, determining the underlying genetics of my populations using mtDNA sequences, was necessary for me to fully understand why patterns of geographic variation in traits observed in Parts 3-5 may exist. By knowing the origin and history of the Beaver Archipelago populations, I was better able to explain why I saw some of the differences in behavior, morphology, and life-history traits that I observed for *T. sirtalis* from this system. Specifically, I found that the Beaver Archipelago was most likely colonized by populations from both the lower and upper peninsula of Michigan, and that island populations are, genetically, more similar to upper peninsula populations than to lower peninsula populations, which contradicts past hypotheses that suggest the opposite. In the process of collecting tissue samples for Part 2, this portion of the study grew much larger than previously planned with samples being collected and sequenced from across most of the Great Lakes region. As a result, not only did I determine the origin of Beaver Archipelago *T. sirtalis* populations, but I was able to provide support for the recolonization hypotheses for the Great Lakes region in general (i.e., the two front recolonization hypothesis). In addition, data from Part 2 established that the *T. sirtalis* populations of the Beaver Archipelago, despite originating from surrounding mainland populations, are genetically dissimilar from other Great Lakes *T. sirtalis* (being characterized by seven unique haplotypes, 18.9 % of observed haplotypes) possibly due to a founder effect, genetic drift, or natural selection.

Parts 3, 4, and 5 of my dissertation all focused on phenotypic traits that I thought would be most likely to vary among the islands of the Beaver Archipelago and between the islands and the mainland. Specifically, I focused on traits that were related to

differences in *T. sirtalis* predator and prey composition, as differences in both are documented for this system. In addition, both predator pressures and resource availability are major influences on the daily activities and fitness of organisms, and, therefore, often require change in phenotypic traits to counter the specific predator- and prey-related selection pressures that may occur in a particular population. The most stereotypical characteristics attributed to island species or island populations are "island tameness" and either "island dwarfism" or "island gigantism." Specifically, popular belief holds that organisms become less responsive to potential predators, smaller, or larger on islands due to decreased predator pressures and little to no exposure to mankind. In addition, differences in prey availability are also indicated as influencing size shifts on islands. The data collected for my study indicate that these stereotypical characteristics are not always seen in island populations, despite differences in selection pressures that one would expect to allow for the development of such characteristics.

Specifically, in Part 3, I found no general island/mainland trends in antipredator behavior, and in Part 5 I found no island/mainland trends in body size. However, for antipredator behavior, at least, there was some indication that predator pressures play a role in the antipredator behavior variation I observed with snakes from sites with fewer predators being less reactive than snakes from sites with more predators. Two such sites were located in the Beaver Archipelago, but the fact that they occurred on the islands seemed to be less important than the fact that they occurred with fewer predators. Variation in the number of ventral scales, which is related to speed and the ability to escape from predators, (Part 3), head morphology (Part 4), responses to prey chemical

cues (Part 4), and reproductive life-history traits (Part 5) were also observed, but again, none appear to be specifically due to island/mainland differences. Instead, the majority of differences I observed appear to be related to the underlying genetics and origins of my populations with differences that are not congenital most likely resulting from phenotypic plasticity. The lack of general island/mainland differences may be due to a variety of factors including: 1) lack of sufficient time for island populations to diverge, 2) gene flow among the islands and between the islands and mainland, 3) lack of significant differences in selection pressures, 4) the ability of *T. sirtalis*, being the generalist that it is, to adjust to differences in selection pressures without experiencing visible phenotypic changes. Regardless, variation in many of the traits I examined did exist and appeared to be the result of interactions in the underlying genetics and history of the populations in question and phenotypic plasticity with little if any variation being linked to evolutionary change.

Overall, for Parts 3, 4, and 5 two main results can be seen: 1) differences among sites and 2) differences between neonates and adults (Table 6.1). For Part 3 (antipredator-related traits) and Part 4 (foraging/feeding-related traits) the main foci of both (i.e., antipredator behavior and head morphology) vary considerably among sites for adults, but not for neonates indicating that differences in adults may be either due to selection, maturational changes, or phenotypic plasticity; the latter of which is tested and supported by my experiments. However, in terms of mass and length, neonate characteristics were more variable expressing some island/mainland trends. In terms of ventral scale counts, neonates and adults show the same trends in geographic variation

indicating the influence of historical processes on a fixed trait. These results indicate that to fully understand geographic variation in phenotypic traits that detailed studies including multiple sites and both babies/neonates and/or juveniles and adults are needed.

My research indicates that the history of Beaver Archipelago populations has had major influences on variation in phenotypic traits despite Beaver Island and High Island being separated from mainland populations for close to 10,000 years. Of course, gene flow may play a major role as well, despite that the islands are separated from the mainland by 25-30 km of water, as even a couple migrants per generation may have significant and lasting effects on the underlying genetics of populations, especially small populations. My work also seems to indicate that phenotypic divergence in island populations may begin as the result of phenotypic plasticity, which may later become fixed due to evolutionary change as suggested by the Baldwin effect. Given the young age of the populations, the differences I noted that may be due to plasticity may not have had time to be acted upon by evolutionary change. This may indicate that phenotypic divergence in other island systems may also begin as the result of plasticity, which later becomes fixed. In fact, this appears to be the case for differences in the body size of insular tiger snake populations; however, much more data on the beginnings of phenotypic divergence are required to confirm that this is indeed how divergence in island and other systems occurs.

