Contextual Flexibility: Reassessing the Effects of Prey Size and Status on Prey Restraint Behaviour of Macrostomate Snakes

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Abstract

Contextual flexibility in prey restraint behaviour has been documented in advanced snakes (Colubroidea), but the degree of flexibility for earlier snake lineages has been largely unstudied. We document the prey restraint behaviour of five snake species belonging to three early macrostomate lineages: Loxocemidae, Erycinae and Boidae. Species from these lineages were chosen for this study because they utilize similar prey resources but exhibit different ecological habits that may have important consequences on prey restraint behaviour. Snakes (n = 27) were studied in a systematic experimental design assessing the effects of mouse size (small and large) and status (live and dead) across a total of 216 feeding trials. *Loxocemus* and Erycine snakes were highly flexible in their prey restraint behaviour patterns and these varied across prey category. Individuals of *Boa constrictor* exhibited very little contextual flexibility in feeding behaviour, confirming earlier reports. Flexibility in prey restraint behaviour corresponded with loop application pattern, whether the snake bent laterally or ventrally when forming a loop around prey. Our study is the first to show that early macrostomate snakes exhibit flexible prey restraint behaviours. Thus, our results suggest that flexibility in predatory behaviour may be more widespread across snake taxa than previously thought and we offer hypotheses for the observed interspecific differences in snake feeding behaviour.

Introduction

Animals interact with their environment in diverse ways. Behavioural flexibility, the ability to vary the deployment of behavioural patterns in response to different situations, is an important aspect of the phenotype of many organisms (Caro & Bateson 1986; Helfman 1990; Gordon 1991; Kieffer & Colgan 1993; Mercier & Lenoir 1999). The aggregate set of available behaviours, commonly known as a behavioural repertoire, represents the variety of ways in which an organism can respond to a particular situation. Understanding when and why specific behaviours are employed as well as their flexibility has the potential to shed light on more general questions such as under what conditions behavioural flexibility evolves (Gordon 1991).

Many predatory organisms have the ability to shift among different behaviours or modes while feeding (Helfman 1990). Flexibility in various aspects of the predatory cycle has been shown to be dependent upon proximate characteristics of the prey as well as ecological conditions that may affect the density of the prey or where prey can be found (Jaeger & Barnard 1981; Formanowicz et al. 1982; Brown 1986). Within the predatory cycle, prey handling is one common axis of behavioural diversification that may reveal interspecific divergence between predators utilizing similar resources. Prey vary in size, shape, elusiveness, antipredator adaptations and location.

Any number of combinations of these prey characteristics can influence the types of prey acquisition behaviours employed and, in turn, shape the behavioural flexibility of predators (Helfman & Clark 1986; Helfman & Winkelman 1991; Burghardt & Krause 1999; Souza et al. 2007). Determining how natural variation in prey characteristics affects prey acquisition by predators is an important step to understanding the development of predatory repertoires and ultimately the evolution of novel feeding strategies.

Snakes comprise a monophyletic clade of obligate predators that exhibit tremendous ecological and evolutionary diversity. The vast majority of the 3000 or so species of snakes are able to consume large and potentially dangerous prey (Cundall & Greene 2000). The widespread presence of constriction, a specialized prey restraint behaviour in snake lineages that are known to consume relatively bulky prey, suggests that large prey species, capable of retaliating against the predator, necessitate specialized restraint tactics.

During constriction a snake restrains prey by applying two or more body loops around the prey while exerting pressure (Greene & Burghardt 1978; Greene 1983a, 1994). Constriction serves as an ideal topic for comparative evolutionary studies as it consists of a readily defined sequential modal action pattern (Burghardt 1973; Barlow 1977), varies across species, and occurs in lineages that are ecologically and morphologically diverse (Greene 1977; Greene & Burghardt 1978; Moon & Mehta 2007). Much of the work on prey restraint repertoires in snakes has focused on members of a large and diverse radiation, the Colubroidea, containing over 80% of all snake species. Many members of the Colubroidea, have a relatively large prey restraint repertoire and are known to vary their restraint behaviours in response to prey size, type and activity level (Greene & Burghardt 1978; Greenwald 1978; De Queiroz 1984; Milostan 1989; Gregory et al. 1980; Mori 1991, 1994, 1995; Rodriguez-Robles & Leal 1993; Mehta 2003). Based on observations of a small number of species, it has been suggested that other snake lineages, such as boas and pythons, are less capable of varying their prey restraint behaviour in response to proximate characteristics of the prey (Greene 1977; Milostan 1989).

