5-2005

Constriction Behavior, a Key Innovation In Snake Evolution: The Integration of Ethology and Physiology

Rita S. Mehta
University of Tennessee - Knoxville

Recommended Citation

Mehta, Rita S., "Constriction Behavior, a Key Innovation In Snake Evolution: The Integration of Ethology and Physiology. " PhD diss., University of Tennessee, 2005.
https://trace.tennessee.edu/utk_graddiss/2262

To the Graduate Council:

I am submitting herewith a dissertation written by Rita S. Mehta entitled "Constriction Behavior, a Key Innovation In Snake Evolution: The Integration of Ethology and Physiology." 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 Psychology.

Gordon M. Burghardt, Major Professor

We have read this dissertation and recommend its acceptance:

Marguerite Butler, Todd_Freeberg, Neil Greenberg, Richard Saudargas

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a dissertation written by Rita S. Mehta entitled "Constriction Behavior, a Key Innovation In Snake Evolution: The Integration of Ethology and Physiology**."** 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 Psychology.

Gordon M. Burghardt
Major Professor

We have read this dissertation
and recommend its acceptance:

Marguerite Butler____________

Todd_Freeberg______________

Neil Greenberg______________

Richard Saudargas___________

Accepted for the Council:

___Anne_Mayhew______
Vice Chancellor and
Dean of Graduate Studies

(Original signatures are on file with student records.)

Constriction Behavior, a Key Innovation in Snake Evolution: The Integration of Ethology and Physiology

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

Rita S. Mehta
May 2005

ACKNOWLEDGEMENTS

In each Part (Part 1: Introduction excluded) I acknowledge the people who helped me in many ways. In addition to help with the individual projects, I received invaluable help and inspiration throughout my education from several people, many of whom contributed to the specific projects. I am grateful to my advisor Gordon Burghardt. Gordon Burghardt was the reason I came to Tennessee. I would also like to thank the members of my committee, Marguerite Butler, Todd Freeberg, Neil Greenberg and Richard Saudargas for their support, guidance and encouragement. Jim Hall also helped review proposals and always provided insightful comments.

I am most indebted to Brad Moon for allowing me to study muscle physiology in his laboratory. Without Brad’s help, this dissertation would not have been as interesting. Brad’s immense support and instruction with EMG experiments as well as our conversations about constriction behavior in snakes enabled me to better understand the importance of combining physiology with behavior. I made many trips to conduct EMG experiments at the University of Louisiana. During these trips I was invited to sit in on valuable graduate student seminars and was also given an outlet to present my work. Bob Jaeger always made it a point to try and touch basis with me and talk to me about “the next step” in my research career. Ali Rabatsky not only provided much help during experiments but her friendship has been invaluable. I sincerely thank Kate Wadsworth for welcoming me and giving me a comfortable and enjoyable place to stay while visiting Lafayette, Louisiana.

I would especially like to acknowledge Tracy and David Barker, Brad Moon and Brian Ott. These individuals provided the animals used in this study. Tracy and David

also helped me develop a better understanding of snake behavior. The summer I spent with them has been one of my most valuable and memorable research experiences. Little did I know that all those late night conversations with Tracy would help me formulate both my masters and doctoral research. Brian Ott, on more than one occasion, allowed me to photograph snakes from his personal collection as well as the collection at the University of Alabama. Brian was also enthusiastic and great to talk to about snake feeding biology.

There are many other people I would like to thank: Marguerite Butler for her helpful insight and encouragement, Karen Davis and Lauren Kirby for being excellent lab mates and friends and for covering my classes while I was in Louisiana. Sarah Connor, Laurianne Hebb, and Ellen Mahurin made my experience in the program more fun. Catherine Mc. Millen helped me build the spectacular plexi glass feeding arena used during my stimulus control experiments in Part 2. Dr. David and Sharon Kirby loaned me their family Hi-8mm video recorder when mine broke so I could continue to collect videos of snake feeding for my research. I also received help and support from many undergraduate students: Cassandra Fenner, Jessica Graham, Jennifer Miller, Brooke Patrick, Shahrzad “Sherry” Rahbar and Samuel Riggan. These students were not only enthusiastic and hardworking but were able to quickly see the benefit as well as the enjoyment that comes from carefully observing animals. Many others have provided wonderful support, encouragement and scintillating conversation throughout my education: David Cundall, Harry Greene, Javier Rodriguez-Robles and Randall Reiserer. My husband Jonathon Redwine, snake enthusiast at heart, was always ready to talk and listen about snake feeding biology.

Equipment for this study was funded by the American Psychological Association (APA), the Department of Psychology at the University of Tennessee, Knoxville (UT), the U.T Scholarly Activities Research Incentive Fund (SARIF), and the Reptile Behavior Fund of the Burghardt Lab. Presentations of parts of this study to the Society of Integrative and Comparative Biology (SICB) and at the VII International Conference of Vertebrate Morphology (ICVM) were supported by the Department of Psychology at UT and the U.T Graduate Student Association. A sincere thank you is reserved for Dr. Jim Lawler, our Psychology Department head. On many occasions, Dr. Lawler helped me find a source of funding for needed laboratory equipment.

I would like to thank my parents, Dr. Onkar and Dr. Sooi Mehta. Without their help, love, support and encouragement, I would be lost. I thank my brother Roy for his encouragement, ideas and editorial help. I am so grateful for my husband Jonathon for being a terrific sport in a long distance relationship. Jack has been a constant source of ideas, strength, encouragement and comic relief throughout my graduate career. I also must acknowledge my father, mother, and sister in-law: Dr. William and Shirley Redwine and Cynthia Redwine. I am extremely blessed to be married into such a wonderful and loving family. Last but not least, I thank my dog Marley for guarding the backyard when I had to work late and for always being happy to see me regardless of how late I came home.

ABSTRACT

Among living tetrapod vertebrates, snakes exhibit the most radical shifts in feeding biology and among limbless squamate reptiles, only snakes have undergone a substantial adaptive radiation. The behavioral innovation, constriction, has been associated with the success of this clade. Constriction is a prey restraint behavior that enabled snakes to immobilize and subdue extremely large prey items relative to their own body mass. This behavior pattern is associated with the incredible shifts observed in snake feeding biology from consuming small meals frequently to less frequent feeding on large prey. Although constriction is an ethological homology for the majority of snakes, variations of constriction postures have been documented in many derived snake lineages. Nevertheless, the mechanisms driving behavioral variation are not well understood. In this dissertation, I attempt to use a comparative hierarchical approach to examine constriction behavior at both the ethological and physiological levels in order to better understand the behavioral variation of this key innovation.

As reviewed in Part 1, derived snake lineages seem to have several methods with which to restrain prey. Prey restraint methods appear to vary with respect to prey characteristics (size, shape, activity level). On the other hand, intermediate taxa (boas and pythons) are thought to be less variable in the prey restraint phase of feeding. The kinematics of loop application pattern also appears to differ between intermediate and derived snake groups. Derived snakes use the lateral part of their body to wind prey whereas boas tend to bend ventrally around prey. The polarity for variable prey restraint behavior and loop application patterns have not been determined as observations on feeding behavior for basal snake taxa are lacking.

I report on stimulus control studies evaluating prey restraint behavior and loop application pattern for basal and intermediate snake taxa in Part 2. Testing for the effects of prey size and status on the prey restraint behavior enabled me to polarize variable prey restraint behavior and loop application pattern. Prey size and status had varying effects on the capture position, prey restraint method, prey restraint time and swallowing time for basal and semi-fossorial boas while individuals of *B. constrictor* only constricted prey. Looping one or more times around prey was observed during the intraoral transport (swallowing) phase of feeding in the majority of trials for *L. bicolor* and Erycine snakes (*Eryx muelleri*, *Charina bottae*, *Lichanura triviragata*). Loop application patterns varied across snake taxa with basal and semi-fossorial boas applying loops laterally around prey. Individuals of *B. constrictor* bent ventrally around prey. The ability to vary prey restraint behavior, in response to prey characteristics and applying loops laterally around prey is probably the ancestral condition in snakes. Intermediate taxa, such a boas exhibit a derived simplified behavioral repertoire.

Examining the underlying physiology of a complex motor pattern, such as constriction behavior, can provide a better understanding of the hierarchical structure of organisms in nature. As an ethological homology, constriction behavior provides us with the opportunity to trace evolutionary change at other levels of biological organization and to examine how various levels within a hierarchy relate to one another.

Although constriction is an important key innovation associated with the adaptive radiation of snakes, few studies have examined the underlying physiological patterns of this complex motor pattern that may account for the kinematic variability of constriction postures among snakes. In Parts 3 & 4, I comparatively examine the muscle activity patterns during constriction for basal and intermediate snake lineages. I specifically investigated how the underlying physiological mechanisms of constriction correspond to the postural changes observed at the behavioral level using electromyography. Lateral bending and unilateral muscle activity patterns were predominant in the basal taxon, *Loxocemus bicolor.* Lateral bending and unilateral muscle activity patterns were also observed in derived snake taxa previously documented. Ventral bending and bilateral epaxial muscle activity patterns were predominant in intermediate lineages and present in derived snake lineages. Therefore, similar to prey restraint behaviors, three epaxial muscle activity patterns were observed: 1) mostly lateral bending with unilateral epaxial muscle activity, 2) mostly ventral bending with bilateral muscle activity and 3) mostly lateral and some bilateral bends associated with both unilateral and bilateral epaxial muscle activity, “mixed”. The kinematic and muscle activity patterns correspond with the ethological data in Part 2.

Lateral bending and unilateral epaxial muscle activity support the more variable prey restraint behaviors observed in basal and derived snake taxa. Ventral bending and bilateral activity supports the highly stereotyped behavior patterns observed in intermediate snake taxa. A ‘mixed’ kinematic and epaxial activity pattern supports highly variable prey restraint methods as observed from previous research on gopher snakes and kingsnakes. Thus the patterns of epaxial muscle activity underlying constriction behavior can be correlated with the variability in prey restraint postures.
In Part 5, I integrate the behavioral, physiological, and ecological differences reported for *L. bicolor* and Boid snakes, from the stimulus control data and the physiological

data collected in this study, to further discuss the origin and evolution of feeding behavior among basal, intermediate and derived snake taxa.

TABLE OF CONTENTS

PARTS **PAGE**

LIST OF TABLES

PART 2 **PAGE**

PART 3

PART 4

LIST OF FIGURES

PART 1 **PAGE**

PART 2

PART 3

PART 4

PART 5

PART 1

INTRODUCTION

BEHAVIORAL VARIATION AND HIERARCHY OF ORGANISMAL DESIGN

The idea that a particular trait or a suite of characters may be correlated with an increase in species diversification has a long tradition in evolutionary biology (Simpson, 1953; Mayr, 1969). Evolutionary novelties, otherwise known as key innovations, are adaptations enabling a clade to utilize a resource from which the ancestors of the clade were previously excluded (Liem, 1974; Futuyma, 1998). Traditionally, key innovations were viewed as newly acquired physical structures that potentially permitted a new function. However, recent attention has been directed to other types of key innovations, such as novel dietary habits and behavior patterns (Wainwright et al., 2002; Alfaro et al. 2001). Although these behavioral innovations are dependent upon underlying physiological traits involving, but not limited to, the musculoskeletal system, sensory systems, and the brain, the phylogenetic analysis of these traits has been little used in comparative behavior studies (Lauder & Reilly, 1996). Physiological traits may be especially important in examining behavioral innovations within a clade when they can be associated with the clade's success.

The re-evaluation of the key innovation concept is reflected in recently proposed definitions (for review see Muller and Wagner, 1991). According to Hunter (1998), key innovations are aspects of organismal phenotypes important to the origin or subsequent success of a taxonomic group. This definition highlights the idea that specific attributes of organisms have been especially important and relatively stable over evolutionary time. I interpret Hunter's definition to include behavioral, morphological, and physiological characters. By extending the key innovation concept to traits that are not just structural helps further integrate the concepts of ecology and macroevolution to better understand the differential performance among clades. When investigated from a hierarchy of levels, key innovations can potentially provide a better understanding of the evolutionary processes acting on different aspects of the organism (Lauder & Schaffer, 1993; Lauder & Reilly, 1996; Hunter 1998).

Understanding the mechanisms that drive behavioral similarities and differences among animals has long inspired ethologists and evolutionary biologists (Tinbergen, 1963; Mayr, 1969). Current views in evolutionary biology strongly suggest that thorough comparative analysis of behavioral variation among species requires that the character traits under examination be broken down and then reassembled into a hierarchy of levels (Lauder, 1994). In other words, behavioral differences among closely related taxa within a clade may be sorted into the functional interrelationships between morphology, muscle topology and central nervous system output. This hierarchy of data, which incorporates characters grouped into structural or functional classes, reflects proximate causes for variation at the behavioral level (Lauder and Reilly, 1996; Lauder, 1991). The study of the proximate mechanisms that may drive behavioral differences across groups of organisms is especially important when clades exhibit differential success (species diversity) as measured by number of species or new adaptive zones as defined by their set of related ecological niches.

The study of the evolutionary patterns of congruence among functional classes of characters at different hierarchical levels raises several general questions (Lauder, 1994): 1) do traits at some levels tend to be more conservative and show relatively little interspecific variation?, 2) do traits at some levels tend to be more interspecifically labile?, and 3) is variation at one level correlated with variation at another level? For example, taxa may reveal homologous patterns of muscle activity but divergent behavior patterns due to changes that may have occurred in musculoskeletal topology (Lauder, 1991). These patterns of discordance among levels present interesting problems in the evolution of organismal design. The phylogenetic perspective combined with the analysis of organismal traits at several hierarchical levels allows these questions to be addressed (Striedter & Northcutt, 1991; Lauder 1991, 1994).

Although studies of a single species are valuable, they only address questions concerning character maintenance of that species (McLennan, 1991). In order to reveal the processes involved in character transformation, information from at least two other species, preferably the closest extant taxon to our focal group and an outgroup, are necessary. Phylogenetic comparative methods (PCM) can be used to infer the ancestral states of characters and to suggest patterns of character transformation. Specifically, PCM enables researchers to infer patterns and processes of character evolution from the patterns observed in extant species (Martins & Hansen, 1996).

In this dissertation, I comparatively examine constriction behavior, a key behavioral innovation in snakes. Snakes have inspired studies in an array of fields such as evolutionary biology (Greene, 1983; 1997; Cundall & Greene, 2000), comparative psychology (Burghardt, 1991; Chiszar et al., 1992), functional morphology, and physiology (Cundall, 1987; Kardong, 1998). This undoubtedly reflects the considerable biological diversity encompassed in these externally simplified vertebrates. Some of the most fascinating characteristics of this group of reptiles centers around their unique feeding behaviors. Among living tetrapod vertebrates, snakes exhibit the most radical shifts in feeding biology and among limbless squamate reptiles (lizards and snakes) only snakes have achieved substantial adaptive radiation and high species richness (Cundall & Greene, 2000).

I use a hierarchical perspective to examine constriction behavior at both the ethological and physiological levels. Constriction, a complex feeding behavior in snakes, serves as an ideal topic for interesting comparative evolutionary studies since it 1) is a key behavioral innovation that has been correlated with the success of a vertebrate clade, 2) consists of a readily defined sequential modal action pattern (Burghardt, 1973; Barlow, 1977), 3) varies interspecifically, and 4) reveals great diversity in ecological and morphologic adaptations for assessment of similarities and differences (Greene, 1977). Since Greene & Burghardt's (1978) study on the homology of constriction behavior in snakes, little research has focused on the biomechanics or physiology of this behavior pattern. The overall goal of this dissertation is to trace the transformation of constriction, a key behavioral innovation, across ethological and physiological levels. My aims are to:

1) comparatively examine the effects of prey characteristics (prey size and status) on the constriction behavior of basal and intermediate snakes, 2) document the kinematics and epaxial muscle activity patterns during constriction in a basal snake, and 3) examine the kinematics and epaxial muscle activity patterns during constriction in two intermediate snake lineages and compare the patterns to those of basal and derived snakes. In the following pages, I provide a brief summary of the work on constriction behavior in snakes that led to the aims of this study and present a summary of my dissertation experiments for Parts 2-4.

CONSTRICTION BEHAVIOR IN SNAKES

A Brief Overview

Accounts of snake feeding behavior, particularly descriptions of prey handling/ restraint, can be found in the literature as early as the late1800's (Hopley, 1882; Wall, 1911; Boulenger, 1912; Mole, 1924). Ditmars (1914) must have been one of the first reptile enthusiasts to separate snakes into, "constrictors, semi-constrictors, and non-constrictors." Constrictors were defined as, "serpents of all sizes that kill their prey by coiling about it and squeezing it to death" (p.199). According to Ditmars (1914), constrictors could be found within the families Boidae and Colubridae. Semi-constrictors were those snakes that subdued prey by holding it within a single coil or pressing it firmly against the ground by a fold of the body while attempting to swallow it. Since Ditmar's review on the feeding habits of serpents, several short descriptions of snakes constricting prey appeared in the literature (Loveridge, 1928; Pope, 1935; Axtell, 1951; Myers, 1965). However, it was not until the contributions of Pope (1961), Frazzetta (1966) and Shrewsbury (1969), that constriction was recognized as an innovation worthy of comparative behavioral (Willard, 1977) and kinematic (Greenwald, 1978) analyses.

Shortly after, constriction was not only identified as a key behavioral innovation in snakes, but was recognized as the first behavioral homology documented at the familial level (Greene, 1977; Greene & Burghardt, 1978). Greene (1977) was the first to examine constriction behavior from a phylogenetic perspective, which led to a better understanding of the distribution and evolution of the behavior pattern. Constriction behavior, and the role of prey handling in snake feeding biology, would soon be recognized as a worthy topic for comparative evolutionary studies.

One of the most interesting observations about constriction feeding behavior that stemmed from the work of Greene (1977) is the diversity of loop application patterns that can be observed while snakes form a constriction coil. Greene (1977) documented slight individual variability and no interspecific and intergeneric variability in coil application movements for intermediate snake taxa (Boas and pythons) but highly variable coil application patterns in derived snake taxa. Although Greene's initial assessment of constriction behavior was superimposed onto a phylogeny that is no longer supported, current views on the evolutionary relationship of snakes support Greene's findings that constriction is a shared modal action pattern for the majority of snake taxa (Greene, 1994). This variation in constriction behavior is reflected in the definition of constriction posed by Greene & Burghardt (1978). Constriction was defined as, "a behavior pattern in which prey is immobilized by pressure exerted by two or more points on a snake's body" (p. 74). This definition is broad and general, encompassing the great variability in constriction postures, and remains the accepted definition for this unique prey restraint behavior today.

Greene (1977) reported that 19 out of the 27 possible permutations of constriction postures could be observed in colubroid snakes. Since then, studies have attempted to examine how different stimuli may contribute to the variability of constriction across colubroid snake taxa. Specifically, prey size (Mori, 1991, 1994; Mehta, 2003), prey type (Mori, 1991) and prey activity level (de Queiroz, 1984) have been correlated with the incredible diversity in prey handling behavior observed in colubroid lineages. Much work has also focused on the interspecific variation of constriction patterns (de Queiroz & Groen, 2001, Milostan, 1989; Mori, 1994; Rudolph et al., 2003) and the ontogeny of constriction (Milostan, 1989; Mori, 1991, 1993 a, b; Waters, 2000; Mehta, 2001).

The ontogeny of constriction has encouraged interesting questions related to innate and learned behavior patterns, and the role of maturation in learning (Mori, 1993 a,b, 1994, 1995; Milostan, 1989; Mehta, 2001). Subsequent experience with prey is said to affect prey restraint behavior in snakes as well as overall response to prey items (Fuchs & Burghardt, 1971). Mori (1993a) documented how feeding experience with different sized prey can influence subsequent prey restraint behavior in *Elaphe climacophora*, a derived snake. Mehta (2001) documented how experience and maturation can affect prey restraint behavior in young trinket snakes, *Elaphe helena*.

In addition to the studies dealing with the ontogeny of constriction, the recent contributions in the herpetological literature on prey handling observations for derived snake taxa (Gregory et al., 1980; Waters, 2000; de Queiroz & Groen, 2001; Rudolph et al., 2003) further support the importance of phylogenetic history in behavioral variation. Intermediate snake taxa, such as boas and pythons, do not exhibit considerable variation in their constriction postures throughout ontogeny, and are observed to be highly stereotyped as adults (Greene, 1977; Greene & Burghardt, 1978; Milostan, 1989). In fact, boas and pythons constrict with the same prowess as the adults of their species on their first encounter with prey (Greene, 1977; pers. obs.). On the other hand, colubroid lineages exhibit variation in their prey handling repertoire throughout ontogeny (Milostan, 1989; Mori, 1991; Mehta, 2001, 2003) and evidence suggests that strong selection pressures for prey immobilization can lead to the evolution of constriction behavior in non-constricting lineages (Gregory et al., 1980; de Queiroz & Groen, 2001). Eventually, these newly selected behaviors can be refined over evolutionary time. Thus, in a habitat in which one must specialize on a particular prey item, it might be expected that neonate snakes have an innate prey-handling behavior, whereas a more unpredictable environment may favor more flexible and plastic behavior.

Today, there is still a major need for the assessment of constriction coil application pattern for many species of snakes (neonates and adults), primarily basal alethinophidian taxa and highly derived snake species that are members of colubroid lineages. Whether the colubroid coil application pattern and variable prey handling behavior is derived remains unknown. In fact, assessment of the polarity of variable prey restraint methods is not possible without the examination of basal snake taxa that are known to constrict prey and a reexamination of basal and intermediate macrostomate constriction behavior. Although Greene (1977) examined the constriction postures of boas and pythons on various substrates and with various prey items, a stimulus control design was not used. Stimulus control studies consist of a series of experiments in which the stimulus expected to elicit or control the behavior under investigation is varied by a single parameter. This not only allows for close examination of any variability in behavior, but this standard experimental design is ideal for comparative studies.

As the literature on prey handling behavior increases, the terminology used to describe constriction postures needs to be evaluated. Assessment of loop application with the current terminology is becoming increasingly challenging. This challenge may be partially due to the fact that as more descriptions of constriction behavior are obtained, particularly from derived snake lineages, some loop application patterns will not easily or neatly fit into the categories of descriptors proposed. This is especially true if constriction patterns are continuous rather than discrete patterns, particularly in derived snake taxa that re-evolved constriction (de Queiroz & Groen, 2001). Also, as different researchers contribute to our understanding of constriction behavior, some of the terminology can potentially be misinterpreted. This phenomenon has already been documented in the antipredator literature but fortunately, newly proposed terminology will help clarify behaviors observed for future antipredator studies (Mori & Burghardt, 2004). Thus, it is becoming more evident that we need to do the following: 1) streamline characters and character states, and identify only the crucial characters important for comparative studies, 2) agree on simple descriptors that are easily understood in the absence of diagrams or photos and 3) continue to provide diagrams, photos and video of constriction behavior whenever possible.

Willard (1977) examined the constriction behavior for 43 species of snakes. In his analyses three methods of loop application were recognized: 1) those with venter (stomach scales) facing forward, 2) those with venter facing backward, and 3) irregular coils with no consistent surface against the prey. Greene (1977) adopted the first two character states but renamed them as (twist [1] and no twist [2]) which is less descriptive but is clear when photos or diagrams are available. Although studies have adopted the terminology proposed by Greene (1977) and Willard (1977), I argue that if constriction is to be studied from other levels, other than the ethological level, the 'twist' may not be as crucial of a character state in understanding constriction but rather, which part of the snake's surface is in contact with the prey. The terminology I use (ventral and lateral) that should replace descriptors that are commonly associated with the character state ‘twist,’ (venter facing forward and venter facing backward) was suggested and applied earlier by Moon (2000).

Moon (2000), the first to examine constriction from a physiological perspective, described constriction postures in terms of the axial skeleton and musculature. The axial musculature is separated into two parts, the epaxial (dorsal) and hypaxial (ventral) regions, and these regions play important roles in constriction behavior (Cundall, 1987). The elongate Bauplan of snakes enables them to form small arcs in their body by bending laterally and ventrally. In relation to the definition of constriction (Greene & Burghardt, 1978), I describe a bend to be lateral when the side of the snake comes in contact with the prey. During lateral bending, one can easily see not only the dorsal part of the snake but also the ventral part (Fig. A-1; photos also available in Greene & Burghardt, 1978; Shine & Schwaner, 1985)[1]. During a ventral bend, the ventral side of the snake is pressed against the prey so one can see *mostly* the dorsal side of the snake. These bending postures that correlate with the underlying muscle activity patterns observed during constriction (Moon, 2000), are less elusive than presence or absence of a ‘twist,’ proposed by Greene (1977), and will be used throughout this dissertation.

OBJECTIVE AND SUMMARY OF PARTS

Part 2: A Re-examination of the Evolution of Constriction Patterns in Snakes

Snakes comprise a monophyletic group of obligate predators. Behavioral, structural, and physiological innovations enabled snakes to achieve an exceptionally speciose and

---

[1] All figures are located in the Appendix.

diverse adaptive radiation (Cundall and Greene, 2000). Constriction of prey appears to have been the key behavioral innovation behind the success of this reptilian group (Greene, 1983, 1994). Snakes can be categorized into seven major groups with respect to their feeding biology (Fig A-2): Group I- those that feed on very small prey and frequently, Group II-VI-those that feed relatively infrequently on large prey items, and Group VII- (Colubroidea) reflects many feeding shifts from piscivorous snakes to snakes that feed on prey that can exceed their own body mass by as much as 50% (Viperidae) and snakes that approach lizards in feeding biology (some lineages in the Colubridae).

As shown in Fig. A-2, the earliest snakes, blind snakes (Scolecophdia), are Group I snakes. Blind snakes are restricted to eating small invertebrates, such as termites, and have a heavily ossified skull for burrowing (Kley, 2001). All other snakes belong to the Alethinophidia and can be classified in one of the six feeding groups. Alethinophidians contain three major snake groupings: Basal Alethinophidia, Macrostomata and Colubroidea. Basal alethinophidians are the least studied of all snake lineages (Cundall & Greene, 2000). Within the Family Loxocemidae (one species), is the oldest extant taxon capable of consuming and immobilizing mammalian prey matching or slightly exceeding its own head width. Only one study (Greene, 1977) has documented constriction behavior in *Loxocemus bicolor*.

The next group of snakes, the Macrostomata (i.e., Boines - next four families in Fig. A-2, are characterized by a morphological key innovation, the streptostylic quadrate. This change in the lower jaw allowed snakes to swallow prey much larger in diameter than their own heads. Several studies have shown that constriction in the macrostomata is highly stereotyped (Greene, 1977; Willard, 1977; Milostan, 1989). Moreover, constriction patterns of boas and pythons do not appear to be affected by varying prey characteristics, although more empirical evidence is necessary (Greene, 1977; Milostan, 1989).

Colubroid snakes (last four families in Fig. A-2), comprising over 90% of all extant snake taxa, represent such a diversity of shapes, sizes, habitats, diets, and behavior that generalizations of the group are difficult (Greene, 1997). Constriction evolved multiple times in the colubroid snakes, although it seems completely absent from the Viperidae (Greene, 1994). Unlike boas and pythons, colubroid constriction is more variable in form and deployed selectively in response to prey characteristics (type: Mori, 1991, 1993 a, b; size: Mori, 1991, 1993 a, b; Mehta, 2003; and activity level: De Queiroz, 1984). At least five different prey restraint behaviors have been described for colubroid snakes (Greene, 1977; Greenwald, 1978; Mori, 1994; De Queiroz & Groen, 2001; Mehta, 2003**.** Although it has been assumed that the colubroid constriction pattern is derived, and the boine pattern ancestral, this has not been confirmed through careful study of basal snakes.

The goal of my experiment in Part 2 is to describe in detail the prey restraint behavior and loop application pattern for *Loxocemus bicolor*, *Boa constrictor*, and four species of Erycine snakes and to test for the effects of prey size and status on their feeding behaviors. The phylogenetic position of *L. bicolor*, sister taxon to boas and pythons, and the morphological data that suggests *L. bicolor* has some boid–like characteristics, lead me to hypothesize that constriction behavior for *L. bicolor* will be highly stereotyped and that prey characteristics will not affect the prey restraint behavior for any of these three lineages.

Part 3: The Kinematics and Epaxial Muscle Activity Patterns During Constriction in the Neotropical Sunbeam Snake (Loxocemus bicolor)

Bilateral and unilateral muscle activity patterns are common across vertebrates, especially in those taxa that have undergone limb reduction or limb loss. A recent study examining the role of the epaxial musculature during constriction in derived snake taxa revealed mostly unilateral patterns of epaxial muscle activity during constriction (Moon, 2000). Whether epaxial muscle activity patterns are homologous across snake lineages is unknown, as only derived species have been examined. The purpose of this experiment is to examine the epaxial muscle activity pattern of a basal alethinophidian snake, *Loxocemus bicolor.* This experiment, along with the behavioral studies for *L. bicolor* I present in Part 2 of this dissertation, will help polarize constriction postures in snakes as well as muscle activity patterns during constriction. From morphological data *L. bicolor* has been regarded as either a primitive boid, (Frazzetta, 1966,1970; Rieppel, 1978) or as a member of a distinct family intermediate between basal and derived snakes. As current taxonomy suggests *L. bicolor* is the sister taxon to intermediate lineages (boas and pythons), I hypothesize that *L. bicolor* will exhibit highly stereotyped constriction postures. Constriction with high stereotypy seems to be associated with ventral bending rather than lateral bending. Ventral bending, unlike lateral bending, requires that the ventral scales of the snake be in contact with the prey. In lateral bending, one side of the snake is in contact with the prey, and epaxial muscles are active on the side of the body that comes in contact with the prey. Therefore, I hypothesized that *L. bicolor* will exhibit bilateral epaxial muscle activity patterns during constriction.

This implies that unilateral epaxial muscle activity is the derived condition in snakes that constrict prey.

Part 4: The Evolution of Constriction Patterns in Snakes: A Physiological Homology

Constriction, a prey restraint behavior in which prey is immobilized and subdued via pressure, was the first behavioral homology to be identified at the familial level (Greene & Burghardt, 1978; Greene, 1994). However, only recently have the underlying muscle activity patterns and kinematics been assessed for this key behavioral innovation (Moon, 2000). The purpose of this study is to examine whether constriction behavior is homologous at the physiological and kinematic levels across snake taxa. Moon (2000) and Mehta, in collaboration with Moon (Part 3), examined the epaxial motor patterns for two derived and one basal snake taxa. In order to use the comparative method to assess homology across the Serpentes, a third taxon, intermediate in phylogenetic position, must be examined. I examine constriction at the physiological and biomechanical levels for two intermediate taxa, *Python molurus* and *Boa constrictor*. *Python molurus* and *B. constrictor* are basal macrostomate snakes that are known to constrict prey items comprising significant portions of their own body mass. Earlier studies suggested that the kinematics of intermediate snake taxa differed from highly derived snake lineages (Frazzetta, 1970). More recent behavioral and electromyographic studies (Parts 2 & 3) revealed that an intermediate snake taxon, *Boa constrictor*, exhibited highly stereotyped feeding patterns and did not vary constriction behavior in response to prey size or status. Basal snake lineages, on the other hand, appear to share similar behavior and epaxial motor patterns with highly derived snake lineages. Based on my experiments that analyzed constriction behavior in intermediate snake taxa, I hypothesize that boas and pythons will exhibit derived constriction patterns at the behavioral, biomechanical, and physiological levels. Comparisons of epaxial muscle activity patterns in behaviorally homologous characters across three major lineages of snakes will help reveal whether numerous functional specializations have occurred in muscle activity patterns and how these transformations relate to variability in constriction postures.

Part 5: How Deep Is Constriction Behavior?

In this dissertation I explore the levels of homology for a key behavioral innovation, constriction. This phylogenetic concept of homology has important implications for evolutionary biology and can help address proximate as well as ultimate causation of character variability (Lauder, 1990, 1994). Restricting the use of the term homology to monophyletic clades enables a better understanding of the covariation that may take place between several characters. Many interesting trends in the evolution of organismal design appear when characters are examined from a hierarchy of levels (Lauder, 1994). In particular, multilevel studies illustrate the complexity of the relationship between structure, function and behavior. Several studies to date have illustrated that the reorganization at one level of organismal design may not necessarily lead to changes at other levels of design (Lauder, 1990, 1991; Wainwright & Lauder, 1992). Traits that are homologous at one level of organismal design need not be homologous at other levels. In Part 5, I summarize the findings of my study and discuss the implications of my work to the understanding of the evolution of feeding behavior in snakes. I also discuss how the ideas in this study can be used to better understand character transformation and organismal design.

LITERATURE CITED

Alfaro, M. E., J. Janovetz, and M.W.Westneat. 2001. Motor control across trophic strategies: Muscle activity of biting and suction feeding fishes. American Zoologist 41: 1266-1279.

Axtell, R.W. 1951. An additional specimen of *Lampropeltis blairi* from Texas. Copeia 1951: 313.

Barlow, G.W. 1977. Modal action patterns. In: How animals communicate (T.A. Sebeok, ed.), University of Indiana Press, Bloomington. Pp. 94-125.

Boulenger, G.A. 1912. A vertebrae fauna of the Malay Peninsula from the Isthmus of Kra to Singapore including the adjacent islands. Reptilia and Batrachia. Taylor and Francis, London.

Burghardt, G.M. 1973. Instinct and innate behavior: toward an ethological psychology. In: The study of behavior. (J.A.Nevin, ed.), Scott, Foresman and Co., Glenview, Illinois. Pp. 322-400.

Burghardt, G.M. 1991. Cognitive ethology and critical anthropomorphism: a snake with two heads and hognose snakes that play dead. In: Cognitive ethology: the minds of other animals: essays in honor of Donald R. Griffin.Lawrence Erlbaum Associates. Pp. 53-90.

Cadle, J.E. 1994. The colubrid radiation in Africa (Serpentes: Colubridae): phylogenetic relationships and evolutionary patterns based on immunological data. Zoological Journal of the Linnaean Society 110: 103-140.

Cadle, J.E., H.C. Dessauer, C. Gans, and D.F. Gartside. 1990. Phylogenetic relationship and molecular evolution in uropeltid snakes (Serpentes: Uropeltidae): allozymes and albumin immunology. Biological Journal of the Linnean Society 40: 293-320.

Chiszar, D., R. K. Lee, C. W. Radcliffe, and H. M. Smith. 1992. Searching behaviors by rattlesnakes following predatory strikes. In: Biology of the Pitvipers. (J.A. Campbell and E.D. Brodie, Jr., eds.). Selva, Tyler, TX. Pp.369-382.

Cundall, D. 1987. Functional morphology. In: Snakes: ecology and evolutionary biology. (R.A.Seigel, J.T. Collins & S.S. Novak, eds.), New York, McGraw-Hill. Pp. 106-140.

Cundall, D.and H. W. Greene. 2000. Feeding in snakes. In: Feeding: form, function, and evolution in tetrapod vertebrates.(K. Schwenk, ed.), Academic Press, San Diego. Pp. 293-333.

Cundall,D., V. Wallach, and D.A. Rossman. 1993.The systematic relationships of the Snake genus *Anomochilus.* Zoological Journal of the Linnean Society 109: 275-299.

De Queiroz, A.1984. Effects of prey type on the prey-handling behavior of the Bull snake, *Pituophis melanoleucus*. Journal of Herpetology 18: 333-336.

De Queiroz, A. and R. R. Groen. 2001. The inconsistent and inefficient constricting behavior of Colorado western terrestrial garter snakes, *Thamnophis elegans*. Journal of Herpetology 35: 450-456.

Ditmars, R. L.1914. The feeding habits of Serpents. Zoologica.1: 197-238.

Frazzetta, T.H. 1966. Studies of the morphology and function of the skull of the Boidae (Serpentes). Part II. Morphology and function of the jaw apparatus in *Python sebae* and *Python molurus*. Journal of Morphology 118: 217-296.

Frazetta, T.H. 1970. Studies on the fossil snake Dinilysia patagonica Woodward.II. Jaw machinery I the earliest snakes. Forma et Function 3: 205-221.

Fuchs, J.L.and G.M. Burghardt. 1971. Effects of early feeding experience in responses of garter snakes to food chemicals. Learning and Motivation. 2: 271-279.

Futuyma, D.J. 1998. Evolutionary biology. Third edition. Sinauer Associates.

Greene, H.W. 1977. Phylogeny, convergence, and snake behavior. Unpublished P.h.D dissertation, University of Tennessee, Knoxville, Tennessee.

Greene, H.W. 1983. Dietary correlates of the origin and radiation of snakes. American Zoologist 23: 431-441.

Greene, H.W. 1994. Homology and behavioral repertoires. In: Homology, the Hierarchical Basis of Comparative Biology. (B.K. Hall, ed.).Academic Press, San Diego. Pp.369-391.

Greene, H.W. 1997. The Evolution of Mystery in Nature. University of California Press.

Greene, H.W. and G. M. Burghardt. 1978. Behavior and Phylogeny: Constriction in Ancient and Modern snakes. Science, v. 200: 74-76.

Greenwald, O.E. 1978. Kinematics and time relations of prey capture by gopher snakes. Copeia. 1978: 263-268.

Gregory, P. T., J. M. Macartney, and D. H. Rivard. 1980. Small mammal predation and prey-handling behavior by the Garter snake, *Thamnophis elegans.* Herpetologica, 36: 87-93.

Hopley, C. 1882. Snakes: Curiosities and Wonders of Serpent Life. Griffin and Farran.

Hunter, J.P. 1998. Key innovations and the ecology of macroevolution. Trends in Ecology and Evolution 13: 31-36.

Kardong, K. V. 1998. Rattlesnake strike behavior: kinematics. Journal of Experimental Biology 201: 837-850.

Kley, N.J. 2001. Prey transport mechanisms in blindsnakes and the evolution of unilateral feeding systems in snakes. American Zoologist 41: 1321-1337.

Kluge, A.G. 1991. Boine phylogeny and research cycles. Miscellaneous Publications of the Museum of Zoology, University of Michigan 178: 1-58.

Kluge, A.G. 1993. *Calabaria* and the phylogeny of erycine snakes. Zoological Journal of the Linnean Society 107: 293-351

Lauder, G.V. 1990. Functional morphology and systematics: studying functional patterns in an historical context. Annual Review of Ecology and Systematics 21: 317-340.

Lauder, G.V. 1991. Biomechanics and evolution: integrating physical and historical Biology in the study of complex systems. In: Biomechanics and evolution. (J.M.V. Rayner and R.J. Wootton, eds.). Cambridge University Press, Cambridge. Pp.1-19.

Lauder, G.V. 1994. Homology, Form, and Function. In: Homology: The hierarchical basis of comparative biology.(BK. Hall ed.), Academic Press. Pp. 155-196.