It would also be ideal to sample more species from the Beaver Archipelago and both *T. sirtalis* and other species from other island systems both in the Great Lakes region

and elsewhere, as while I hope it is possible that my work will generally apply to a variety of fauna found in a variety of island systems, the mechanisms driving and maintaining phenotypic change in island faunas may vary considerably. However, it is my hope that at least the methodologies I employed are duplicated elsewhere, as few researchers know the underlying genetics of their systems, are able to work with individual animals that are naïve to environmental conditions (e.g., lab-born neonate gartersnakes), and examine more than one type of phenotypic trait at any one time. More data such as those presented herein are needed to fully understand the divergence of organisms living on islands and to understand the beginning of speciation/macroevolution. If phenotypic plasticity is key to such processes, it has been underestimated, except by only a few, and deserves more attention in future research.

APPENDIX VI

TABLES AND FIGURES

Table 6.1. Summary of morphological and behavioral data collected for adult and neonate common gartersnakes (*Thamnophis sirtalis*) for Parts III, IV, and V. Means for SVL (mm), mass (g), condition index (CI), jaw length (JL) (cm), head length (HL) (cm), head width (HW) (cm), interocular distance (IOD) (cm), eye diameter (ED) (cm) and flee for both neonates and adults from the lower (LP) and upper (UP) peninsulas of Michigan, the Miller's Marsh (MM) and Sawmill (SM) sites from Beaver Island, Garden Island (GI) and High Island (HI). For bite, strike, tail-wag, head-hide, urinate, defecate, musk, and flatten, frequencies are given. Rows in bold print for a particular age class and measure indicate a significant site effect.

	Adults						Neonates					
Measure	LP	UP	MM	SM	GI	HI	LP	UP	MM	SM	GI	HI
SVL (mm)	524.59	518.25	447.59	437.77	476.45	449.34	**143.29**	**148.14**	**164.28**	**158.88**	**153.46**	**154.57**
Mass (g)	**89.59**	**69.06**	**39.07**	**42.99**	**58.80**	**39.25**	**1.37**	**1.61**	**2.01**	**1.90**	**1.79**	**2.00**
CI	**0.82**	**0.76**	**0.74**	**0.79**	**0.79**	**0.74**	**0.77**	**0.78**	**0.77**	**0.78**	**0.79**	**0.81**
JL (cm)	**20.29**	**19.02**	**17.81**	**18.30**	**18.67**	**17.50**	9.44	9.19	9.78	9.63	9.59	9.43
HL (cm)	**17.23**	**17.38**	**15.75**	**15.97**	**16.47**	**15.72**	9.11	8.97	9.44	9.37	9.35	9.12
HW (cm)	**12.10**	**11.30**	**9.59**	**9.14**	**10.17**	**9.36**	5.11	5.17	5.34	5.33	5.18	5.37
IOD (cm)	**7.56**	**7.33**	**7.07**	**7.07**	**7.39**	**7.14**	4.17	4.03	4.35	4.48	4.32	4.38
ED (cm)	**3.83**	**3.80**	**3.66**	**3.75**	**3.77**	**3.59**	2.24	2.29	2.32	2.32	2.24	2.19
Flee	**27.35**	**28.67**	**24.96**	**20.79**	**5.26**	**2.61**	19.81	21.9	22.89	14.81	13.78	15.25
Bite	**0.15**	**0.33**	**0**	**0.02**	**0**	**0**	0.10	0.10	0.08	0.19	0.18	0.06
Strike	**0.23**	**0.33**	**0.01**	**0.09**	**0**	**0**	0.17	0.24	0.22	0.06	0.28	0.19
Tail-wag	**0.12**	**0**	**0.12**	**0.14**	**0**	**0**	0.54	0.57	0.51	0.56	0.58	0.75
Head-hide	0.12	0	0.11	0.09	0.01	0.02	0.15	0.10	0.11	0.13	0.08	0.06
Urinate	**0.04**	**0.33**	**0.12**	**0.14**	**0.03**	**0**	0.34	0.33	0.34	0.31	0.36	0.44
Defecate	**0.08**	**0.33**	**0.07**	**0.12**	**0.01**	**0**	0.0	0.05	0.03	0.0	0.06	0.06
Musk	**0.08**	**0.67**	**0.15**	**0.05**	**0.03**	**0**	**0.51**	**0.67**	**0.72**	**0.69**	**0.56**	**0.75**
Flatten	**0.08**	**0**	**0**	**0**	**0**	**0**	**0.0**	**0.10**	**0.05**	**0.06**	**0.04**	**0.13**

Appendix

Table A.1. Common gartersnake (*Thamnophis sirtalis*) neonates remaining in the control (n = 55), gently handled (n = 53), and shaken (n = 51) treatment groups used to examine phenotypic plasticity of antipredator behavior after the 42 week treatment period. Litters from the lower peninsula (LP) of Michigan, the Miller's Marsh (MM) and Sawmill (SM) site on Beaver Island, Garden Island (GI), and High Island (HI) were represented in each treatment group.