The observation that prey restraint behaviour exhibits some degree of flexibility and that this flexibility varies interspecifically in colubrid snakes, suggests that factors such as phylogenetic history may shape the prey restraint repertoire of snakes. The extent of flexibility in prey restraint behaviour, however, has not been determined for most snake lineages, and it is premature to evaluate whether the present observations are due to shared descent or other factors such as physiology or ecology. Our goal here was to empirically evaluate the contextual flexibility of prey restraint behaviour for representatives of three early macrostomate lineages: *Loxocemus bicolor*, New and Old World Erycines (*Charina trivirgata*, *Charina bottae* and *Eryx muelleri*) and *Boa constrictor*. These five taxa were chosen for this study because they exhibit different ecological habits and molecular and morphological phylogenies indicate that two of these lineages, *L. bicolor* and 'Erycines' (*sensu latu*), may be potentially interesting for future comparative studies attempting to polarize behavioural characters in snakes (Tchernov et al. 2000; Scanlon 2006). Recent molecular studies suggest that *L. bicolor* is the sister taxon to pythons (Vidal & David 2004; Vidal & Hedges 2004; Vidal et al. 2007) while Old World and New World Erycines are boid snakes but are not each others closest relatives (Vidal et al. 2007). *Loxocemus bicolor* and Erycines exhibit both semi-fossorial and terrestrial habits, while *B. constrictor* is semi-arboreal and terrestrial. Despite these ecological differences, dietary data reveals considerable dietary overlap across these five species. Both *L. bicolor* and Erycines consume lizards, squamate eggs and small mammals (Mora & Robinson 1984; Mora 1987, 1991; Rodriguez-Robles et al. 1999; Rodriguez-Robles 2003), while larger Erycine snakes also tend to add larger mammals and birds in their diet (Rodriguez-Robles et al. 1999). *Boa constrictor* consumes mostly lizards, birds and mammals (Greene 1983a,b; Smith 1994; Sironi et al. 2000; Greene et al. 2003; Boback 2004). Therefore, any interspecific differences in the prey restraint repertoire of these boid predators, may suggest that other factors may shape the evolution of feeding behaviour in early macrostomate snakes.

Although Greene & Burghardt (1978) examined the constriction postures of boas and pythons on various substrates and with various prey items, a systematic stimulus control design was not used. A stimulus control study not only allows for close examination of any variability in behaviour, but this standard experimental design is ideal for comparative studies. This study is the first to systematically address the contextual flexibility, or lack thereof, in prey restraint behaviour for non-colubroid snakes. To examine the effects of prey characteristics on the predatory cycle, we varied two aspects of mammalian prey (*Mus musculus*) previously shown to affect

prey restraint behaviour in snakes: size (Mehta 2003) and status (De Queiroz 1984).

Materials and Methods

Twelve adult sunbeam snakes, *L. bicolor*, six subadult sand boas, *E. muelleri*, two neonate rubber boas, *C. bottae*, two adult rosy boas, *C. trivirgata* and five adult boa constrictors, *B. constrictor imperator,* on loan from commercial breeders and private collectors, were housed in the Ethology Lab at the University of Tennessee, Knoxville. Measurements of all snakes are shown in Table 1. Snakes were maintained individually in plastic containers (ranging from 260 × 180 to 460 × 240 mm) lined with 10 cm of shredded aspen substrate with water in ceramic bowls, available *ad libitum*. Larger animals were housed in larger containers. As rodents are included in the diet for all snake species used in this study, snakes were fed laboratory mice (*M. musculus)* biweekly. Mice (live and dead) comprised between 6% and 30% of an individual snake's relative mass. Room temperature was maintained at 28°C and photoperiod was on a 14L:10D cycle.