Lauder, G.V. and B.H. Shaffer. 1993. Design of feeding systems in aquatic vertebrates: major patterns and their evolutionary interpretations. The skull. Volume 3. Functional and evolutionary mechanisms. (J. Hanken, B.K. Hall, eds.). University of Chicago Press. Chicago & London. Pp.113-149

Lauder,G.V. and S.M. Reilly.1996. The mechanistic bases of behavioral evolution:

a multivariate analysis of musculoskeletal function. In: Phylogenies and the comparative method in animal behavior. (Emilia P. Martins, ed.), Oxford University Press, Pp 104-137.

Liem, K. F. 1974. Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. Systematic Zoology 20: 425-441.

Loveridge, A. 1928. Notes on snakes and snake bites in east Africa. Bulletin of the Antivenin Institute of Amererica 2: 32-41.

Martins, E. P. and Hansen, T. F. 1997.Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. American Naturalist 149: 646-667.

Mayr, E. 1969. Principles of systematic zoology. McGraw-Hill Book Co., New York.

McLennan, D.A. 1991 Integrating phylogeny and experimental ethology: from pattern to process. Evolution 45: 1773-1789.

Mehta, R.S. 2001. Prey-handling behavior in newly hatched trinket snakes, *Elaphe helena.* Unpublished Masters Thesis, University of Texas, Tyler, Texas.

Mehta, R. S. 2003. The effects of prey-size on the prey-handling behavior of hatchling *Elaphe helena*. Herpetologica 4: 471-476.

Milostan, M. 1989. The comparative ontogeny of prey capture and immobilization in *Boa constrictor* and *Elaphe obsolete*, with comments on the origin and evolution of constriction in boid and colubrid snakes. Unpublished M.S. thesis, Central Michigan University, Mt. Pleasant.

Mole, R.R. 1924. The Trinidad snakes. Proceedings of the Zoological Society, London 1924: 235-278.

Moon, B.R. 2000. The mechanics and muscular control of constriction in gopher snakes (*Pituophis melanoleucus*) and a king snake (*Lampropeltis getula*). Journal of Zoological Society, London 252(1): 83-98.

Mori, A. 1991. Effects of prey size and type on prey-handling behavior in *Elaphe quadrivirgata.* Journal of Herpetology 25: 160-166.

Mori, A.1993a. Does feeding experience with different size of prey influence the subsequent prey-handling behavior in *Elaphe climacophora* ? Journal of Ethology 11: 153-156.

Mori, A.1993b. Prey handling behavior of neonatal rat snakes, *Elaphe taeniura* and

E.dione (Colubridae). Japanese Journal of Herpetology 15**:** 59-63.

Mori, A.1994. Prey-handling behavior of newly hatched snakes in two species of the genus *Elaphe* with comparison to adult behavior. Ethology 97: 198-214.

Mori,A.1995. Prey handling behavior of the young rat snake *Elaphe taeniura* (Squamata:Reptilia).Mem.Fac.Sci. Kyoto Univ. (Ser. Biol), 16: 43-47.

Mori, A. and G.M.Burghardt. 2004. Thermal effects on the antipredator behaviour of snakes: A review and proposed terminology. Herpetological Journal 14: 79-87.

Muller, G.B. and Wagner, G. P. 1991. Novelty in evolution: restructuring the concept. Annual Review of Ecology and Systematics 22: 229-256.

Myers, C.W. 1965. Biology of the ringneck snake, *Diadophis punctatus*, in Florida. Bulletin of the Florida State Museum 10: 43-90.

Pope, C.H. 1935. The reptiles of China. Natural History of Central Asia 10: 1-604.

Pope, C.H. 1961. The giant snakes. A.A. Knopf, New York.

Rieppel, O. 1978. A functional and phylogenetic interpretation of the skull of the Erycinae (Reptilia, Serpentes). Journal of Zoology (London) 186: 185-208.

Rieppel, O.1988. A review of the origin of snakes. In: Evolutionary Biology. (Hecht, B. Wallace, and G.T. Prance, eds.). Plenum, New York. Pp. 37-130.

Rudolph, D.C., Burgdorf, S.J., Conner, R.N., Collins, C. S., Saenez, D. Schaefer, R.R., Trees, T., Duran M., Ealy, M, and Himes, G. 2002. Prey handling and diet of Louisiana pine snakes (*Pituophis ruthveni*) and black pine snakes (*P. melanoleucus lodingi*), with comparisons to other selected colubrid snakes. Herpetological Natural History 9: 57-62.

Shrewsbury, K. 1969. The constriction habits of Lampropeltis (kingsnakes) and Elaphe (ratsnakes). Proceedings of the Oklahoma Academy of Sciences 48: 274-276.

Shine, R and Schwaner, T. 1985. Prey constriction by venomous snakes: a review, and new data on Australian species. Copeia 1985: 1067-1071.

Simpson, G.G. 1953. Tempo and mode in evolution. New York, Columbia University Press.

Striedter, G.F. and R.G. Northcutt. 1991. Biological hierarchies and the concept of Homology. Brain, Behavior, and Evolution 38: 177-189.

Tinbergen, N. 1963. On aims and methods of ethology. Zeitschrift fur Tierpsychology 20: 410-433.

Wainwright, P. C. and G. V. Lauder. 1992. The evolution of feeding biology in sunfishes (Centrarchidae). In: Systematics, Historical Ecology, and North American Fishes. American Society of Ichthyologists and Herpetologists. (R. L. Mayden, ed.), Stanford University Press, Stanford. Pp. 472-491.

Wainwright, P. C, D. R. Bellwood and M. Westneat. 2002. Ecomorphology of locomotion in labrid fishes. Environmental Biology of Fishes 65: 47-62.

Wall, F. 1911. A popular treatise on the common Indian snakes. Part XXIII. Journal of Bombay Natural History Society 23: 206-215.

Waters, M.R. 2000. Feeding behavior of crayfish snakes (*Regina*): Allometry, ontogeny and adaptations to an extremely specialized diet. Unpublished P.h.D dissertation, University of Tennessee, Knoxville, Tennessee.

Willard, D.E. 1977. Constricting methods of snakes. Copeia 1977: 379-382.

APPENDIX

A.

B.

Figure A-1 Bending patterns of snakes during the prey restraint phase of feeding. An adult *Loxocemus bicolor* constricts large live prey by using the lateral (side) of its body to apply loops to form a coil (A). A juvenile *Eunectes murinus* uses ventral bending to apply loops around a small dead prey item (B). In ventral bending the snake bends forward so the belly scales of the snake are pressed up against the prey.

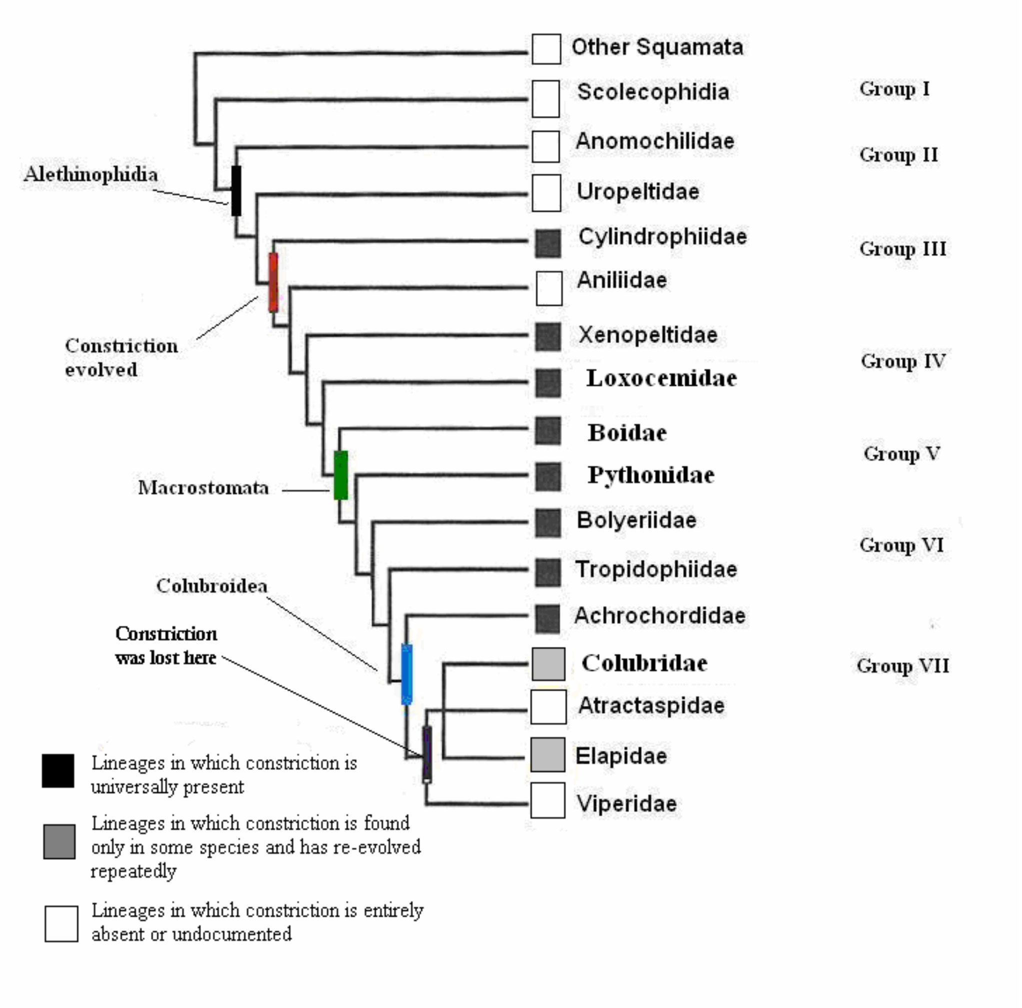


Figure A-2 Phylogeny of the Serpentes compiled from morphological (Cundall et al., 1993; Kluge, 1991, 1993; Rieppel, 1978, 1988) and molecular data (Cadle, 1994; Cadle et al., 1990). Families in bold are the focus of my dissertation.

PART 2

A RE-EXAMINATION OF THE EVOLUTION OF CONSTRICTION PATTERNS IN SNAKES

ABSTRACT

The selection for multiple prey restraint methods helped snakes shift from feeding on small prey items to relatively larger prey. Derived snake taxa exhibit more than one prey restraint method and empirical studies reveal that prey characteristics (size, status, type and activity level) influence which prey restraint behavior is employed. Intermediate taxa are presumed to have fewer restraint behaviors that are highly stereotyped, although empirical evidence is lacking. Whether variable or stereotyped prey restraint behavior is the derived condition is unknown as data for basal lineages are lacking. I provide empirical evidence for the feeding stereotypy of intermediate snake taxa and examine the polarity of prey restraint behaviors. I also attempt to polarize another behavior pattern that may be directly related to a snake's ability to vary prey restraint behavior during feeding, loop application pattern. I comparatively examined the effects of prey size and status (dead and live), using laboratory mice (*Mus musculus*), on the predatory cycle of *Loxocemus bicolor*, *Boa constrictor* and three species of Erycine snakes (sand boas). Prey size and status affected capture position, prey restraint method, prey restraint time and swallowing (intraoral transport) time for *L. bicolor* and Erycines. Individuals of *B. constrictor* exhibited very few changes in feeding behavior. *Loxocemus bicolor* and Erycine snakes employed three restraint methods: Simple seizing, constriction, and looping behavior while individuals of *Boa constrictor* were only observed coiling prey. During looping and coiling, *L. bicolor* and Erycines applied loops laterally around prey while *B. constrictor* wound around prey using ventral and ventral-lateral bends of the body. These comparative differences in prey restraint behaviors and loop application

pattern may represent different selection regimes related to the transition from feeding on small prey items frequently to larger prey items less frequently.

INTRODUCTION

Snakes are a monophyletic group of obligate predators that exhibit tremendous ecological and evolutionary diversity. The diversity, in part, is related to behavioral and morphological key innovations that allowed snakes to evolve specialized prey acquisition behaviors. Specialization of prey acquisition behaviors such as prey capture, immobilization and consumption techniques enabled snakes to shift from consuming tiny prey items frequently to feeding infrequently upon larger prey. The multiple shifts in foraging strategies accompanied with the ability to feed on prey items as diverse as insects, vertebrate eggs and large mammals, contributed to the substantial adaptive radiation of snakes and enabled snakes to be the most successful among limbless squamate reptiles (Cundall & Greene, 2000; Fig A-1)[1].

Unlike most tetrapods, the snake predatory cycle alone reflects incredible physiological, morphological and behavioral innovations. Four phases have been identified: 1) prey capture, 2) prey restraint, 3) prey manipulation, and 4) intraoral transport and swallowing (Cundall & Greene, 2000). Prey capture in many species consists of extremely fast striking movements with the anterior portion of the trunk (Cundall & Deufel, 1999; Kardong, 1998; Frazzetta, 1966)**.** Prey restraint includes three main strategies: simply seizing prey with the jaws, constriction, and envenomation. Prey manipulation involves orienting prey in preparation for intraoral transport. Intraoral

---

[1] All figures and tables are located in the Appendix.

transport and swallowing involves moving entire prey items through the oral cavity using alternating movements of the left and right jaw elements and pushing the prey further down the trunk using concertina-like trunk movements (Moon and Gans, 1998).

Of the four phases of the predatory cycle, the prey restraint phase seems to best exemplify evolutionary and ecological adaptations, illustrating the transition between lizard-like feeding habits (Group I) to consuming much larger prey but less frequently (Groups II-VII; Fig.A-1). In simple seizing, a restraint technique that may be indicative of more basal snakes, the snake holds the prey item in its jaws until the struggling of the prey diminishes. This is mostly performed with smaller prey items. Constriction and envenomation, not always mutually exclusive, are strategies that are used for restraint of relatively large prey (Savitzky, 1980; Shine & Schwaner, 1985).

Constriction, defined as a prey-handling method in which pressure is exerted from two or more points on a snake's body, evolved early in snake evolution and is an ethological homology for the majority of snake taxa (Greene & Burghardt, 1978; Fig. A-1). Constriction behavior provides material for comparative evolutionary studies since constriction 1) is a readily defined sequential behavior pattern (Burghardt, 1973; Barlow, 1977), 2) varies interspecifically, and 3) reveals great diversity in ecological and morphologic adaptations for assessment of similarities and differences (Greene 1977). Since Greene & Burghardt's (1978) study on the homology of constriction, much work has focused on the interspecific variation of constriction patterns (de Queiroz & Groen, 2001; Milostan, 1989; Mori, 1994) and the ontogeny of constriction (Milostan, 1989; Mori, 1991, 1993a, b).

For example, early macrostomate snakes (Group V; Fig. A-1), specifically boas and pythons, that are able to consume prey items weighing more than their own body mass have been documented using one main constriction pattern (Greene, 1977; Greene & Burghardt, 1978). In colubroid snakes (last four families in Fig. A-1), comprising over 90% of all extant snake taxa, (Greene, 1997), constriction was lost and regained multiple times independently in many lineages (Greene, 1994). Colubroid lineages that constrict prey exhibit variability in constriction behavior. Greene (1977) observed that the majority of variation in constriction postures could be observed in colubroid snakes. Others have documented the incredible variability in colubroid prey restraint behavior with respect to prey size (Mori, 1991, 1993a, 1995; Mehta, 2003), type (Mori, 1991) and activity level (de Queiroz, 1984). Studies on the ontogeny of constriction have revealed that juvenile boas constrict with the same prowess as adults (Greene, 1977; Milostan, 1989) whereas hatchlings of some colubroid genera exhibit more variability in constriction postures that may be linked to development, muscle maturation and experience (Mori, 1994, 1995; Mehta, 2003).

Another variable behavior state of the prey restraint phase that necessitates further attention is loop application pattern during constriction. In the first analyses of loop application, three behavioral states were recognized: 1) loops with venter facing forward, 2) loops with venter facing backward, and 3) irregular coils with no consistent surface against the prey (Willard, 1977). Greene (1977) adopted the first two character states but renamed them as (twist [1] and no twist [2]). The above terms are purely descriptive and if constriction is to be studied from other levels, other than the ethological level, the 'twist' may not be as crucial of a character state in understanding constriction but rather, which part of the snake’s surface is in contact with the prey. The terms used in this paper (ventral and lateral) that replace descriptors commonly associated with the character state ‘twist and no twist’ (venter facing forward and venter facing backward) was applied earlier by Moon (2000).

Moon (2000), examined the colubroid loop application pattern and found that gopher snakes (*Pituophis melanoleucus*) and a king snake (*Lampropeltis getulus*) used the lateral portion of their body to loop around prey. Both of these species, similar to many other colubroids, have been documented exhibiting variable prey restraint behavior. Whether lateral bending during loop application and variable prey restraint behavior are derived conditions remains unknown. Assessment of the polarity of these two character states is not possible without the examination of basal snake taxa that are known to constrict prey as well as a re-examination of basal macrostomate constriction behavior. Although Greene (1977) examined the constriction postures of boas and pythons on various substrates using diverse prey items, a stimulus control design was not used. Stimulus control studies consist of a series of trials in which the stimulus expected to release the behavior under investigation is varied by a single parameter. This standard experimental design is ideal for comparative studies and allows for close examination of variability in behavior.

In this paper I perform a comparative analysis of constriction behavior to further examine and polarize variable prey restraint behavior and loop application pattern. The main subjects of my study are *Loxocemus bicolor*, Erycine snakes (*Eryx muelleri, Charina triviragata* and *Charina bottae*) and *Boa constrictor* (Fig.2). *Loxocemus bicolor* is the most basal extant taxon capable of constricting large endothermic prey slightly exceeding its own head width (Mehta, unpublished data). Although the phylogenetic position of *Loxocemus* is argued (Frazzetta, 1970; McDowell, 1975; Rieppel, 1978, 1988; Pough et al., 1998), the most accepted view is that it is the sister taxon to the macrostomata, the large mouth snakes (Cundall et al**.** 1993; Cundall & Greene, 2000; Fig.A-2).

The family, Boidae, an early lineage of macrostomates, is characterized by morphological innovations of the skull enabling increased gape size (Rieppel, 1988). Boidae includes two subfamilies, Boinae and Erycinae (Kluge, 1991). The species *Boa constrictor* (Boinae) is examined in this study because it is both terrestrial and semi-arboreal. Thus the behavior patterns that will be revealed from stimulus control studies may be driven by proximate mechanisms such as variable ecology. The Erycinae, both Old and New World forms, are semi-fossorial and terrestrial species with macrostomate skull characteristics that enable them to consume large prey items. In general, Erycines are smaller boas that are typically <1 m in total length (Stebbins, 1985; Greene, 1997). Collectively, Erycines appear to share feeding habits that are more similar to basal alethinophidian snakes (Rodriguez-Robles et al., 1999). Therefore, examining the effects of prey characteristics on the predatory cycle of *L. bicolor, Boa constrictor,* and Erycine snakes will add to the understanding of the feeding transitions that may have taken place in the evolution of snakes.

Specifically, my goals are to: 1) examine the effects of prey size and status on the predatory cycle in these three phylogenetically important snake lineages, 2) polarize the character variable prey restraint behavior and 3) polarize loop application behavior during constriction. Based on the phylogenetic relationship of *L. bicolor* to the Boidae, I predict that the three lineages will not vary prey restraint behaviors with respect to prey size and status and that coiling will be the most commonly observed prey restraint behavior. Based on my preliminary observations, loop application pattern will differ across the three lineages examined.

MATERIALS AND METHODS

General Methods

Subjects and maintenance- Twelve adult *Loxocemus bicolor*, six subadult *Eryx muelleri*, two neonate *Charina bottae*, two adult *Charina triviragata*, and five adult *Boa constrictor imperator,* obtained from commercial breeders or private collectors, were housed in the Ethology Lab at the University of Tennessee, Knoxville. Measurements of all snakes are shown in Table A-1. Snakes were maintained individually in plastic containers (ranging from 260 x 180 to 460 x 240 mm). Larger animals were housed in larger containers. All containers were lined with 10 cm of shredded aspen substrate. Water was available *ad libitum*. Snakes were fed laboratory mice biweekly. Mice (live and dead) comprised anywhere from 6 - 30% of an individual snake's body mass (BM). Room temperature was maintained at 28°C with minimal variation and photoperiod was on a 14L:10D cycle.

Experimental design - To examine the effects of prey characteristics on the predatory cycle, I varied prey characteristics which served as the stimuli, in a controlled fashion. The general testing method was as follows: Large snakes (> 600mm, N = 17), were placed in a 1206mm x 584 mm x 457 mm plexiglass terrarium which served as the feeding arena. Smaller snakes (< 600mm, N = 10) were placed in a 914 mm x 457 mm x

457 mm plexiglass feeding arena. I varied two aspects of mammalian prey (*Mus musculus*) that have been shown to affect prey restraint behavior in snakes: size (Mehta, 2003) and status (de Queiroz, 1984). I used a 2x2 factorial design (small prey versus large prey x live versus dead) where prey were administered using a latin square cyclic matrix. The latin square cycle consisted of an 8 x 8 matrix because each individual snake received two trials in each of the four categories. Trials were initiated by introducing live prey or positioning dead prey into the terrarium. After a 5 minute period, an individual snake was introduced into the feeding arena. Prey items were placed into the arena first since pilot observations revealed that snakes tended to explore the new terrarium more when prey were absent. In fact, snakes would take as long as 6 hours to begin the predatory cycle when introduced first. A 10 -14 day interval between feeding trials was maintained for the majority of snakes. However, based on pilot observations, individuals of *E. muelleri* fed less frequently compared to the other snake species used in this study especially when feeding upon large prey.

Relative prey size- Although snakes are gape-limited predators, they are capable of consuming individual prey items that comprise anywhere from 20% to 100% of their own body mass (Cundall & Greene, 2000). In the reptile literature it is standard practice to express prey size relative to size of the predator. Two measurements are used to express this relationship: Weight Ratio (WR) and Ingestion Ration (IR). WRs are calculated by dividing the weight of the prey by the weight of the predator whereas IRs are determined by dividing the prey’s largest diameter by the head width of the snake. It is generally thought that both WR and IR give relatively similar results because as weight increases, head width or gape increases. I specifically use IR in this study because I was interested in the effects of size on the predatory behavior of snakes and using WRs may be misleading when studying the prey: predator relationship. This is because WR can be quite large, regardless of IR (Greene, 1983a) as exemplified in snakes that consume elongate organisms such as eels and also with regard to neonate and hatchling snakes. However, when feeding on mammalian prey or some reptilian prey (e.g. birds), the WRs must be very large to approach even 10% of a predator's body mass (BM) for adult snakes. This may not just pertain to snakes. Taking mass into consideration is especially complex when dealing with elongate organisms, because predators such as snakes have their mass spread across a greater length than most vertebrates with comparable weights. This definitely has substantial implications from a sensory feedback perspective and estimating prey size becomes more complex. For example a large rat may take up a greater area along the trunk of a snake, but may have a WR of only 3% for an adult snake. However, the IR would be 70%. Therefore, IRs would have greater implications for feeding and are used as a measure of prey in this study. Prey were considered small if their IRs were between 40-60% and large when IRs approached 80-100%. The large prey category increased the possibility of observing and evaluating constriction, especially with live prey.

Behaviors recorded- All feeding trials were recorded behind one-way glass with an 8-mm Sharp video recorder VL-E43U (30 fps) until the mouse was completely swallowed. The feeding behaviors recorded were modified from Greene, (1977), de Queiroz (1984), Milostan, (1989), and Mori (1991, 1994):

1) <u>Capture position</u>: the part of the prey's body first grasped by the snake. Three states were recorded: a) anterior (head and shoulder), b) middle (abdomen and

forelegs), or c) posterior (pelvic region, hind legs, and tail).

2) Prey restraint method: based on pilot observations of feeding for *L. bicolor*, Erycine snakes and *Boa constrictor*, four states were recorded: a) Simple Seizing (SS): grasping the prey in its jaws without subduing it with the body; b) Loop (L): winding one encircling loop around prey, c) Coiling (C): using two or more fully encircling loops around a prey, and d) Pinion (P): one or more non-encircling loops that push prey against some surface of the feeding arena or the prey can be wedged between non-encircling loops. Each of these behaviors can be performed immediately (I) after capture, or delayed (D), 1 or more seconds after prey capture. The behaviors, L, C, and P, are shown in Fig. A-3.

3) Loop orientation: Greene (1977) described loop orientation in terms of passing an imaginary line through the long axis of a loop or coil and the relationship of this line to the substrate. Three states could be observed: a) Horizontal (H): the imaginary line runs relatively parallel to the substrate; b) Vertical (V): the imaginary line runs relatively perpendicular through the long axis of the prey and the substrate; c) Mixed (M): there are two imaginary lines. One line runs parallel to the substrate and the other runs perpendicular to the substrate. Loop orientation is shown in Fig. A-4.

4) Loop application pattern: the method by which a loop was applied around prey during loop and coil. Three states were observed: a) Lateral (L): only one side of the body was used press up against prey; b) Ventral (V): the belly scales of the snake were pressed up against the prey; c) Ventral- lateral (VL): in the first loop the belly of the

snake was pressed up against the prey and in the 2nd loop the side of the snake was pressed against the prey.

5) Condition of prey before ingestion: after the prey restraint phase and just before swallowing, two states were observed: a) Dead (D) or b) Live (L).

6) Swallowing Position: there were two directions in which prey could be swallowed. Either the head and neck region of the prey could enter the mouth of the snake first: a) Anterior (A) or the tail end could be ingested first: b) Posterior (P).

7) Whether a loop is present during swallowing: one or more loops are wound around the prey and act to stabilize the prey while the snake is swallowing. Only two possible states were recognized: a) Presence of loop or b) Absence of loop

8) Prey restraint time: the elapsed time from the moment the prey was struck or seized to the commencement of swallowing.

9) Swallowing (Intraoral transport) time: the period from the commencement of swallowing to the point at which the snake began pushing the prey down toward its mid body and the snake’s mouth could completely close.

10) Total feeding time: the time from when the snake captured or seized the prey until the snake finished swallowing.

Analyses

I report the effects of prey size and status on the predatory cycle of snakes and implications (if any) of these analyses in association with the between species comparison for each variable. Since all species were subject to the same feeding regime before the experiment and the same stimulus control experiments during this study, the summary values obtained from each species group are comparable.

I present percentages of trials in which particular behaviors are shown and means for continuous data, so overall trends can be observed. It has been shown that experience with one trial can play a role in subsequent trials (Fuchs & Burghardt, 1971). Therefore, I used the McNemar Test of Significant Changes for categorical data to test for whether individuals changed behaviors within a prey category across trials 1 and 2 (Sokal & Rohlf, 1995). If trials 1 & 2 did not differ, I presented Chi-squared results for trial 1. If there were significant differences between trials 1 & 2, I present the Chi-squared results for both trials. For species in which certain behaviors were observed 80% or more, the McNemar test was not used. The 80% cut-off was chosen because when a particular behavior was observed 80% of the time, more than 2/3 of the individuals were observed performing the behavior. Therefore, although there may have been some variation, it was slight.

This experiment was designed to examine the effects of prey size and status on the various phases of the feeding cycle. Individuals were subject to only two trials across the four prey categories, therefore individual variation could not be examined. If one or more individuals appear to consistently exhibit differences from the majority in their response to the different prey categories, I discuss the variations observed.

Categorical data (capture position, restraint method, loop formation and swallowing position) were coded before analyses and I used the Pearson’s Chi-squared test to examine the effects of the four prey categories (LA, SA, LD, and SD) on these behaviors. I used the Kruskal-Wallis test to examine continuous variables (prey restraint time, swallowing time, total feeding time) because these data were demonstrably non-normal and the groups I compared had unequal sample sizes. The means for continuous data were ranked. Non-parametric Tukey-type multiple comparisons were used to determine significant differences between samples.

I used SPSS version 12.0 (2003) to perform descriptive statistics and nonparametric tests. All tests are two-tailed. A Monte Carlo significance level was used to give a precise estimate since small sample sizes were used in this study. Significance levels were set at $P < 0.05$. Marginal significance refers to $0.05 < P < 0.08$.

RESULTS

From July 2002 – September 2004, I recorded and analyzed 96 feeding trials for *Loxocemus bicolor* (N = 12), 16 trials for *Charina triviragata* (N = 2), 16 trials for *Charina bottae* (N = 2), 48 trials for *Eryx muelleri* (N = 6), and 40 trials for *Boa constrictor* (N = 5). I examined the effects of prey category on specific feeding behaviors for the three lineages of snakes. The following results are organized by dependent variables. Dependent variables are presented in the order in which they would appear in the predatory cycle.

Prey capture

Capture behavior did not change significantly between trials 1 and 2 in any of the four prey categories (SA: $G_{adj} = 1.18$, df = 1, $P > 0.10$; SD: $G_{adj} = 1.31$, df = 1, $P > 0.10$; LA: $G_{adj} = 0.89$, df = 1, $P > 0.10$; LD: $G_{adj} = 1.46$, df = 1, $P > 0.10$). A Pearson's Chi-squared test revealed a difference in capture position between the four prey categories for all 27 snakes ($X^2_{0.05, 3} = 25.142$, $P < 0.001$). Within prey categories there were significant differences in capture position. With the exception of small live prey, the majority of prey were captured by the anterior (Table A-2). The part of the prey item's body initially captured by *L. bicolor* was affected by prey category while the capture responses for *B. constrictor* and Erycines were not (Table A-3). Individuals of *L. bicolor* mostly captured SA and LA prey by the posterior while SD and LD prey were captured by the anterior. The two boid lineages mostly captured prey by the anterior irrespective of size or status.

Prey restraint method

Out of the four possible prey restraint methods observed during pilot observations, three prey restraint behaviors were recorded during this experiment: simple-seizing (SS), coil (C) and loop (L). Two behavioral states were possible for the C and L restraint methods: delayed coil (DC), and delayed loop (DL). However, DC and DL occurred in fifteen or less trials out of the 216 trials. Due to these low frequencies I collapsed DC with C and DL with L for the following analyses. Snakes did not significantly change their prey restraint behavior between trials 1 and 2 across prey categories (SD: G_{adj} = 0.065, df = 1, P >0.10; LA: G_{adj} = 0.152, df = 1, P > 0.10;LD: G_{adj} = 1.46, df = 1, P >0.10: G_{adj} = 0.28, df = 1, P > 0.10).

The three lineages differed in their distribution of prey restraint behaviors ($X^2_{0.05, 6}$ = 18.273, P < 0.006). Prey size and status affected the prey restraint responses observed for individuals of *L. bicolor* and Erycine snakes but these prey attributes did not seem to affect the prey restraint behavior for individuals of *Boa constrictor* (Fig. A-5). Individuals of *L. bicolor* exhibited significantly differed prey restraint behaviors across prey categories ($X^2_{0.05, 6}$ = 39.79, P < 0.001). *Loxocemus bicolor* mostly constricted SA and LA prey by looping or coiling. SD prey were simply-seized or looped while the behaviors SS, C/DC, or L/DL were used to restrain LD prey. Individuals of *B. constrictor* did not exhibit significantly differed prey restraint behaviors across prey categories ($X^2_{0.05, 6} = 3.07$, $P < 0.380$). *Boa constrictor*, on the other hand, consistently coiled around prey irrespective of prey size and status. Only once was an individual *B. constrictor* observed simply- seizing prey. In this particular trial, the SD prey offered to the snake was just 40% of the snake's head width and did not exceed the length of the snake's jaw. Erycine snakes exhibited significantly differed prey restraint behaviors across prey categories ($X^2_{0.05, 6} = 24.204$, $P < 0.001$). Erycines mostly coiled SA and LA prey while SD and LD prey were either simply-seized or coiled. The behaviors L/DL were also observed for SD and LD prey.

Loop orientation during prey restraint

Snakes did not significantly change loop orientation when performing the prey restraint behaviors L/DL and C/DC between trials 1 and 2 for all four prey categories (SA: $G_{adj} = 0.02$, df = 1, $P > 0.10$; SD: $G_{adj} = 0.82$, df = 1, $P > 0.10$; LA: $G_{adj} = 1.41$, df = 1, $P > 0.10$; LD: $G_{adj} = 1.63$, df = 1, $P > 0.10$).

Significant differences in loop orientation were observed across species ($X^2_{0.05, 2} = 32.75$, $P < 0.001$) (Fig. A-6). Individuals of *L. bicolor* used mostly horizontal loops while coiling or looping around SA, LA and LD prey. Prey in the SD category were coiled using a combination of horizontal and vertical loops. Individuals of *B. constrictor* only wound horizontal loops around SD, LA, and LD prey. In the SA prey category, individuals of *B. constrictor* either wound horizontal (50%) or vertical (50%) loops around prey during coiling. Erycine snakes mostly applied horizontal loops around SA, LA, and LD prey. In trials with SD prey, Erycines mostly applied a mix of both horizontal and vertical coils (60%).

Loop application pattern

Three different loop application patterns were observed: lateral, ventral, and a combination of ventral and lateral bending. In 100% of the trials during which C/DC and L/DL behaviors were used to restrain prey, *L. bicolor* and Erycine snakes used lateral bends to loop around prey. Individuals of *B. constrictor* used ventral bends and ventral-lateral bends when applying loops around SA, LA and LD prey (Fig. A-7). In all trials with SD prey, individuals of *B. constrictor* applied loops using the ventral part of their body.

Condition of prey prior to ingestion in live trials

Prey items were dead prior to ingestion for almost all live prey trials across species and prey categories (99.5%). In all SA and LA trials, *L. bicolor* and *B. constrictor* successfully immobilized and killed live prey prior to ingestion. Only in 1 out of the 40 Erycine feeding trials with live prey was prey still alive prior to swallowing. During this trial *Charina bottae* was the predator and the prey was in the SA category with an IR of 42%. This prey item was small but was not active at the beginning of the trial. The *C. bottae* simply seized and consumed the live prey posterior first.

Prey swallowing position

There were no significant differences observed in swallowing position between snake lineages ($X^2_{0.05,\,2} = 2.98$, $P < 0.001$). In 98% of feeding trials, all snakes consumed prey, irrespective of category, head first.

Mean prey restraint time

Mean prey restraint times demonstrated a non-normal distribution. The deviation from normality resulted from trials in which snakes immediately swallowed prey after seizing.

In these specific cases, prey restraint times were zero. Therefore, only prey restraint times for the behaviors C/DC and L/DL were used in the following analysis. The three lineages differed significantly in mean prey restraint times (SA: $H_{0.05, 2} = 14.23$, $P < 0.001$; SD: $H_{0.05, 2} = 12.16$, $P < 0.002$; LA: $H_{0.05, 2}$,= 11.82, $P < 0.003$; LD $H_{0.05, 2}$,= 11.84, $P < 0.003$). Mean prey restraint times for individuals of *B. constrictor* were significantly longer across prey categories compared to *L. bicolor* and Erycine snakes (Fig. A- 8) (Table A-4: A). Mean prey restraint times did not differ across prey categories for *Boa constrictor* ($H_{0.05, 3} = 3.8$, $P = 0.284$) whereas mean restraint times significantly differed across prey categories for *L. bicolor* ($H_{0.05, 3} = 20.19$, $P < 0.001$) and Erycine snakes ($H_{0.05, 3} = 20.15$, $P < 0.001$).

Presence of a loop while swallowing

Prey restraint method did not affect whether snakes looped around prey while swallowing (*L. bicolor*: $X^2_{0.05, 2} = 3.556$, $P = 0.169$; *B. constrictor*: $X^2_{0.05, 2} = 1.3888$, $P = 0.425$; Erycine snakes: $X^2_{0.05, 2} = 0.081$, $P = 0.624$). Individuals of *L. bicolor* looped around prey to facilitate swallowing during 98% of the feeding trials. Looping while swallowing occurred in 91% of the trials in which constriction or a single loop was not used to immobilize prey. Boas used loops during swallowing in 30% of their trials with LA and LD prey but in none of the trials with SA and SD prey. In trials in which a loop was used during swallowing, loops were mostly maintained from the prey restraint phase. Occasionally, (15%) loops were wound around prey after constriction. Erycine snakes used loops during swallowing in all four prey categories. Loops were generally applied around SD and LD prey items. With SA and LA prey, Erycines maintained their coil from the constriction posture and often changed looping behavior along the body of the prey during swallowing.

Mean swallowing / intraoral transport time

The three lineages differed significantly in swallowing times in the SD ($H_{0.05, 2} = 10.36$, $P = 0.006$), LA ($H_{0.05, 2} = 12.84$, $P = 0.002$), and LD prey categories ($H_{0.05, 2}$,= 8.68, $P = 0.013$). Swallowing times significantly differed across prey categories for all three snake lineages (*L. bicolor*: $H_{0.05, 3} = 26.7$, $P < 0.001$; *B. constrictor*: $H_{0.05, 3} = 10.15$, $P = 0.017$; Erycine snakes: $H_{0.05, 3} = 22.68$, $P < 0.001$) (Figure A-9) (Table A-4:B).

Mean total feeding time

In addition to prey restraint and swallowing times, total feeding time included other behaviors for which durations were recorded. The time it took for some snakes to unwind around prey before swallowing and the time some snakes took to locate the anterior portion of the prey were included in the total feeding time. Total feeding times marginally differed across prey categories for *Boa constrictor* ($H_{0.05, 3} = 7.96$, $P = 0.06$) whereas total feeding times were significantly different across prey categories for *L. bicolor* ($H_{0.05, 3} = 25.90$, $P < 0.001$) and Erycine snakes ($H_{0.05, 3} = 18.36$, $P < 0.001$) (Figure A-10) (Table A-4:C).

DISCUSSION

The effects of prey size and status

This study suggests that prey size and status affects the predatory cycle for basal alethinophidian and basal macrostomate snakes. Variations in seven out of the ten characters examined were associated with at least one of the prey categories during feeding for *L. bicolor* and the Erycines (Table A-5). Individuals of *B. constrictor* revealed little change in feeding behavior with respect to prey size and status. This stimulus control experiment supports the earlier claims that *B. constrictor* is highly stereotyped in feeding behavior irrespective of prey characteristics (Willard, 1977; Greene, 1977). Broader evolutionary comparisons are discussed below.

Prey restraint behavior and loop application pattern were of specific interest in this study. My hypothesis, based on the phylogenetic relationship of *L. bicolor* to *B. constrictor* and Erycine snakes, was rejected. Contrary to my predictions, this study demonstrated that prey category affected the prey restraint behaviors for *L. bicolor* and Erycine snakes and that coiling (C) was not the predominant restraint behavior in all prey categories. Two handling behaviors other than C were observed: SS and L. Individuals of *L. bicolor* and Erycine snakes restrained active prey using mostly coiling but also looping behavior. Dead prey, irrespective of size, were restrained using any of the three restraint methods: SS, C, and L. For the most part, *B. constrictor* only restrained prey via C. Thus, the semi-fossorial snakes in this study were able to recognize when constriction or other behaviors may be appropriate to restrain prey. The observation that individuals of *L. bicolor* and Erycines exhibited different prey restraint behaviors with respect to prey size and status has important evolutionary implications.