	Control		Gently Handled		Shaken	
Litter	male	female	male	female	male	female
LP0002	1	0	1	0	1	0
LP0007	0	6	4	3	4	2
LP0008	1	0	0	2	1	1
LP0010	3	4	5	2	1	4
LP0011	2	2	1	3	3	1
LP0012	1	4	2	3	0	4
LP0013	0	3	1	0	1	3
LP0015	0	1	0	0	0	0
MM0916	0	2	0	2	2	0
MM0966	1	2	3	2	1	2
MM0967	1	1	1	2	2	3
SM0011	0	1	1	1	0	2
SM0067	0	1	1	0	1	1
GI0004	1	2	1	1	1	1
GI0042	3	0	1	2	0	3
GI0062	1	2	1	3	0	3
GI0063	2	0	0	2	2	0
HI0002	1	5	0	2	1	0
HI0020	0	1	0	0	0	0
Total =	18	37	23	30	21	30

Table A.2. Common gartersnake (*Thamnophis sirtalis*) neonates remaining in the treatment group exposed to ophiophagous snake chemical cues (n = 26) and the treatment group not exposed to ophiophagous snake chemical cues (n = 23) used to examine phenotypic plasticity of responses to ophiophagous snakes after the 42 week treatment period. Litters from the lower peninsula (LP) of Michigan, the Miller's Marsh (MM) and Sawmill (SM) site on Beaver Island, Garden Island (GI), and High Island (HI) were represented in each treatment group.

	Exposed		Not exposed	
Litter	male	female	male	female
LP0020	3	0	2	1
LP0027	0	1	0	2
LP0030	1	1	0	2
LP0031	2	1	2	2
LP0032	1	0	0	1
MM0504	1	0	2	2
MM0965	0	1	0	1
MM1030	0	1	0	0
MM1039	1	0	0	0
MM1068	1	1	0	1
MM1076	2	2	1	1
MM1080	1	1	0	0
SM0086	0	1	0	1
GI0075	1	1	1	1
HI0075	0	1	0	0
Total =	14	12	8	15

Table A.3. Result of grouping adult common gartersnakes, *Thamnophis sirtalis*, based on their site of origin when using antipredator behavioral data and ventral scale counts (VSC) in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown.

	Function				
Variable	1	2	3	4	5
Flee	**0.955**	-0.216	-0.004	0.050	-0.048
Strike	-0.101	0.038	**0.641**	-0.065	0.428
Bite	0.106	**0.970**	-0.102	0.056	0.085
Tail wag	0.118	-0.095	0.057	-0.071	0.088
Head hide	0.144	-0.204	0.188	0.114	0.007
Urinate	0.132	-0.103	-0.317	-0.259	0.043
Defecate	0.055	-0.089	0.146	-0.369	0.494
Musk	0.175	0.411	-0.395	0.109	-0.422
Flatten	-0.086	0.225	0.394	0.570	-0.313
VSC	0.101	-0.138	-0.496	0.693	0.521
Eigenvalue	1.029	0.421	0.194	0.042	0.023
% of Variance	**60.2**	**24.6**	**11.4**	2.5	1.4
Wilks' Lambda	0.272	0.553	0.785	0.938	0.977
X^2	387.56	176.71	71.98	19.13	6.83
df	50	36	24	14	6
P-Value	< **0.001**	< **0.001**	< **0.001**	0.16	0.34

Table A.4. Classification/Prediction table resulting from grouping adult common gartersnakes, *Thamnophis sirtalis*, based on their site of origin when using antipredator behavioral data and ventral scale counts in a multiple discriminant function analysis (MDA). Snakes were collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). The MDA classified 50.8 % of original cases correctly.

		Predicted Group Membership for Adult Snakes						
	Site	GI	HI	SM	MM	LP	UP	
								Total
Original Count	GI	**39**	23	3	3	0	0	68
	HI	20	**34**	0	0	0	0	54
	SM	8	7	**15**	10	3	0	43
	MM	9	9	32	**63**	0	0	113
	LP	1	0	10	8	**4**	3	26
	UP	0	0	0	2	0	**1**	3
								307
%	GI	**57.4**	33.8	4.4	4.4	0	0	
	HI	37.0	**63.0**	0	0	0	0	
	SM	18.6	16.3	**34.9**	23.3	7.0	0	
	MM	8.0	8.0	28.3	**55.8**	0	0	
	LP	3.8	0	38.5	30.8	**15.4**	11.5	
	UP	0	0	0	66.7	0	**33.3**	

Table A.5. Result of grouping adult common gartersnakes, *Thamnophis sirtalis*, based on if they were from an island of the Beaver Archipelago or from mainland Michigan when using antipredator behavioral data and ventral scale counts (VSC) in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown.

	Function
Variable	1
Flee	0.303
Strike	0.357
Bite	**0.649**
Tail wag	0.007
Head hide	0.057
Urinate	-0.251
Defecate	-0.012
Musk	0.115
Flatten	0.437
VSC	-0.271
Eigenvalue	0.297
% of Variance	**100.0**
Wilks' Lambda	0.771
X^2	77.98
df	10
P-Value	**< 0.001**

Table A.6. Classification/Prediction table resulting from grouping adult common gartersnakes, *Thamnophis sirtalis*, based on if they were from an island of the Beaver Archipelago or from mainland Michigan when using antipredator behavioral data and ventral scale counts in a multiple discriminant function analysis (MDA). Snakes were collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). The MDA classified 89.9 % of original cases correctly.

		Predicted Group Membership for Adult Snakes		
	Group	Islands	Mainland	
Original Count	Islands	**267**	11	Total 278
	Mainland	20	**9**	29
				307
%	Islands	**96.0**	4.0	
	Mainland	69.0	**31.0**	

Table A.7. Result of grouping adult common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that some Beaver Archipelago islands were submerged, while others were not when using antipredator behavioral data and ventral scale counts (VSC) in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown.