Feeding Trials

The general testing method was as follows: large snakes (>600 mm, n = 17), were placed in a 1206 × 584 × 457 mm plexiglass terrarium which served as the feeding arena. Smaller snakes (<600 mm, n = 10) were placed in a 914 × 457 × 457 mm plexiglass feeding arena. We used a 2 × 2 factorial design (small prey vs. large prey × live vs. dead) in which prey were administered using an 8 × 8 Latin square cyclic matrix. Snakes were tested twice in each of the four prey categories.

Experiments were initiated by placing live prey or positioning dead prey in the arena. After a 5-min period, an individual snake was introduced into the arena. Prey items were introduced first as pilot observations revealed that snakes explored more and fed less when prey were introduced second. A 10- to 14-d interval between feeding trials was maintained for the majority of snakes with the exception of *E. muelleri*. Individuals of *E. muelleri* fed less frequently, adopting a 17- to 20-d interval between trials with small prey and up to a 42-d interval when feeding upon large prey.

Ethical Note

Live prey are a critical component for laboratory-based snake feeding studies. Live prey offer both the chemosensory stimulation that snakes naturally encounter in the wild and tactile stimulation as snakes physically respond to live prey (Moon 2000). Pilot observations revealed that the snake species examined in this study behaved similarly towards thawed rodent prey purchased from commercial suppliers and mice that were killed in a nitrous oxide chamber 10–15 min prior to feeding. Therefore, to minimize the number of mice killed for our study, we purchased frozen mammalian prey from local pet stores and warmed them on an electric heating pad

Table 1: Sample sizes, mean ± SE for snout-vent length (SVL), mass, head width and mandible length for 27 individual snakes recorded at the start of our study, as well as mean ingestion ratio (IR) and mean mandible (MLR) ratio offered to each individual throughout the study

	SVL (mm)	Mass (g)	Head width (mm)	Mandible length (mm)	Small live IR (MLR)	Small dead IR (MLR)	Large live IR (MLR)	Large dead IR (MLR)
Loxocemus bicolor (n = 12)								
Mean	878.11	400.62	15.65	24.93	51.19 (50)	51.32 (50)	90.63 (89)	92.11 (90)
SE	186.42	112.74	0.65	1.06	1.31 (0.72)	1.52 (0.63)	5.6 (1.33)	3.24 (1.24)
Boa constrictor (n = 5)								
Mean	1475.33	1792.79	22.78	41.63	54.41 (50)	55.42 (50)	96.23 (90)	97.03 (90)
SE	367.40	60.31	1.20	0.43	3.22 (0.62)	4.12 (1.42)	4.44 (4.32)	3.14 (4.04)
Eryx muelleri (n = 6)								
Mean	453.34	102.61	7.71	17.56	52.11 (50)	54 (48)	91.53 (86)	93.41 (88)
SE	135.20	6.32	0.73	0.60	0.3 (0.34)	1.24 (2.31)	1.13 (0.8)	2.23 (1.14)
Charina bottae (n = 2)								
Mean	307.20	126.83	8.92	10.12	54.2 (51)	55 (50)	92.34 (85)	95.12 (88)
SE	12.50	9.44	0.41	0.34	0.45 (0.21)	0.32 (0.41)	1.21 (0.83)	1.01 (1.31)
Charina trivirgata (n = 2)								
Mean	363.10	74.42	9.89	11.15	53.81 (50)	54.31 (49)	93.11 (89)	95.32 (90)
SE	43.30	13.41	1.02	0.26	1.11 (0.12)	0.45 (0.33)	0.74 (0.58)	0.53 (0.71)

(to roughly $30 \pm 2°C$) before offering them to snakes.