Based on these data, the ability to vary prey restraint method appears to be a basal character for snakes. This finding is supported by feeding observations for *Xenopeltis unicolor*, another semi-fossorial basal taxon which is the sister lineage to *L. bicolor* (Cundall & Greene, 2000). During feeding observations of four adult *X. unicolor* I observed looping and coiling behavior with prey of varying size (Mehta, unpubl. data).

Not only does variable prey restraint behavior seem to be the basal condition, but these data suggest that variable prey restraint is especially important for semi-fossorial species and relatively small basal macrostomates. More than one prey restraint behavior in the behavioral feeding repertoire for basal and intermediate semi-fossorial species as well as many derived colubroids may enable these snakes to take on a wider size range of prey.

My second hypothesis, based on preliminary observations of feeding behavior, predicted that loop application pattern will differ across the three lineages examined. The results of this study support my hypothesis. Individuals of *L. bicolor* and Erycine snakes applied loops laterally around prey whereas *B. constrictor* applied loops by ventral bending and ventral-lateral bending around prey.

In this study, semi-fossorial species, varied their prey restraint behaviors with respect to prey category and used lateral bending to apply loops around prey. In a recent study (Rudolph et al., 2002), gopher snakes (*Pituophis ruthveni*) pinioned gophers (*Geomys breviceps*) in burrow systems but constricted prey during open situations (eg. laboratory arena). Gopher snakes (*Pituophis melanoleucus*) use lateral bends when applying loops around prey (Moon, 2000). Another semi-fossorial Old World Erycine snake, *Calabaria reinhardtii,* was observed applying lateral loops around prey and has also has been observed using the prey restraint behaviors loop and pinion (pers. obs.). These studies and observations support the idea that lateral bending is associated with variable prey restraint behaviors which seem to be particularly useful for snakes inhabiting or hunting in subterranean or leaf litter environments.

Lateral bending also appears to allow flexibility in which portion of the body can be used to restrain prey: anterior or posterior. Individuals of *L. bicolor* mostly used the anterior portion of their body to loop around prey but were also observed using the posterior portion of their body (Fig. A-11). The ability to apply loops laterally with either the anterior or posterior portion of the body may be adaptive. By releasing a portion of the body from engaging in prey restraint with a single prey, some snakes may be able to subdue a second or even third prey item with unoccupied parts of the trunk. Earlier accounts of snake feeding behavior support this idea (Hopley, 1882). Alternatively, looping around prey using the posterior portion of the body allows the snake to still be vigilant while restraining prey (Mehta, 2001, 2003). Increased vigilance may be especially important for hatchlings or neonates since snakes predating upon prey are susceptible to their own predators. Ventral bending or ventral-lateral bending does not seem to allow for the same behavioral flexibility as lateral bending. On the other hand, ventral benders are usually much larger snakes and don’t seem to have the same predatory pressures as smaller snake species.

Prey restraint times varied greatly between the different taxa studied and further reflect variations in prey restraint behavior in response to prey categories. *Loxocemus bicolor* and Erycine snakes took longest to restrain SA and LA prey. When controlling for relative prey diameter across species, overall mean restraint times for *Boa constrictor* were significantly longer and these mean prey restraint times did not differ across prey categories. This suggests that regardless of prey size and status, prey restraint durations for *B. constrictor* are relatively constant, supporting the idea that boas do not greatly vary prey restraint behavior. A recent study examining the aerobic metabolism of *B. constrictor amarali* during constriction revealed that neither time spent during constriction or metabolic rate was correlated with prey size (Cajani, et al., 2003).

SA prey seemed easier for *L. bicolor* to handle compared to Erycines, because individuals were longer and were able to use more of their body to initially position the prey before applying loops or a coil. During SA feeding trials, subadult Erycines often took longer to form a stable constriction posture and during four trials, SA prey which were very active, escaped while snakes were trying to position their loops. On the other hand, once a stable constriction posture was maintained, Erycines spent less time coiling around prey compared to *L. bicolor*.

In only one trial was prey swallowed alive posterior first. This was a trial with SA prey in which a *Charina bottae* was the predator. The prey, 1-2 days old, had an anterior and posterior that was similar in size and was relatively inactive. The *C. bottae* may have misjudged the SA prey for dead. The prey was short in length and ½ of the prey was already in the snake's mouth before any prey activity was observed. Nestling mammals comprise a significant portion of the diet for adult *C. bottae* and the relative increase in the incidence of posterior-first ingestion reflects predation on relatively small prey items relative to snake head size (Rodriguez-Robles & Leal, 1993; Rodriguez-Robles et al., 1999).

I expected, large prey, irrespective of status, took longer to swallow than small prey for both *L. bicolor* and Erycine species. Although relative prey size was controlled across these three snake lineages, individuals of *Boa constrictor* still exhibited the longest times to swallow prey across all categories compared to *L. bicolor* and Erycine snakes. The fact that individuals of *Boa constrictor* took a longer time to swallow prey is interesting. Perhaps because boas are large in size, they may have less predators compared to these semi-fossorial species (i.e *L. bicolor* and the Erycines) and thus there may not be strong selection for boas to swallow prey items rapidly. Selection pressures, however, change throughout ontogeny. Future studies examining the ontogeny of swallowing behavior while controlling for prey size are necessary. Intraoral transport times may also be longer for *B. constrictor* because the swallowing phase of feeding proceeds a very energetically taxing phase, prey restraint. As prey size increases, ingestion time increases, although metabolic effort remains constant (Cajani, et al., 2003).

Prey manipulation in snakes

Greene (1977) mentioned that the swallowing phase is sometimes accompanied by the application of “post-constriction” loops that help reduce the diameter of the prey prior to swallowing. Gopher snakes and king snakes pull on prey with their jaws while prey is still in their loops or coil as if straightening or slightly stretching prey in preparation for swallowing (Moon, 2000). Young rat snakes (*Elaphe obsolete*) and boas (*Boa constrictor*) sometimes hold prey within their coils as they attempt ingestion (Milostan, 1989). As far as I am aware, these are the only references of post prey restraint looping behavior as most studies document how loops are applied during the prey restraint phase (Ditmars, 1914; Greenwald, 1978; Willard, 1977; Greene & Burghardt, 1978; de Queiroz, 1984; Gregory et al., 1980; Shine & Schwaner, 1985; Mori, 1991, 1993, 1994; de Queiroz & Groen, 2001; Mehta, 2003).

In this study, I observed post constriction loops in many feeding trials. In half of the trials with *B. constrictor* a vertical or horizontal loop was maintained around prey while swallowing. For *L. bicolor* and Erycine snakes, loops were present during swallowing even during trials when SS was employed. In trials in which the snake’s body was not used to restrain prey, loops were applied either during the beginning of the swallowing phase or shortly thereafter.

The lack of data explaining the function of looping while swallowing may be due to the challenge of measuring prey during this feeding phase. During preliminary observations with *L. bicolor* I took measurements of prey that snakes were in the process of swallowing in order to test the hypothesis that post constriction loops reduced prey diameter (Greene, 1977). Two problems arose while taking measurements: 1) my disturbance confounded swallowing times and 2) snakes would drop or regurgitate their 1/2 swallowed prey item which impacted future feedings.

From this study and my previous observations on feeding behavior (Mehta, 2003), I provide some evidence that may help assess the function of post constriction loops during swallowing. Firstly, although constriction during prey restraint is energetically expensive (Canjani et al., 2003), my data reveals that snakes take much longer to swallow prey than to restrain prey. Secondly, in many feeding trials (*L. bicolor*: 92 %; *Boa constrictor*: 58%; Erycine: 93%) loops were applied during swallowing, irrespective of prey restraint behavior. Semi-fossorial species, *L. bicolor* and Erycine snakes, exhibited post constriction loops for small and large prey. Individuals of *Boa constrictor* maintained loops around the majority of large prey items (90%). Therefore, the frequency at which snakes were applying loops may be correlated with the degree of difficulty snakes may have had swallowing prey.

Often, snakes were observed using a part of the loop, the substratum, or the side of the arena to anchor prey items while pulling their body over prey. Although these observations took place under more or less unnatural conditions, the natural environment is complex and multidimensional. If snakes, in an open arena, use the substratum or the side of the arena to assist in intraoral transport, chances are that they probably use objects in their natural environment to aid in swallowing. Unfortunately, detailed observations of snakes consuming prey in the wild are relatively rare.

There may be three functional consequences of looping or coiling around prey while swallowing: 1) immobilization (if the prey is still alive), 2) reducing diameter of the prey so it is easier to swallow, and 3) anchoring prey so it does not slide while swallowing. Could looping while swallowing have been a protoadaptation for the evolution of constriction or is looping around prey while swallowing derived from constriction?

Early alethinophidians consuming high WR but low IR prey, would have needed a way to stabilized prey while pulling their musculature over prey. Without a way to stabilize prey, snakes may have spent a long time in the swallowing phase as prey would have slid while the snake pushed. I did not find any significant differences in swallowing time between trials in which small prey were looped and those in which small prey were swallowed without loops for *L. bicolor* ($P>0.05$). This lack of significance may be due to the low frequency of trials in which prey were not looped while swallowing ($N = 8$). Currently, constriction is recognized as serving two functions: immobilization and subduing prey. For some snakes, constriction may serve a third function: anchoring the prey while swallowing. In this study, snakes that did not employ constriction as a prey restraint technique applied loops around prey while swallowing (*L. bicolor*: 82%; Erycines: 95%).

Evolutionary implications

The ability to vary prey restraint behavior and laterally bend around prey appears to be the basal condition for snakes based on data for *L. bicolor*. Early semi-fossorial snakes, that began shifting to prey with higher WRs but low IRs probably pinioned or looped prey by bending laterally. How far back variable prey restraint behavior may have evolved is unknown as little behavioral data has been gathered for Group III snakes (Fig. A-1). Based on these data, the first snakes able to consume high WR and IR prey, probably exhibited more than one prey restraint method and used the side of their body to immobilize prey. Thus, variable prey restraint behavior and lateral bending may be two important character states in snake evolution that enabled semi-fossorial species to gradually shift their feeding biology from consuming small prey frequently to larger prey less frequently. *Xenopeltis unicolor* and *Loxocemus bicolor*, the two most extant lineages capable of subduing relatively large prey slightly exceeding their own head width, vary prey restraint behaviors and bend laterally around prey. Erycines, basal macrostomates, vary prey restraint behavior and laterally bend around prey, further emphasizing the importance of variable restraint behavior and lateral bending for early semi-fossorial snake species. The ability to vary prey restraint behaviors either re-evolved or was retained by Erycines and suggests that ecological pressures have played an important role in shaping the prey restraint phase of feeding.

Large macrostomate snakes, such as boas and pythons, have one prey restraint behavior which was frequently used and over time, became highly stereotyped. This stereotypy may have been more advantageous for capturing and constricting very large prey and may be associated with a more stable environment. The colubroid prey restraint pattern appears to exhibit the most variability. The fact that colubroid snakes, the group to which 90% of all snake species belong, vary prey restraint behavior suggests that variable prey restraint behavior has played a significant role in snake evolution.

ACKNOWLEDGEMENTS

This research was approved by the Institutional Animal Care and Use Committee, University of Tennessee, Knoxville. I am indebted to Gordon M. Burghardt, Marguerite Butler, Todd Freeberg, Neil Greenberg, and Richard Saudargas for helpful suggestions on experimental design and for helpful comments on this chapter. I thank Carey Springer for statistical consultation. I thank Tracy and David Barker of Vida Preciosa International (VPI) and Brad Moon for providing the animals used in this study. Karen Davis, Cassandra Fenner, Lauren Kirby, Brooke Patrick and Samuel Riggan on numerous occasions provided help with animal care or helped digitize video. This study was funded in part by the American Psychological Association (APA), the Department of Psychology at the University of Tennessee, Knoxville (UT) and the U.T Scholarly Activities Research Incentive Fund (SARIF). Presentations of parts of this study to the Society of Integrative and Comparative Biology (SICB) and the Society of Animal Behavior (ABS) were supported by the Department of Psychology at UT and the U.T Graduate Student Association.

LITERATURE CITED

Barlow, G. 1977. Modal action patterns. In: How animals communicate.(T.A. Sebeok, Ed.).University of Indiana Press, Bloomington. Pp 94-125.

Burghardt, G.M. 1973. Instinct and innate behavior: toward an ethological psychology. In: The study of behavior. (J.A. Nevin, ed.). Scott, Forseman and Co., Glenview, Illinois.

Canjani,C., D.V. Andrade, A.P. Cruz-Neto, and A.S. Abe. 2003. Aerobic metabolism during predation by a boid snake. Comp. Biochemistry and Physiology Part A.133: 487-498.

Cundall,D., V. Wallach, and D.A. Rossman. 1993.The systematic relationships of the Snake genus *Anomochilus.* Zoological Journal of the Linnean Society 109: 275-299.

Cundall, D., and A. Deufel. 1999. Striking patterns in booid snakes. Copeia 1999: 868-883.

Cundall, D. and H. W. Greene. 2000. Feeding in snakes. In: Feeding: Form, Function, and Evolution in Tetrapod Vertebrates. (K. Schwenk ed.), Academic Press, Sand Diego. Pp. 293-333

De Queiroz, A. 1984. Effects of prey type on the prey-handling behavior of the Bull Snake, *Pituophis melanoleucus*. Journal of Herpetology 18: 333-336.

De Queiroz and R. R.Groen. 2001. The inconsistent and inefficient constricting behavior of Colorado western terrestrial garter snakes, *Thamnophis elegans*. Journal of Herpetology 35: 450-460.

Ditmars, R.L.1914. The feeding habits of Serpents. Zoologica. 1: 197-238.

Frazzetta, T.H. 1966. Studies on the morphology and functions of the skull in the Boidae (Serpentes). 2. Morphology and function of the jaw apparatus in *Python sebae* and *Python molurus*. Journal of Morpholology 118: 217-296.

Frazzetta, T.H. 1970. Studies on the fossil snake *Dinilysia patagonica* Woodward. II. Jaw machinery in the earliest snakes. Forma et function 3: 205-221.

Fuchs, J.L.and G.M. Burghardt. 1971. Effects of early feeding experience in responses of garter snakes to food chemicals. Learning and Motivation. 2: 271-279.

Greene, H.W. 1977. Phylogeny, convergence, and snake behavior. Unpublished

P.h.D dissertation, University of Tennessee, Knoxville, Tennessee.

Greene, H.W. 1983a. Dietary correlates of the origin and radiation of snakes. Amer. Zool. 23: 431-441.

Greene, H.W.1983b. *Boa constrictor* (Boa, Bequer, Boa Constrictor). In: Cost Rican Natural History. (D.H. Janzen, ed.), University of Chicago Press, Chicago. Pp. 380-382.

Greene, H.W. 1997. The Evolution of Mystery in Nature. University of California Press.

Greene, H. W. and G. M. Burghardt 1978. Behavior and Phylogeny: Constriction in Ancient and Modern snakes. Science,v. 200: 74-76.

Greenwald, O.E. 1978. Kinematics and time relations of prey capture by gopher snakes. Copeia. 1978: 263-268.

Gregory, P.T., J. M. Macartney, and D.H. Rivard. 1980. Small mammal predation and prey-handling behavior by the Garter snake, *Thamnophis elegans.* Herpetologica, 36: 87-93.

Kardong, K. V. 1998. Rattlesnake strike behavior: kinematics. Journal of Experimental Biology 201: 837-850.

Kluge, A.G. 1991. Boine phylogeny and research cycles. Miscellaneous Publications of the Museum of Zoology, University of Michigan 178: 1-58.

Kluge, A.G. 1993. *Calabaria* and the phylogeny of erycine snakes. Zoological Journal of the Linnean Society 107: 293-351

McDowell, S.B. 1975. A catalogue of snakes of New Guinea and the Solomons, with special reference to those in the Bernice P. Bishop Museum, III. Anilioidea and Pythoninae. Journal of Herpetology 9: 1-79.

Milostan, M. 1989. The comparative ontogeny of prey capture and immobilization in *Boa constrictor* and *Elaphe obsolete*, with comments on the origin and evolution of constriction in boid and colubrid snakes. Unpublished M.S. thesis, Central Michigan University, Mt. Pleasant.

Mehta, R.S. 2001. Prey-handling behavior in newly hatched trinket snakes, *Elaphe helena.* Unpublished Masters Thesis, University of Texas, Tyler, Texas.

Mehta, R.S. 2003. The effects of prey-size on the prey-handling behavior of hatchling *Elaphe helena*. Herpetologica 4: 471-476.

Moon, B.R. 2000. The mechanics and muscular control of constriction in gopher snakes (*Pituophis melanoleucus*) and a king snake (*Lampropeltis getula*). Journal of Zoology, London 252: 83-98.

Moon, B.R and C. Gans.1998. Kinematics, muscular activity and propulsion in gopher snakes. The Journal of Experimental Biology 201: 2669-2684.

Mori, A. 1991. Effects of prey size and type on prey-handling behavior in *Elaphe quadrivirgata.* Journal of Herpetology 25: 160-166.

Mori, A.1993a. Does feeding experience with different size of prey influence the subsequent prey-handling behavior in *Elaphe climacophora* ? Ethology 11: 153-156.

Mori, A.1993b. Prey handling behavior of neonatal rat snakes, *Elaphe taeniura* and *E.dione* (Colubridae). Japanese Journal of Herpetology 15**:** 59-63.

Mori, A.1994. Prey-handling behavior of newly hatched snakes in two species of the genus *Elaphe* with comparison to adult behavior. Ethology 97: 198-214.

Mori, A.1995. Prey handling behavior of the young rat snake *Elaphe taeniura* (Squamata:Reptilia). Memoirs of the Faculty of Science. Kyoto Univ. Serial Biology 16: 43-47.

Rieppel, O. 1978. A functional and phylogenetic interpretation of the skull of the Erycinae (Reptilia, Serpentes). Journal of Zoology, London 186: 185-208.

Rieppel, O. 1988. A review of the origin of snakes. In: Evolutionary Biology, Vol. 22. (M. K. Hecht, B. Wallace, and G.T. Pance, eds.). Plenum, New York. Pp. 37-130.

Rodriguez-Robles, J. and Leal, M. 1993. Effects of prey type on the feeding behavior of *Alsophis portoricensis* (Serpentes: Colubridae). Journal of Herpetology 27: 163-168.

Rodriguez-Robles, J., C.J. Bell, and H.W. Greene. 1999. Gape size and evolution of diet in snakes: feeding ecology of erycine boas. Journal of Zoology (London) 248: 49-58.

Rudolph, D.C., S.J. Burgdorf, R.N. Conner, C.S.Collins, D.Saenez, R.R.Schaefer, T. Trees, M. Duran, M. Ealy, G.Himes. 2002. Prey handling and diet of Louisiana pine snakes (Pituophis ruthveni) and black pine snakes (P. melanoleucus lodingi), with comparisons to other selected colubrid snakes. Herpetological Natural History 9: 57-62.

Savitsky, A.H. 1980. The role of venom delivery strategies in snake evolution. Evolution 34: 1194-1204.

Shine, R and Schwaner, T. 1985. Prey constriction by venomous snakes: a review, and new data on Australian species. Copeia 1985: 1067-1071.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry. W.H. Freeman and Co., San Francisco,CA.

Stebbins, R.C. 1985. A field guide to the Western reptiles and amphibians. Boston: Houghton and Mifflin Co.

Willard, D.E. 1977. Constricting methods of snakes. Copeia 1977: 379-382.

APPENDIX

Table A-1 Measurements for 27 individual snakes used in this study. Measurements were taken at the start of my study. Abbreviations: SVL = length of snake from rostrum to cloaca, TL = Tail length of snake measured from cloaca to tail tip, WT = weight of snake and HW = head width of snake.

Species	**SVL (cm)**	**TL (cm)**	**WT (g)**	**HW (cm)**
***Loxocemus bicolor* (N = 12)**				
Mean	66.79	10.37	400.62	1.51
SE	114.00	3.98	112.74	0.02
***Boa constrictor* (N = 5)**				
Mean	1475	17.04	1792.79	2.78
SE	3670	0.93	60.31	0.12
***Erycines* (N = 10)**				
***Charina bottae* (N = 2)**				
Mean	426.83	60	30.72	8.92
SE	9.83	5.014	1.26	0.41
***Charina triviragata* (N = 2)**				
Mean	557.5	87.5	114.23	9.89
SE	44.33	1.77	13.41	1.017
***Eryx muelleri* (N = 6)**				
Mean	180	33.6	102.61	9.71
SE	13.52	2.698	6.32	0.73

Table A-2 The results of Chi-square analysis for capture behavior within each prey category across all snakes (Table A-1). Chi-square tests were performed on frequency data. Abbreviations: SA = small alive, SD = small dead, LA = large alive, LD = large dead.

	Prey Categories				Expected Value
	SA[a]	SD[b]	LA[c]	LD[d]	
Posterior	44.0%	24.0%	35.0%	11.0%	42.2%
Anterior	44.0%	76.0%	59.0%	83.0%	44.2%
Middle	12.0%	.0%	6.0%	6.0%	8.3%

a. Chi-square = 12.23, df =2, P < 0.05

b. Chi-square = 14.52, df = 1, P < 0.001

c. Chi-square = 23.44, df = 2, P < 0.001

d. Chi-square = 61.44, df = 2, P < 0.001

Table A-3 The percentage of prey captured by the anterior, posterior, and middle across the four prey categories for the three snake lineages examined. Each animal was tested twice in all four conditions. Chi-square tests reveal significant differences in capture position across prey categories only for *L. bicolor*. Tests were performed on frequency data. Abbreviations: SA = small alive, SD = small dead, LA = large alive, LD = large dead.

		Prey Categories				Expected Value
Species		SA	SD	LA	LD	
Loxocemus bicolor[a] (N = 12)	Posterior	79.2%	33.3%	62.5%	16.7%	47.9%
	Anterior	8.3%	66.7%	33.3%	75.0%	45.8%
	Middle	12.5%	.0%	4.2%	8.3%	6.3%
Boa constrictor[b] (N = 5)	Posterior	.0%	10.0%	20.0%	10.0%	10.0%
	Anterior	90.0%	90.0%	80.0%	80.0%	85.0%
	Middle	10.0%	.0%	.0%	10.0%	5.0%
Erycine Snakes[c] (N = 10)	Posterior	25.0%	20.0%	10.0%	5.0%	15.0%
	Anterior	65.0%	80.0%	80.0%	95.0%	80.0%
	Middle	10.0%	.0%	10.0%	.0%	5.0%

a. Chi-square = 30.16, df = 6, $P < 0.001$

b. Chi-square = 4.12, df = 6, $P = 0.661$

c. Chi-square = 8.46, df = 6, $P = 0.206$

Table A-4 Post hoc non-parametric Tukey-type multiple comparisons between three different lineages following Kruskall-Wallis analyses for prey restraint time (A), swallowing time (B), and total feeding time (C) within each prey category. Pairwise differences between rank sums were tabulated for each of the dependent variables.

A.

Prey Category	**Species Comparisons**	**P -values**
SA	*L. bicolor* x *B. constrictor*	$P < 0.001$*
	B. constrictor x Erycine Snakes	$P < 0.001$*
	L. bicolor x Erycine Snakes	$P > 0.05$
SD	*L. bicolor* x *B. constrictor*	$P < 0.001$*
	B. constrictor x Erycine Snakes	$P < 0.001$*
	L. bicolor x Erycine Snakes	$P > 0.05$
LA	*L. bicolor* x *B. constrictor*	$P < 0.001$*
	B. constrictor x Erycine Snakes	$P < 0.001$*
	L. bicolor x Erycine Snakes	$P > 0.05$
LD	*L. bicolor* x *B. constrictor*	$P < 0.001$*
	B. constrictor x Erycine Snakes	$P < 0.001$*
	L. bicolor x Erycine Snakes	$P > 0.05$

B.

Prey Category	**Species Comparisons**	**P –values**
SA	*L. bicolor* x *B. constrictor*	$P > 0.05$
	B. constrictor x Erycine Snakes	$P > 0.05$
	L. bicolor x Erycine Snakes	$P > 0.05$
SD	*L. bicolor* x *B. constrictor*	$P < 0.001$*
	B. constrictor x Erycine Snakes	$P < 0.001$*
	L. bicolor x Erycine Snakes	$P > 0.05$
LA	*L. bicolor* x *B. constrictor*	$P < 0.001$*
	B. constrictor x Erycine Snakes	$P < 0.001$*
	L. bicolor x Erycine Snakes	$P > 0.05$
LD	*L. bicolor* x *B. constrictor*	$P < 0.001$*
	B. constrictor x Erycine Snakes	$P < 0.001$*
	L. bicolor x Erycine Snakes	$P > 0.05$

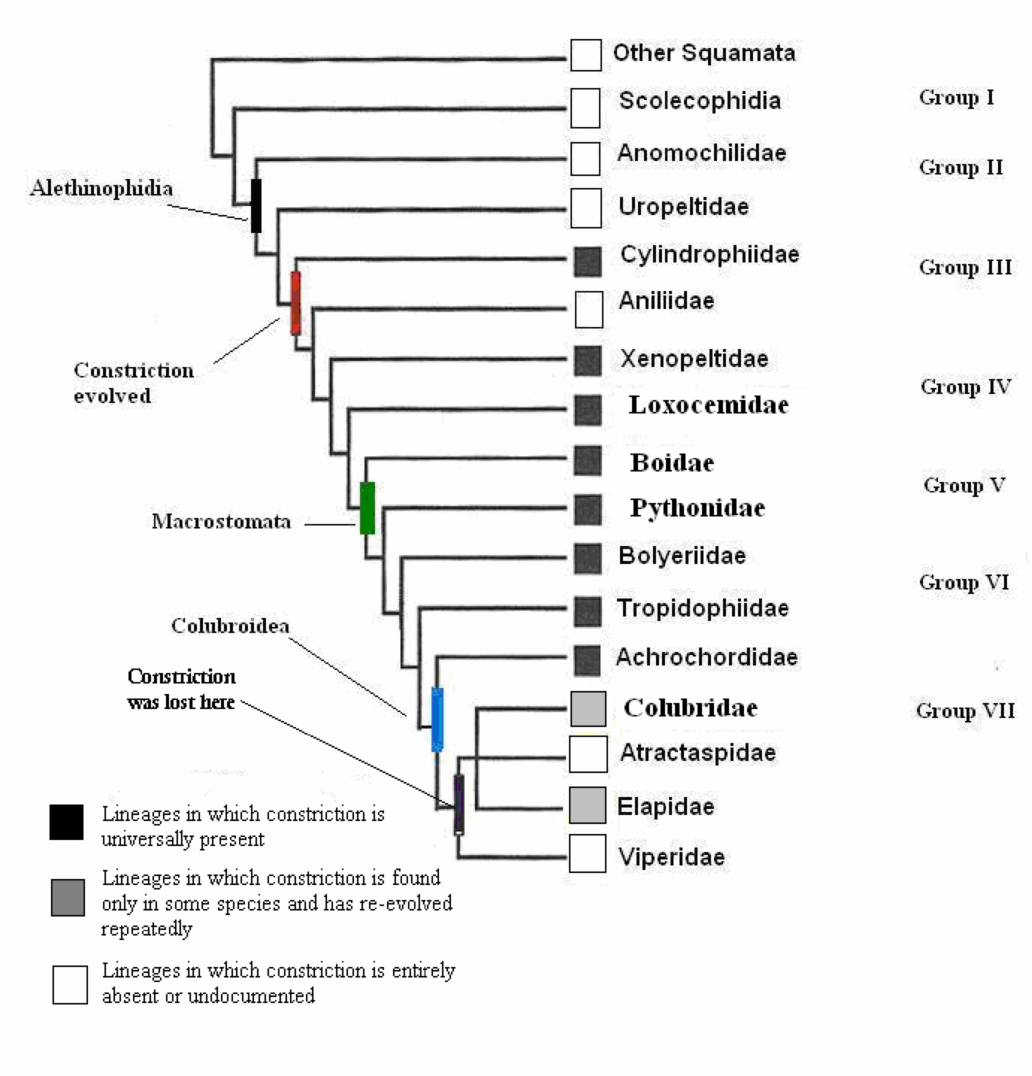
C.

Prey Category	**Species Comparisons**	**P –values**
SA	*L. bicolor* x *B. constrictor*	$P < 0.05$*
	B. constrictor x Erycine Snakes	$P < 0.05$*
	L. bicolor x Erycine Snakes	$P > 0.05$
SD	*L. bicolor* x *B. constrictor*	$P < 0.05$*
	B. constrictor x Erycine Snakes	$P < 0.05$*
	L. bicolor x Erycine Snakes	$P < 0.05$*
LA	*L. bicolor* x *B. constrictor*	$P < 0.05$*
	B. constrictor x Erycine Snakes	$P < 0.05$*
	L. bicolor x Erycine Snakes	$P < 0.05$*
LD	*L. bicolor* x *B. constrictor*	$P <0.05$*
	B. constrictor x Erycine Snakes	$P > 0.05$
	L. bicolor x Erycine Snakes	$P < 0.05$*

Table A-5 The effects of prey characteristics (size and status) on various aspects of the predatory cycle for *Loxocemus bicolor* (N = 12, 96 feedings), *Boa constrictor* (N= 5, 40 feedings), and Erycine snakes (N = 10, 80 feedings). (*) Changes in Behavior, (-) No detectable changes in behavior.

	Snake Species Examined		
Prey Category	***Loxocemus bicolor***	***Boa constrictor***	**Erycine snakes**
Prey Size			
Capture Position	-	-	-
Prey Restraint Method	-	-	-
Loop Orientation	-	-	*
Condition Before Ingestion	-	-	-
Restraint Time	*	-	*
Swallowing Position	-	-	-
Loop Present	-	-	*
Swallowing Time	*	-	*
Prey Status			
Capture Position	*	-	-
Prey Restraint Method	*	* (small sample size)	-
Loop Orientation	*	-	-
Condition Before Ingestion	-	-	-
Restraint Time	*	-	*
Swallowing Position	-	-	-
Loop Present	-	-	-
Swallowing Time	*	-	*

Figure A-1 Phylogeny of the Serpentes compiled from morphological (Cundall et al., 1993; Kluge, 1991, 1993; Rieppel, 1978, 1988) and molecular data (Cadle, 1994; Cadle et al., 1990). *Loxocemus bicolor* (Loxocemidae) is the sister taxon to boas and pythons. The lineages Boinae (*Boa constrictor*) and Erycinae (*Charina bottae*, *C. triviragata*, and *Eryx muelleri*) are in the Boidae. Families in bold (Loxocemidae, Boidae, Pythonidae, and Colubridae) are discussed in this study. Groups I –VII refer to shifts in feeding behavior during snake evolution (Cundall & Greene, 2000). Groups I-V reveal the transition from feeding often on lots of small prey items (Scolecophidia: blind snakes) to feeding on prey items that comprise at least 50% of a snake's own body mass (Boidae & Pythonidae). Group VI snakes feed on both relatively small and large prey. Group VII (Colubroidea) reflects many feeding shifts from piscivorous snakes to snakes that feed on prey that can exceed their own body mass by as much as 50% (Viperidae) and snakes that approach lizards in feeding biology (some lineages in the Colubridae).


Other Squamata
Scolecophidia
Group I
Anomochilidae
Group II
Alethinophidia
Uropeltidae
Cylindrophiidae
Group III
Aniliidae
Constriction evolved
Xenopeltidae
Loxocemidae
Group IV
Boidae
Group V
Macrostomata
Pythonidae
Bolyeriidae
Group VI
Tropidophiidae
Colubroidea
Achrochordidae
Constriction was lost here
Colubridae
Group VII
Atractaspidae
Elapidae
Viperidae
Lineages in which constriction is universally present
Lineages in which constriction is found only in some species and has re-evolved repeatedly
Lineages in which constriction is entirely absent or undocumented

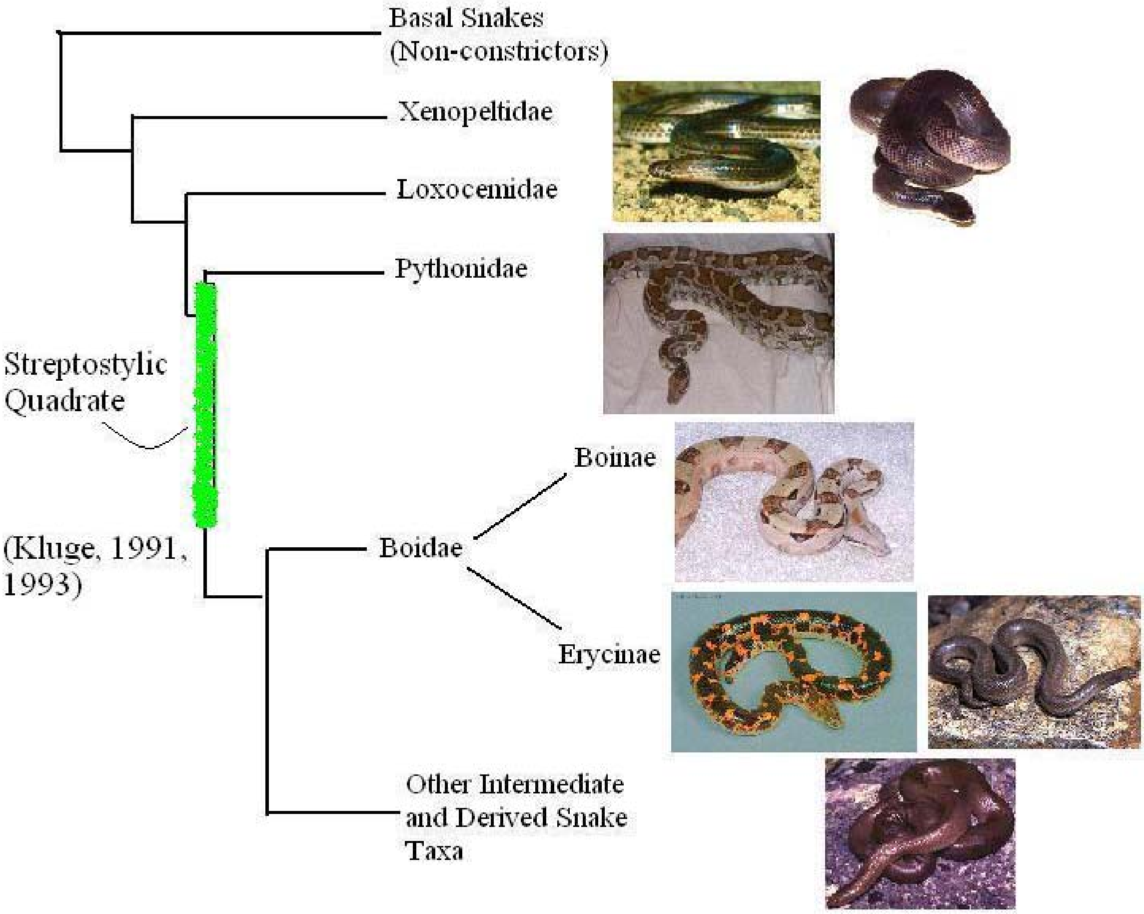


Figure A-2 Snake taxa of interest in this study and their phylogenetic relationship.

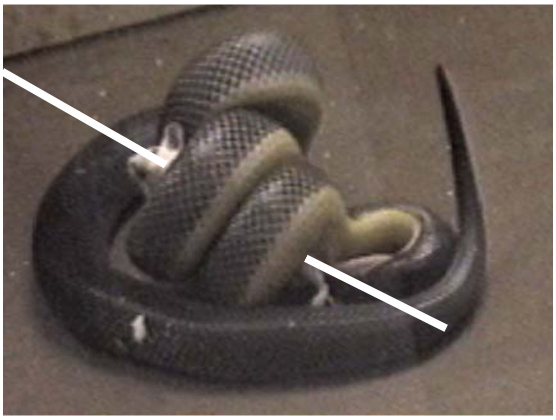
A. Constriction

B. Looping

C. Pinion

Figure A-3 Three out of the four different prey restraint behaviors (simple seizing (SS), constriction (C), loop (L), and pinion (P)) exhibited during pilot observations and stimulus control experiments. Adult *L. bicolor* constricts large live prey (1.A), loops (1.B), and pinions (1.C) small live prey.

A.



B.

Figure A-4 These photos exhibit the two loop orientation patterns observed during constriction behavior. In 4A, horizontal loops are applied around prey while in 4B, vertical loops are wound around prey during constriction. The loop orientation patterns correspond to the relationship between the substrate and an imaginary axis running through the loops of the snake.

Figure A-5 The percentage of trials in which prey from each of the four prey categories were restrained using SS, C/DC, L/DL for the three lineages examined. A: *Loxocemus bicolor*, B: *Boa constrictor* and C: Erycine snakes. Abbreviations for restraint behaviors are: SS = simple seizing, C = coil, DC = delayed constriction, L = loop, and DL = delayed loop. Abbreviations for prey categories are: SA = small live; SD = small dead, LA = large alive, and LD = large dead.