	Function		
Variable	1	2	3
Flee	0.283	**0.851**	0.168
Strike	0.027	0.112	-0.551
Bite	**0.895**	-0.378	0.051
Tail wag	-0.029	0.127	-0.010
Head hide	-0.103	0.258	-0.155
Urinate	-0.055	0.002	0.360
Defecate	-0.046	0.096	-0.009
Musk	0.415	-0.217	0.293
Flatten	0.187	0.020	-0.503
VSC	-0.026	0.319	**0.536**
Eigenvalue	0.470	0.240	0.163
% of Variance	**53.9**	**27.5**	**18.7**
Wilks' Lambda	0.472	0.694	0.860
X^2	224.64	109.43	45.19
df	30	18	8
P-Value	< **0.001**	< **0.001**	< **0.001**

Table A.8. Classification/Prediction table resulting from grouping adult common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that some Beaver Archipelago islands were submerged while others were not when using antipredator behavioral data and ventral scale counts in a multiple discriminant function analysis (MDA). Snakes were collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). The MDA classified 65.7 % of original cases correctly.

		Predicted Group Membership for Adult Snakes				
	Site	GI	BI & HI	LP	UP	
						Total
Original Count	GI	**60**	8	0	0	68
	BI & HI	64	**135**	11	0	210
	LP	4	13	**6**	3	26
	UP	0	2	0	**1**	3
						307
%	GI	**88.2**	11.8	0	0	
	BI & HI	30.5	**64.3**	5.2	0	
	LP	15.4	50.0	**23.1**	11.5	
	UP	0	66.7	0	**33.3**	

Table A.9. Result of grouping adult common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that the lower peninsula of Michigan was connected to the Beaver Archipelago via a land bridge using antipredator behavioral data and ventral scale counts (VSC) in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown.

	Function
Variable	1
Flee	0.033
Strike	-0.156
Bite	**0.958**
Tail wag	-0.070
Head hide	-0.215
Urinate	0.041
Defecate	-0.074
Musk	0.520
Flatten	0.036
VSC	0.012
Eigenvalue	0.397
% of Variance	**100.0**
Wilks' Lambda	0.716
X^2	100.22
df	10
P-Value	**< 0.001**

Table A.10. Classification/Prediction table resulting from grouping adult common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that the lower peninsula of Michigan was connected to the Beaver Archipelago via a land bridge when using antipredator behavioral data and ventral scale counts in a multiple discriminant function analysis (MDA). Snakes were collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). The MDA classified 98.4 % of original cases correctly.

		Predicted Group Membership for Adult Snakes		
	Group	LP & islands	UP	
Original Count	LP & islands	**301**	3	Total 304
	UP	2	**1**	3
				307
%	LP & islands	**99.0**	1.0	
	UP	66.7	**33.3**	

Table A.11. Result of grouping adult common gartersnakes, *Thamnophis sirtalis*, based on molecular phylogenetic data when using antipredator behavioral data and ventral scale counts (VSC) in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown.

	Function
Variable	1
Flee	**0.338**
Strike	**0.474**
Bite	**0.330**
Tail wag	0.038
Head hide	0.156
Urinate	-0.306
Defecate	0.017
Musk	-0.089
Flatten	**0.488**
VSC	-0.317
Eigenvalue	0.822
% of Variance	**100.0**
Wilks' Lambda	0.822
X^2	58.83
df	10
P-Value	< **0.001**

Table A.12. Classification/Prediction table resulting from grouping adult common gartersnakes, *Thamnophis sirtalis*, based on molecular phylogenetic data when using antipredator behavioral data and ventral scale counts in a multiple discriminant function analysis (MDA). Snakes were collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). The MDA classified 88.3 % of original cases correctly.

		Predicted Group Membership for Adult Snakes		
	Group	UP & islands	LP	
Original Count	UP & islands	**260**	21	Total 304
	LP	15	**11**	3
				307
%	UP & islands	**92.5**	7.5	
	LP	57.7	**42.3**	

Table A.13. Result of grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on their site of origin when using antipredator behavioral data and ventral scale counts (VSC) in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown.

	Function				
Variable	1	2	3	4	5
Flee	0.335	**0.866**	0.196	0.034	-0.115
Strike	-0.277	-0.236	1.146	0.468	0.421
Bite	-0.061	0.255	-0.633	-0.252	-0.251
Tail wag	-0.017	-0.081	-0.212	0.584	-0.091
Head hide	-0.189	0.118	-0.029	0.203	0.031
Urinate	-0.044	0.105	-0.123	0.638	0.093
Defecate	0.137	-0.343	0.476	-0.138	-0.435
Musk	0.236	-0.152	0.082	-0.280	0.873
Flatten	0.431	-0.215	-0.579	0.092	-0.141
VSC	**0.857**	-0.079	0.017	0.156	-0.199
Eigenvalue	0.323	0.148	0.019	0.014	0.010
% of Variance	**62.9**	**28.8**	3.7	2.7	1.9
Wilks' Lambda	0.631	0.835	0.959	0.977	0.990
X^2	150.33	58.89	13.82	7.60	3.14
df	50	36	24	14	6
P-Value	< **0.001**	**0.009**	0.951	0.909	0.791

Table A.14. Classification/Prediction table resulting from grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on their site of origin when using antipredator behavioral data and ventral scale counts in a multiple discriminant function analysis (MDA). Neonates were born to mothers from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). The MDA classified 41.4 % of original cases correctly.