Relative Prey Size

We were interested in the effects of prey size on the prey restraint behaviour of snakes that also vary in gape limitation (Cundall & Greene 2000) and size. Therefore, we use relative prey size to test for inter-specific differences in prey restraint behaviour. Although our study spanned a considerable amount of time and snakes grew during the course of the study, relative prey size allowed us to test for the effects of prey size on behaviour by keeping the predator–prey relationship consistent across time. We measured head width (HW; to the nearest 0.1 mm) and right mandible length (ML; to the nearest 0.1 mm) for all snakes. We used two methods for assigning mouse prey to size categories: ingestion ratios (IR) and mandible ratios (MLR). Ingestion ratios were calculated by dividing the largest circumference of the prey by the predator's HW (Loop & Bailey 1972; Greene 1983a). Mandible ratios were calculated by dividing the largest circumference of the prey by snake ML. We measured the circumference of the prey at the shoulder girdle and the pelvic girdle by placing the prey on a measuring tape and firmly wrapping the tape around the opposite side of the prey. The larger of these two measures were used to calculate IR and MLR. Although HW may be considered highly variable because of the flexibility of the quadrate-mandibular joint in snakes, we used HW in this study so that our data could be compared to other published studies on snake feeding behaviour that describe the predator–prey relationship in terms of IR. However, ML may be more informative for maximum performance than HW because it is a more realistic measure of the size of the oral cavity in snakes and is easier to measure. Aside from prey circumference, prey length is also an important variable that influences both prey restraint behaviour (Cundall & Greene 2000) and swallowing performance in snakes (Pough & Groves 1983; Cundall & Deufel 2006). During pilot studies we measured largest circumference of the prey and prey length and found that prey circumference is highly correlated with prey length ($r^2 = 0.96$). Therefore, we use prey circumference in this study. Prey were considered small if IRs fell between 40% and 60%, which was equivalent to MLRs ranging between 33% and 50%. Prey were considered large when IRs approached 80–100% of a predator's HW (Loop & Bailey 1972), which was

equivalent to MLRs ranging between 72% and 91%. Mean IRs and MLRs for each snake taxon across prey categories are listed in Table 1.

Feeding Behaviours

Seven behaviour patterns in the feeding repertoire for snakes were examined. These were modified from Greene (1977), De Queiroz (1984), Milostan (1989) and Mori (1991, 1994) and are the following:

Capture position

The part of the prey's body first grasped by the snake. Three states were recorded: (1) anterior (A; head and shoulder), (2) middle (M; abdomen and forelegs) or (3) posterior (P; pelvic region, hind legs and tail).

Prey restraint method

Four states were recorded: (1) simple-seizing (SS): grasping the prey in its jaws without subduing it with the body; (2) loop (L): winding one encircling loop around prey, (3) coiling (C): using two or more fully encircling loops around a prey and (4) 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 behaviours can be performed immediately (I) after capture or delayed (D), 1 or more seconds after prey capture.

Loop orientation

An imaginary line is drawn through the long axis of a loop or coil and the relationship of this line to the substrate characterizes loop orientation (Greene 1977). Three states were recorded: (1) horizontal (H): the imaginary line runs relatively parallel to the substrate; (2) vertical (V): the imaginary line runs relatively perpendicular through the long axis of the prey and the substrate and (3) mixed (M): there are two imaginary lines (one for each loop). One line runs parallel to the substrate and the other runs perpendicular to the substrate (Fig. 1).

Loop application pattern

The method by which a loop was applied around prey during loop and coil. Three states were observed: (1) lateral (L): only one side of the body was pressed up against prey; (2) ventral (V): the
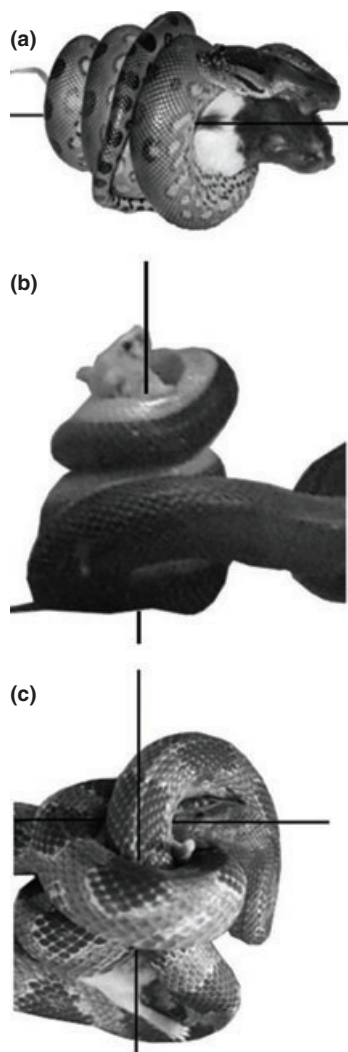
Fig. 1: Examples of the three character states for loop orientation observed in snakes: (a) horizontal – the imaginary line runs relatively parallel to the substrate, (b) vertical – the imaginary line runs relatively perpendicular through the long axis of the prey and the substrate and (c) Mixed – there are two imaginary lines (one for each loop). One line runs parallel to the substrate and the other runs perpendicular to the substrate.

belly scales of the snake were pressed up against the prey and (3) ventral–lateral (VL): in the first loop the belly of the snake was pressed up against the prey and in the second loop the side of the snake was pressed against the prey.