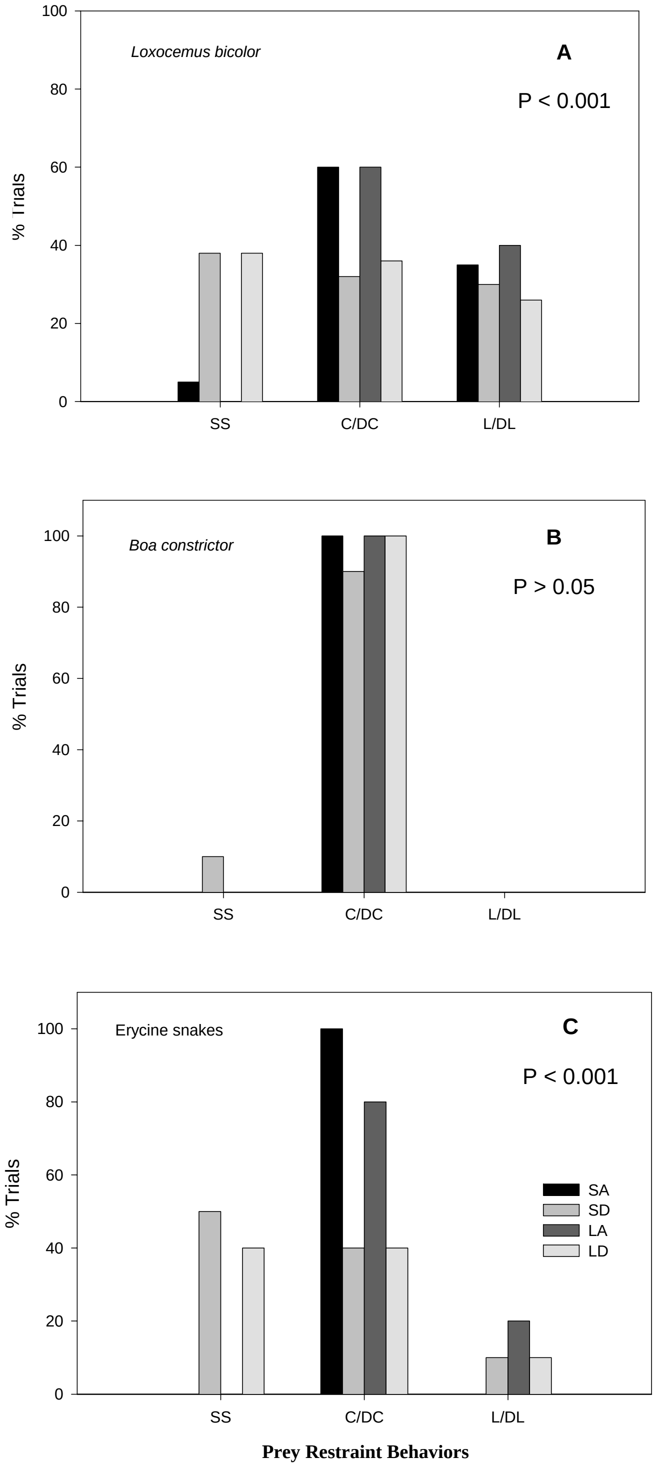

Loxocemus bicolor
A
P < 0.001
% Trials
100
80
60
40
20
0
SS
C/DC
L/DL
Boa constrictor
B
P > 0.05
% Trials
100
80
60
40
20
0
SS
C/DC
L/DL
Erycine snakes
C
P < 0.001
% Trials
100
80
60
40
20
0
SA
SD
LA
LD
SS
C/DC
L/DL
Prey Restraint Behaviors

Figure A-6 The percentage of trials in which prey from each of the four prey categories were restrained using either horizontal, vertical, or mixed loops when performing the behaviors C/DC and L/DL. A: *Loxocemus bicolor*, B: *Boa constrictor* and C: Erycine snakes. Abbreviations for prey categories are: SA = small live; SD = small dead, LA = large live, and LD = large dead.

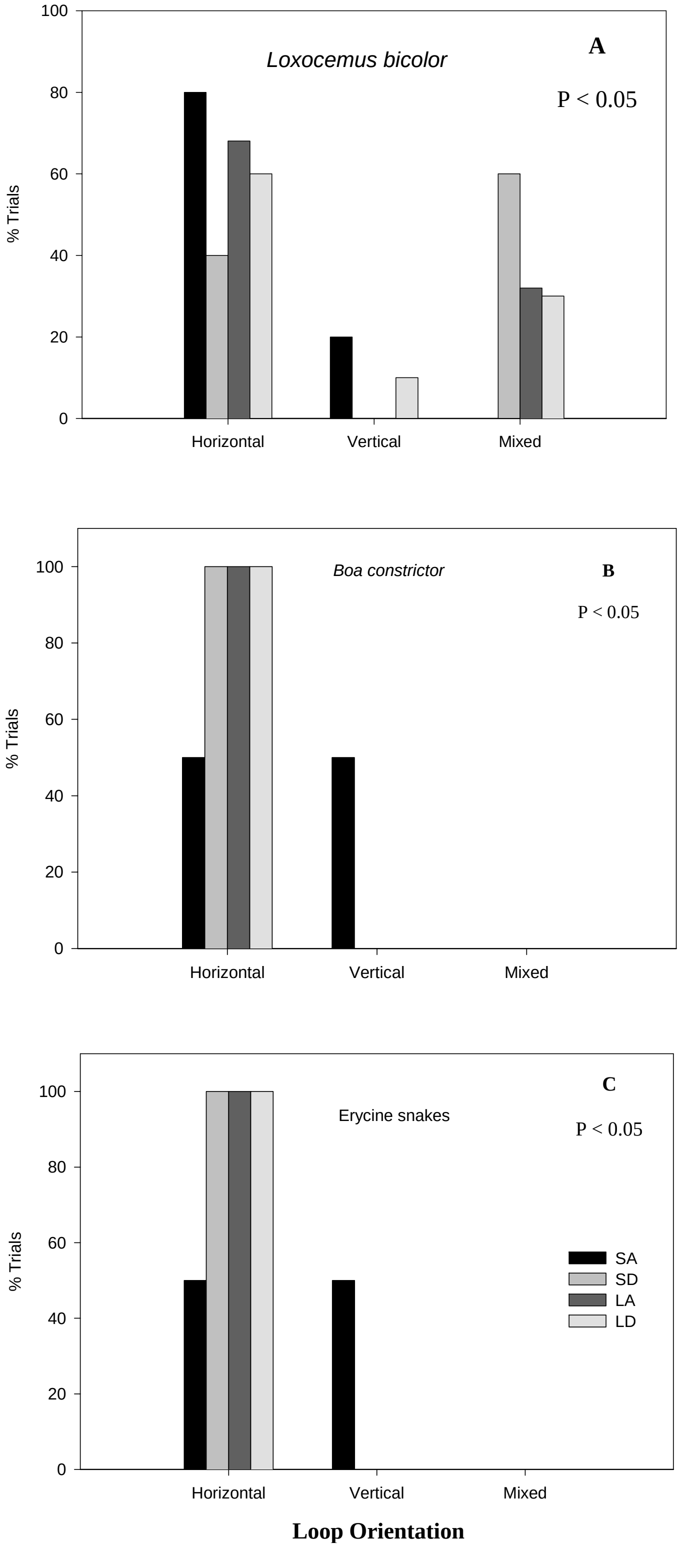
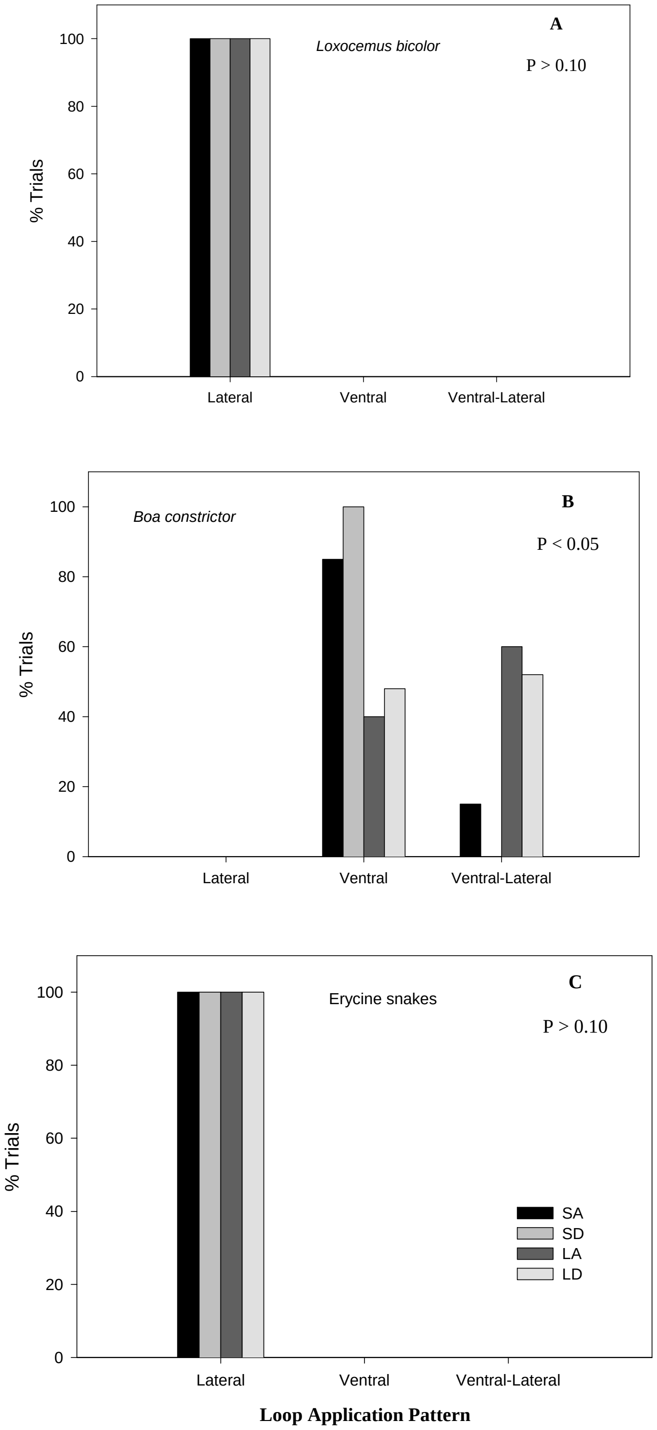
A
Loxocemus bicolor
P < 0.05
% Trials
0
20
40
60
80
100
Horizontal
Vertical
Mixed
B
Boa constrictor
P < 0.05
% Trials
0
20
40
60
80
100
Horizontal
Vertical
Mixed
C
Erycine snakes
P < 0.05
% Trials
0
20
40
60
80
100
SA
SD
LA
LD
Horizontal
Vertical
Mixed
Loop Orientation

Figure A-7 The percentage of trials in which lateral, ventral, and ventral-lateral loop application patterns was observed in each of the four prey categories for the three lineages examined. A: *Loxocemus bicolor*, B: *Boa constrictor* and C: Erycine snakes. Abbreviations for prey categories are: SA = small live; SD = small dead, LA = large live, and LD = large dead.


A
Loxocemus bicolor
P > 0.10
% Trials
0
20
40
60
80
100
Lateral
Ventral
Ventral-Lateral
B
Boa constrictor
P < 0.05
% Trials
0
20
40
60
80
100
Lateral
Ventral
Ventral-Lateral
C
Erycine snakes
P > 0.10
% Trials
0
20
40
60
80
100
SA
SD
LA
LD
Lateral
Ventral
Ventral-Lateral
Loop Application Pattern

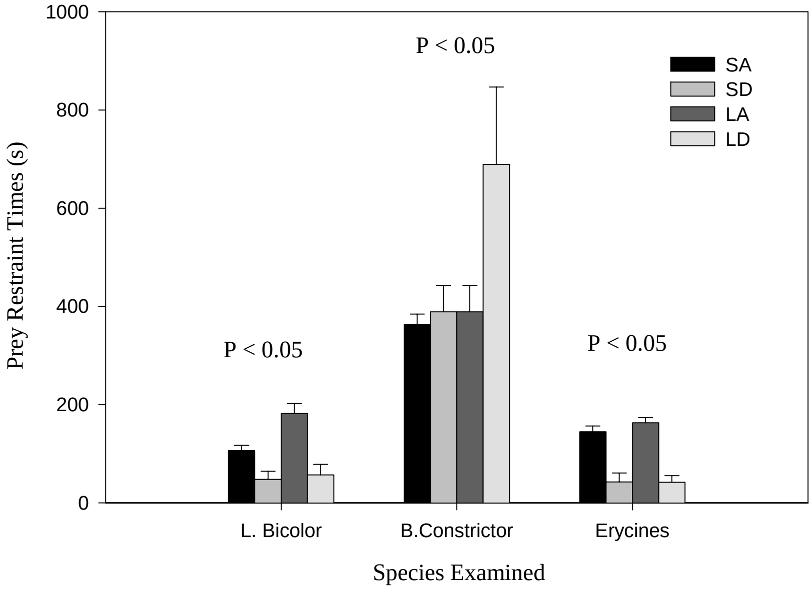


Figure A-8 Mean prey restraint times across prey categories for each of the three lineages examined. P-values indicate significant differences across prey categories within species.

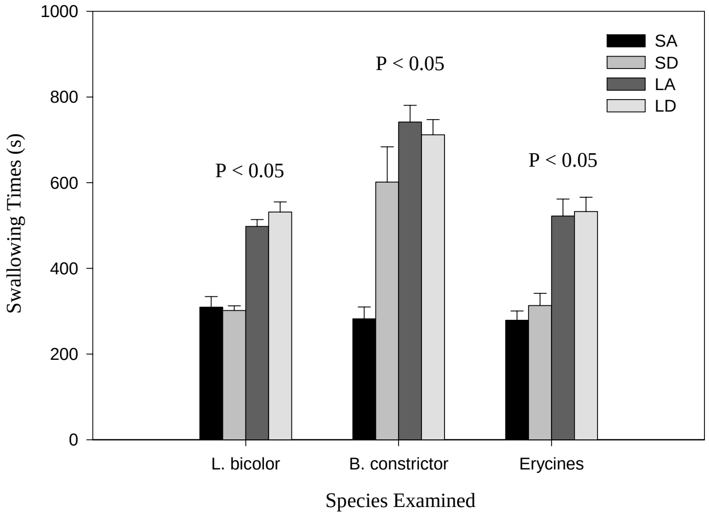


Figure A-9 Mean swallowing times across prey categories for each of the three lineages examined. P-values indicate significant differences across prey categories within species.

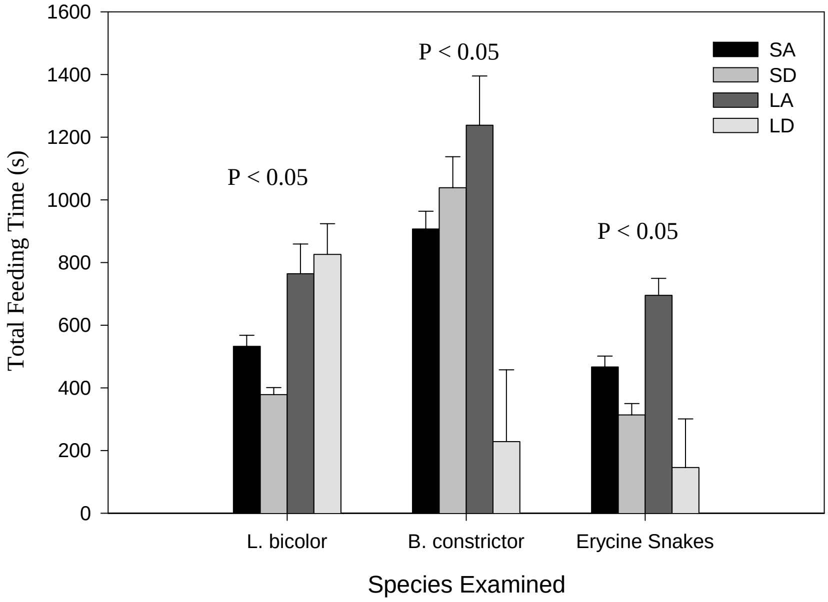


Figure A-10. Mean total feeding times across prey categories for each of the three lineages examined. P-values indicate significant differences across prey categories within species.

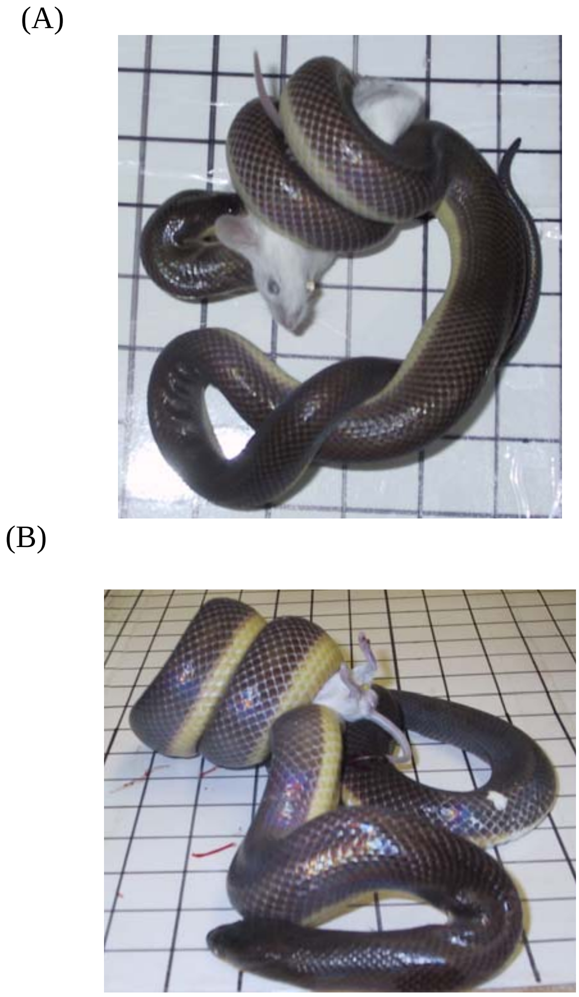


Figure A-11 Coiling behaviors observed for *Loxocemus bicolor*. Coiling with the anterior portion of the body (A) and coiling with the posterior portion of the body (B).

PART 3

The Kinematics and Epaxial Muscle Activity Patterns During Constriction in the Neotropical Sunbeam Snake (*Loxocemus bicolor)*

ABSTRACT

Constriction, a prey restraint method, is an important key innovation associated with the adaptive radiation of snakes and is homologous for the majority of snake taxa. Although studies have documented the variability of constriction patterns among snakes, how the underlying physiological mechanisms of constriction correspond to variation observed at the behavioral level remains poorly studied. I describe the kinematics and epaxial muscle activity patterns of constriction in a basal snake, *Loxocemus bicolor*. I also quantify pressure exerted on small mammalian prey during constriction. Loops wound or wrapped around prey during a constriction coil were formed using the lateral portion of the snake's trunk. Lateral bending around live and dead prey corresponded with unilateral epaxial activity. Constriction postures, the duration of epaxial muscle activity and pressure exertion were variable in feeding trials with both live and dead mice (*Mus musculus)* comprising 8-18.3% of the snake's body mass. Although snakes maintained constriction postures for several minutes, epaxial muscle activity and force exerted on prey were intermittent. Epaxial muscles, specifically the LD and IL, were highly active during initial coil formation and in response to prey movements. Pressure exerted on prey ranged from 6-54 kPa. Epaxial muscle activity patterns of *L. bicolor* appear similar to derived snake species suggesting that epaxial muscle activity patterns may be homologous across the diverse lineages of constricting snakes.

INTRODUCTION

Tracing the transformation of character traits and determining the mechanisms responsible for present day phenotypes are current challenges for those interested in evolutionary patterns. As organismal design is hierarchical in nature, it is critical to not only recognize that distinct levels of biological design exist, but to understand how these levels may be causally interrelated (Striedter & Northcutt, 1991; Lauder, 1994). For example, it is recognized that complex characters such as behavioral traits are dependent upon diverse anatomical and physiological traits such as the musculoskeletal system, sensory systems, and the brain. However, to understand evolutionary change as well as the conservation of specific behavioral phenotypes, an examination of the behavioral character along with underlying anatomical and physiological mechanisms is necessary. A phylogenetic concept of homology provides us with the opportunity to trace evolutionary change at various levels of organization and to examine how these levels relate to one another (Patterson, 1982; Striedter & Northcutt, 1991; Lauder, 1986, 1994). Behavioral traits, subject to selection, are correlated with specific functional units or character complexes, which, in turn, are susceptible to their own evolutionary pressures and constraints (Schwenk and Wagner, 2001).

Constriction is an ancient behavioral homology that is shared across the majority of snake taxa, and is associated with the adaptive radiation of snakes (Greene & Burghardt, 1978; Greene, 1983, 1994). During constriction, a snake restrains prey by looping around it, thus limiting the ability of the prey to escape or retaliate against the predator (Cundall & Greene, 2000). Looping during prey restraint is a highly coordinated behavior pattern which requires small-radius bends of the axial skeleton. Similar to other types of bending in vertebrates, the bends in the axial skeleton during constriction are produced by the axial musculature.

The axial musculature is divided into the epaxial (dorsal) and hypaxial (lateral and ventral) muscles. The epaxial muscles produce the broad lateral bends observed during locomotion in fishes (Williams et al., 1989), amphibians (Frolich and Biewener, 1992) and snakes (Jayne, 1988 a, b; Moon and Gans, 1998). In snakes, the epaxial musculature includes the (muscles) Mm. spinalis-semispinalis (SP-SSP), longissiums dorsi (LD), and iliocostalis (IL). Collectively, the epaxials consist of overlapping segments that are bound by connective tissue into longitudinal columns along the body (Gans, 1962). These three muscles (SP-SSP, LD, IL) are interconnected and form a chain of muscle-tendon segments. Specifically, the large superficial epaxial muscles are thought to support the exertion of large forces during axial bending (Gasc, 1981) and have been hypothesized to produce large bends in the snake's trunk during locomotion and constriction (Mosauer, 1932 a, b; Ruben, 1977). A recent electromyography study confirmed that the epaxial muscles are highly active during striking and coil formation and intermittently active during sustained constriction (Moon, 2000).

Although constriction is homologous for the majority of snake taxa, the behavior pattern was lost and then re-evolved multiple times independently in derived snake lineages, collectively termed the colubroidea (Greene, 1994; Fig A-1)[1]. Unlike intermediate snake taxa (i.e, boas and pythons), constriction behaviors observed in colubroid lineages vary through ontogeny (Greene, 1977; Milostan, 1989; Mehta, 2001) as well as in response to prey characteristics (type: Mori, 1991, 1993; size: Mori, 1991, 1993; Mehta, 2003; and activity level: de Queiroz, 1984). At least three different prey restraint postures that directly exert pressure on prey have been described for colubroid snakes (Greene, 1977; Greenwald, 1978; Mori, 1994; de Queiroz & Groen, 2001; Mehta, 2003).

---

[1] All figures and tables are located in the Appendix.

Two hypotheses for the differences in prey restraint behaviors between intermediate and derived snake taxa have been suggested. Ruben (1977) documented that the epaxial muscles for derived taxa span more vertebrae and are not able to produce the small radius bends necessary for the constriction postures observed in intermediate snakes. Three lines of evidence refute this hypothesis. Firstly, there are many derived snake lineages that exhibit constriction behavior and are capable of forming constriction postures similar to intermediate snake groups (Shine & Schwaner, 1985). Another comparative study documenting differences in epaxial musculature noted that epaxial muscles and tendon lengths did not differ between constrictors and highly derived non-constrictors (Jayne, 1982). Lastly, it has been shown that the epaxial muscles of gopher snakes, *Pituophis melanoleucus* and king snakes, *Lampropeltis getula*, two highly derived snake species, are intermittently active during constriction (Moon, 2000). Therefore, the underlying mechanisms driving behavioral variation in prey restraint behaviors for intermediate and derived snakes remains largely unknown.

Loop formation during constriction behavior also seems to vary between basal, intermediate and derived snake taxa, as indicated in Part 2. Boas and pythons appear to form loops around prey using the ventral portion of their body while derived constrictors seem to loop prey using the lateral portion of their trunk. Lateral bending, in which only one side of the body is pressed up against the prey, is associated with unilateral muscle activity patterns during constriction. Unilateral lateral epaxial muscle activity, alternating activity between the left-and right-side, is common during undulatory locomotion in fishes, amphibians and snakes, and is considered to be the ancestral pattern of locomotor control (Ritter, 1995). In snakes that use ventral bending to loop around prey, we would expect to observe bilateral muscle activity patterns. Bilateral epaxial activity, which is synchronous activation of both left and right muscles, is characteristic of birds and mammals and has been proposed to provide postural stability of the trunk (Gatesy and Dial, 1993; English, 1980). Therefore, two epaxial muscle activation patterns have been documented within diverse vertebrate clades but how these two patterns relate to a major behavioral innovation in snake feeding behavior has not been examined.

Ventral bending may be correlated with the high stereotypy documented for constriction in intermediate taxa while lateral bending may be associated with variable prey restraint postures. Whether lateral bending and variable prey restraint behaviors or ventral bending and high stereotypy during constriction is the derived or ancestral condition is unknown, as constriction kinematics has not been examined for basal snake groups.

For understanding the physiology underlying constriction behavior in snakes, only two species have been tested for underlying muscle activity patterns. However, two taxa and an outgroup taxon that represents the ancestral condition of the traits under investigation are minimal for making evolutionary inferences. At least one outgroup comparison is necessary for detecting which aspects of this key behavioral innovation are ancestral and which are derived. Sampling from basal and intermediate lineages is necessary for making inferences about the evolution and diversification of constriction postures and their corresponding motor patterns.

In addition to examining the effects of prey characteristics on constriction (Part 2), I examined the kinematics and epaxial muscle activity of constriction in the neotropical sunbeam snake, *Loxocemus bicolor*. *Loxocemus bicolor* can contribute important information from ethological and physiological perspectives because as the sister taxon to boas and pythons (Fig. A-1), it represents the oldest extant taxon capable of both constricting and ingesting endothermic prey that matches or slightly exceeds its own head width. *Loxocemus bicolor* represents an important outgroup to most of the other constricting snakes species, and by studying this basal snake I will be able to make sound evolutionary inferences about constriction, its epaxial muscle activity patterns, and its homology among the diverse lineages of snakes. To my best understanding, no experimental data to date have been gathered on *L. bicolor,* and only a few natural history accounts have been published on this species (Merchan & Mora, 2001; Mora, 1991, 1987).

In this paper, I documented the kinematics and epaxial muscle activity patterns during constriction subsequent to the strike and initial formation of a coil. Based on earlier observations of constriction behavior for this species in a laboratory setting, I hypothesize that *L. bicolor* will exhibit variable prey restraint behavior which will be reflected in changes in epaxial muscle activity patterns and pressure exerted on prey. I tested the following predictions: (1) *Loxocemus bicolor* bends laterally when forming a loop or coil around the body of the prey. (2) Epaxial muscles exhibit unilateral activity during lateral looping around prey. (3) Epaxial muscle activity and pressure exertion are intermittent during a sustained constriction event even though the coils during constriction are continuously maintained. (4) Epaxial muscle contraction contributes to pressure exertion during constriction. (5) During a constriction event, peak pressure exertion will be elicited in response to struggling movements by prey. I manipulated some prey movements during constriction in attempt to elicit maximal pressures; hence, the data allow comparisons of the effects of different kinds of prey movements on constriction pressures and duration.

MATERIALS AND METHODS

General Methods

Subjects and maintenance-I recorded the kinematics, epaxial muscle activity patterns and pressure exerted on prey during constriction for 5 adult neotropical sunbeam snakes (*Loxocemus bicolor*). All snakes were housed individually in plastic containers with water *ad libitum* and maintained on a 14L:10D cycle. Temperature ranged from 23-26º. Laboratory mice (*Mus musculus*) of various size ranges were offered to snakes weekly.

General experimental design-Snakes were fasted for 10 days before use in feeding trials. For all trials, I placed an individual snake in a 30 x 50 cm lidless glass terrarium. Electrode leads and pressure tubing exited through the top of the terrarium, allowing free movement of snakes that were attached to wire and tubing. I maintained the ambient and surface temperatures under the video spotlights at 23ºC over the course of the experiments.

In all experiments I offered mice to the snakes using 30 cm long forceps, and recordings were made of constriction prior to swallowing. Dead and live mice offered as prey ranged from 8–18.3 % of the snakes body mass (BM) and 32-41 % of the snakes head width (IR). Pre-killed mice, maintained at body temperatures of 38-40°C, were used to control for cues produced by the constricted prey. In roughly half of the feeding trials with dead prey, a small rubber bulb connected to a pressure transducer (described below) was attached to a mouse. To simulate prey movements, I tugged on mouse limbs protruding from the snake's coil with forceps once the snake had formed a stable coil during the beginning of constriction. Live mice were offered to snakes to elicit constriction in response to natural movements by the prey. To record constriction pressures in these trials, the rubber bulb was lightly taped to the mouse's fur.

To describe constriction postures for *L. bicolor,* I adopted three behavioral states from Greene (1977), Greene & Burghardt (1978), de Queiroz (1984), and Mori (1991):

Loop- winding one encircling loop around prey
Coil- winding more than one loop around the prey
Pinion- one or more non-encircling loops that push prey against some surface of the feeding arena or the prey can be wedged between non-encircling loops

During the initial constriction posture, two discrete movements were used to apply a loop or coil around the prey:

Winding-prey were turned about their long axis while loops were applied like a rope on a windlass (Shrewsbury, 1969)
Wrapping- consisted of one to several loops applied over, under, and around the stationary prey

Three states of loop application/ bending patterns were observed for each of these behaviors. The terminology used here is modified from Greene (1977) and follows Moon (2000):

Lateral-only one side of the body was used to press against prey
Ventral- the belly scales of the snake were pressed against the prey
Ventral- lateral- in the first loop the belly of the snake was pressed against the prey and in the 2nd loop the side of the snake was pressed against the prey.

Videography and electromyography-All feeding trials were videotaped (30 fps) using a standard Hi- 8mm Sharp video camera VL-E43U positioned approximately 1.5 m from the terrarium containing the snake. The terrarium was lined with a 2-cm grid for scale and contained a mirror angled at 45° from the horizontal to reflect a dorsal view into the video camera.

I used liquid paper to paint a horizontal mark on snakes at every 10 vertebrae. The number of vertebrae were determined using ventral scale counts (Alexander & Gans, 1966). At every 25 vertebrae, a mark was painted on the snake's midline. These markings served as landmarks on the video and enabled me to determine the number of vertebrae used in loops during constriction and average loop radius. For video data acquisition, Pinnacle Software and Peak Motus were used to digitize selected images to evaluate whether ventral or lateral bending occurred directly after the snake seized the prey. By slowing down the digital images to < 20 (fps), I could also determine whether there was a side bias during lateral bending. Peak Motus software uses the horizontal and vertical scales in the video field to correct for camera angle and adjust the image shape to acquire a 3-D position of the subject when making measurements. In order to measure vertebral bending angles, I used the vertebral midline of the animal as the starting point and measured the distance between adjacent ventral scales from the midline using Image J. I then divided the total degree of bending by the number of vertebrae used in the loop to get an estimate of bending angle per vertebral joint. Vertebral bending angles were calculated from 1-2 digitized frames for all 5 individuals.

To record epaxial muscle activity patterns, I implanted bipolar hook electrodes (Loeb & Gans, 1986) into the semispinalis portion (SSP) of the Mm. spinalis-semispinalis, the M. longissimus dorsi (LD), and the M. iliocostalis (IL) in all five individuals. I implanted the electrodes bilaterally in the epaxial muscles at vertebrae 50 and 80. This vertebral spacing enabled me to detect any bilateral differences in muscle activity and any longitudinal propagation of muscle activity. Table A-1 depicts snake size and electrode placements for individuals of *Loxocemus bicolor* from which epaxial muscle activity was recorded.

I made bipolar-hook electrodes out of polyimide-coated (H-ML) stainless-steel wire (316 LVM, California Wire Co.) with a bipole spacing of 1.0 mm and bare recordings tips of 0.5 mm. Because the animals were on loan, with some restrictions on use, from a private breeder, I was unable to implant electrodes surgically and to kill specimens after the experiments for determining electrode placement. Therefore, I dissected a preserved museum specimen and practiced inserting a needle into the target muscles. This practice allowed me to determine the proper sites and depths of electrode implantation for each muscle by counting scale rows from the dorsal midline and inserting the needle to depths that worked for the preserved specimen. Furthermore, the epaxial muscles of *Loxocemus* form clearly visible bulges along the body while contracting during handling, which allowed me to confirm the intended implantation sites on each individual prior to implantation.

To implant electrodes, I made short (1-mm) longitudinal incisions in the skin with a scalpel, and then inserted the electrodes using a 23-gauge hypodermic needle. The incisions were kept very shallow and were intended only to ease the needle insertion through the thick, keratinous outer scales. I used tiny droplets of cyanoacrylate surgical glue to seal each implantation site. I bundled together the electrode leads from each implantation site and fixed them to the skin with small patches of tape so that all leads could exit as one bundle in the middle of the two implantation sites. Once each electrode was implanted, a gentle pull on the leads produced a slight bulge under the scales that indicated that the electrode was in the correct position.

To remove the electrodes at the end of each experiment, I confirmed electrode placement as closely as possible using a microscope and visual inspection of electrode position and depth. Although it was possible that the electrode tips may have spanned more than one serial segment of each muscle, this was not problematic because the measurements were intended only to reveal gross timing of muscle activity throughout constriction; thus, potential errors of a few milliseconds were highly unlikely to bias my measurements of EMG bursts lasting several seconds or longer.

During the experiments, I recorded the EMG signals in real time at 10,000 Hz using a BIOPAC MP150 data acquisition system. I set the EMG 100C differential amplifiers to a gain of 1000 and a band pass of 100-5000 Hz. After data acquisition, I digitally filtered the data using a Finite Impulse Response (FIR) filter created using 200 filter coefficients and set to a band pass of 100-1000 Hz. Thus, the analog to digital sampling rate was 10 times higher than the highest frequencies analyzed, which gave accurate digital reproduction of the EMG signals without aliasing. I then analyzed the EMG signals using Acqknowledge (version 3.7.1) software.

Plethysmography-To measure constriction pressure, I used a Harvard Apparatus physiological blood pressure transducer connected to a small water-filled rubber bulb. The bulb was lightly taped to fur of live mice or implanted in the body cavity of dead mice. The pressure signals were digitized simultaneously with the EMG signals. I recorded constriction pressures from 5 snakes and 8 readings (Table 2).

During feeding trials, the transducer was connected to the BIOPAC150 EMG system. The transducer outputs were calibrated at 32-40kPa (4-300 mmHg) above atmospheric pressure, which encompassed the pressures recorded during the constriction events. Pressure signals were recorded and analyzed together with the EMG signals. I synchronized the video, EMG, and pressure recordings by simultaneously video taping a flashing LED (10 Hz) and recording the LED voltage spikes along with the EMG and pressure signals.

Analysis

I examined kinematic (N =17), electromyographic (N = 17), and pressure (N = 8) data for 17 constriction events from 5 neotropical sunbeam snakes (Table 2). From video data, I was able to describe how *L. bicolor* captured live and dead prey. I also measured the duration of three stages of prey restraint, including (1) the time from capture/contact to the initiation of coiling, (2) the time required to form a stable coil posture, and (3) the duration of coiling from the beginning of the stable coil posture to the visible loosening of the loops wound around the prey. I tested the effects of prey condition (live *vs* dead) on these components of prey restraint time using paired T-tests with significant values set at $P \leq 0.05$. I also recorded whether the snakes looped around the prey using ventral or lateral bending. If the snakes bent laterally, I recorded whether the right or left side of the body was used to contact the prey. Kinematic measurements were made from digitized video. Vertebral curvature was calculated while snakes formed constriction coils as well as average number of vertebrae used to form a loop.

From EMG data I measured mean duration (ms) of epaxial muscle activity, timing (ms) of epaxial muscle activity (the on-set/off-set of the left and right Ssp, LD and IL in relation to one another), mean intensity (mV) of each burst of activity, and rectified area. Although electrode construction and implantation were standardized as much as possible, comparison of signal amplitudes or rectified areas between muscles and between individuals is confounded by potential variation in electrode structure and EMG implant position.

From pressure data, I recorded the following variables: (1) maximum pressures (kPa) exerted on the prey at any point during constriction, (2) changes in pressure (kPa) during a constriction event, and (3) changes in pressure in relation to epaxial muscle activity.

RESULTS

Prey capture and coil formation

In a previous study, mice that were offered to snakes via forceps precluded the need for snakes to extend the anterior portions of their body to strike at prey (Moon, 2000). However, the snakes used in this study do not usually extend their bodies to capture prey from any major distances (pers. obs., RSM). Therefore, descriptions of the initial capture behavior are mentioned. Seizing prey involved the anterior neck region (before vertebrae 25), which did not contain electrodes. The head was elevated above the substratum (N = 17) before capture and snakes would make 2-3 open-mouth sweeps towards the prey. Immediately after seizing prey, the head of the snake would bend ventrally and then turn laterally.

Individual snakes used two strategies for capturing prey: 1) the snake would stop ~2 cm from the prey item and tongue-flick. After tongue-flicking, a snake would slowly move towards the prey and seize the prey with its jaws. 2) If the snake had already detected the prey item, then it would move quickly towards the prey, stopping abruptly once the rostrum touched the prey. Then, the snake would either quickly grasp the prey with its jaws or the snake would burrow under the prey using its rostrum to slowly lifting the prey up off the substratum to seize it. In 9 trials, snakes missed the prey on the first (N = 9) or second (N = 12) attempt but eventually captured prey.

Coil duration, from the beginning of the stable coil posture to visible loosening, lasted from 49- 830s for live prey and 130-568.17s for dead prey (Table A-3). Coil durations for dead prey were confounded by manipulations during a constriction event that were intended to both elicit maximal pressures and epaxial muscle activity. Prey restraint times did not differ when immobilizing dead and live prey (paired samples t – tests: capture-coil delay, $t_{16} = 1.48$, $P > 0.05$; coil formation time, $t_{16} = -0.53$, $P > 0.05$ and coil duration: $t_{16} = 0.14$, $P > 0.05$).

Kinematics

Upon capturing live mice, snakes formed one to three loops around prey (N = 17) (Fig A-2). Loop formation was relatively variable and on several occasions (N = 9), coils would overlap one another. Overlapping coils were often unstable and snakes changed restraint posture when coils overlapped in order to exert necessary pressures on the prey. Snakes immediately wound (N = 11) or wrapped (N = 6) prey and loops were applied by lateral bending (N = 17). During lateral bending, snakes either bent to the right or left side when applying loops. No individual snake revealed a side bias while bending (Table A-4). Initial tightening of coils was observed during coil formation and occurred by reducing coil diameter. This patterns was occasionally visible (N = 5) in digitized video. Due to the nature of their variable constriction patterns, individuals of *L. bicolor often* changed handling postures during a single feeding event. This made it difficult to obtain more than two still frames that revealed a clear dorsal and lateral view of constriction postures for each individual. The average number of vertebrae used in a coil ranged from 37-59 (Table A-4).

Muscle activity patterns

The dominant muscles active during constriction were the left and right LD and IL at v50 and v80. These are the most lateral muscles of the epaxial complex and are mechanically suited to produce sinusoidal waves along the body in lateral undulation as well as during constriction. The LD and IL muscles were active for longer durations compared to the Ssp. muscles at v50, and the LD and IL muscles also exhibited higher rectified areas (Table A-5).

Muscle activity pattern was unilateral which correlates with the lateral bending in constriction postures observed for this species.

Muscle activity and pressure exertion

Epaxial muscle activity and pressure exertion were pronounced during coil formation and in response to natural and simulated struggling movement by the prey. Muscle activity during coil formation was typically unilateral. Although snakes maintained coil postures for up to several minutes, epaxial muscles ceased activity after initial coil formation in all 17 trials. This muscle activity pattern indicates that epaxial muscles contract intermittently during constriction.

I observed gradual as well as abrupt changes in pressure throughout the 8 constriction events for which pressure data were acquired. Epaxial muscle contractions were associated with increases in pressure. However, pressure increases were not correlated with the rectified integrated area of EMG bursts during coil formation ($r = 0.20$, $P > 0.05$, $N = 8$, Fig A-4) and during prey struggles ($r = -0.13$, $P > 0.05$, $N = 8$; Fig A-5). When muscle activity ceased after coil formation or after responding to prey movements, moderate to high pressures were nonetheless sustained. The timing and magnitude of pressure exertion varied considerably within and among constriction events. Constriction pressures ranged from 6-54 kPa in live and dead mice.