		Predicted Group Membership for Neonate Snakes						
	Site	GI	HI	SM	MM	LP	UP	
Original Count	GI	**17**	4	17	5	7	0	Total 50
	HI	2	**6**	2	3	1	2	16
	SM	3	1	**8**	2	2	0	16
	MM	8	10	9	**29**	11	12	79
	LP	16	14	17	20	**71**	16	154
	UP	1	2	0	9	1	**8**	21
								336
%	GI	**34.0**	8.0	34.0	10.0	14.0	0.0	
	HI	12.5	**37.5**	12.5	18.8	6.3	12.5	
	SM	18.8	6.3	**50.0**	12.5	12.5	0.0	
	MM	10.1	12.7	11.4	**36.7**	13.9	15.2	
	LP	10.4	9.1	11.0	13.0	**46.1**	10.4	
	UP	4.8	9.5	0.0	42.9	4.8	**38.1**	

Table A.15. Result of grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on if they were from an island of the Beaver Archipelago or from mainland Michigan when using antipredator behavioral data and ventral scale counts (VSC) in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown.

	Function
Variable	1
Flee	-0.324
Strike	0.109
Bite	-0.286
Tail wag	-0.090
Head hide	-0.229
Urinate	-0.188
Defecate	0.312
Musk	0.458
Flatten	0.316
VSC	**0.610**
Eigenvalue	0.107
% of Variance	**100.0**
Wilks' Lambda	0.903
X^2	33.42
df	10
P-Value	< **0.001**

Table A.16. Classification/Prediction table resulting from grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on if they were from an island of the Beaver Archipelago or mainland Michigan when using antipredator behavioral data and ventral scale counts in a multiple discriminant function analysis (MDA). Neonates were born to mothers from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). The MDA classified 63.1 % of original cases correctly.

		Predicted Group Membership for Neonate Snakes		
	Group	Islands	Mainland	
Original Count	Islands	**101**	60	Total 161
	Mainland	64	**111**	175
				336
%	Islands	**62.7**	37.3	
	Mainland	36.6	**63.4**	

Table A.17. Result of grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that some Beaver Archipelago islands were submerged while others were not when using antipredator behavioral data and ventral scale counts (VSC) in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown.

	Function		
Variable	1	2	3
Flee	0.262	**0.821**	-0.011
Strike	-0.350	-0.344	0.758
Bite	-0.027	0.296	-0.434
Tail wag	-0.016	0.070	-0.182
Head hide	-0.199	0.147	0.013
Urinate	-0.061	0.245	0.025
Defecate	0.123	-0.448	-0.252
Musk	0.256	-0.168	0.855
Flatten	0.478	-0.062	-0.336
VSC	**0.847**	-0.011	-0.181
Eigenvalue	0.296	0.095	0.010
% of Variance	**73.8**	**23.6**	2.6
Wilks' Lambda	0.698	0.904	0.990
X^2	117.95	33.02	3.35
df	30	18	8
P-Value	< **0.001**	**0.017**	0.91

Table A.18. Classification/Prediction table resulting from grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that some Beaver Archipelago islands were submerged while others were not when using antipredator behavioral data and ventral scale counts in a multiple discriminant function analysis (MDA). Neonates were born to mothers from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI) and High Island (HI). The MDA classified 47.0 % of original cases correctly.

		Predicted Group Membership for Neonate Snakes				
	Site	GI	BI & HI	LP	UP	
						Total
Original Count	GI	**29**	8	11	2	50
	BI & HI	26	**41**	17	27	111
	LP	31	21	**78**	24	154
	UP	1	9	1	**10**	21
						336
%	GI	**58.0**	16.0	22.0	4.0	
	BI & HI	23.4	**36.9**	15.3	24.3	
	LP	20.1	13.6	**50.6**	15.6	
	UP	4.8	42.9	4.8	**47.6**	

Table A.19. Result of grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that the lower peninsula of Michigan was connected to the Beaver Archipelago via land bridge when using antipredator behavioral data and ventral scale counts (VSC) in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown.

	Function
Variable	1
Flee	0.319
Strike	-0.566
Bite	0.135
Tail wag	0.047
Head hide	-0.164
Urinate	-0.038
Defecate	0.143
Musk	-0.050
Flatten	0.519
VSC	**0.833**
Eigenvalue	0.088
% of Variance	**100.0**
Wilks' Lambda	0.919
X^2	27.88
df	10
P-Value	**< 0.01**

Table A.20. Classification/Prediction table resulting from grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that the lower peninsula of Michigan was connected to the Beaver Archipelago via a land bridge when using antipredator behavioral data and ventral scale counts in a multiple discriminant function analysis (MDA). Neonates were born to mothers from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). The MDA classified 74.1 % of original cases correctly.

		Predicted Group Membership for Neonate Snakes		
	Group	LP & islands	UP	
Original Count	LP & islands	**232**	83	Total 315
	UP	4	**17**	21
				336
%	LP & islands	**73.7**	26.3	
	UP	19.0	**81.0**	

Table A.21. Result of grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on molecular phylogenetic data when using antipredator behavioral data and ventral scale counts (VSC) in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown.

	Function
Variable	1
Flee	-0.143
Strike	-0.115
Bite	-0.177
Tail wag	-0.055
Head hide	-0.238
Urinate	-0.162
Defecate	0.295
Musk	0.343
Flatten	0.429
VSC	**0.756**
Eigenvalue	0.205
% of Variance	**100.0**
Wilks' Lambda	0.830
X^2	61.39
df	10
P-Value	< **0.001**

Table A.22. Classification/Prediction table resulting from grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on molecular phylogenetic data when using antipredator behavioral data and ventral scale counts in a multiple discriminant function analysis (MDA). Neonates were born to mothers from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). The MDA classified 65.5 % of original cases correctly.

		Predicted Group Membership for Neonate Snakes		
	Group	UP & islands	LP	
				Total
Original Count	UP & islands	**117**	65	182
	LP	51	**103**	154
				336
%	UP & islands	**64.3**	35.7	
	LP	33.1	**66.9**	

Table A.23. Result of grouping adult common gartersnakes, *Thamnophis sirtalis*, based on their site of origin when using head measurements in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown. See Table 4.1 in Appendix IV for head measurement abbreviations.

	Function				
Variable	1	2	3	4	5
IOD	-0.580	**1.263**	**1.356**	-0.505	1.158
ED	-0.537	-0.306	-0.968	0.640	1.394
HL	-0.118	**1.223**	-0.834	2.349	-2.415
HW	0.807	0.710	-0.742	-0.187	0.349
JL	**1.151**	-2.698	**1.360**	-1.648	0.128
Eigenvalue	0.172	0.129	0.033	0.020	0.006
% of Variance	**47.7**	**35.8**	**9.3**	5.6	1.6
Wilks' Lambda	0.713	0.835	0.943	0.975	0.994
X^2	118.00	62.73	20.40	8.95	2.01
df	25	16	9	4	1
P-Value	**< 0.001**	**< 0.001**	**0.016**	0.062	0.156

Table A.24. Classification/Prediction table resulting from grouping adult common gartersnakes, *Thamnophis sirtalis*, based on their site of origin when using head measurements in a multiple discriminant function analysis (MDA). Snakes were collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). The MDA classified 27.9 % of original cases correctly.

		Predicted Group Membership for Adult Snakes						
	Site	GI	HI	SM	MM	LP	UP	
								Total
Original Count	GI	**15**	24	17	6	16	16	94
	HI	5	**18**	2	5	1	7	38
	SM	6	7	**34**	6	8	8	69
	MM	14	33	29	**20**	17	13	126
	LP	3	2	1	2	**9**	5	22
	UP	0	2	0	0	1	**3**	6
								355
%	GI	**16.0**	25.5	18.1	6.4	17.0	17.0	
	HI	13.2	**47.4**	5.3	13.2	2.6	18.4	
	SM	8.7	10.1	**49.3**	8.7	11.6	11.6	
	MM	11.1	26.2	23.0	**15.9**	13.5	10.3	
	LP	13.6	9.1	4.5	9.1	**40.9**	22.7	
	UP	0.0	33.3	0.0	0.0	16.7	**50.0**	

Table A.25. Result of grouping adult common gartersnakes, *Thamnophis sirtalis*, based on if they were from an island of the Beaver Archipelago or from mainland Michigan when using head measurements in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown. See Table 4.1 in Appendix IV for head measurement abbreviations.

	Function
Variable	1
IOD	-0.892
ED	-0.506
HL	0.008
HW	**0.824**
JL	**1.201**
Eigenvalue	0.147
% of Variance	**100.0**
Wilks' Lambda	0.871
X^2	48.21
df	5
P-Value	< **0.001**

Table A.26. Classification/Prediction table resulting from grouping adult common gartersnakes, *Thamnophis sirtalis*, based on if they were an island of the Beaver Archipelago or from mainland Michigan when using head measurements in a multiple discriminant function analysis (MDA). Snakes were collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). The MDA classified 75.8 % of original cases correctly.

		Predicted Group Membership for Adult Snakes		
	Group	Islands	Mainland	
				Total
Original Count	Islands	**249**	78	327
	Mainland	8	**20**	28
				355
%	Islands	**76.1**	23.9	
	Mainland	28.6	**71.4**	

Table A.27. Result of grouping adult common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that some Beaver Archipelago islands were submerged while others were not using head measurements in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown. See Table 4.1 in Appendix IV for head measurement abbreviations.

	Function		
Variable	1	2	3
IOD	-0.542	**1.019**	**1.608**
ED	-0.607	0.226	-0.849
HL	-0.041	**1.823**	-1.972
HW	**0.779**	0.251	-0.607
JL	**1.142**	-2.663	**1.857**
Eigenvalue	0.168	0.034	0.028
% of Variance	**73.3**	**14.6**	**12.1**
Wilks' Lambda	0.806	0.941	0.973
X^2	75.44	21.09	9.55
df	15	8	3
P-Value	**< 0.001**	**0.007**	**0.023**

Table A.28. Classification/Prediction table resulting from grouping adult common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that some Beaver Archipelago islands were submerged while others were not using head measurements in a multiple discriminant function analysis (MDA). Snakes were collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI) and High Island (HI). The MDA classified 44.5 % of original cases correctly.

		Predicted Group Membership for Adult Snakes				
	Site	GI	BI & HI	LP	UP	
						Total
Original Count	GI	**29**	31	18	16	94
	BI & HI	54	**116**	31	32	233
	LP	4	3	**10**	5	22
	UP	2	0	1	**3**	6
						355
%	GI	**30.9**	33.0	19.1	17.0	
	BI & HI	23.2	**49.8**	13.3	13.7	
	LP	18.2	13.6	**45.5**	22.7	
	UP	33.3	0.0	16.7	**50.0**	

Table A.29. Result of grouping adult common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that the lower peninsula of Michigan was connected to the Beaver Archipelago via a land bridge when using head measurements in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown. See Table 4.1 in Appendix IV for head measurement abbreviations.

	Function
Variable	1
IOD	-1.367
ED	0.454
HL	**2.095**
HW	1.000
JL	-1.662
Eigenvalue	0.037
% of Variance	**100.0**
Wilks' Lambda	0.965
X^2	12.57
df	5
P-Value	**0.028**

Table A.30. Classification/Prediction table resulting from grouping adult common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that the lower peninsula of Michigan was connected to the Beaver Archipelago via a land bridge when using head measurements in a multiple discriminant function analysis (MDA). Snakes were collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). The MDA classified 77.2 % of original cases correctly.