DISCUSSION

Prey capture and coil formation – Loxocemus bicolor has a slow and relatively imprecise capture behavior. Individuals often missed prey more than once even though the prey item may have been roughly 2 cm from the snake’s rostrum. Once captured, the capture-coil phase was very quick for live prey ($x < 2.00$ s). The coil formation phase, which entailed looping around the prey until a stable coil was formed took much longer (live prey :$\bar{x} = 44.3 \pm 28.29$ s; dead prey: $\bar{x} = 32.50 \pm 14.77$ s). The length of time it took the snakes to form a stable coil may have been affected by a few variables such as 1) how or where the prey was originally seized, 2) whether the snakes wound or wrapped the prey, 3) how motivated the snake was to consume the prey, and 4) how much the mouse struggled once seized. Regardless, the more erratic the loops, the more time the snake had to spend readjusting the coil. Coil duration was the longest phase of prey restraint ($\bar{x} = 530.8 \pm 244$ s). None of the three prey restraint stages: capture-coil, coil formation and coil duration were significantly affected by prey status (live vs dead).

In an earlier study (Moon, 2000), coil duration phase for gopher snakes and king snakes was directly correlated with relative prey mass. Due to a limited number of animals, I chose to examine feeding behavior with a narrow size range of prey items in order to maximize the number of feeding trials per individual. Based on my behavioral observations in Part 2, I would expect coil duration to also increase with relative prey mass for *L. bicolor*.

Kinematics- Loxocemus bicolor applied coils to the prey item by either winding or wrapping movements as described by Greene (1977). In some trials overlapping coils were observed. Overlapping coils are usually not observed in boas and pythons, but have been observed in derived snake lineages (Willard, 1977; Greenwald, 1978; Mori, 1991; Mehta, 2003).

Snakes bent laterally when forming loops around prey items, which supported earlier findings in Part 2. Lateral bending was also observed in two derived snake taxa, gopher snakes and king snakes, during constriction with live and dead mice (Moon, 2000). Lateral bending is most probably the ancestral bending pattern in snakes and boas and pythons may exhibit a derived posture, although further kinematic studies for intermediate snake taxa are necessary.

The majority of prey restraint postures involve some vertebral curvature. The vertebral curvature for *L. bicolor* ranged from 5.8-8.6° which is relatively small compared to some of the bending angles reported in the literature (44°: Cundall, 1995; 33°: Moon, 2000). Degree of vertebral curvature appears to vary across different snake groups (Mosauer, 1932 a, b; Gasc, 1974, 1976; Jayne, 1988a; Cundall, 1995; Moon, 2000) and may be due to the morphological variation that has been documented along the vertebral column (Gasc, 1974, 1976; Moon, 1999).

Muscle activity patterns – Although prey restraint postures for *L. bicolor* were relatively variable, the epaxial muscle activity patterns during snake movement were consistent. The epaxial muscles revealed discrete phases of activity during a constriction event. Muscles activity was present during coil formation and then again in response to natural and artificial struggling movements of the prey.

I observed unilateral epaxial muscle activity during constriction in all feeding trials. Unilateral epaxial muscle activity corresponded to left and right lateral bending on the side of the snake that was directly pressed against the prey. This pattern was similar to epaxial muscle activity patterns observed for derived snake taxa (Moon, 2000). The unilateral muscle activity patterns for both *L. bicolor* and derived snake taxa, which also correspond to lateral bending suggests that lateral bending, unilateral muscle activity patterns and variable constriction behavior may be more indicative of ancestral prey restraint methods and supports the stimulus control studies I performed in Part 2. Examining intermediate taxa will help determine whether muscle activity patterns during constriction are conserved across snake taxa although variable prey restraint behavior and lateral bending are not.

Muscle activity and pressure exertion-Pressure changes were abrupt during the initial coil formation phase while gradual increases and decreases were observed throughout the feeding trial. Epaxial muscles appeared to be active when pressure increased during coil formation and in response to natural and artificial struggling movement of prey. However, pressure increases were not correlated with the rectified integrated area of EMG bursts during coil formation and during prey struggles. Closer examination of epaxial activity indicated that when muscle activity ceased moderate to high pressures were sustained. One interesting observation was that in trials in which reduction of coil diameters were observed, the epaxial muscles were active. This may imply that the epaxial muscles play a role in tightening the coils which may directly or indirectly affect pressure exertion. It was also apparent that snakes were not exerting maximum pressure on small prey. Artificial manipulations of the prey with forceps while the prey was in the snakes' coil did elicit higher pressures however these still may not reflect maximum pressure exertion against prey.

On the basis of my kinematic, electromyographic and pressure data, I can draw the following conclusions concerning the kinematics and epaxial muscle activity during constriction behavior in *Loxocemus bicolor*. First, the kinematics of prey restraint behavior for this basal taxon exhibited variability but not as much variability as observed in physiological (Moon, 2000) and behavioral studies (Greenwald, 1978; Mori, 1991, 1993) with derived snakes. The variation in constriction postures and the range of overall prey restraint times supports earlier observations of feeding behavior reported for *L. bicolor* (Part 2).

Loxocemus bicolor bends laterally when forming a loop or coil around the body of the prey and the underlying epaxial muscles exhibit unilateral activity during lateral bending. Thus lateral bending and epaxial muscle activity patterns for *L. bicolor* were similar to those of gopher snakes and king snake, two derived snake taxa studied by Moon (2000). These similarities suggest that the basal *L. bicolor* and the two highly derived snake species employ similar mechanisms of bending and epaxial muscle activity when handling small endothermic prey. Lateral bending appears to be associated with variable pretty restraint behavior. Variability in prey restraint behavior is thought to have functional significance and may be a way snakes minimize handling prey and decrease overall feeding duration.

Epaxial muscle activity and pressure exertion for *L. bicolor* are intermittent during a sustained constriction event even though the coils during constriction are continuously maintained. Three lines of evidence suggest that the epaxial muscle may not be the only muscle involved during constriction. Firstly, although the coil posture was maintained for several minutes, the epaxial muscles ceased firing directly after coil formation. Secondly, during two observations with two different individual snakes, loops were applied around dead prey by wrapping. Wrapping movements resulted in overlapping, unstable coils that snakes readjusted throughout the feeding trials. During these postural adjustments the epaxials were not active. Thirdly, although epaxial muscles were active during slight increases in pressure, epaxials ceased activity while pressures were still sustained.

This is the first physiological study documenting variable constriction behavior in a basal snake taxon and comparisons with derived taxa can be discussed. In derived snakes, prey restraint behavior will vary with respect to prey characteristics, particularly prey size and status (Milostan, 1989; Mehta, 2003). In this study, I tested the effects of prey status on prey restraint durations while keeping prey size relatively constant. Prey status may not have affected the duration of the three prey-handling stages because of the manipulations I performed on dead prey while in the snakes coils. Larger prey would probably influence any one of the three handling durations. Overall mouse size is probably more influential on constriction behavior since the snake's trunk is in direct contact with the surface of the mouse. Snakes are able to respond to movement cues by the prey once the prey is in the snakes' coil as exhibited by both epaxial muscle activity and increases in pressure exertion. Prey restraint method, specifically in species that exhibit both winding and wrapping motions around prey may be in response to struggles elicited by prey upon capture. Prey items in this study were relatively small in relation to the snakes. Therefore, snakes may have had a difficult time initially immobilizing prey and employed overlapping coils in order to properly hold prey and keep the prey in one position. This work contributes to an understanding of constriction at the physiological level and helps shed light on the underlying mechanisms driving variation at the behavioral level. Sampling additional taxa will help document the diversity of epaxial muscle activity patterns within a successful monophyletic group of obligate predators. Within the clade Serpentes, a more indepth examination of epaxial muscle activity and further examination of how motor patterns correlate with constriction postures will allow us to assess the homology of constriction behavior at the physiological level.

ACKNOWLEDGEMENTS

This research was approved by the Institutional Animal Care and Use Committee, University of Louisiana at Lafayette. I am indebted to Gordon Burghardt, Marguerite Butler, Todd Freeberg, Neil Greenberg, Brad Moon, and Richard Saudargas for helping to improve the manuscript. I thank Tracy Barker for allowing me to borrow animals. I am especially grateful for the following people who whose help enabled me to travel to Louisiana to conduct these experiments: Karen Davis, Cassandra Fenner, Lauren Kirby, Brad Moon, Brian Ott, Ali Rabatsky, and Kate Wadsworth. This study was funded in part by the American Psychological Association (APA), the Department of Psychology at the University of Tennessee, Knoxville (UT) and the U.T Scholarly Activities Research Incentive Fund (SARIF). Presentations of parts of this study to the Society of Integrative and Comparative Biology (SICB) and at the VII International Conference of Vertebrate Morphology (ICVM) were supported by the Department of Psychology at UT and the U.T Graduate Student Association.

LITERATURE CITED

Alexander, A. A. and Gans, C.1966. Then pattern of dermal-vertebral correlation in snakes and amphisbaenians. Zoological Mededelingen 41: 17-190.

Cadle, J.E. 1988. Phylogenetic relationships among advanced snakes: a molecular perspective. University of California Publications in Zoology 119: 1-70.

Cundall,D., V. Wallach, and D.A. Rossman. 1993.The systematic relationships of the Snake genus *Anomochilus*. Zoological Journal of Linnean Society 109: 275-299.

Cundall, D. and H. W. Greene. 2000. Feeding in snakes. In K. Schwenk (ed.), Feeding: Form, Function, and Evolution in Tetrapod Vertebrates., pp. 293-333. Academic Press, San Diego.

De Queiroz, A.1984. Effects of prey type on the prey-handling behavior of the Bull snake, *Pituophis melanoleucus*. Journal of Herpetology 18: 333-336.

De Queiroz, A. and R. R. Groen. 2001. The inconsistent and inefficient constricting behavior of Colorado western terrestrial garter snakes, *Thamnophis elegans*. Journal of Herpetology 35: 450-456.

English, A.W. 1980. The functions of the lumbar spine during stepping in a cat. Journal of Morphology 165: 55-66.

Frolich, L.M. and A.A. Biewener. 1992. Kinematic and electromyographic locomotion in the salamander *Ambystoma tigrinum*. Journal of Experimental Biology 1 62: 107-130.

Gans, C. 1962. Terrestrial locomotion without limbs. American Zoologist 2: 167-182.

Gasc, J.P. 1974. L'interprétation fonctionnelle de l'appareil musculo-squelettique de l'axe vertebral chez les Serpents (Reptilia). Mémoirs of the Museum of Natural History Nat., Paris, Ser. A 83: 1-82.

Gasc, J.P. 1981. Axial Musculature. In (Gans,C. and T.S. Parsons), Biology of the Reptilia, Vol. 11, Pp. 355-435. New York, New York: Academic Press.

Gatesy, S. M. and Dial, K.P. 1989.Tail muscle activity patterns in walking and flying in pigeons (*Columba livia*). Journal of Experimental Biology 176: 55-76.

Greene, H.W. 1977. Phylogeny, convergence, and snake behavior. Unpublished Ph.D dissertation, University of Tennessee, Knoxville.

Greene, H. W. 1983. Dietary correlates of the origin and radiation of snakes. American Zoologist 23: 431-441.

Greene, H. W. 1994. Homology and behavioral repertoires. In B. K. Hall (ed.), Homology: the hierarchical basis of comparative biology, Pp. 369-391. Academic Press, San Diego.

Greene, H.W. and G.M. Burghardt. 1978.Behavior and phylogeny: constriction in ancient and modern snakes. Science Vol.200: 74-76.

Greenwald, O.E. 1978. Kinematics and time relations of prey capture by gopher snakes. Copeia. 1978: 263-268.

Kluge, A.G. 1991. Boine phylogeny and research cycles. Miscellaneous Publications of the Museum of Zoology, University of Michigan 178: 1-58.

Kluge, A.G. 1993. *Calabaria* and the phylogeny of erycine snakes. Zoological Journal of the Linnean Society 107: 293-351

Lauder, G.V. 1994. Homology, Form, and Function. In BK. Hall (ed.), Homology: The Hierarchical Basis of Comparative Biology, Pp.155-196.Academic Press.

Lauder, G.V. 2003. The intellectual challenge of biomechanics and evolution. In Vincent L.Bels, Jean-Pierre Gasc and Adria Casinos (eds.),Vertebrate Biomechanics and Evolution, Pp. 319-325. Scientific Publishers Ltd, Oxford.

Loeb, G.E. and Gans, C. 1986. Electromyography for Experimentalists. Chicago, Illinois: University of Chicago Press.

McDowell, S. B. 1975. A catalogue of snakes of New Guinea and the Solomons, with special reference to those in the Bernice P. Bishop Museum, III. Anilioidea and Pythoninae. Journal of Herpetology 9:1-79.

Mehta, R.S. 2001. Prey-handling behavior in newly hatched trinket snakes, *Elaphe helena.* Unpublished Masters Thesis, University of Texas, Tyler, Texas.

Mehta, R.S. 2003. The effects of prey-size on the prey-handling behavior of hatchling *Elaphe helena*. Herpetologica 4: 471-476.

Merchan, M. and Mora, J.M. 2001.Loxocemus bicolor, biology and distribution of the Mexican burrowing python in Costa Rica. Reptilia (GB) 15: 50-55.

Milostan, M. 1989. The comparative ontogeny of prey capture and immobilization in *Boa constrictor* and *Elaphe obsolete*, with comments on the origin and evolution of constriction in boid and colubrid snakes. Unpublished M.S. thesis, Central Michigan University, Mt. Pleasant.

Moon, B.R. 2000. The mechanics and muscular control of constriction in gopher snakes (*Pituophis melanoleucus*) and a king snake (*Lampropeltis getula*). Journal of Zoological Society, London 252: 83-98.

Mora, J.M.1987. Predation by *Loxocemus bicolor* on the eggs of *Ctenosaura imiles* and *Iguana iguana.* Journal of Herpetology 21: 334-335.

Mora, J.M.1991. *Loxocemus bicolor* (burrowing python). Feeding behavior. Herpetological Review 22: 61.

Mori, A. 1991. Effects of prey size and type on prey-handling behavior in *Elaphe quadrivirgata.* Journal of Herpetology 25: 160-166.

Mori, A.1993. Does feeding experience with different size of prey influence the subsequent prey-handling behavior in *Elaphe climacophora* ? Journal of Ethology 11: 153-156.

Mori, A.1994. Prey-handling behavior of newly hatched snakes in two species of the genus *Elaphe* with comparison to adult behavior. Ethology 97: 198-214.

Mosaur, W. 1932. On the locomotion of snakes. Science 76: 583-585.

Mosaur, W. 1935. The mycology of the trunk region of snakes and its significance for ophidian taxonomy and phylogeny. University of California Publications in Biological Sciences 1: 81-120.

O'Reilly, J.C., A.P. Summers and D.A. Ritter. 2000. The evolution of the function of the trunk muscles during locomotion in adult amphibians. American Zoologist 40: 123-135.

Rieppel, O. 1978. A functional and phylogenetic interpretation of the skull of the Erycinae (Reptilia, Serpentes). Journal of Zoology, London 186: 185-208.

Rieppel, O. 1988. A review of the origin of snakes. In: Evolutionary Biology, Vol. 22. (M. K. Hecht, B. Wallace, and G.T. Pance, eds.). Plenum, New York. Pp. 37-130.

Ritter, D.A. 1995. Epaxial muscle function during locomotion in a lizard (*Varanus salvator*) and the proposal of a key innovation in vertebrate axial musculoskeletal system. Journal of Experimental Biology 198: 2477-2490.

Ritter, D.A. 1996. Axial muscle function during lizard locomotion. The Journal of Experimental Biology 199: 2499-2510.

Ruben, J.A. 1977. Morphological correlates of predatory modes in the Coachwhip (*Masticophis flagellum*) and Rosy boa (*Lichanura rosefusca*). Herpetologica, 33: 1-6.

Schwenk, K. and G. P. Wagner.2001. Function and the evolution of phenotypc stability: connecting pattern to process. American Zoologist 41: 552-563.

Shrewsbury, K. 1969. The constricting habits of Lampropeltis (kingsnakes) and Elaphe (ratsnakes). Proceedings of the Oklahoma Academy of Sciences 48: 274-276.

Willard, D.E. 1977. Constricting methods of snakes. Copeia 1977: 379-382.

Williams, T.L., S. Grillner, V.V. Smolianinov, P.Wallen, S. Kashin, and R. Rossignol, 1989. Locomotion in lamprey and trout: the relative timing of activation and movement. Journal of Experimental Biology 143: 559-56.

APPENDIX

Table A-1 Sizes and electrode placements for individuals of *Loxocemus bicolor* (L) from which epaxial muscle activity was recorded. SVL = snout to vent length, TL = tail length, BV = body (trunk) vertebrae from snout to vent, TV = number of tail vertebrae, SSP = M. semispinalis, LD = M. longissimus dorsi, IL = M. iliocostalis, V = vertebrae.

Snake	**Mass (g)**	**SVL + TL (mm)**	**BV + TV (number)**	**Muscles**	**Electrode**
L-01	571.2	975 + 87	268 + 46	SSP,LD,IL	Bilateral V50, V80
L-03	388.9	1035 +129	190 + 46	SSP,LD,IL	Bilateral V50, V80
L-04	530.9	1025 +125	235 + 47	SSP,LD,IL	Bilateral V50, V80
L-07	402.6	833 + 80	238 + 50	SSP,LD,IL	Bilateral V50, V80
L-16	1169.0	1311 +149	266 + 51	SSP,LD,IL	Bilateral V50, V80

Table A-2: Number of snakes and feeding events used in quantitative analysis of electromyographic (EMG), pressure, and handling times. *Loxocemus bicolor* = L. The complete data column indicates the number of feeding trials for which combined EMG, pressure, and handling time data were available for each snake. The bottom row indicates the total number of snakes and constriction events represented in each data set.

Snake	Total No. of Feedings	EMG	Pressure	Restraint Time	Complete Data
L-01	5 (3D, 2L)	5	1	5	1(0D, 1L)
L-03	4 (2D,2L)	4	2	4	2(2D, 0L)
L-04	2 (2D, 0L)	2	2	2	2(2D, 0L)
L-07	1 (0D, 1L)	1	1	1	1(0D,1L)
L-16	5 (2D, 3L)	5	2	5	2(2D, 0L)
Total	**17**	**17**	**8**	**17**	**8(6D, 2L)**

Table A-3 Prey handling times for five adult *Loxocemus bicolor*. Values given are $\bar{x}$ ± SD and range in s (N = number of constriction events); degrees of freedom were obtained by paired student t- tests. See text for details.

Prey	**Capture-coil (s)**	**Coil Formation (s)**	**Coil Duration (s)**	**Peak Pressure (kPa)**
Live	1.41 ± 0.52	44.3 ± 28.29	530.58 ± 244	38.22 ± 11.68
	0.15-1.45	3.3-177.2	49-829.55	8-50
	(N = 8)	(N = 8)	(N = 8)	(N = 2)
Dead	11.17 ± 9.92	32.50 ± 14.77	557.74 ± 177	40.02 ± 9.11
	0.8-28.5	4.75-47.85	130-568.17	6-56
	(N = 9)	(N = 9)	(N = 9)	(N = 6)
	P = 0.19	P = 0.61	P = 0.89	

Table A-4 Kinematic measurements for constriction loops taken from five adult *Loxocemus bicolor* during constriction with live and dead prey (*Mus musculus*). Coil direction refers to whether snakes turned to the left (L) or right (R) to make a lateral bend. Number of loops in a coil refers to how many loops were applied around the prey item. Average Number of vertebrae in loop refers to how many vertebrae were used to form 1 loop around a prey item. Vertebral curvature refers to the average degree of vertebral bending that occurs in 1 loop.

Snake #	**Total Number of Feedings**	**Coil Direction Left/Right**	**Number of Loops in a Coil**	**Range of Vertebrae in a Loop for each snake**	**Vertebral Bending Angle per Joint in a Single Loop (º)**
L-01	5	(3L/2R)	1-2	37-42	8.6
L-03	4	(3L/1R)	2	38-40	8.2
L-04	1	(1L/0R)	2	40	7.9
L-07	2	(1L/1R)	2	37-39	8.2
L-16	5	(2L/3R)	1-2	53-59	5.8

Table A-5 Mean, standard deviation, minimum, and maximum of EMG variables: duration of muscle activity (ms), amplitude (mV) of each burst of activity, and area under the rectified (absolute value) EMG trace (mV) calculated for five adult *Loxocemus bicolor*.

Variable	N	Mean	SD	Min.	Max.
		Duration of Muscle Activity (ms)			
LILv50	14	14.21	12.44	1.2	23.45
LLDv50	16	16.33	10.45	1.6	31.22
LSspv50	17	10.11	3.4	2.2	16.21
RSspv50	17	12.43	6.5	1.3	19.3
RLDv50	17	18.13	17.31	2.2	40.2
RILv50	17	12.44	12.3	3.1	26.3
LILv80	17	16.12	8.2	2.4	36.1
RILv80	16	10.41	9.1	1.2	25.1
		Amplitude of Muscle Activity (mV)			
LILv50	14	0.03	0.01	0.002	0.04
LLDv50	16	0.04	0.01	0.002	0.06
LSsp v50	17	0.01	0.01	0.002	0.06
RSsp v50	17	0.06	0.02	0.001	0.10
RLD v50	17	1.12	0.01	0.002	1.63
RIL v50	17	0.02	0.02	0.003	0.05
LIL v80	17	0.03	0.01	0.002	0.10
RIL v80	16	0.02	0.01	0.001	0.05
		Rectified Area (mV)			
LILv50	14	2.27	0.88	0.09	2.39
LLD v50	16	3.43	0.74	0.22	4.28
LSsp v50	17	1.12	0.56	0.32	1.37
RSsp v50	17	2.33	0.35	0.35	2.43
RLD v50	17	2.62	0.33	0.51	3.03
RIL v50	17	2.11	0.43	0.56	2.63
LIL v80	17	1.41	0.59	0.19	2.22
RIL v80	16	1.33	0.31	0.44	1.94

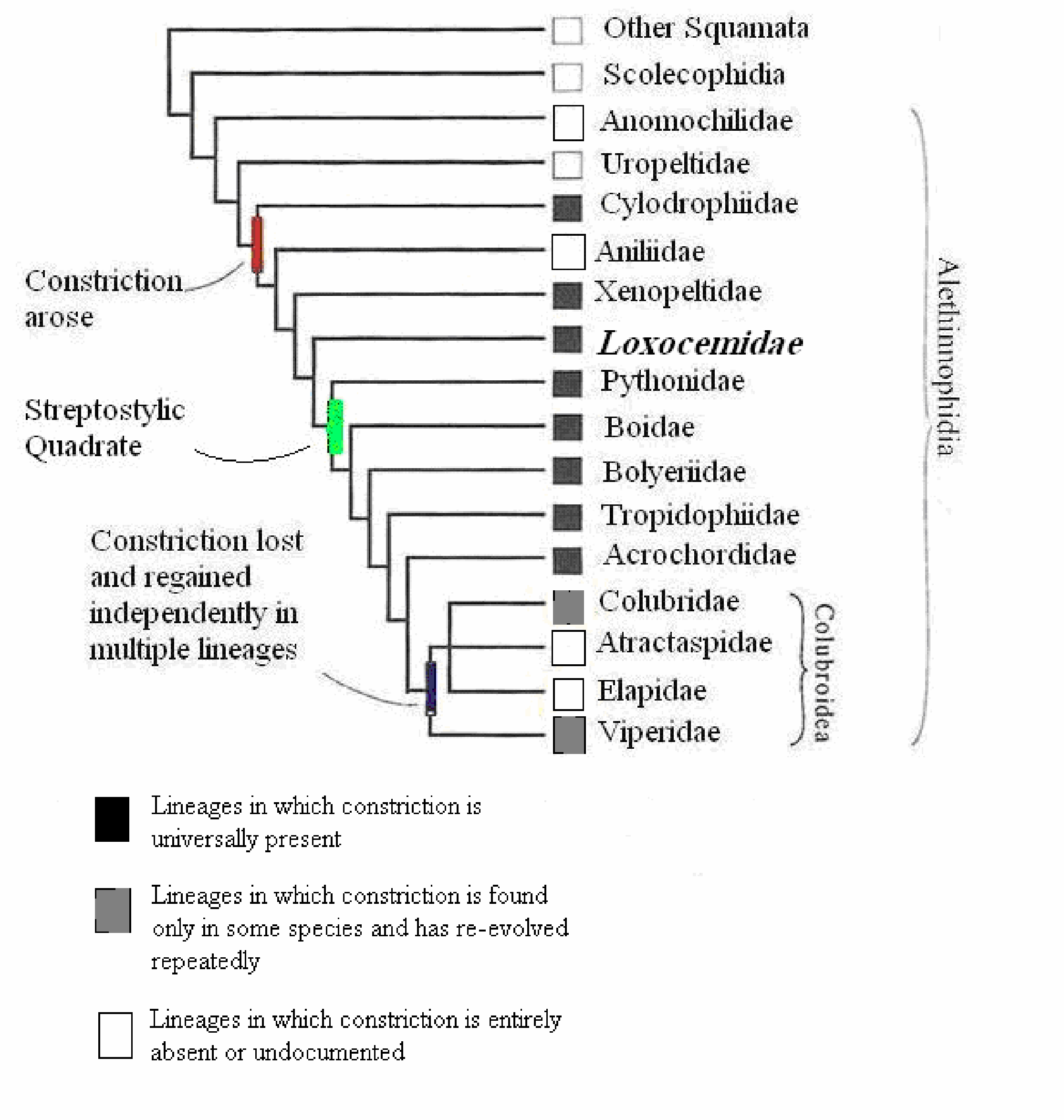


Figure A-1 Phylogeny of the Serpentes compiled from morphological (Cundall et al., 1993; Kluge, 1991, 1993; Rieppel, 1978, 1988) and molecular data (Cadle, 1994; Cadle et al., 1990). Loxocemidae (in italics) represents the sister taxon to boas and pythons and is the focus of this study.

Figure A-2 Image of *Loxocemus bicolor* bending laterally to apply loops around a small live mouse. Top: Two lateral loops are applied to large live prey during a behavioral experiment. Bottom: Three lateral loops are applied to small dead prey during an EMG trial.

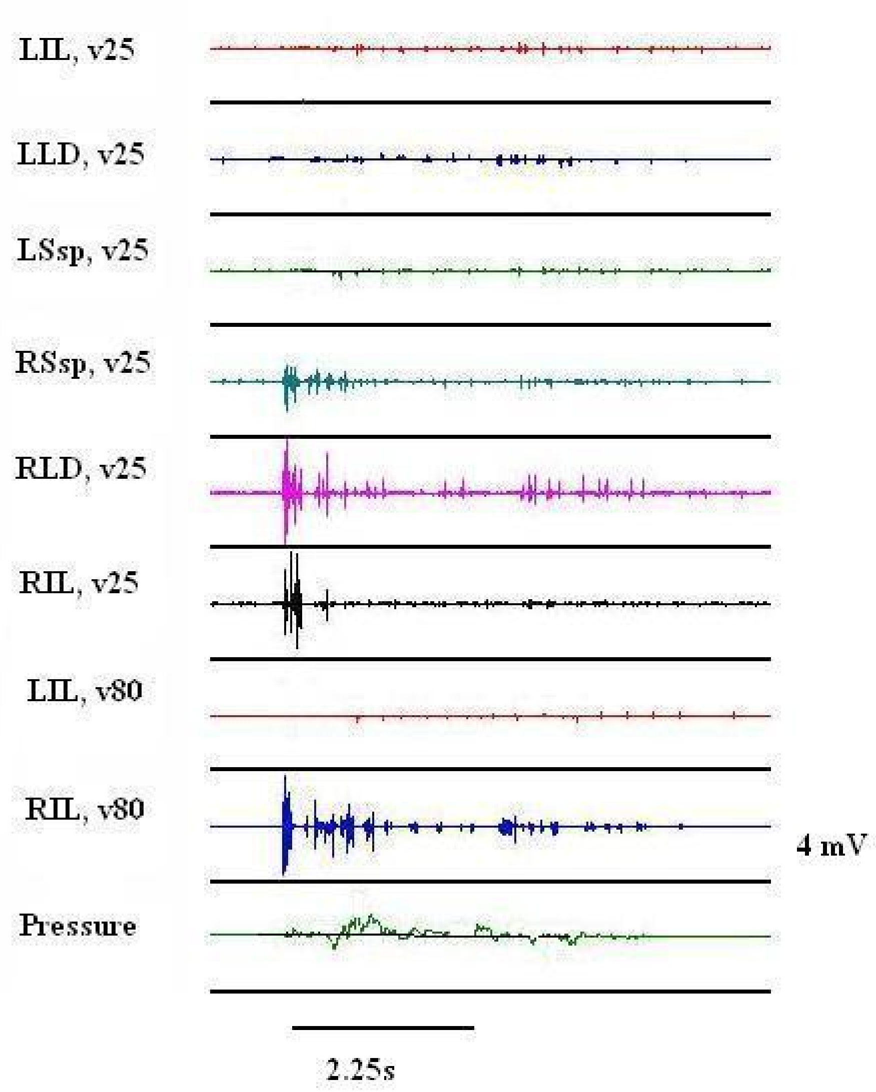


Figure A-3 Epaxial muscle activity of lateral bending during the initial coil formation phase of constriction for *Loxocemus bicolor* (L-03) with live prey.

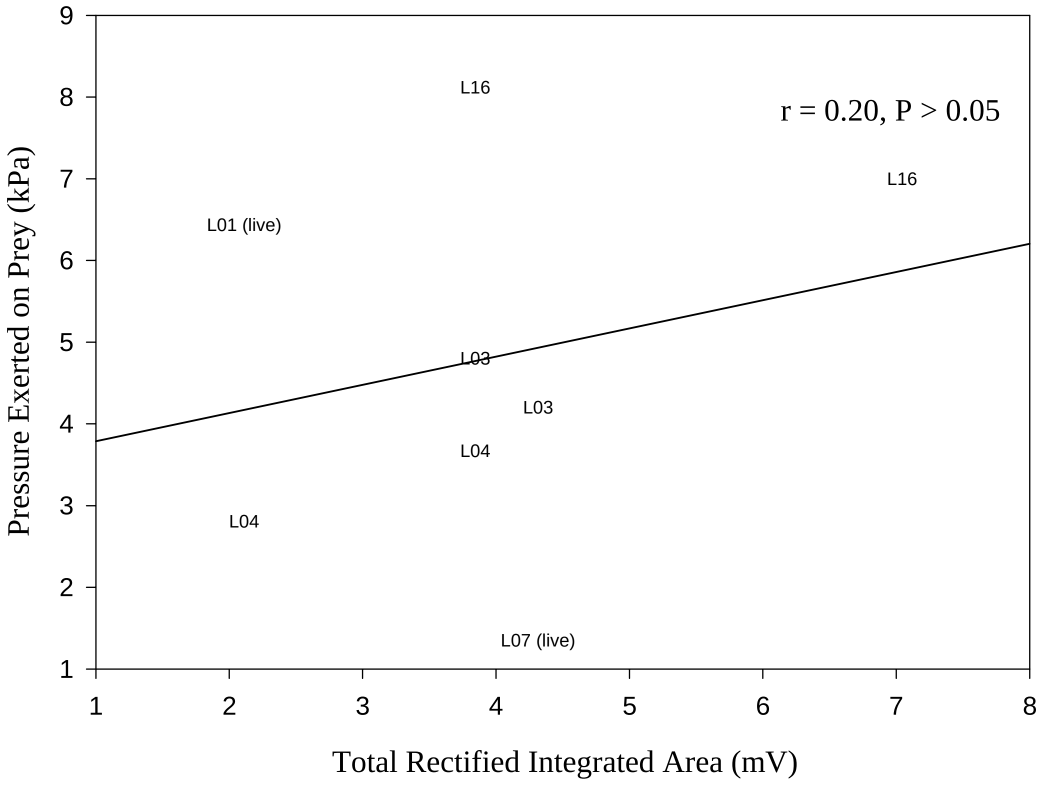


Figure A-4 This figure depicts the relationship between total epaxial area active (total rectified area) and pressure exerted on prey during the initial coil formation phase for prey trials (live and dead) for *Loxocemus bicolor*. Live prey trials are indicated in parenthesis.

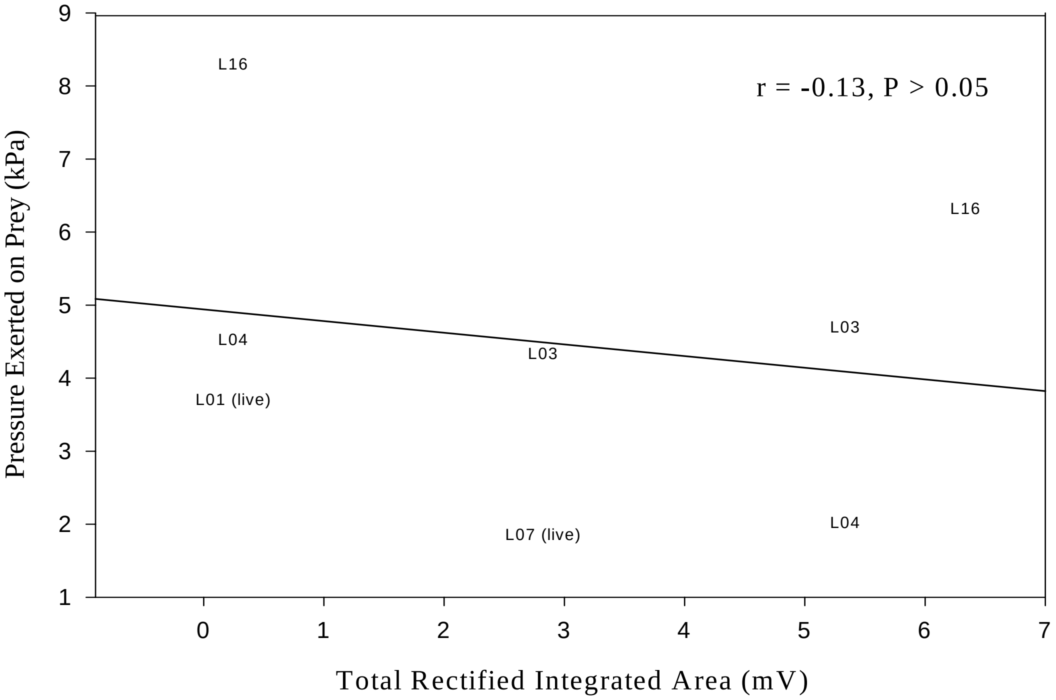


Figure A-5. This figure depicts the relationship between epaxial area active (total rectified area) and pressure exerted on prey in response to prey movements for prey trials (live and dead) for *Loxocemus bicolor*. Live prey trials are indicated in parenthesis.

PART 4

THE EVOLUTION OF CONSTRICTION MOTOR PATTERNS IN SNAKES: A PHYSIOLOGICAL HOMOLOGY

ABSTRACT

Constriction is a prey restraint behavior that enabled snakes to immobilize and subdue extremely large prey items relative to their own body mass. This behavior pattern is associated with major shifts observed in snake feeding biology from consuming small meals frequently to less frequent feeding on large prey. Although constriction is an ethological homology for at least eight lineages within the basal Alethinophidia, the underlying physiological patterns of this complex motor pattern have yet to be examined from a comparative perspective. The epaxial motor patterns during constriction behavior were investigated in two species of snakes (*Boa constrictor* and *Python molurus*) belonging to two intermediate snake lineages. Kinematic data revealed that *P. molurus* bent the long axis of the body ventrally when winding two or more loops around a prey item while *B. constrictor* bent ventrally to wind the first loop around the prey but would either bend ventrally or laterally to apply the second loop around prey. EMGs recorded bilaterally from the epaxial muscles, semi-spinalis spinalis (SSP), longissimus dorsi (LD) and iliocostalis (IL) for both snake species revealed an intermittent muscle activity pattern. The epaxial muscles exhibited the most activity during the brief coil-formation phase of constriction. Bursts of epaxial muscle activity were also present in response to the struggling of the prey while in the coil of the snake. During a constriction event, the timing of the epaxial muscles observed in these two species is similar to previous EMG recordings for one basal and two derived snake taxa. However, the epaxial muscle activity patterns of constriction behavior differ. The epaxial muscle activity patterns, unilateral activity, are homologous across snake taxa and seem to correlate with lateral

bending around prey. Boas and pythons appear to exhibit bilateral muscle activity which is related to ventral bending around prey.

INTRODUCTION

The term homology pervades the scientific literature well before the 18th century (Panchen, 1994). Character traits that can be traced back to a common ancestor are termed homologous (Mayr, 1982). The delineation of homology from analogy, similar function in convergent characters, is credited to Owens (1843), although Owen based his ideas of relationships only on external characters. The idea that homologous features are primarily structural has a long pedigree in biology (Owens, 1843; de Beer, 1971). More recently, as whole organisms are considered to comprise a hierarchy of characters, some have questioned whether the property of homology resides at any one hierarchical level (Roth, 1991). Current views of homologous traits abandon the idea that structures or any other specific class of data (i.e. genetic, developmental, behavioral) serve as the locus of homology (Lauder, 1990, 1991). These perspectives consider a monophyletic multilevel view of homology. In a monophyletic view of homology, all classes of data are considered equally important as the organization of biological systems is hierarchical by nature (Salthe, 1994; Striedter & Northcutt, 1991). The study of the depth of homology between classes of characters permits proximate analyses of underlying mechanisms driving character variability within clades. In this paper, I use the hierarchical approach to further examine variability in a behavioral character that has been correlated with the success of the vertebrate clade: the Serpentes.

Snakes are a monophyletic group of obligate predators that have achieved substantial adaptive radiation and high species richness. There are over 2500 species of snakes and the majority of these species are capable of consuming individual prey weighing 20% or more of their own mass (Cundall & Greene, 2000). This exceptional feeding biology is correlated with the appearance of two key innovations that evolved early on in the history of snakes. The first innovation is behavioral and the second is a suite of morphological characters that include elongation of the quadrate, moveable suspension of the supratemporal, and greater mobility of the snout complex (Franzetta, 1970). Although the morphological characters enabled intermediate snake taxa to consume prey items exceeding their own head width, it is the behavioral innovation, constriction, which revolutionized the dietary habits of snakes (Fig A-1)[1]. Greene (1983) pointed out that although extant basal alethinophidian snakes may feed exclusively on vertebrates with small diameters, the prey are relatively heavy in relation to the snake. Therefore the diet of extinct basal snakes was probably taxonomically diverse, but narrow in terms of prey shape, as indicated by extant basal snake groups. Basal snakes would have been behaviorally capable of restraining heavy prey. This would have led to profound shifts in feeding biology as functional innovations can provide a new selective advantage for subsequent structural changes during the origins of adaptive radiations (Gans, 1974).