		Predicted Group Membership for Adult Snakes		
	Group	LP & islands	UP	
Original Count	LP & islands	**270**	79	Total 349
	UP	2	**4**	6
				355
%	LP & islands	**77.4**	22.6	
	UP	33.3	**66.7**	

Table A.31. Result of grouping adult common gartersnakes, *Thamnophis sirtalis*, based on molecular phylogenetic data when using head measurements in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown. See Table 4.1 in Appendix IV for head measurement abbreviations.

	Function
Variable	1
IOD	-0.607
ED	-0.713
HL	-0.613
HW	0.658
JL	**1.856**
Eigenvalue	0.140
% of Variance	**100.0**
Wilks' Lambda	0.877
X^2	45.93
df	5
P-Value	< **0.001**

Table A.32. Classification/Prediction table resulting from grouping adult common gartersnakes, *Thamnophis sirtalis*, based on molecular phylogenetic data when using head measurements in a multiple discriminant function analysis (MDA). Snakes were collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). The MDA classified 76.9 % of original cases correctly.

		Predicted Group Membership for Adult Snakes		
	Group	UP & islands	LP	
				Total
Original Count	UP & islands	**256**	77	333
	LP	5	**17**	22
				355
%	UP & islands	**76.9**	23.1	
	LP	22.7	**77.3**	

Table A.33. Result of grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on their site of origin when using head measurements in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown. See Table 4.1 in Appendix IV for head measurement abbreviations.

	Function				
Variable	1	2	3	4	5
IOD	**0.824**	-0.731	0.216	-0.388	**0.578**
ED	-0.176	**0.789**	0.382	-0.465	0.417
HL	0.385	0.153	-0.144	-0.655	-1.164
HW	-0.087	0.228	**0.868**	0.690	-0.435
JL	0.048	0.427	-0.902	**0.915**	**0.634**
Eigenvalue	0.204	0.097	0.052	0.013	0.010
% of Variance	**54.2**	**25.8**	**13.9**	**3.5**	**2.7**
Wilks' Lambda	0.703	0.846	0.928	0.977	0.990
X^2	148.73	70.38	31.28	9.77	4.27
df	25	16	9	4	1
P-Value	< **0.001**	< **0.001**	< **0.001**	**0.045**	**0.039**

Table A.34. Classification/Prediction table resulting from grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on their site of origin when using head measurements in a multiple discriminant function analysis (MDA). Neonates were born to mothers collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) sites on Beaver Island, Garden Island (GI) and High Island (HI). The MDA classified 34.1 % of original cases correctly.

		Predicted Group Membership for Neonate Snakes						
	Site	GI	HI	SM	MM	LP	UP	
								Total
Original Count	GI	**20**	13	5	13	9	4	64
	HI	1	**8**	2	4	0	3	18
	SM	4	2	**7**	3	1	0	17
	MM	8	6	16	**27**	9	12	78
	LP	30	27	22	35	**71**	46	231
	UP	0	1	1	1	4	**13**	20
								428
%	GI	**31.3**	20.3	7.8	20.3	14.1	6.3	
	HI	5.6	**44.4**	11.1	22.2	0.0	16.7	
	SM	23.5	11.8	**41.2**	17.6	5.9	0.0	
	MM	10.3	7.7	20.5	**34.6**	11.5	15.4	
	LP	13.0	11.7	9.5	15.2	**30.7**	19.9	
	UP	0.0	5.0	5.0	5.0	20.0	**65.0**	

Table A.35. Result of grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on if they were from an island of the Beaver Archipelago or from mainland Michigan when using head measurements in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown. See Table 4.1 in Appendix IV for head measurement abbreviations.

	Function
Variable	1
IOD	**0.738**
ED	-0.118
HW	0.066
HL	0.475
JL	-0.075
Eigenvalue	0.177
% of Variance	**100.0**
Wilks' Lambda	0.850
X^2	68.86
df	5
P-Value	< **0.001**

Table A.36. Classification/Prediction table resulting from grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on if they were from an island of the Beaver Archipelago or from mainland Michigan when using head measurements in a multiple discriminant function analysis (MDA). Neonates were born to mothers collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI) and High Island (HI). The MDA classified 65.4 % of original cases correctly.

		Predicted Group Membership for Neonate Snakes		
	Group	Islands	Mainland	
Original Count	Islands	**117**	60	Total 177
	Mainland	88	**163**	251
				428
%	Islands	**66.1**	33.9	
	Mainland	35.1	**64.9**	

Table A.37. Result of grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that some Beaver Archipelago islands were submerged while others were not using head measurements in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown. See Table 4.1 in Appendix IV for head measurement abbreviations.

	Function		
Variable	1	2	3
IOD	**0.789**	-0.337	-0.060
ED	-0.210	**0.775**	0.195
HW	-0.068	**0.854**	-0.172
HL	0.391	-0.049	-1.121
JL	0.092	-0.343	1.355
Eigenvalue	0.199	0.064	0.013
% of Variance	**72.0**	**23.2**	4.7
Wilks' Lambda	0.774	0.928	0.987
X^2	108.43	31.77	5.50
df	15	8	3
P-Value	**< 0.001**	**< 0.001**	0.14

Table A.38. Classification/Prediction table resulting from grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that some Beaver Archipelago islands were submerged while others were not when using head measurements in a multiple discriminant function analysis (MDA). Neonates were born to mothers collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI), and High Island (HI). The MDA classified 41.4 % of original cases correctly.

		Predicted Group Membership for Neonate Snakes				
	Site	GI	BI & HI	LP	UP	
						Total
Original Count	GI	**30**	14	15	5	64
	BI & HI	33	**48**	14	18	113
	LP	50	48	**85**	48	231
	UP	0	2	4	**14**	20
						428
%	GI	**46.9**	21.9	23.4	7.8	
	BI & HI	29.2	**42.5**	12.4	15.9	
	LP	21.6	20.8	**36.8**	20.8	
	UP	0.0	10.0	20.0	**70.0**	

Table A.39. Result of grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that the lower peninsula of Michigan was connected to the Beaver Archipelago via a land bridge when using head measurements in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown. See Table 4.1 in Appendix IV for head measurement abbreviations.

	Function
Variable	1
IOD	**0.646**
ED	-0.178
HW	0.029
HL	0.438
JL	**0.597**
Eigenvalue	0.077
% of Variance	**100.0**
Wilks' Lambda	0.929
X^2	31.31
df	5
P-Value	< **0.001**

Table A.40. Classification/Prediction table resulting from grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on geological data which indicate that the lower peninsula of Michigan was connected to the Beaver Archipelago via a land bridge using head measurements in a multiple discriminant function analysis (MDA). Neonates were born to mothers collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI) and High Island (HI). The MDA classified 74.1 % of original cases correctly.

		Predicted Group Membership for Neonate Snakes		
	Group	LP & islands	UP	
Original Count	LP & islands	**302**	106	Total 408
	UP	5	**15**	20
				428
%	LP & islands	**74.0**	26.0	
	UP	25.0	**75.0**	

Table A.41. Result of grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on molecular phylogenetic data when using head measurements in a multiple discriminant function analysis. Standardized canonical discriminant function coefficients, Eigenvalues, % of variance explained, and significance of each function are shown. See Table 4.1 in Appendix IV for head measurement abbreviations.

	Function
Variable	1
IOD	**0.603**
ED	0.067
HW	0.294
HL	**0.553**
JL	-0.317
Eigenvalue	0.113
% of Variance	**100.0**
Wilks' Lambda	0.898
X^2	45.35
df	5
P-Value	< **0.001**

Table A.42. Classification/Prediction table resulting from grouping neonate common gartersnakes, *Thamnophis sirtalis*, based on molecular phylogenetic data using head measurements in a multiple discriminant function analysis (MDA). Neonates were born to mothers collected from the lower (LP) and upper peninsula (UP) of Michigan, Miller's Marsh (MM) and Sawmill (SM) on Beaver Island, Garden Island (GI) and High Island (HI). The MDA classified 62.6 % of original cases correctly.

		Predicted Group Membership for Neonate Snakes		
	Group	UP & islands	LP	
Original Count	UP & islands	**145**	86	Total 231
	LP	74	**123**	197
				428
%	UP & islands	**62.8**	37.2	
	LP	37.6	**62.4**	

VITA

John S. Placyk, Jr. was born in South Jersey, considered a suburb of Philadelphia, in 1975 where the majority of his family remains today. He spent his boyhood exploring the wild areas surrounding his house including an overgrown lot by his home ("The Shade"), the outskirts of the local sewer plant ("The Desert"), and the local park ("Green Acres") for amphibians and reptiles. John maintained a large collection of freshwater and marine fish and a variety of amphibians and reptiles from middle school until the late 1990's. While attending Triton Regional High school, John began working at a local pet store (Zoo's Pet Center), eventually becoming its manager while also pursuing a Bachelor's degree in Marine Science from the Richard Stockton College of New Jersey from which he graduated in 1998. Upon the creation of the now famous "super" pet stores (e.g., PetSmart, Petco), John's "mom and pop" pet store quickly went out of business and he spent the late 90's driving a forklift and stocking gigantic freezers and coolers at the local Sam's Club.

Looking for greener and more stimulating pastures, John enlisted in graduate school in 1998 when he stumbled upon his Master's advisor, Brent Graves, at Northern Michigan University (NMU) while conducting a search of graduate programs with herpetology emphases in a magazine for herp hobbyists. At NMU, he had great plans of studying kin recognition in amphibians for his thesis research, but ended up examining how salamanders use there special senses to find food. Upon finishing his M.S. in Biology at NMU in May of 2000, John briefly worked as a research assistant for the Manomet Center for Conservation Sciences in Manomet, Massachusetts where he studied

shorebird and wetland bird behavior. In August of 2000, John began his doctoral research with Gordon Burghardt at the University of Tennessee studying geographic variation in the phenotypic traits of gartersnakes. Despite being bitten and musked upon several hundred times by his test subjects, John surfaced with his Doctor of Philosophy degree in Ecology and Evolutionary Biology in August of 2006. While in east Tennessee, he met and eloped with the love of his life (Lana) and replaced his scaly menagerie by acquiring more creatures with fur then he would ever have imagined.

Currently, John is a postdoctoral fellow in Ben Fitzpatrick's lab at the University of Tennessee. He lives happily in Caryville, Tennessee with Lana, Maggy, Buffy, Raney, Jelly Bean, Gizmo, Phoebe, Mooch, Willa, Georgiana, Cindy Lou Who, Creamy, Susie (a.k.a., Kitten), Dora, Poppy, Howard, Flopsy, Mopsy, Pippin, Dickens, Ash, Ellie, and Uncle Willie the latter three of which have scales.