Constriction is a prey handling method in which a relatively large prey is immobilized using pressure exerted from two or more points on a snake’s body (Fig A-2). Constriction as a prey restraining technique is a behavioral innovation that is shared

---

[1] All figures and tables are located in the Appemdix.

among the majority of snake lineages (Green & Burghardt, 1978). Greene (1977) documented 27 possible state combinations for constriction behavior. Of these, nineteen were observed in taxa belonging to the derived family colubridae. Colubrids exhibited intergeneric and interspecific variability in constriction behavior. In contrast, intermediate taxa, boas and pythons, exhibited very little variability in the behavior pattern, as their constriction posture was described by a single coil application pattern and 9 out of 27 possible state combinations (Greene, 1977; Mehta, Part 2). Since Greene's seminal work on constriction behavior, more empirical evidence of prey-handling behavior has been gathered for different snake taxa and currently broad generalizations of constriction motor patterns can be found in the literature (see de Queiroz & Groen, 2001).

Although constriction is homologous among major snake taxa and has been correlated with the success of the Serpentes, behavioral variations of the behavior pattern and the underlying physiology are not well understood. Interspecific and intraspecific variation in constriction postures can be observed among different lineages of snakes. Comparative studies examining the underlying physiological mechanisms of complex behavior patterns can be especially informative for behaviors exhibiting variability within lineages of a clade. As shown in Part 2, basal snake taxa (early Alethiniphidia) constrict in a relatively variable pattern whereas intermediate snakes constrict in a highly stereotyped pattern. Constriction behavior was lost and then revolved multiple times independently in colubroid lineages. The colubroids that do constrict have evolved multiple ways of restraining prey items (Mori, 1991, 1994; Mehta, 2003).

This variability in constriction patterns represents a unique challenge in the realm of evolutionary biology and biomechanics. Ethological innovations, especially complex movement patterns, are dependent upon physiological traits such as the musculoskeletal system, sensory systems, and the brain. Although constriction as a motor pattern has been established as an ethological homology for the majority of snake taxa, there are other levels of homology that necessitate consideration. The most interesting patterns of form and function are revealed when examining homologous traits across a hierarchy of levels (Lauder, 1994). Variability in constriction postures may be driven by variation in muscle activity patterns across different taxonomic groups.

The elongate limbless *Bauplan* of snakes precludes the use of the body for motion other than various bends and twists. Nevertheless, although externally simplified, snakes exhibit extremely complex axial musculature (Mosaur, 1935; Auffenberg, 1958, 1961, 1962; Gasc, 1974, 1981; Pregill, 1977). The axial musculature, separated into the epaxial (dorsal) and hypaxial (ventral) muscle groups, support a variety of movements (Moon & Gans, 1998). The epaxial muscles, specifically the muscles (mm.) of the semispinalis-spinalis (Ssp), longissimus dorsi (LD), and iliocostalis (IL), are the most well-studied muscles in snakes. This is because the epaxials are superficial muscles with large cross-sectional areas. These muscles span many vertebrae and are mechanically suited to produce small radius bends and control lateral flexion of the vertebral column in snakes.

Most studies of motor control in snakes have focused on the mechanics and underlying epaxial muscle activity of locomotor behaviors (Jayne, 1988; Gans, 1986, 1994; Moon and Gans, 1998). Only recently, has the role of the epaxial muscles during constriction been of interest (Moon, 2000; Mehta, see Chapter 3). Moon (2000) documented intermittent epaxial muscle activity patterns and both unilateral and bilateral epaxial muscle activity in two derived snake taxa: gopher snakes (*Pituophis melanoleucus* and

Lampropeltus getulus). In Part 3 of this dissertation, I recorded epaxial muscle activity during constriction for a basal snake, *Loxocemus bicolor*. I described epaxial muscle activity as intermittent and variable. The epaxial muscles exhibited strong unilateral muscle activity during initial coil formation and in response to prey movements, suggesting that epaxial muscle activity patterns may be homologous across the diverse lineages of constricting snakes. In order to determine whether the underlying muscle activity patterns of constriction behavior are homologous at the physiological level, the epaxial muscle activity patterns of intermediate snake taxa are in need of examination.

Members of the families Boidae and Pythonidae are excellent snakes with which to further examine epaxial muscle activity patterns during constriction. Boas and pythons, heavy-bodied snakes with strong feeding responses, exhibit high stereotypy in their constriction behavior compared to the proboids (Mehta, Part 2) and derived snakes (Greene, 1977). Specifically, boas are said to wind anterior, using horizontal coils with an initial twist (Greene & Burghardt, 1978). The character state, initial twist, means that during the first loop, snake bends ventrally. This character state looks like ½ of a loop. The next full loop encircling the prey may either be achieved by ventral bending or lateral bending (Franzetta, 1970). Colubrid snakes sometimes exhibit ventral bending during prey capture and the initiation of prey coiling (Moon, 2000). *Loxocemus bicolor*, a basal snake, exhibits lateral bending and unilateral epaxial activity (Mehta, Part 3). Moon (2000) described bilateral epaxial activity during the initial coil formation when gopher snakes bent ventrally. In Part 3, I described some capture postures for *L. bicolor* that looked like ventral bending above vertebrae 25 but were not. These bends that appeared to be ventral would quickly turn lateral, and since I did not inject electrodes before v25, I was unable to trace muscle activity during this quick postural change. In *Loxocemus bicolor* the looping around the prey is accomplished by bending laterally around prey. During lateral bending, the epaxial muscles exhibit unilateral activity.

The purpose of this study is to determine whether the variation in constriction postures observed at the behavioral level correspond to underlying muscle activity patterns. These results will allow me to evaluate whether epaxial muscle activity patterns are homologous across the diverse groups of snakes that use constriction as a means to restrain prey during feeding. Specifically, I examined the mechanics and epaxial muscle activity patterns during constriction for four subadult *Python molurus* and three subadult *Boa constrictor imperator*. Because striking (Cundall & Deufel, 1999) and the effects of prey characteristics on constriction behavior (Mehta, Part 2) has been addressed previously, I emphasized the kinematics, epaxial muscle activity in relation to constriction postures, and whether muscle activity is sustained during constriction. The following hypotheses were tested: 1) Bending patterns when forming loops around prey and epaxial muscle activity patterns for boas and pythons differ from those of basal and advanced snakes and 2) The epaxial muscle activity patterns for boas and pythons correspond with the high stereotypy observed during the prey restraint phase.

MATERIALS AND METHODS

General Methods

Subjects and maintenance-I recorded the kinematics and epaxial muscle activity patterns during constriction for 4 subadult burmese pythons (*Python molurus*) and 3 adult boa constrictors (*Boa constrictor imperator*) (refer to Table A-1). Whenever possible, I also measured pressure exerted on both live and dead prey items during constriction. Before experiments commenced, snakes were housed individually in plastic containers lined with corrugated cardboard for substrate. Water was available *ad libitum* and live and dead laboratory mice (*Mus musculus*), of varying relative prey mass, were offered to snakes bi-weekly. Photoperiod was maintained on a 14L: 10D cycle and room temperature was maintained at 24° C.

General experimental design-Snakes were fasted for 10 days before use in experiments. For all experiments, I placed an individual snake in a 300 x 500 mm lidless glass terrarium. Electrode leads and pressure tubing exited through the top of the terrarium, allowing free movement of snakes that were attached to wire and tubing. I maintained ambient and surface temperatures under the video spotlights at 23ºC over the course of the experiments.

In all experiments I offered mice or rats to the snakes using forceps. I recorded muscle activity patterns during constriction and other movement patterns prior to swallowing. Dead and live mice and rats offered as prey ranged from 2–12% of the snake’s body mass and 32-61% of the snakes head width. Pre-killed mice and rats were used to control for cues produced by the constricted prey. In half of the feeding trials with dead prey, prey were instrumented with a small pressure transducer in the body cavity. To simulate prey movements, I tugged on the limbs of the prey that protruded from the snake’s coil with forceps during the course of a constriction event. In order to compare my results between the two snake species and to ensure consistency during simulating natural prey movements, I waited at least 15 seconds after the initial coil application to simulate prey movements.

Live mice were offered to snakes to elicit constriction in response to natural movements by the prey. To record constriction pressures in these trials, the rubber bulb was lightly taped to the mouse's fur (see Part 3 for details).

Coil Application/ Terminology: To describe constriction postures for snakes, I adopted standard terminology from Greene (1977), Greene & Burghardt (1978), Mori (1991) and Shrewsbury (1969). I also adopt terminology used by Moon (2000) to describe the movement patterns of the axial skeleton during constriction:

Loop: a bend of the body pressed against the prey.

Pinion: 1 or more loops that push the prey against the surface but do not encircle the prey (de Queiroz, 1984).

Non-overlapping loop: a loop that partially encircles the prey (Mori, 1991).

Coil: 1 or more loops that fully encircle the prey (Greene & Burghardt, 1978).

In forming loops, Greene (1977) recognized three character states that described the way loops could be applied around a prey item. Loops could be *wound* around prey. During winding movements the prey is turned along its axis as the loops are applied in 1 direction. Loops could also be *wrapped* around prey. During wrapping, loops could be placed from more than 2 directions around prey. The resulting coils could be vertical or horizontal relative to the substratum, resulting in the lateral or ventral surface of the snake pressed against the prey.

Lateral bend: A bend is considered lateral when only one side of the snake's body is in contact with the prey.

Therefore only the right or left side of the snake can form an encircling loop around prey. When the axial skeleton bends laterally to form a coil, the dorsal as well as part of the ventral scales are visible.

Ventral bend: A bend is considered ventral when the ventral or belly scales of the snake make full contact with the prey.

When the axial skeleton bends ventrally to form a coil, only the dorsal scales of the snake are visible as the ventral side of the snake is facing the prey.

Ventral-lateral bend: When two loops are wound around the prey, the first loop can be applied by ventral bending and the second loop can be applied by lateral bending.

Videography and Electromyography

All feeding trials were videotaped (30 fps) using an 8-mm Sharp video camera VL-E43U positioned approximately 1.5 m from the terrarium containing the snake. The terrarium was lined with a 2-cm grid for scale and contained a mirror angled at 45^{o} from the horizontal to reflect a dorsal view into the video camera. Experiments were recorded at 30 fps.

For video data acquisition, Pinnacle Software was used to digitize selected images to determine the number of vertebrae used in loops during constriction and average loop radius. Snakes were marked every 10 vertebra (indicated by ventral scale counts; Alexander and Gans, 1966) with tape in order to count vertebrae on video. The vertebral midline and a cross-bar at every tenth vertebra were painted on the snake to aid in measuring average loop radius. Measurements were made using Image J which uses the horizontal and vertical scales in the video field to correct for camera angle and adjust the image shape.

To record epaxial muscle activity patterns, I implanted bipolar hook electrodes (Loeb & Gans, 1986) into the semispinalis portion (SSP) of the muscles (mm). spinalis-semispinalis, the m. longissimus dorsi (LD), and the m. iliocostalis (IL) in all five individuals. I implanted the electrodes bilaterally in the epaxial muscles at two points along the snake's trunk (Table A-1). Electrode spacing was between 30-40 vertebrae. This vertebral spacing enables detection of any bilateral differences in muscle activity and any longitudinal propagation of muscle activity. Table A-1 depicts snake size and electrode placements for individuals of *Python molurus* (P) and *Boa constrictor* (B) from which epaxial muscle activity was recorded.

Electrodes were constructed from polyimide-coated (H-ML) stainless-steel wire (California Wire Co., 316LMG) with a bipole spacing of 1.0 mm and bare recordings tips of 0.5 mm. I injected electrodes with a 23-gauge hypodermic needle into the target muscle. Entry wounds were sealed with small amounts of cyanoacrylate glue and a surgical wound dressing. Electrode leads were fixed to the snake's skin with tape. Small patches of tape were placed at intervals along the trunk. The leads were bound into one bundle and loose ends were connected to the amplifiers beyond the posterior electrode insertion site. Electrodes remained in place for 48 hours. Upon removal of electrodes, I used a microscope for visual inspection of electrode position and depth to confirm electrode placement.

During the experiments, I recorded the EMG signals in real time at 10,000 Hz using a BIOPAC MP150 data acquisition system. I set the EMG 100C differential amplifiers to a gain of 1000 and a band pass of 100-5000 Hz. After data acquisition, I digitally filtered the data using a Finite Impulse Response (FIR) filter created using 200 filter coefficients and set to a band pass of 100-1000 Hz. Thus, the analog to digital sampling rate was 10 times higher than the highest frequencies analyzed, which gave accurate digital reproduction of the EMG signals without aliasing. I then analyzed the EMG signals using Acqknowledge (version 3.8.1) software.

Plethysmography-To measure constriction pressure, I used a Harvard Apparatus physiological blood pressure transducer connected to a small water-filled rubber bulb. The bulb was lightly taped to fur of live mice or implanted in the body cavity of dead mice. The pressure signals were digitized simultaneously with the EMG signals. I recorded constriction pressures from 4 snakes and 11 readings (Table A-2).

During feeding trials, the transducer was connected to the BIOPAC150 EMG system. The transducer outputs were calibrated at 32-40kPa (4-300 mmHg) above atmospheric pressure, which encompassed the pressures recorded during the constriction events. Pressure signals were recorded and analyzed together with the EMG signals. I synchronized the video, EMG, and pressure recordings by simultaneously video taping a flashing LED (10 Hz) and recording the LED voltage spikes along with the EMG and pressure signals.

Analysis

I examined the kinematic (N = 22), electromyographic (N = 22), and pressure (N = 11) data for 22 constriction trials from 7 individuals representing two intermediate snake taxa (4 *Python molurus*: 3 *Boa constrictor imperator*) (Table A-2). From video data, I was able to describe how *P. molurus* and *B. constrictor* handled live and dead prey. I measured the duration of three stages of prey handling, including (1) the time from capture/contact to the initiation of coiling, (2) the time required to form a stable coil posture, (3) the duration of coiling from the beginning of the stable coil posture to the visible loosening of the loops wound around the prey. I tested the effects of prey condition (live *vs* dead) on these components of handling time using paired T-tests. I also recorded whether the snakes looped around the prey using ventral or lateral bending. If the snakes bent laterally, I recorded whether the right or left side of the body was used to contact the prey. Kinematic measurements were made from digitized video. Vertebral curvature was calculated while snakes formed constriction coils as well as average number of vertebrae used to form a loop.

From EMG data I measured mean duration (ms) of muscle activity, timing (ms) of epaxial muscle activity (on-set/off-set) in relation to one another, mean intensity (mV) of each burst of activity, and area under the rectified (absolute value) EMG trace in mV·ms (computed by multiplying the mean signal amplitude of the rectified spikes by the duration of the burst). Although electrode construction and implantation were standardized as much as possible, comparison of signal amplitudes or rectified areas between muscles and between individuals is confounded by potential variation in electrode structure and EMG implant position.

From pressure data, I recorded the following variables: (1) maximum pressures (kPa) exerted on the prey at any point during constriction, (2) changes in pressure (kPa) during a constriction event, and (3) changes in pressure in relation to epaxial muscle activity during the initial coil formation phase and in response to prey struggles.

RESULTS

Capture and coil formation

Mice were offered to snakes via forceps, precluding the need for snakes to extend the anterior portions of their body to strike at prey, as previously observed by Moon (2000). Therefore, snakes did not demonstrate the driving scissors striking behavior indicative of pythons and boas (Cundall & Deufel, 1999). Rather than strike, all individuals simply seized prey. Although seizing behavior was much slower than strikes, snakes seized prey in less than 2 s. Seizing involved the anterior neck region (before vertebrae 25) or just the head of the snake, neither of which contained electrodes.

Python molurus: After seizing prey, individual pythons would bend the anterior portion (up to vertebrae 77) of their bodies ventrally so that once a coil was formed, it was difficult to see the ventral scales of the snake because they were up against the prey item. From video frames, ventral bending was initiated by the head of the snake rolling forward towards the substrate. If pythons formed a coil, two or more encircling loops wound around prey; the second loop of the coil would also be wound ventrally.

Boa constrictor: Individual boas would also bend ventrally after seizing prey. Video analysis for boas also revealed the head of the snake rolling forward and toward the substrate to initiate a ventral bend. If boas formed a coil around prey, the second loop of the coil would either be ventral or lateral. During a lateral bend to form a loop only one side (left/right) of the snake would be in contact with the prey. For trials in which boas bent laterally (N = 7 trials, 3 individuals), individuals alternated between the right and left sides**.** The data for the kinematics of loop application for both species are given in Table A-4.

Prey restraint times – I used mean capture-coil time, mean coil formation time, and mean coil duration time for individuals with more than one trial for dead prey in order to examine prey restraint times between species. An ANOVA revealed no significant differences between capture-coil time ($F_{(1, 4)} = 1.114$, $P = 0.351$), coil formation time ($F_{(1,4)} = 1.156$, $P = 0.343$) and coil duration ($F_{(1,4)} = 2.40$, $P = 0.196$) between boas and pythons when restraining dead prey**.** Interspecific handling differences with live prey could not be assessed. Only one boa received a trial with a live prey item.

Python molurus: Prey restraint times, specifically the initial coil formation time and time to form a stable coil, did not differ significantly when pythons immobilized dead and live prey (capture-coil delay, $t_2 = -8.05$, $P = 0.51$; coil formation time, $t_2 = 8.41$, $P = 0.48$). There were significant differences ($t_{2\,=}\,3.02$; $P<0.001$) in average coil duration between dead and alive mice. On average, coil duration was longer when handling dead mice ($N = 3$). Coil duration, from the beginning of the stable coil posture to visible loosening, lasted from 30-238s for live prey and 118-188s for dead prey (Table A-3).

Boa constrictor: Boas were subject to more trials with dead prey and only one individual boa was offered live prey for a single feeding trial. This was mainly due to the difficulty I had in finding live prey that would not be too small for the boas to handle. Although boas exhibit high stereotypy during the prey restraint phase (Greene, 1977; Mehta, Part 2), prey needs to be long enough to extend beyond the jaws to be constricted (Cundall & Greene, 2000). Due to the single trial with live prey for *Boa constrictor*, the effects of prey status on handling times could not be examined.

Constriction postures- I describe constriction postures for snakes using standard terminology from Shrewsbury (1969), Greene (1977), Greene & Burghardt (1978), Mori

(1991), and Moon (2000). In all 22 trials, patterns of loop formation were relatively stereotyped and coils did not overlap for *P. molurus* and *B. constrictor* (Fig. A-3). I measured the number of vertebrae used in a coil from 1 frame for each individual in each trial (N =22). The average number of vertebrae used in a coil was 96. The kinematic measurements of constriction loops can be found in Table A-4.

Python molurus: Upon capturing mice, pythons formed 1.5 to 2 encircling loops around prey (N = 11). Pythons usually wound prey immediately after seizing it (N = 10). During winding movements, mice were turned about their long axis. Due to the short length of many of the prey items, loops that were formed past vertebrae 50 were not around the mouse. All individual pythons applied loops around the prey by bending ventrally (N = 11).

Boa constrictor: Boas formed 1.5 to 2 encircling loops around prey (N = 11). Similar to pythons, all prey were encircled using winding movements. Boas mostly exhibited ventral bending (N = 8). In two trials with two *B. constrictor*, the second encircling loop in a coil was formed laterally.

Epaxial muscle activity

In general, the muscle activity patterns for boas and pythons exhibited great similarity in this study so I state the general patterns I found for both of these species in a combined fashion. The epaxial muscles of boas and pythons were active during the initial coil formation phase of constriction. For all trials (N = 22), epaxial muscle activity ceased directly after snakes formed a stable coil. Bilateral biphasic activity, in which both sides of epaxial muscles are active, was observed during the initial coil formation phase for pythons handling both live and dead prey (Figs. A-4 & A-5). Boas exhibited both, uniphasic lateral activity, where only one side of the epaxial muscles are active, and biphasic bilateral activity patterns during initial coil formation (Figs. A-6 & A-7).

Muscle activity ceased directly after coil formation in both boas and pythons and epaxial muscle activity was not observed unless the prey struggled in the snake's coils (Figs. A-8 & A-9). Muscle activity was present in response to both natural prey movements and artificially induced prey movements for both species. Some muscle activity patterns in response to prey movements were unilateral for boas. In trials in which ventral-lateral bending was used to initially wind loops around the prey (N = 11), the right and left IL v25 would exhibit bilateral activity while the right or left LD and IL v50 would be unilaterally active. Ventral-lateral bending with bilateral-unilateral muscle activity patterns was observed during 6 trials. In the lateral loop, v25 and v50 were almost always active, a pattern mostly exemplified in *Boa constrictor* (Fig. A-10).

Relationship between epaxial muscle activity and pressure exertion

Epaxial muscle activity and pressure exertion were pronounced during coil formation and in response to natural and simulated struggling movement by the prey. Bilateral and unilateral muscle activity during coil formation was synchronous with pressure exertion, although in 8 out of the 11 pressure trials, peak pressures were maintained when epaxials ceased actvity. Although boas and pythons maintained coil postures for up to several minutes, epaxial muscles ceased activity directly after initial coil formation (N =22; Figs. A-6, A-7 & A-10) and in relation to prey struggles. This pattern indicates that epaxial muscles contract intermittently during constriction.

Epaxial muscle contractions were associated with increases in pressure during coil formation and in response to prey movements. I did not analyze two out of the eleven pressure trials due to odd pressure readings that became apparent after the initial coil formation phase. These odd pressure recording were observed in trials with *P. molurus*. Of the nine trials I did examine, pressure increases were not correlated with the rectified integrated area of EMG bursts during the initial coil formation phase ($r = 0.193$, $P > 0.620$; Fig. A-11) and during prey struggles ($r = 0.269$, $P > 0.49$; Fig. A-12). When muscle activity ceased quickly after coil formation or after responding to prey movements, moderate to high pressures were sustained. These high pressures (30-56) would last anywhere from 5-12.36 s after epaxial muscles ceased activity. The timing and magnitude of pressure exertion varied considerably within and among constriction events. Pythons exerted pressures which ranged from 23-69 kPa for alive mice and 15-45 kPa for dead mice. Boas exerted pressures that ranged from 24.5 -70 kPa during the single constriction event with alive prey and 23-52 kPa for dead mice (Table A-3; Figs. A-11 & A-12).

DISCUSSION

On the basis of electromyographic and kinematic data, *Python molurus* and *Boa constrictor* exhibit similar patterns of muscle activity during constriction. The initial capture-coil and initial coil formation phases were quicker for boas compared to pythons. Although I controlled for relative prey mass, boas exhibited longer durations of coiling during constriction. Both species wound loops around prey items and coiling consisted of 1.5-2 loops. Both species bent ventrally when initiating the first loop. When a second loop was wound around the prey, the loop could either be lateral or ventral. In two trials, with two boas the second encircling loop was formed using a lateral bend. Franzetta

(1966) described coil formation for *Python molurus* and *Python sebae* based on film analysis and concluded that the ventral side is pressed against the victim, seldom the lateral side. My reports support this observation, however boas seem to also be able to wind laterally around prey. Lateral looping in boas was mostly performed with the second loop while ventral bending was observed in the first 1.5 loops, a continuation of the capture-coil phase.

Three major conclusions can be drawn from examining the epaxial muscle activity patterns of boas and pythons during constriction. Firstly, during the capture-coil phase, boas and pythons bend ventrally and the epaxial muscles exhibit bilateral muscle activity patterns. These activity patterns are usually strong bursts of muscle activation that last anywhere from 2.5 – 6.8 s. Secondly, epaxial muscles cease activity when the coil is fully formed. Although snakes maintain their constriction posture, epaxial muscles are not active. Thirdly, epaxial muscles become active in response to prey struggles. Epaxial muscle activity patterns in response to natural or simulated prey movement were mostly bilateral for boas and pythons. However, in 2 trials with *Boa constrictor* no.2 and no 4, epaxial muscles exhibited unilateral muscle activity. In these two trials the epaxial muscle activity was unilateral because the second loop of the coil was laterally placed (Fig.A-10). The epaxial muscles responded to prey movements as natural and artifical prey struggles were followed by bursts in epaxial muscle activity. This was particularly interesting because no changes in constriction posture were observed. Prey characteristics such as size and activity level are considered to have very little affects on the constriction postures for intermediate snake taxa (Greene, 1977; Greene & Burghardt, 1978; Milostan, 1989). My data in Part 2 support these observations. As the epaxial muscles become active the muscular contraction reduces the diameter of the coil. Unfortunately, the contraction for some of these trials was so subtle that coil diameter reduction was not easily observed. Canjani et al. (2003) examined the aerobic metabolism of constriction and revealed that metabolic rate did not correspond to the size of prey constricted. It appears that boas have highly specialized physiological mechanisms that enable them to constrict extremely large prey without undergoing too many observable postural shifts as exhibited by highly derived snakes.

Lastly, all snakes exerted high pressures during the initial coil formation phase of constriction and pressure recordings continued to remain steady throughout constriction although epaxial muscles ceased activity. This finding suggests two things: 1) boas and pythons squeeze prey hard during the initial capture phase that leads into the formation of the first loop via ventral bending and 2) other muscles are contributing to holding the stable coil posture as well as contributing to pressure exertion. The musculature of snakes is highly complex and any one of the estimated 15 muscles comprising the axial musculature in snakes could be active during constriction (Cundall, 1987). However, from my study, I can only describe the epaxial musculature activity patterns.

Physiological homology

Moon (2000) was the first to examine the highly complex three dimensional movement pattern, constriction, from a physiological perspective. Specifically, the muscle activity patterns in relation to constriction kinematics was described using two derived snake taxa, gopher snakes (*Pituophis melanoleucus*) and king snakes (*Lampropeltis getula*). In Part 3 of this dissertation I examined the epaxial muscle activity patterns for a basal snake taxon, *Loxocemus bicolor*. These studies, along with the research just described, enables a phylogenetic approach to the analysis of homology for constriction behavior at the physiological level.

In all three studies, four general trends were observed: 1) snakes exhibited intermittent epaxial muscle activity, 2) during the initial coil formation phase of constriction, epaxial activity was strong but abrupt in duration, 3) epaxial muscle activity always ceased after the coil was formed, and 4) epaxial muscles were active in response to natural and artificial prey movements. The derived snakes, gopher snakes and king snakes used lateral bends to wind around prey as did the basal taxon, *Loxocemus bicolor*. This suggests that the ancestral constriction posture involved lateral bending and unilateral muscle activity. Boas and pythons, as discussed earlier in this chapter, primarily wound around prey by ventral bending of the trunk. These results suggest that ventral bending with bilateral muscle activity is a derived constriction pattern.

The amount of pressure exerted on prey during constriction varied between the four lineages examined. Derived snake taxa exerted 6.1- 30.9 kPa (Moon, 2000) while the basal snake, *Loxocemus bicolor* exerted anywhere from 8-50 kPa for alive prey and 6-56 kPa for dead prey. Higher pressures appear to have been exerted on dead prey items. These trials with dead prey, however, were compromised by the need to artificially manipulate prey movements in an attempt to elicit maximal pressures by snakes. Boas and pythons exhibited similar pressures for live prey 23-70 kPa. Trials with dead prey exhibited more variability. Boas can consume large meals in the wild; although the variability of prey struggling while prey is in the coils of a snake has never been studied, I speculate that larger prey may struggle more vigorously than smaller prey. I also speculate that snakes perceive the struggles of larger prey more easily and have ways to incorporate these struggles into their muscle activity patterns.

In general, these pressure trials did not reveal maximal pressures for any of the species examined. This statement is based on the fact that small prey items were primarily used in this study and that larger pressures would be necessary to immobilize and kill larger prey. Also, natural as well as manipulated prey movements elicited increases in pressure for all three taxa I examined. Further examination of the effects of prey activity and prey size would be useful in determining whether some taxa are capable of exerting more force on prey than others. Future studies should also consider the relationship between snake diameter and force exerted on large prey.

Structural and functional classes of data reflect proximate causes for variation at the behavioral level (Lauder, 1991, Lauder & Reilly, 1996). The study of the proximate mechanisms that may drive behavioral differences across groups of organisms is especially important when monophyletic groups exhibit differential success (i.e. species diversity and richness). The underlying epaxial muscle patterns, bilateral and lateral activity, in basal, intermediate and derived snake taxa exemplify the variability in constriction postures. My data in Parts 2 & 3 of this dissertation reveal that basal snakes such as *Loxocemus bicolor* vary prey restraint methods with respect to prey size and status and use the lateral portion of their body to apply encircling loops around prey. This behavior pattern is reflected in the underlying epaxial muscle patterns, which reveal unilateral muscle activity during constriction. Intermediate taxa, boas and pythons, constrict in a highly stereotyped manner which corresponds with ventral bending around prey. Ventral bending is controlled by bilateral epaxial activity. Derived snake taxa exhibit the greatest variability in prey restraint postures which is reflected in variable epaxial muscle activity patterns. Moon (2000) documented that gopher snakes and king snakes exhibit both bilateral and unilateral muscle activity patterns when handling prey. From these studies, I can conclude that the epaxial muscle activity patterns are homologous across snake taxa. Unilateral muscle activity is most probably the basal condition while intermediate and advanced snake lineages exhibit a more derived epaxial muscle pattern. These different muscle patterns suggest that numerous functional specializations have occurred in the underlying activity of homologous behaviors, a topic that has been gaining more attention (Schwenk & Wagner, 2001).

Moon (1998) studied the interrelationships of locomotion, constriction, and swallowing in snakes. Each of these behaviors is subject to their own selection pressures and the underlying axial skeleton is subject to internal selection which most probably helps to support these diverse behaviors. This illustrates that all levels of organismal design can be subject to extensive transformation.

ACKNOWLEDGEMENTS

This research was approved by the Institutional Animal Care and Use Committee, University of Louisiana at Lafayette. I am indebted to Brad Moon for his expertise in muscle physiology and the use of EMG equipment. I am grateful to Gordon Burghardt, Marguerite Butler, Todd Freeberg, Neil Greenberg and Richard Saudargas for helpful comments on this chapter. Brian Ott and Stephen Secor provided animals used in this study. Joey Bennett, Brad Moon and Ali Rabatsky helped immensely during electrode placement and EMG experiments. Karen Davis, Cassandra Fenner, Lauren Kirby, and Brooke Patrick helped prepare electrodes, digitize video and took over teaching responsibilities while I was away. Kate Wadsworth provided a place to stay and statistical consulation. This study was funded in part by the American Psychological Association (APA), the Department of Psychology at the University of Tennessee, Knoxville (UT) and the U.T Scholarly Activities Research Incentive Fund (SARIF). Presentations of parts of this study to the Society of Integrative and Comparative Biology (SICB) and at the VII International Conference of Vertebrate Morphology (ICVM) were supported by the Department of Psychology at UT and the U.T Graduate Student Association.

LITERATURE CITED

Alexander, A. A. and Gans, C. 1966. Then pattern of dermal-vertebral correlation in snakes and amphisbaenians. Zoological Mededelingen 41: 17-190.

Cadle, J.E. 1988. Phylogenetic relationships among advanced snakes: a molecular perspective. University of California Publications in Zoology 119: 1-70.

Carrier, D.1989.Ventilatory action of the hypaxial muscles of the lizard *Iguana iguana*: a function of slow muscle. Journal of Experimental Biology 143: 435-457.

Cundall,D., V. Wallach, and D.A. Rossman. 1993.The systematic relationships of the Snake genus *Anomochilus*. Zoological Journal of Linnean Society 109: 275-299.

Cundall, D. and H. W. Greene. 2000. Feeding in snakes. In K. Schwenk (ed.), Feeding: Form, Function, and Evolution in Tetrapod Vertebrates., Pp. 293-333. Academic Press, San Diego.

de Beer, G.R. 1971. “Some general biological principles illustrated by the evolution of Man,” Oxford Biological Readers No. 1. Oxford University Press, London.

English, A.W. 1980. The functions of the lumbar spine during stepping in a cat. Journal of Morphology 165: 55-66.

Gans, C. 1962. Terrestrial locomotion without limbs. American Zoologist 2: 167-182.

Gasc, J.P. 1974. L’interprétation fonctionnelle de l’appareil musculo-squelettique de l'axe vertebral chez les Serpents (Reptilia). Mémoirs du Museum d’Histoire Naturelle, Paris, Serial.83: 1-182.

Gasc, J.P. 1981. Axial Musculature. In (Gans,C. and T.S. Parsons), Biology of the Reptilia, Vol. 11, Pp. 355-435. New York, New York: Academic Press.

Gatesy, S. M. and Dial, K.P. 1989.Tail muscle activity patterns in walking and flying in pigeons (*Columba livia*). Journal of Experimental Biology 176: 55-76.

Greene, H.W. 1977. Phylogeny, convergence, and snake behavior. Unpublished Ph.D dissertation, University of Tennessee, Knoxville.

Greene, H. W. 1983. Dietary correlates of the origin and radiation of snakes. American Zoologist 23: 431-441.

Greene, H. W. 1994. Homology and behavioral repertoires. In B. K. Hall (ed.), Homology: the hierarchical basis of comparative biology, Pp. 369-391.

Academic Press, San Diego.

Greene, H.W. and G.M. Burghardt. 1978.Behavior and phylogeny: constriction in ancient and modern snakes. Science Vol.200: 74-76.

Greenwald, O.E. 1978. Kinematics and time relations of prey capture by gopher snakes. Copeia. 1978: 263-268.

Jayne, B.C. 1982. Comparative morphology of the semispinalis-spinalis muscle of snakes and correlations with locomotion and constriction. Journal of Morphology 172: 83-96.

Jayne, B.C.1988a. Muscular mechanisms of snake locomotion: an electromyographic study of sidewinding and concertina modes of *Corallus cerastes, Nerodia fasciata,* and *Elaphe obsolete.* Journal of Experimental Biology 140: 1-33.

Jayne, B.C.1988b. Muscular mechanisms of snake locomotion: an electromyographic study of lateral undulation of the Florida banded water snake (*Nerodia fasciata*) and the yellow rat snake (*Elaphe obsoleta*). Journal of Morphology 197: 159-181.

Jayne, B.C., Lauder,G.V., Reilly, S.M. and Waiwright, P.C. 1990. The effect of sampling rate on the analysis of digital electromyograms from vertebrae muscle. Journal of Experimental Biology 154: 557-565.

Lauder, G.V. 2003. The intellectual challenge of biomechanics and evolution. In Vincent L.Bels, Jean-Pierre Gasc and Adria Casinos (eds.),Vertebrate Biomechanics and Evolution, Pp. 319-325. Scientific Publishers Ltd, Oxford.

Loeb, G.E. and Gans, C. 1986. Electromyography for Experimentalists. Chicago, Illinois: University of Chicago Press.

Mayr, E. 1982. The Growth of Biological Thought: Diversity, evolution, and inheritance. Harvard University Press, Cambridge, MA.

Mehta, R.S. 2003. The effects of prey-size on the prey-handling behavior of hatchling *Elaphe helena*. Herpetologica 4:4 71-476.

Moon, B.R. 2000. The mechanics and muscular control of constriction in gopher snakes (*Pituophis melanoleucus*) and a king snake (*Lampropeltis getula*). Journal of Zoological Society, London 252: 83-98.

Moon, B.R. 1996. Sampling rates, aliasing, and the analysis of electrophysiological signals. In IEEE Proceedings of the 15th Southern Biomedical Engineering Conference. Bajpai, P.K. (Ed). Pp. 401-404.

Moon, B.R and C. Gans.1998. Kinematics, muscular activity and propulsion in gopher snakes. The Journal of Experimental Biology 201: 2669-2684.

Mori, A. 1991. Effects of prey size and type on prey-handling behavior in *Elaphe quadrivirgata*. Journal of Herpetology 25: 160-166.

Mosaur, W. 1932. On the locomotion of snakes. Science 76: 583-585.

Mosaur, W. 1935. The mycology of the trunk region of snakes and its significance for ophidian taxonomy and phylogeny. University of California Publications in Biological Sciences 1: 81-120.

O'Reilly, J.C., A.P. Summers and D.A. Ritter. 2000. The evolution of the function of the trunk muscles during locomotion in adult amphibians. American Zoologist 40: 123-135.

Owen, R. 1843. Lectures on the comparative anatomy and physiology of the invertebrate Animals, Delivered at the Royal College of Surgeons, in 1843. Longman, Brown, Green, and Longmans, London.

Panchen, A.L. 1994. R. Owen and the concept of homology. In B. K. Hall (ed.), Homology: the hierarchical basis of comparative biology, Pp. 22-62. Academic Press, San Diego.

Ritter, D.A. 1995. Epaxial muscle function during locomotion in a lizard (*Varanus salvator*) and the proposal of a key innovation in vertebrate axial musculoskeletal system. Journal of Experimental Biology 198: 2477-2490.

Ritter, D.A. 1996. Axial muscle function during lizard locomotion. The Journal of Experimental Biology 199: 2499-2510.

Roth, V.L. 1991. Homology and hierarchies: problems solved and unresolved. Journal of Evolutionary Biology 4: 167-194.

Ruben, J.A. 1977. Morphological correlates of predatory modes in the Coachwhip (*Masticophis flagellum*) and Rosy boa (*Lichanura rosefusca*). Herpetologica, 33: 1-6.

Schwenk, K. and G. P. Wagner (2001) Function and the evolution of phenotypic stability: connecting pattern to process. American Zoologist 41: 552-563.

Shrewsbury, K. 1969. The constricting habits of Lampropeltis (kingsnakes) and Elaphe (ratsnakes). Proceedings of the Oklahoma Academy of Sciences 48: 274-276.

Willard, D.E. 1977. Constricting methods of snakes. Copeia 1977: 379-382.

Williams, T.L., Grillner, S., Smolianinov,V.V., Wallen, P., kashin, S. and Rossignol, S. 1989. Locomotion in lamprey and trout: the relative timing of activation and movement. Journal of Experimental Biology 143: 559-566.

APPENDIX

Table A-1 Sizes and electrode placements for individuals of *Python molurus* (P) and *Boa constrictor* (B) from which epaxial muscle activity was recorded. SVL = snout to vent length, TL = tail length, BV = body (trunk) vertebrae from snout to vent, TV = number of tail vertebrae, SSP = M. semispinalis, LD = M. longissimus dorsi, IL = M. iliocostalis, V = vertebrae. All snakes were measured at the start of the experiment.

Snake	**Mass (g)**	**SVL + TL (mm)**	**Muscles**	**Electrode**
P-01	585.3	1135 + 143	SSP, LD, IL	Bilateral V50,V80
P-02	589.0	1035 + 139	SSP, LD, IL	Bilateral V50,V80
P-03	647.2	1058 + 149	SSP, LD, IL	Bilateral V50,V80
P-05	591.1	1025 + 125	SSP, LD, IL	Bilateral V50,V80
B-01	722.1	1237 + 180	SSP, LD, IL	Bilateral V25,V75
B-02	812.4	1305 + 167	SSP, LD, IL	Bilateral V25,V75
B-04	592.9	1024 + 096	SSP, LD, IL	Bilateral V25,V75

Table A-2 Number of snakes and feeding events used in quantitative analysis of electromyographic (EMG), pressure, and handling times for *Python molurus* (P) and *Boa constrictor* (B). The complete data column indicates the number of feeding trials for which combined EMG, pressure, and handling time data were available for each snake. The bottom row indicates the total number of snakes and constriction events represented in each data set.

Snake	**Total No. of Feedings**	**EMG**	**Pressure**	**Handling Time**	**Complete Data**
P-01	3(1D, 2L)	3	2	3	2(1D, 1L)
P-02	1(0D, 1L)	1	0	1	0(0D, 0L)
P-03	5(1D, 4L)	5	4	5	4(0D, 4L)
P-05	2(1D, 1L)	2	1	2	1(1D, 0L)
B-01	3(3D, 0L)	3	0	3	0(0D,0L)
B-02	3(3D, 0L)	3	0	3	0(0D,0L)
B-04	5(4D, 1L)	5	4	5	4(3D,1L)
7	**22**	**22**	**11**	**22**	**11**

Table A-3 Prey handling times for a) four subadult *Python molurus* and b) three *Boa constrictor imperator.* Values given are mean ± SD and range in s (N = number of constriction events); Degrees of freedom were obtained by paired student t- tests. See text for details.

a) ***Python molurus***

Prey	**Capture-coil (s)**	**Coil Formation (s)**	**Coil Duration (s)**	**Peak Pressure (kPa)**
Live	1.59 ± 1.45	10.19 ± 0.08	1.00-3.32	3.0 - 8.62
	138.63 ± 71.50	40.64 ± 18.83	30- 238	23-69
	(N = 8)	(N = 8)	(N = 8)	(N=5)
Dead	1.88 ± 1.24	9.11 ± 6.98	161.00 ± 41.72	25.33 ± 11.50
	1.00- 3.20	4.75 – 47.85	118-188	15 -45
	(N = 3)	(N = 3)	(N = 3)	(N=2)
	P = 0.57	P = 0.48	P < 0.001	

b) ***Boa constrictor imperator***

Prey	**Capture-coil (s)**	**Coil Formation (s)**	**Coil Duration (s)**	**Peak Pressure (kPa)**
Live	2.3	12	120	70
	-	-	-	-
	(N = 1)	(N = 1)	(N = 1)	(N = 1)
Dead	1.092 ± 0.07	5.45 ± 0.16	282.5 ± 164.76	40.14 ± 5.56
	0.2 - 2.24	0.03 – 54.5	111 -652	23-52
	(N = 10)	(N = 10)	(N = 10)	(N=3)

Table A-4 Kinematic measurements for constriction loops taken from four subadult *Python molurus* and three subadult *Boa constrictor* with live and dead prey (*Mus musculus*). Initial Bend/1st loop and 2nd loop, refers to whether the loop was applied by bending ventrally (V) or laterally (L). Average Number of vertebrae in loop refers to how many vertebrae were used to form 1 loop around a prey item. Vertebral curvature refers to the average degree of vertebral bending that occurs in 1 loop.

Snake #	**Number of Feedings Analyzed**	**Initial Bend/ 1st loop**	**2nd loop V/L**	**Average Number of Vertebrae in Loop**	**Vertebral Curvature for a single loop (°)**
P-01	3	3V:0L	3V:0L	103 ± 5	3.42
P-02	1	1V:0L	1V:0L	100 ± 0	3.91
P-03	5	5V:0L	5V:0L	89 ± 1	2.86
P-05	2	2V:0L	1V:1L	96 ± 13	4.21
B-01	3	3V:0L	1V:2L	108 ± 11	4.32
B-02	3	3V:0L	2V:1L	111 ± 6	5.21
B-04	5	3V:2L	0V:5L	104 ± 7	5.87

Table A-5 Mean, standard deviation, minimum, and maximum of EMG variables: duration of muscle activity (ms), intensity (mV) of each burst of activity, and area under the rectified (absolute value) EMG trace (mV) calculated for four subadult *Python molurus.*

Variable	**N**	**Mean**	**SD**	**Min.**	**Max.**
Duration of Muscle Activity (ms)					
LILv50	11	16.51	8.32	11.2	25.65
LLDv50	11	14.23	4.13	6.6	19.32
LSspv50	11	10.41	1.4	4.7	12.08
RSspv50	11	10.84	3.5	2.1	15.27
RLDv50	11	15.04	4.24	11.2	20.82
RILv50	11	6.51	2.3	3.1	9.3
LILv25	11	11.24	7.4	6.4	19.3
RILv25	11	10.01	5.4	5.8	20.6
Amplitude of Muscle Activity (mV)					
LILv50	11	0.06	0.01	0.006	0.09
LLD v50	11	0.04	0.01	0.004	0.06
LSsp v50	11	0.02	0.01	0.002	0.06
RSsp v50	11	0.15	0.02	0.001	0.20
RLD v50	11	2.31	0.06	0.002	3.02
RIL v50	11	0.12	0.02	0.08	0.16
LIL v25	11	0.43	0.01	0.22	0.50
RIL v25	11	0.42	0.01	0.01	0.45
Rectified Area (mV)					
LILv50	11	2.27	0.21	0.19	2.51
LLD v50	11	1.08	0.29	0.35	2.01
LSsp v50	11	1.62	0.10	0.43	1.79
RSsp v50	11	2.88	0.72	0.85	3.93
RLD v50	11	5.01	0.11	0.51	5.27
RIL v50	11	3.66	0.15	0.32	3.88
LIL v25	11	4.11	0.23	0.71	5.22
RIL v25	11	1.34	0.11	1.06	1.67

Table A-6: Mean, standard deviation, minimum, and maximum of EMG variables: duration of muscle activity (ms), intensity (mV) of each burst of activity, and area under the rectified (absolute value) EMG trace (mV) calculated for three subadult *Boa constrictor*.

Variable	N	Mean	SD	Min.	Max.
Duration of Muscle Activity (ms)					
LILv50	**11**	12.34	11.22	1.8	33.67
LLDv50	**11**	18.40	09.15	3.2	42.09
LSspv50	**11**	12.22	4.3	2.8	28.11
RSspv50	**11**	10.13	7.4	3.4	22.41
RLDv50	**11**	19.54	19.21	3.4	26.06
RILv50	**11**	15.62	13.12	3.5	27.67
LILv25	**11**	17.42	11.13	3.2	26.7
RILv25	**11**	11.21	10.21	3.1	22.8
Amplitude of Muscle Activity (mV)					
LILv50	**11**	0.011	0.02	0.03	0.06
LLD v50	**11**	0.08	0.11	0.034	0.02
LSsp v50	**11**	0.07	0.23	0.001	0.09
RSsp v50	**11**	0.05	0.03	0.001	0.07
RLD v50	**11**	2.43	0.41	0.32	0.45
RIL v50	**11**	0.05	0.03	0.005	0.08
LIL v25	**11**	0.07	0.01	0.001	0.12
RIL v25	**11**	0.07	0.01	0.002	0.05
Integrated Rectified Area (mV)					
LILv50	**11**	3.48	1.10	1.33	1.49
LLD v50	**11**	3.63	0.82	2.29	4.11
LSsp v50	**11**	1.42	0.76	0.42	2.87
RSsp v50	**11**	2.04	0.15	2.09	2.19
RLD v50	**11**	2.53	0.11	2.42	2.64
RIL v50	**11**	2.41	0.23	2.18	2.64
LIL v25	**11**	0.41	0.39	0.12	0.69
RIL v25	**11**	1.08	0.41	0.67	1.49

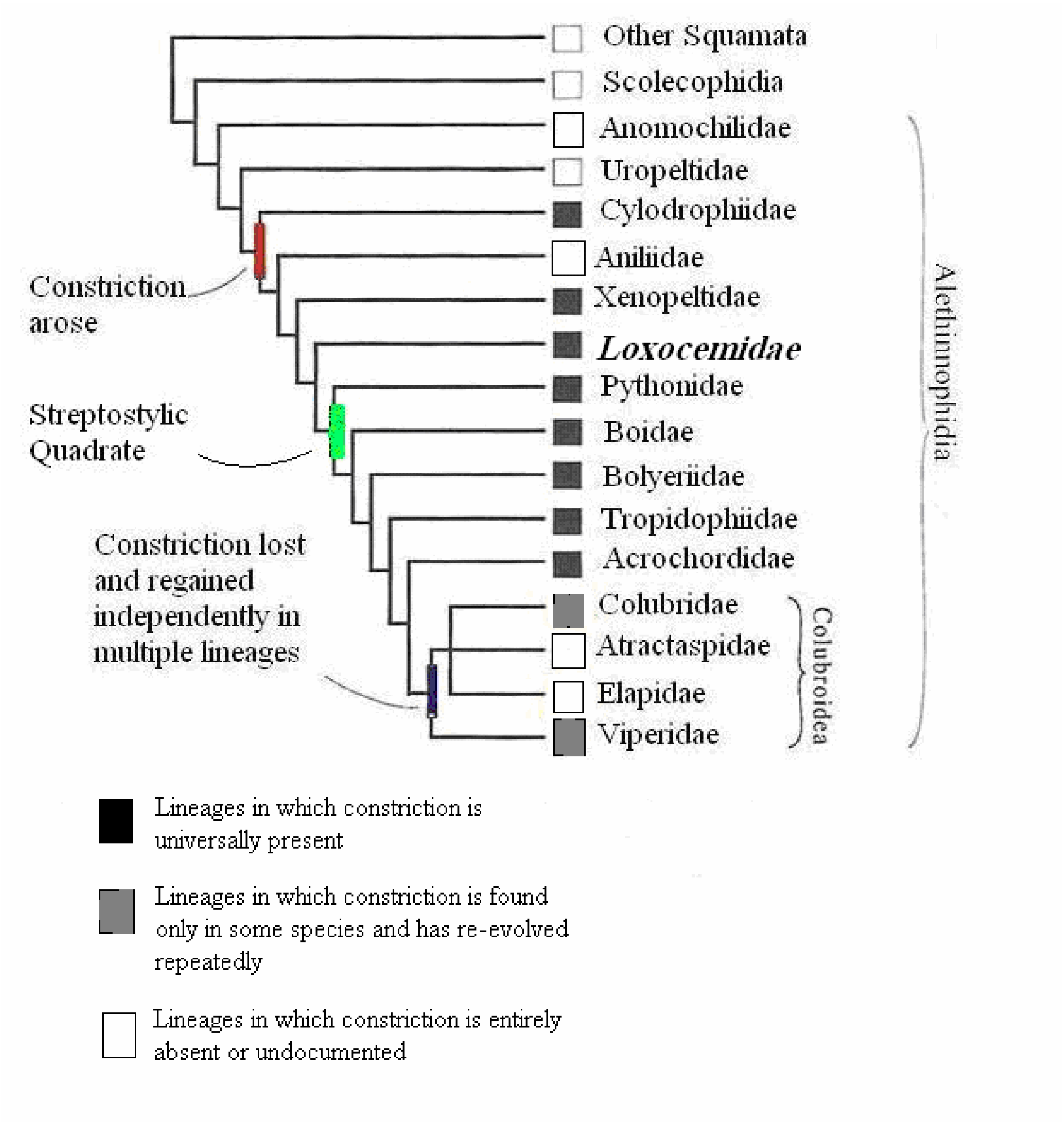


Figure A-1 Phylogeny of the Serpentes compiled from morphological (Cundall et al., 1993; Kluge, 1991, 1993; Rieppel, 1978, 1988) and molecular data (Cadle, 1994; Cadle et al., 1993). Loxocemidae (in italics) represents the sister taxon to boas and pythons. Note the evolution of the key behavioral innovation in snakes, constriction.

Figure A-2 An example of constriction behavior performed by *Eunectes murinus*.

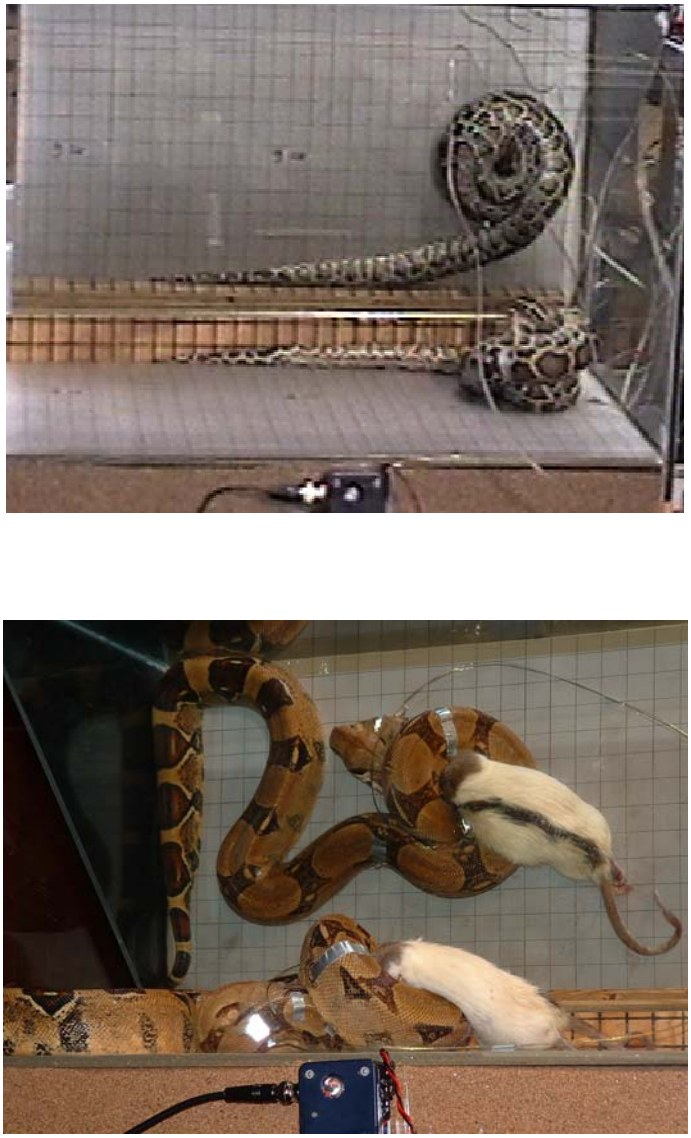


Figure A-3 Examples of *Python molurus* (top) and *Boa constrictor* (bottom) bending ventrally during EMG experiments.

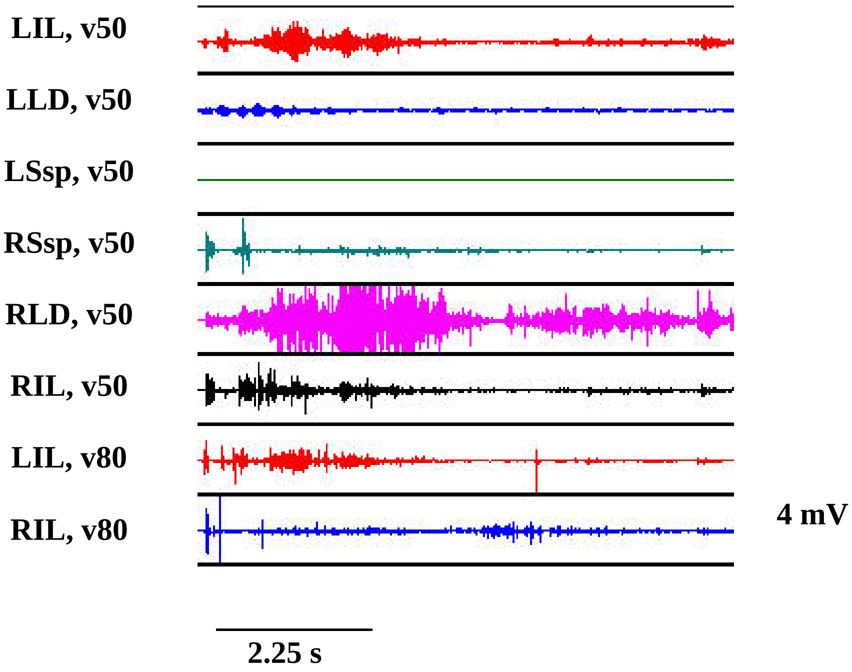


Figure A-4 Epaxial muscle activity of ventral bending during the initial coil formation phase of constriction for *Python molurus* no.1 with live prey.

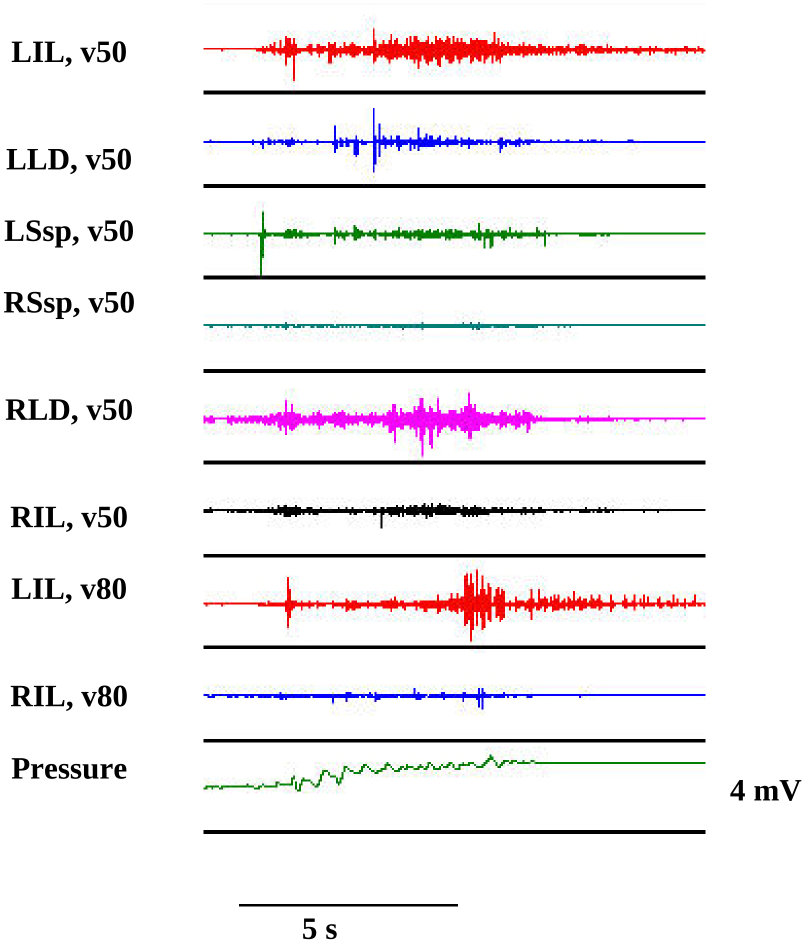


Figure A-5 Epaxial muscle activity of ventral bending during the initial coil formation phase of constriction for *Python molurus* no.1 with dead prey.

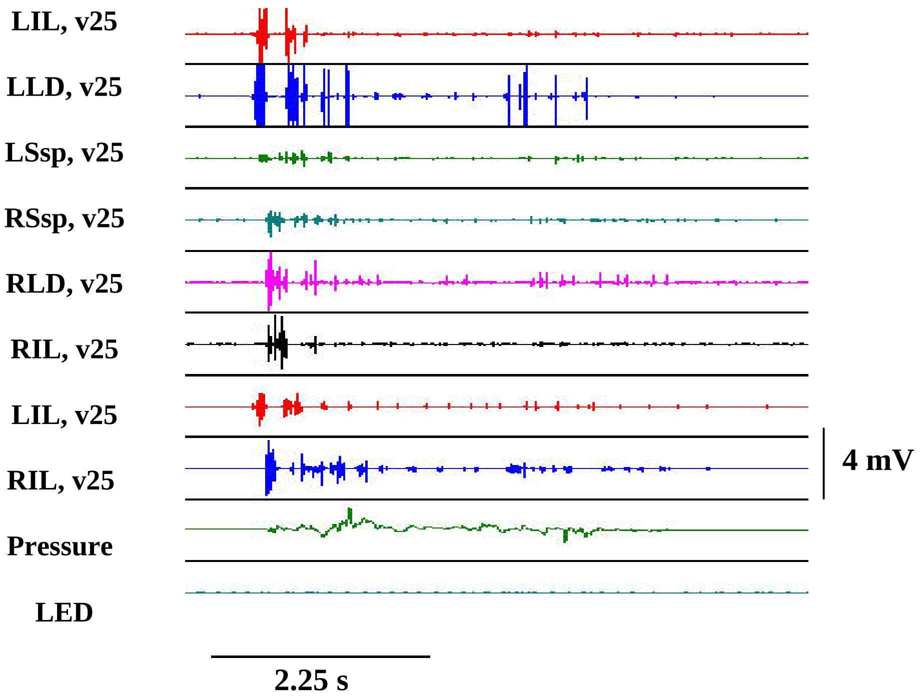


Figure A-6 Epaxial muscle activity of ventral bending during the initial coil formation phase of constriction for *Boa constrictor* no. 4 with live prey.

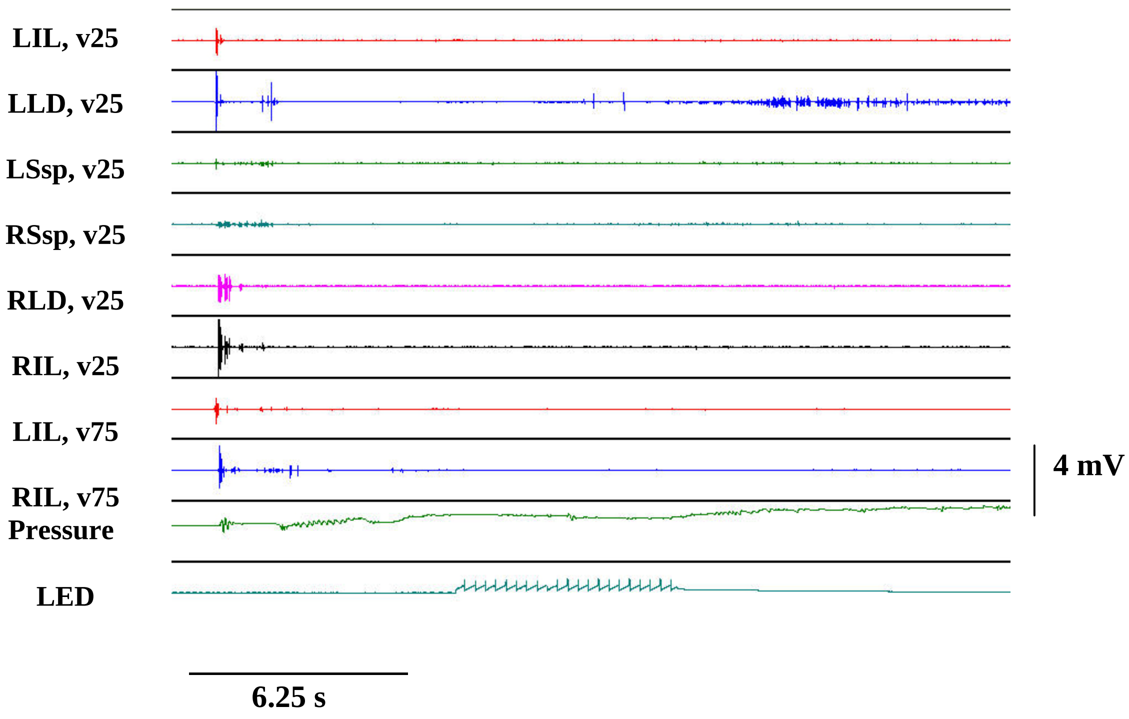


Figure A-7 Epaxial muscle activity o ventral bending during the initial coil formation phase of constriction for *Boa constrictor* no. 4 with dead prey.

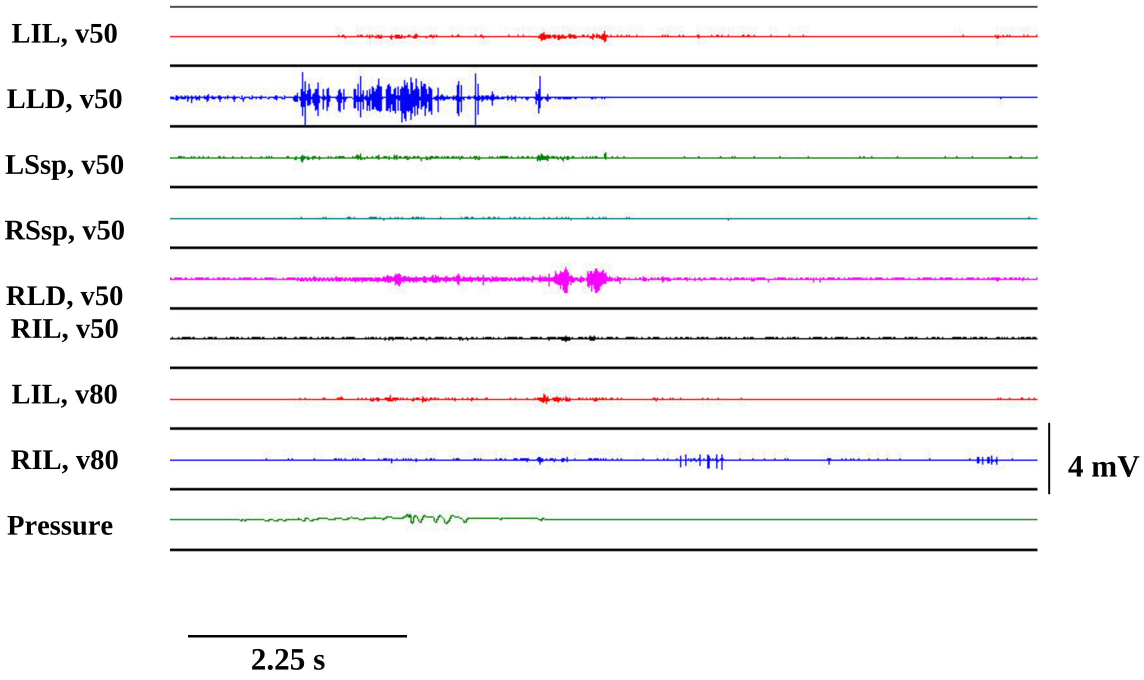


Figure A-8 Intermittent epaxial muscle activity of ventral bending during constriction for *Python molurus* no.5 with dead prey.

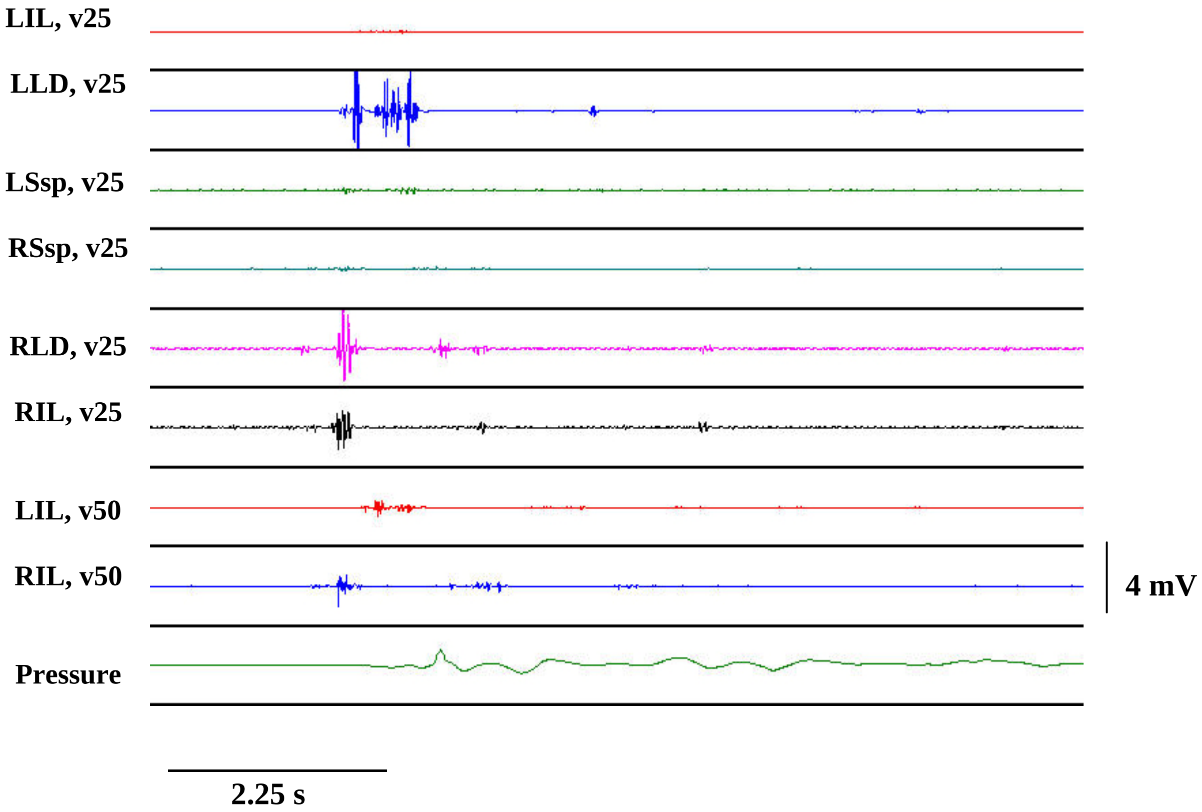


Figure A-9 Intermittent unilateral epaxial activity during response to prey struggles by *Boa constrictor* no. 2.

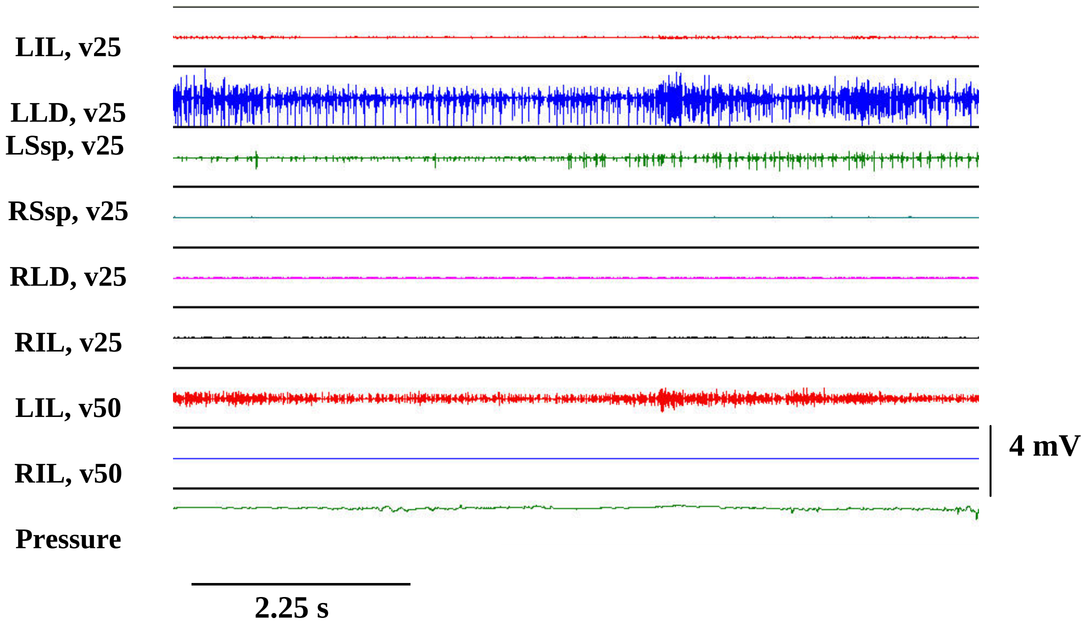


Figure A-10 Intermittent unilateral epaxial activity during response to prey struggles by *Boa constrictor* no. 4.

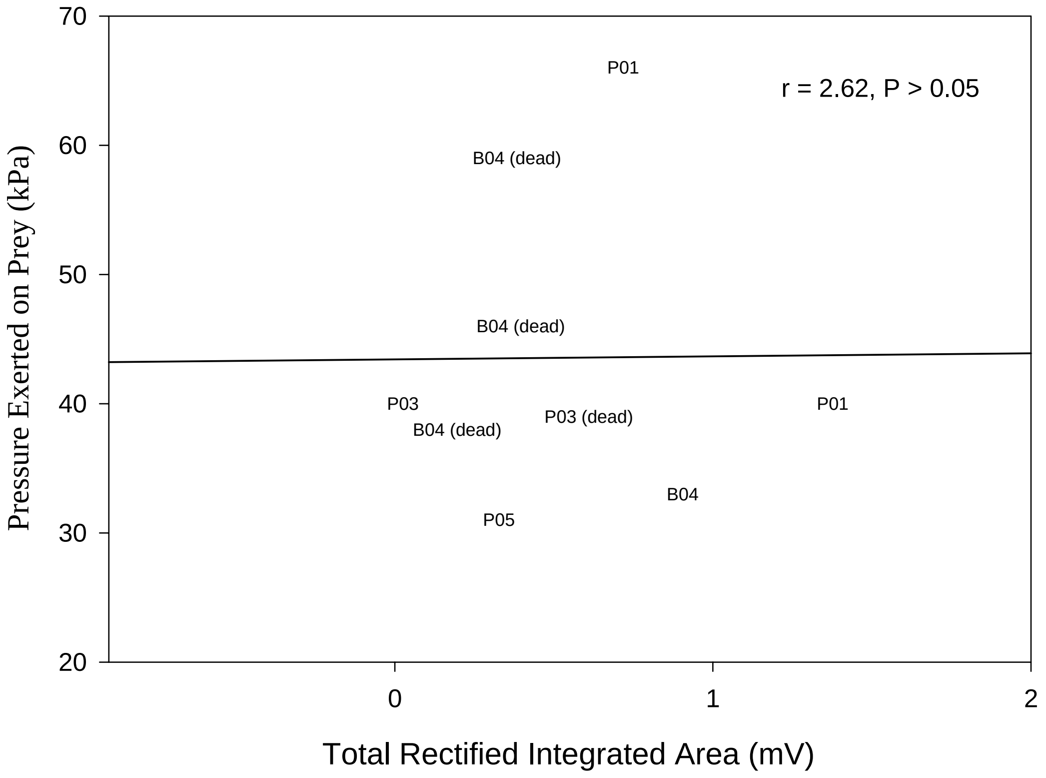


Figure A-11 Relationship between epaxial area active (total rectified area) and pressure during the initial coil formation phase for combined trials with boas and pythons. Trials with dead prey are noted in parenthesis.

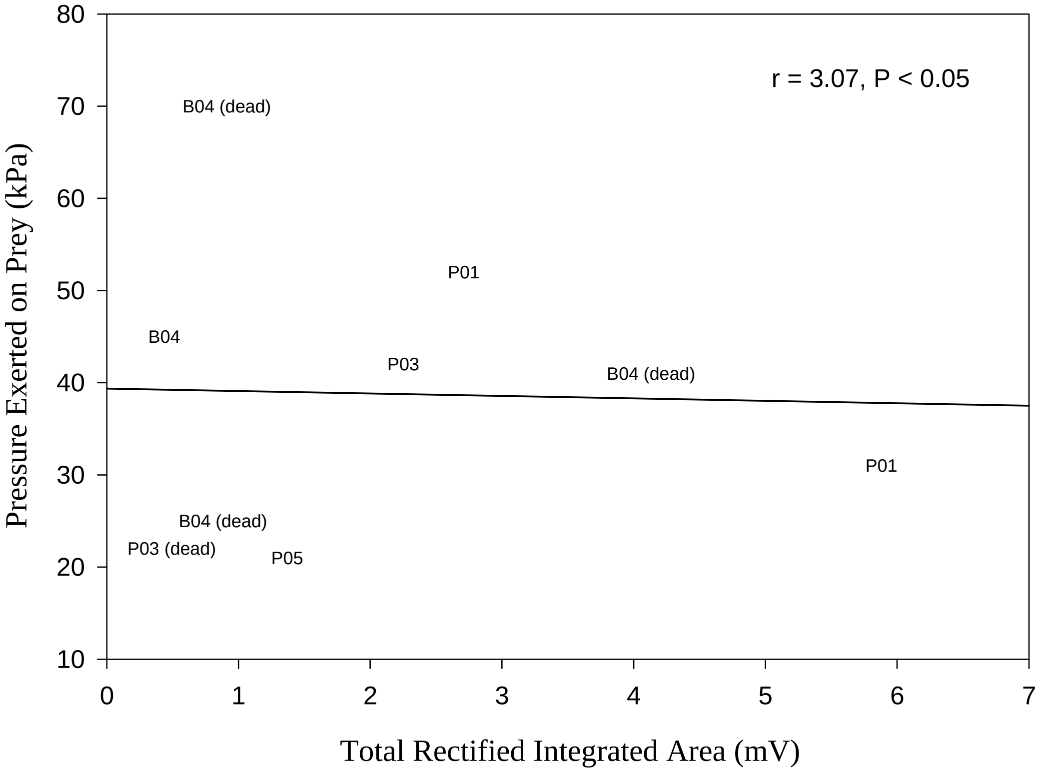


Figure A-12 Relationship between epaxial area active (total rectified area) and pressure during prey struggles for combined trials with boas and pythons. Trials with dead prey are noted in parenthesis.

PART 5

HOW DEEP IS CONSTRICTION BEHAVIOR?

HOMOLOGY FROM A HIERARCHICAL PERSPECTIVE

The concept of homology has long helped biologists describe and compare morphological features across a variety of organisms (Owen, 1843; Moment, 1945). Nonetheless, among concepts in evolutionary biology, the term homology “has a firm reputation as an elusive concept” (Wagner, 1989: 51).

Current understanding of the concept of homology seems to revolve around four major points. Firstly, homologous characters are those that are shared by two or more taxa and can be traced back to a common ancestor (Wiley, 1981; Mayr, 1982; Gans, 1985; Lauder, 1986; Striedter & Northcutt, 1991). Secondly, similarity is not a sufficient criterion of homology, as divergent evolution can produce homologous characters that appear dissimilar (Patterson, 1982). Also, independent transformations from the same ancestral character may produce non-homologous characters that are quite similar (Northcutt, 1984; Wiley, 1981). Thirdly, the evolutionary relationship among taxa is estimated by multiple lines of evidence: this is the core idea behind a phylogenetic approach to the concept of homology (Lauder, 1991, 1994). Lastly, homologous traits of organisms can be genetic, developmental, structural, functional, and behavioral, since whole organisms are comprised of a multitude of characters which can be sorted into a hierarchy of parts.

The points mentioned above help form a hierarchical phylogenetic concept of homology which can be applied to all types of data. In fact, the more levels or classes of that are examined within an organism, the deeper the understanding of character transformation as well as the organism as a whole (Brooks & McLennan, 1991; Vrba & Eldredge, 1984; Lauder, 1981).

PHYLOGENETIC PATTERNS

In this dissertation, I applied a hierarchical phylogenetic concept of homology to examine constriction behavior. The overall goal of my study was to examine the phylogenetic pattern of congruence for constriction behavior at different hierarchical levels. As described earlier, constriction, a key behavioral innovation in snakes, is a specialized prey restraint method. Constriction was the first behavioral homology to be recognized at the familial level and remains an excellent example of how behavior can change over evolutionary time and the importance of historical processes (Greene & Burghardt, 1978). Although constriction is homologous across the majority of snake lineages, there is variation in constriction patterns among basal, intermediate, and derived snake taxa, along with differences in their coil application pattern. The mechanisms of this variation for constriction pattern and coil application have yet to be examined. I hypothesized that the variability in prey restraint behavior and coil application may be linked to different levels of organization. I tested how prey restraint behavior and coil application differed between basal and intermediate snake taxa and whether the underlying physiology could be linked to behavioral variation. Using ethological methods, kinematics and electromyography, I revealed that behavioral variation across snake taxa was concordant with epaxial muscle activity patterns.

In Part 2, I polarized variable prey restraint behavior and coil application pattern by examining one basal and two intermediate snake lineages. Specifically, I found that *Loxocemus bicolor,* a basal snake taxon, is able to employ three different prey restraint behaviors (simple seizing, constriction and looping) in response to prey size and status. These responses are more diverse than intermediate snake lineages but are less diverse compared to prey restraint responses documented for derived snake lineages. Therefore, the ability to vary prey restraint behavior was most likely the basal pattern for snake lineages that constrict prey. The more consistent response to prey evolved in intermediate snake taxa (early macrostomates). Boas and pythons usually capture prey by the anterior and restrain prey by coiling around them, irrespective of prey characteristics.

In Part 3, I documented the kinematics and underlying epaxial muscle activity patterns during constriction for the basal snake *Loxocemus bicolor*. *Loxocemus bicolor* used lateral bends to coil around prey items in a manner similar to colubroid taxa (Greene, 1977; Milostan, 1989). Lateral bending corresponds with unilateral epaxial muscle activity patterns during constriction. In general, epaxial muscle activity was observed in a short burst during the initial coil formation phase of feeding. Two observations suggest that other axial muscles are contributing to the constriction posture: 1) epaxial muscles were not active although constriction postures were maintained, and 2) Pressures exerted on the prey items steadily increased or decreased while the epaxials were not active. Intermittent pressure increases also suggest that snakes only exert as much force as necessary to subdue prey.

In Part 4, I comparatively examined epaxial muscle activity patterns for two intermediate snake lineages, *Boa constrictor* and *Python molurus* and compared these results with my results in Part 3 as well as to the EMG study performed by Moon (2000). Boas and pythons exhibited mostly ventral bending when coiling around prey items. Ventral bending corresponded with bilateral epaxial muscle activity patterns. Sometimes bilateral and unilateral activity patterns were observed during constriction trials with *Boa* *constrictor*. Although boas did not vary their prey restraint behavior with respect to prey size or status in Part 2 of my study, epaxial muscles fired in response to prey struggles. Therefore, boas and pythons can incorporate some sensory feedback into their constriction coils at the physiological level.

From these data, I can draw the following conclusions about the evolution of epaxial muscle activity patterns during constriction: 1) Epaxial muscle activity patterns exhibit intermittent activity patterns during constriction behavior for a basal species, two intermediate snake species and two highly derived snake species suggesting that intermittent epaxial activity is a homologous activity pattern across snakes. 2) Unilateral muscle activity patterns were predominant in the basal taxon, *L. bicolor* and were common in derived snake taxa documented by Moon (2000). Bilateral epaxial muscle activity patterns were predominant in intermediate lineages and present in derived snake lineages. Therefore, similar to prey restraint behaviors, three epaxial muscle activity patterns were observed: mostly unilateral, mostly bilateral and mixed. Lateral bending and unilateral epaxial muscle activity support the more variable prey restraint behaviors observed in basal and derived snake taxa. Ventral bending and bilateral activity support the highly stereotyped behavior patterns observed in intermediate snake taxa. A ‘mixed’ epaxial activity pattern supports highly variable prey restraining methods as observed in gopher snakes and kingsnakes (Moon, 2000; Greenwald, 1978). Thus the diversity of muscle activity underlying prey restraint behavior can be correlated with variability in prey restraint postures.

With these data along with additional observations I have made throughout my studies of feeding behavior, I can piece together a historical scenario for the evolution of prey restraint behavior in snakes. Firstly, I try to emphasize the diversity of prey restraint patterns across the different taxonomic groups of constrictors from an ontogenetic perspective.

Few studies have examined the ontogeny of constriction behavior but those that have, examined intermediate (Greene, 1977; Milostan, 1989) and derived snake groups (Mori, 1991, 1993 a, b, 1994, 1995; Mehta, 2003). From these studies, it is apparent that intermediate snakes, neonate boas and hatchling pythons, constrict prey similar to the adults of the species. On the other hand, new born snakes from derived lineages can exhibit similar constriction patterns to the adults as well as very different prey restraint postures from the adults of the species. Studies that have examined the ontogeny of constriction behavior in derived snakes also reveal that there is a certain amount of variation in hatchling feeding behavior even within the same genus as exhibited in the genus Elaphe (Mori, 1991, 1993, 1994, 1995; Mehta, 2003). Thus, young, inexperienced snakes, mostly from derived snake lineages reveal that specialized motor patterns exhibited in adults can have distinct developmental histories in different taxa (Burghardt, 1978; 1993).

From first-hand observations of *Loxocemus bicolor* and *Xenopeltis unicolor* hatchlings, those that coil prey during their first feeding encounter, constrict with the same prowess as the adults (Mehta, unpubl. data). This suggests there are also distinct differences in the ontogeny of constriction behavior between basal, intermediate, and derived snake lineages.

In Figure A-1[1], I separate snake prey restraint behavior into three responses. In Mode I, basal snake lineages (*Loxocemus bicolor* and *Xenopeltis unicolor*) vary prey restraint behavior with respect to prey size and status. Hatchlings and neonates of these basal taxa can constrict prey during their first encounter, and if constriction is employed, the loop application pattern is similar to adults of the species. Snakes that exhibit Mode II are intermediate snake taxa. In Mode II, coiling around prey is the predominant prey restraint pattern, although simple seizing can be observed infrequently (see Part 2). Hatchlings and neonates of these intermediate lineages constrict prey similar to the adults of the species.

Derived snake taxa fall into the Mode III category. In Mode III, snakes exhibit a relatively large prey restraint repertoire that is highly correlated with prey characteristics (size, activity level, status (live vs dead), and prey type (i.e, mouse, frog, lizard). Neonates and hatchlings of Mode III snakes can exhibit similar prey restraint behaviors as the adult or exhibit highly erratic prey restraint behaviors that gradually become more consistent over time through experience and maturation.

Ecological selection for the evolution of constriction patterns-The prey restraint behavior, constriction, is thought to have evolved prior to the origin of rodents (Greene, 1994). Greene (1983) suggested that early snakes were capable of subduing prey of relatively large weight rations (WR) irrespective of ingestion ration (IR). This is exemplified in snakes that consume elongate organisms such as eels. Early snake lineages that were not insect specialists probably preyed on a variety of heavy bodied prey items that exhibited relatively low IRs or that could be manipulated enough so that

---

[1] All figures are located in the Appendix.

the snake’s skull could pass over the prey. Salamanders, lizards, small fishes and frogs could have easily represented the prey of earlier snake lineages. These prey items, however, vary in shape, length, and activity level, among other characteristics. Thus, it would have been beneficial for earlier snake lineages to develop a relatively diverse repertoire for restraining prey as some prey presumably necessitated more restraint than others. Having a large repertoire of prey restraint techniques may have presumably saved time and energy, although much empirical evidence is necessary to support this idea. A relatively diverse prey restraint repertoire may also have enabled semi-fossorial snakes to feed in the confined spaces that may have been good sources for prey such as burrows, spaces in decaying logs or low crevices found near or in the buttresses of trees.

Early macrostomate snakes, boas and pythons, are characterized by extremely kinetic skulls, fast strikes, a sedentary nature, ambush hunting techniques, and an ability to reach lengths longer than any other snake as well as a maximum mass many times heavier than other snakes. With the evolution of the streptostylic quadrate, a key morphological synapomorphy for the macrostomata, boas as a group are able to consume very large mammalian prey. Although the additional cranial kinesis allows macrostomates to ‘eat big’, immobilizing and subduing relatively large prey poses a different challenge. Prey size, specifically with adult prey, can be correlated with an increase in prey activity. This in turn, increases the chance of prey retaliation on the predator. Since boas and pythons can attain considerable weights and lengths, and are in need of relatively large meals in order to maintain such mass, there may have been increased selection for a more rigorous and almost ‘reflexive’ feeding pattern. In fact, the feeding behavior of extant boas and pythons reflect times that appear energetically expensive when consuming very small and very large prey, suggesting that there may be an optimal prey size for these large snakes (Shine, 1991). Thus, fast strikes and vigorous constriction may be responses to a selection regime favoring a life history tactic for feeding on large prey. These larger snakes tend to drop smaller prey from their diets as average energy content of smaller prey for these large animals are marginal.

Data from Part 2 reveal that mean prey restraint times and total feeding times for subadult boas are longer compared to those of *L. bicolor* and Erycine snakes. Boas did not vary dramatically in prey restraint time across individual prey categories compared to *L. bicolor* and Erycine snakes. When search, capture, and swallowing are energetically costly and time consuming, it is better to choose prey whose profit will outweigh any energy expenditure (Arnold, 1993). Thus, since boas have a narrow size foraging niche, it would be more beneficial to always coil around prey to reduce the risk of getting injured. A consistent constriction response is presumably correlated with physiological specializations such as oxygen capacity and anaerobic muscle output but necessitated further investigation.

Lastly, 80% of all extant taxa are members of the derived snake group, Colubroidea (Greene, 1997; Pough et al., 1998). Colubroids as a group are difficult to generalize as they reflect a diversity of sizes, behaviors, and can be found in a wide array of ecological habitats. Members of this broad group are relatively fast compared to Anilids and Boids and exhibit a large repertoire of feeding tactics. Most colubroid families tend to prey frequently (relative to basal macrostomates) on low WR items. This shift in feeding behavior may be correlated with faster locomotion, more stamina, and diverse locomotor abilities. In contrast to boas and other basal snakes, colubroids have specialized maxillary dentition for coping with a wide array of prey.

More types of prey probably played a role in diversifying the prey restraint repertoire of colubroid snakes. Colubroid snakes arose some time during the Miocene. This epoch is characterized by expansion of grassland habitats. Increased amounts of open habits in concurrence with the fragmentation of forests provided coverage for different types of rodents, birds, and other small organisms (Janis et al., 2000). These grassland inhabitants may have been included in the ancestral colubroid diet. Open grasslands and an increase of grazing mammals may have provided challenges as well as dietary opportunities for Miocene snakes. In fact, extant colubroid snakes that constrict prey may have needed to be hyper vigilant when constricting prey. Constriction in boas and pythons is so reflexive that gradations of external disturbance while these snakes are feeding have little affect on constriction behavior, although more controlled studies are necessary (Mehta, unpubl. data).

Colubroid snakes and some basal species appear to be very sensitive to disturbance while feeding. In fact, it has been suggested, from observations of the prey restraint behavior of a derived hatchling snake, that erratic looping behavior (to form a coil) may be advantageous. Hatchling trinket snakes were observed looping around prey with the posterior portion of their body, thus allowing the hatchling to maintain some vigilance while subduing and immobilizing prey (Mehta, 2001, 2003). Experience with prey has been shown to affect feeding behavior in colubroid snakes, suggesting that learning plays an important role in prey restraint behavior as well as other behaviors related to feeding. A learned response can be more valuable than an automatic/ reflexive response when prey items or prey quantities vary. Derived snake species also exhibit prey restraint patterns that may include many of what may be intermediate forms or precursors to constriction (de Queiroz & Groen, 2001; Gregory et al., 1980).

The erycine paradox-In this dissertation, the ability to vary prey restraint behavior and coil laterally around prey was shown for two basal lineages, Loxocemidae and the Erycinae. Why is the feeding behavior of Erycine snakes more similar to those of *L. bicolor* than to *Boa constrictor*? In general, Erycine snakes, both old and new world forms show specializations for burrowing and are perhaps not closely related to boas (Kluge, 1991, 1993; Greene, 1997). These snakes feed on mainly mammalian prey (Rodriguez-Robles et al, 1999) and possess the key innovation, the streptostylic quadrate which enables them to consume prey greater than their head diameter (Kluge, 1991, 1993), however, these prey items do not seem to approach the IRs for the prey of boas and pythons.

Many smaller macrostomate snakes, about which very little is known, are found in the families Tropidophiidae and Bolyeriidae. Although species in these families are thought to have evolved more recently, they seem to consume smaller prey items (Greene, 1997) which may be attributed to their semi-fossorial nature. Thus selection pressures for subterranean or semi-fossorial lifestyles, may be correlated with a larger prey restraint repertoire in snakes. This has been shown with derived snake lineages such as gopher snakes (Rudolph et al. 2002) as gopher snakes can be observed restraining prey with more than one prey restraint method while foraging in pocket gopher (*Geomys breviceps*) holes.

Phylogenetically meaningful taxa in comparative studies-In Parts 2-4, I studied phylogenetically relevant taxa in order to examine the homology of constriction behavior. My experiments centered around *Loxocemus bicolor*, a basal alethinophidian snake, Old and New World sand boas, the Erycines and two intermediate macrostomate lineages, boas and pythons. The many feeding observations observed under a controlled setting not only shed light on the evolution of constriction behavior but on the prey capture phases of the snake predatory cycle as well as other previously unstudied behavior patterns.

Prey size and status affected the capture behavior for *L. bicolor* suggesting that individuals belonging to this basal lineage can detect differences in prey before capturing and restraining prey. This was easily observed on video, especially with dead prey. Individual *L. bicolor* would come in close contact with prey (~ 2cm in distance) and wait while tongue-flicking. If the prey moved, individuals would retract their head and wait. If no movement was detected, the snakes would approach the prey very slowly and tongue flick so that the tips or tines of their forked tongues touched the prey. If the prey continued to be motionless, snakes would tongue-flick up and down the body of the prey item and then seize the head of the prey. When prey items suddenly became active when snakes were in reaching distance, snakes would employ one of the following two strategies. In the first strategy, snakes would retract their heads and wait in the ambush posture to capture prey as mentioned above. During the second strategy, snakes would circle the arena and try to re-approach the prey using an active searching technique in which the snake would open its mouth and try to grasp the prey when the prey came in reaching distance of the snake (Fig A-2: A). The former of these two strategies seemed more successful. As with the latter technique the snake would miss the prey three or more times.

Regardless of prey size and status, boas and erycines captured mice by the head and shoulders using the fast strikes described by Cundall & Deufel (1999). Both snake lineages would employ an ambush strategy for SA and LA prey, however, Erycines would switch between an actively foraging strategy and an ambush strategy for SA, SD and LD prey (Fig A-2: B & C).

As far as I know, capture behaviors have never been reported for Erycines or *L. bicolor*. Details of these behavior patterns will be addressed elsewhere. In short, Erycine snakes seem to exhibit Driving Scissors Strike (DSC) as described by Cundall and Deufel (1999). *Loxocemus bicolor* does not appear to strike prey but rather, grab prey using a small gape angle compared to macrostomates (Cundall & Deufel, 1999). Striking behavior may not have arisen until the Macrostomata along with the evolution of large the streptostylic quadrate which may allow for the mandibular depression seen in mandibular (MAN) strikes and the gape angles measured in driving scissors strikes (DSC) and palatomaxillary strikes (PMX). My observations suggest that striking may have been a behavioral key innovation in the evolution of macrostomate snakes and probably evolved with the key morphological innovation, the streptostylic quadrate.

Another interesting behavior pattern was observed during feeding in *Loxocemus bicolor*. In April 2002, I observed one of my larger *L. bicolor* individuals hold its tail up in the air and wave it back and forth while attempting to locate the SA prey item in the arena. This behavior was performed 141 s into the feeding trial. The first time the behavior was observed the lower half of the body along with the tail whipped to one side when the prey scampered past the snake. Around 180s into the trial the snake curled its tail in the air and slowly waved it side to side as the mouse approached the lower 1/3 of the snake’s body. Around 262 s the snake waved its tail several times. During this set of waves, the mouse could not be seen in the video frame. At 338 s the snake waved its tail before capturing the mouse. The prey was restrained via constriction. Two horizontal loops were wound around the prey and the snake’s tail, outside of the coil, was elevated.

Since then, six other individuals have been observed performing a similar tail waving behavior. This movement pattern, which was only observed during feeding, was not part of the antipredator repertoire observed for *L. bicolor* (Mehta, unpubl. data). Many species of snakes have been known to caudal lure during feeding (Schuett et al. 1984; Rabatsky & Farrell, 1996; Mullin, 1999; Paralleada, 2002). Prey attraction by luring that is performed only by the caudal portion of the body of some snake species is thought to be an example of feeding mimicry (Neill, 1960; Heatwole & Davidson, 1976; Greene & Campbell, 1972; Schuett et al., 1984). This hypothesis serves well when the prey of the snake consumes worms. However, when the prey item of the snake is a mouse, mimicry seems less feasible. Very little is known about the natural history of *L. bicolor.* Mora (1987, 1991; Mora & Robinson, 1984) documented lizard, lizard eggs and sea turtle eggs to be in the diet of *L. bicolor.* Lizards may very well feed upon insects and other invertebrates, however, the caudal movements were not as slow and steady as the movements described for vipers (Schuett et al., 1984). If anything, the behavior observed in *L. bicolor* seems to be a type of intention movement or displacement behavior (Danje, 1950). An intention movement to displace excitatory behavior during feeding, especially at the crux of the capture phase, may have been the precursor to caudal distraction.

Snakes that exhibited this intention movement when feeding upon prey that consumed invertebrates such as worms may have had increased capture success.

CONCLUSIONS

Constriction behavior provides good material for evolutionary studies and as I have just outlined in this chapter, the behavior itself reflects morphological and ecological adaptations specific to each major snake group as indicated by the three modes (I, II, and III). Ecological variation seems to have historically played an important role in forming the prey restraint repertoire of snakes and continues to play an important role in the feeding behavior of extant snakes (de Queiroz & Groen, 2001; Mori, 1994). Although studies have shown that homologous traits at the morphological or physiological levels need not be homologous at the functional level (Lauder, 1983, 1993), my study revealed how variation of a homologous behavior at the ethological level is concordant with the underlying muscle physiology. As the prey restraint behaviors and coil application patterns changed under different selection regimes, the underlying epaxial muscle activity patterns changed across snake lineages as well.

The key innovation, constriction, is tightly coupled to its underlying physiology. This study illustrates that one possible mechanism driving the differences in constriction postures across snake taxa is underlying muscle activity pattern. When examining epaxial muscle activity across snake lineages, muscle activity appears to also be homologous. Thus constriction is homologous at both the ethological, functional, and physiological levels.

LITERATURE CITED

Arnold, S.J. 1993. Foraging Theory and Prey-size-predator size relations in snakes. In: Snakes: Ecology and behavior (Richard A. Seigel and Joseph T. Collins, eds.). New York: McGraw-Hill. Pp. 87-115.

Burghardt, G.M.1978. Behavioral ontogeny in reptiles: whence, whither, and why? In: The Development of Behavior. (Burghardt, G.M. & Bekoff,M., eds.) Garland, New York. Pp.149-174.

Burghardt, G.M. 1993. Comparative Imperative: genetics and ontogeny of chemoreceptive prey responses in natricine snakes. Brain, Behavior, and Evolution 41:138-146.

Cundall, D., and A. Deufel. 1999. Striking patterns in booid snakes. Copeia 1999: 868-883.

Cundall, D.and H. W. Greene. 2000. Feeding in snakes. In: Feeding: form, function, and evolution in tetrapod vertebrates.(K. Schwenk, ed.), Academic Press, San Diego. Pp. 293-333.

Daanje, A. 1950. On locomotory movements in birds and the intention movements derived from them. Behaviour 3: 49-98.

De Queiroz, A. and R.R. Groen. 2001. The inconsistent and inefficient constricting behavior of Colorado western terrestrial garter snakes, *Thamnophis elegans*. Journal of Herpetology 35: 450-456.

Gans, C. 1985. Differences and similarities: Comparative methods in mastication. American Zoologist 25: 291-301.

Greene, H.W. 1977. Phylogeny, convergence, and snake behavior. Unpublished P.h.D dissertation, University of Tennessee, Knoxville, Tennessee.

Greene, H.W. 1983. Dietary correlates of the origin and radiation of snakes. American Zoologist 23: 431-441.

Greene, H.W. 1994. Homology and behavioral repertoires. In: Homology, the Hierarchical Basis of Comparative Biology. (B.K. Hall, ed.).Academic Press, San Diego. Pp.369-391.

Greene, H.W. 1997. The Evolution of Mystery in Nature. University of California Press.

Greene, H.W. and Campbell, J.A. 1972. Notes on the use of caudal lures by arboreal green pitvipers. Herpetologica 28: 32-34.

Greene, H.W. and G. M. Burghardt 1978. Behavior and Phylogeny: Constriction in Ancient and Modern snakes. Science, v. 200: 74-76.

Greenwald, O.E. 1978. Kinematics and time relations of prey capture by gopher snakes. Copeia. 1978: 263-268.

Gregory, P.T., J. M. Macartney, and D.H. Rivard. 1980. Small mammal predation and prey-handling behavior by the Garter snake, *Thamnophis elegans.* Herpetologica, 36: 87-93.

Heatwole, H. and Davidson, E. 1976. A review of caudal luring in snakes with notes on its occurrence in the Saharan sand viper, *Cerastes vipera*. Herpetologica 32: 332-336.

Janis, C.M., J.D. Theodor, and M. Jessica. 1992. Miocene ungulates and terrestrial primary productivity: Where have all the browsers gone? Proceedings of the National Academy of Sciences of the United States of America 2000, 97: 7899-7904.

Kluge, A.G. 1991. Boine phylogeny and research cycles. Miscellaneous Publications of the Museum of Zoology, University of Michigan 178: 1-58.

Kluge, A.G. 1993. *Calabaria* and the phylogeny of erycine snakes. Zoological Journal of the Linnean Society 107: 293-351

Lauder, G.V. 1986. Homology, analogy, and the evolution of behavior. In: Evolution of animal behavior (M.H. Nitecki and J.A. Kitchell, eds.). Oxford University Press, New York. Pp 9-40.

Lauder, G.V. 1991. Biomechanics and evolution: integrating physical and historical Biology in the study of complex systems. In: Biomechanics and evolution. (J.M.V. Rayner and R.J. Wootton, eds.). Cambridge University Press, Cambridge. Pp.1-19.

Lauder, G.V. 1994. Homology, Form, and Function. In: Homology: The hierarchical basis of comparative biology.(BK. Hall ed.), Academic Press. Pp. 155-196.

Mayr, E. 1982. The growth of biological thought: diversity, evolution, and inheritance. Harvard University Press, Cambridge, MA.

Mehta, R.S. 2001. Prey-handling behavior in newly hatched trinket snakes, *Elaphe helena.* Unpublished Masters Thesis, University of Texas, Tyler, Texas.

Mehta, R.S. 2003. The effects of prey-size on the prey-handling behavior of hatchling *Elaphe helena*. Herpetologica 4:471-476.

Milostan, M. 1989. The comparative ontogeny of prey capture and immobilization in *Boa constrictor* and *Elaphe obsolete*, with comments on the origin and evolution of constriction in boid and colubrid snakes. Unpublished M.S. thesis, Central Michigan University, Mt. Pleasant.

Moment, G.B. 1945. The relationship between serial and special homology and organic similarities. American Naturalist 79: 445-455.

Moon, B.R. 2000. The mechanics and muscular control of constriction in gopher snakes (*Pituophis melanoleucus*) and a king snake (*Lampropeltis getula*). Journal of Zoological Society, London 252: 83-98.

Mora, J.M.1987. Predation by *Loxocemus bicolor* on the eggs of *Ctenosaura similis* and *Iguana iguana*. Journal of Herpetology 21: 334-335.

Mora, J.M. 1991. *Loxocemus bicolor* (burrowing python). Feeding behavior. Herpetological Review 22: 61.

Mora, J.M. and Robinson, D.C. 1984. Predation of sea turtle eggs (*Lepidochelys*) by the snake *Loxocemus bicolor* Cope. Revista de Biologia Tropical 32: 161-162.

Mori, A. 1991. Effects of prey size and type on prey-handling behavior in *Elaphe quadrivirgata*. Journal of Herpetology 25: 160-166.

Mori, A.1993a. Does feeding experience with different size of prey influence the subsequent prey-handling behavior in *Elaphe climacophora* ? Journal of Ethology 11: 153-156.

Mori, A.1993b. Prey handling behavior of neonatal rat snakes, *Elaphe taeniura* and *E.dione* (Colubridae). Japanese Journal of Herpetology 15**:** 59-63.

Mori, A.1994. Prey-handling behavior of newly hatched snakes in two species of the genus *Elaphe* with comparison to adult behavior. Ethology 97: 198-214.

Mori,A.1995. Prey handling behavior of the young rat snake *Elaphe taeniura* (Squamata:Reptilia).Memoirs of the Faculty of.Science, Kyoto University (Serial Biology)16: 43-47.

Mullin, S.J. 1999. Caudal distraction by rat snakes (Colubridae, Elaphe): a novel behavior used when capturing mammalian prey. Great Basin Naturalist 59(4), October 1999: 361-367.

Neill, W.T. 1960. The caudal lure of various juvenile snakes. Quarterly Journal of the Florida Academy of Sciences 23: 173:200.

Northcutt, R.G. 1984. Evolution of the vertebrate central nervous system: patterns and processes. American Zoologist 24: 701-716.

Owen, R. 1843. Lectures on the comparative anatomy and physiology of the invertebrate Animals, Delivered at the Royal College of Surgeons, in 1843. Longman, Brown, Green, and Longmans, London.

Parellada, X; Santos, X. 2002. Caudal luring in free-ranging adult Vipera latasti. Amphibia- Reptilia 23: 343-347.

Patterson, C. 1982. Morphological characters and homology. In: Problems of phylogenetic reconstruction. (K.A. Joysey and A.E. Friday, eds.), Systematics Association Special Volume 25. Academic Press, London. Pp 21-74.

Pough, H. et al. 1998. Herpetology. Prentice Hall. Upper Saddle River, NJ, 07458.

Rabatsky, A.M and Farrell, T. M. 1996. The effects of age and light level on foraging posture and frequency of caudal luring in the rattlesnake, *Sistrurus miliarius barbouri*. Journal of Herpetology 30: 558.

Rodriguez, Robles, J.A., Bell, C.J., and Greene, H.W. 1999. Gape size and the evolution of diet in snakes: feeding ecology of Erycine boas. Journal of Zoology (London) 248: 49-58.

Rudolph, D.C., Burgdorf, S.J., Conner, R.N., Collins, C. S., Saenez, D. Schaefer, R.R., Trees, T., Duran M., Ealy, M, and Himes, G. 2002. Prey handling and diet of Louisiana pine snakes (Pituophis ruthveni) and black pine snakes (P. melanoleucus lodingi), with comparisons to other selected colubrid snakes. Herpetological Natural History 9: 57-62.

Schuett, G W; Clark, D L; Kraus, F. 1984. Feeding mimicry in the rattlesnake *Sistrurus catenatus*, with comments on the evolution of the rattle. Animal Behaviour 32(2) 1984: 625-626.

Shine, R. 1991. Why do larger snakes eat larger prey items? Functional Ecology 5: 493-502.

Striedter, G.F. and R.G. Northcutt. 1991. Biological hierarchies and the concept of Homology. Brain, Behavior, and Evolution 38: 177-189.

Wagner, G.P. 1989. The biological homology concept. Annual Review of Ecology and Systematics. 20: 51-69.

Wiley, E.O. 1981. Phylogenetics: The theory and practice of phylogenetic systematics. Wiley, New York.

APPENDIX

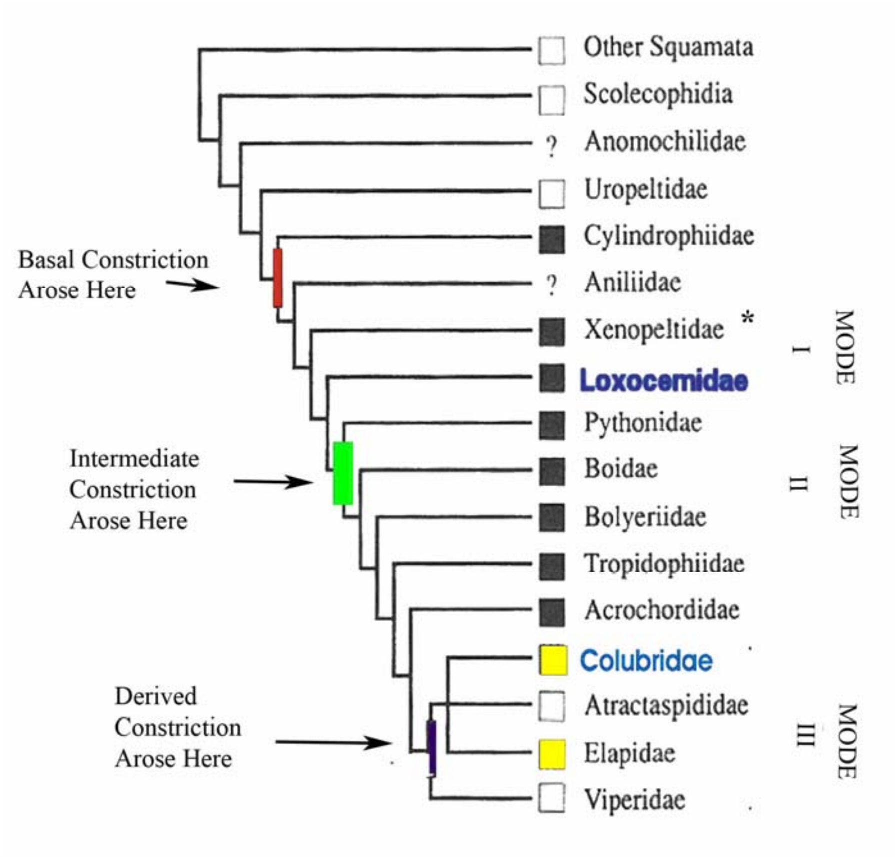
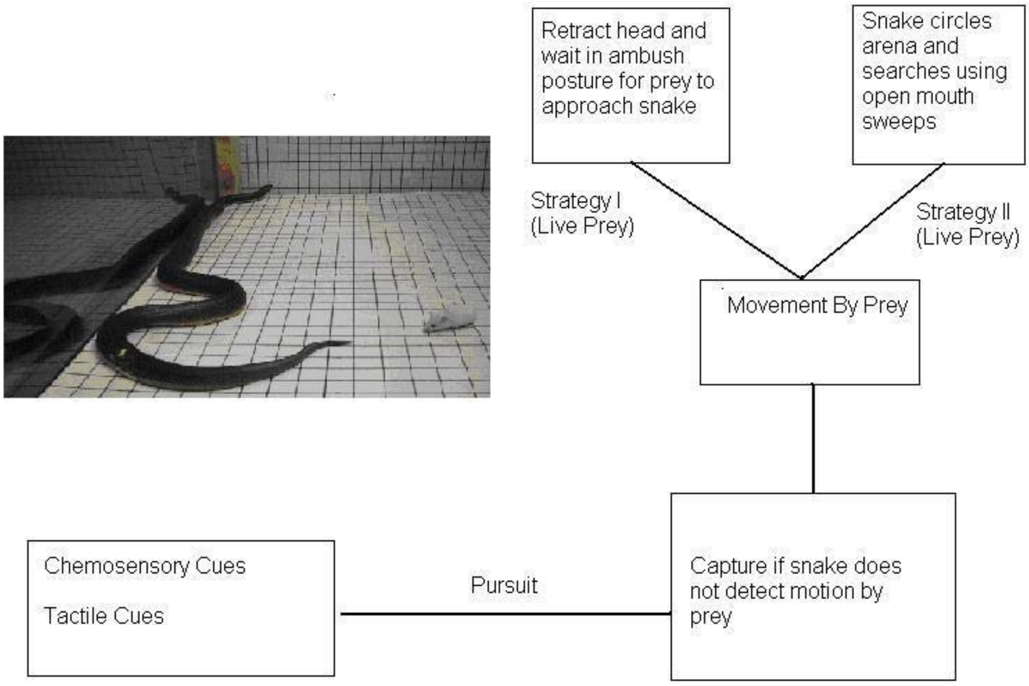


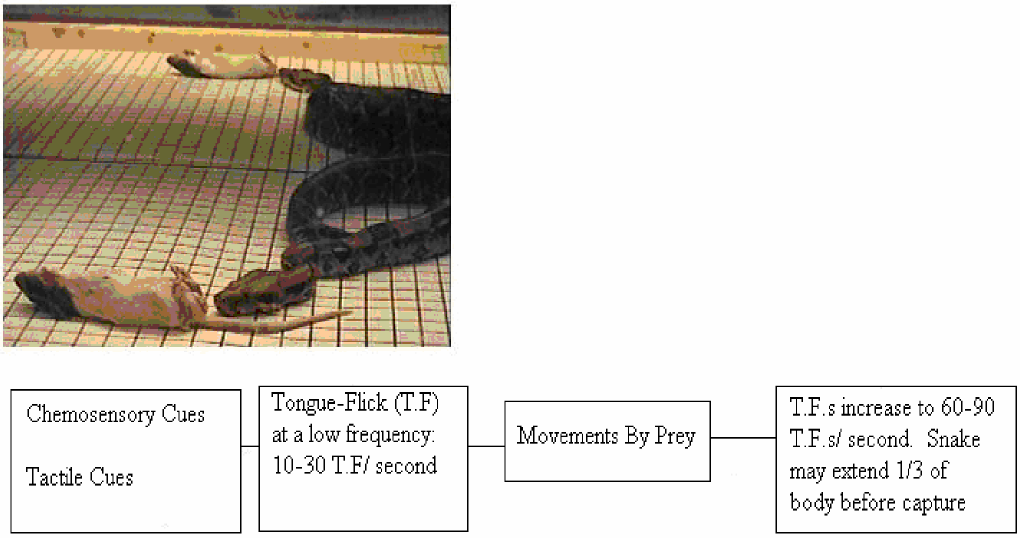
Figure A-1 Phylogeny of the Serpentes compiled from morphological (Cundall et al., 1993; Kluge, 1991, 1993; Rieppel, 1978, 1988) and molecular data (Cadle, 1994; Cadle et al., 1990). The lineage Loxocemidae (in bold) represents the sister taxon to boas and pythons. The three modes (I, II, and III) indicate feeding patterns in snakes. See text for details. *****I observed the effects of prey size and status on prey restraint behavior of four Asian sunbeam snakes, *Xenopeltis unicolor*. Asian sunbeam snakes restrain prey using the behaviors: loop, pinion and coil. These prey restraint behaviors are affected by prey size and status.

Figure A-2 Schematic of the capture strategies for *Loxocemus bicolor, Boa constrictor, and* Erycine snakes from stimulus control studies with small (live and dead) and large (live and dead) prey items

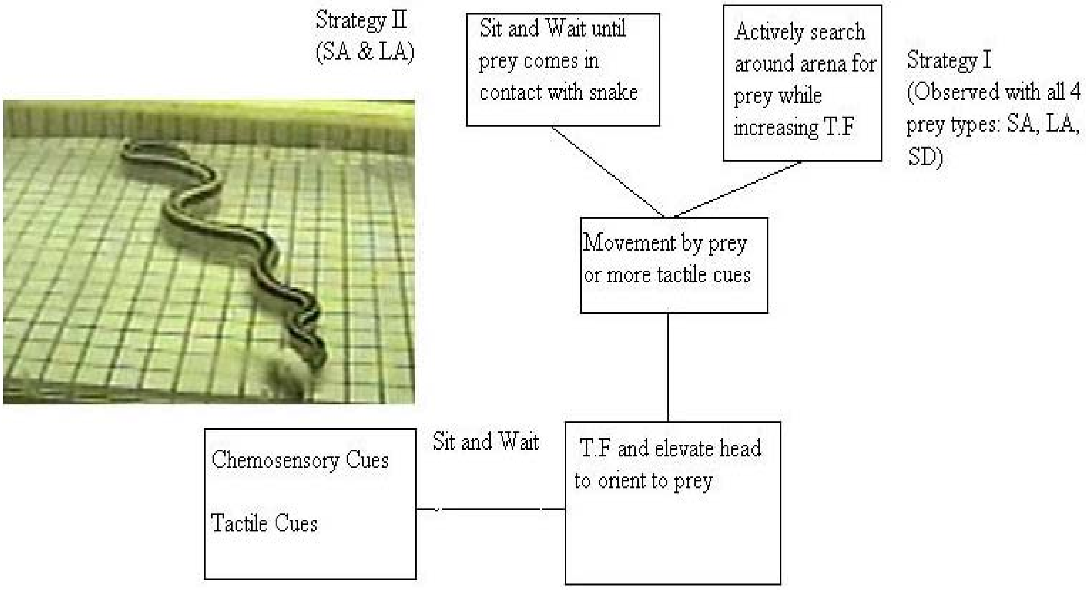
A.


Retract head and wait in ambush posture for prey to approach snake
Snake circles arena and searches using open mouth sweeps
Strategy I (Live Prey)
Strategy II (Live Prey)
Movement By Prey
Chemosensory Cues
Tactile Cues
Pursuit
Capture if snake does not detect motion by prey

B.


Chemosensory Cues
Tactile Cues
Tongue-Flick (T.F) at a low frequency: 10-30 T.F/ second
Movements By Prey
T.F.s increase to 60-90 T.F.s/ second. Snake may extend 1/3 of body before capture

C.


Strategy II
(SA & LA)
Sit and Wait until prey comes in contact with snake
Actively search around arena for prey while increasing T.F
Strategy I
(Observed with all 4 prey types: SA, LA, SD)
Movement by prey or more tactile cues
Sit and Wait
Chemosensory Cues
Tactile Cues
T.F and elevate head to orient to prey

VITA

Rita S. Mehta was born in Fountain Valley, CA. on November 23, 1976. She spent her first five years of life in Calcutta, India and Ipoh, Malaysia. Her family moved back to southern California during the summer of 1981. From 1981 until 1995, Rita grew up in Huntington Beach, CA. She received a Bachelors of Arts from the University of California at Berkeley in 1999. In August of 1999 she entered the Masters program in Biology at the University of Texas, Tyler. She received her Master’s degree in May 2001. In August, 2001 she began to pursue her Doctorate of Philosophy at the University of Tennessee in Knoxville, Tennessee. After graduation she looks forward to a post doctoral appointment at the University of California at Davis.