Condition of prey before ingestion

After the prey restraint phase and just before swallowing, two states were recorded: (1) alive (A) or (2) dead (D).

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: (1) anterior (A) or the tail end could be ingested first and (2) posterior (P).

Prey restraint time (s)

The elapsed time from the moment the prey was struck or seized to the commencement of swallowing.

Statistical Analysis

Categorical data (capture position, prey restraint method, loop formation, loop orientation, loop application and swallowing position) were coded before analyses and Pearson's chi-squared test was used to examine the effects of prey categories on these behaviours. Behavioural states are presented as percentages of trials so overall trends can be observed. We also compared the summary values obtained from each species group as all species were subject to the same feeding regime before the experiment and the same stimulus control experiments during this study. Because of the small sample sizes of some of these hard to obtain species and the variability in age classes, we were conservative in drawing conclusions. In this study, the Erycine lineage is represented by three species, and individuals of each of the species are from different age classes. To increase statistical power, we grouped Erycine snakes for statistical analysis. However, before grouping snakes we used a Kruskall–Wallis test to compare species response across prey categories. Individuals of *E. muelleri*, *C. bottae* and *C. trivirgata* did not significantly differ in their response to prey category and their responses were combined to represent Erycine snakes (Table 2).

As feeding experience can affect subsequent feeding responses (Burghardt & Krause 1999), we used the McNemar test of significant changes to detect differences in behavioural responses between trials 1 and 2 (Sokal & Rohlf 1981). If trials 1 and 2 did not differ, we presented chi-squared results for trial 1. If there were significant differences between trials 1 and 2, we present chi-squared results for both trials. Our experiments were designed to examine the effects of prey size and status on the predatory cycle of snakes and individuals were subject to only two trials across the four prey categories. Therefore, indi-

Table 2: Effects of prey size and status on the predatory cycle of *Loxocemus bicolor* (n = 12, 96 feedings), *Boa constrictor* (n = 5, 40 feedings), *Eryx muelleri* (n = 6, 48 feedings), *Charina trivirgata* (n = 2, 16 feedings) and *Charina bottae* (n = 2, 16 feedings)

	McNemar test (trials 1 and 2)[a]	*Loxocemus bicolor* (n = 12)	*B. constrictor* (n = 5)	*E. muelleri* (n = 6)	*C. trivirgata* (n = 2)	*C. bottae* (n = 2)	Kruskall–Wallis test[c]
Behaviours[b]							
Capture position	0.63	<0.001	0.21	0.26	0.58	0.61	0.83
Restraint behaviour	0.82	<0.001	0.38	<0.001	<0.05	<0.05	0.43
Loop orientation	0.72	<0.001	0.53	<0.05	<0.05	<0.05	0.62
Loop application	0.63	0.73	0.44	0.32	0.38	0.42	0.53
Restraint time (s)	0.51	<0.05	0.28	<0.05	<0.05	<0.05	0.71

The McNemar test of significant changes was used to detect differences in feeding behaviour between trials 1 and 2 within each prey category. Non-parametric statistics were used to evaluate behavioural responses across prey category. Significant p-values under each species column indicate that behavioural responses were significantly different across prey categories, suggesting context-dependent flexibility in prey restraint behaviour.

[a]McNemar's test of changes did not detect any differences in behaviour between trials 1 and 2.

[b]Pearson's chi-squared tests were used for categorical data. Kruskall–Wallis tests were used to examine continuous variables because of small sample sizes.

vidual variation could not be examined. If an individual's response deviated from the mean response, we discuss the behavioural variation observed.

Prey restraint times were log-transformed before analyses. We tested for unequal variances using a Levene's test. Prey restraint times were demonstrably non-normal (p = 0.43) and a Kruskal–Wallis test was then used to examine untransformed prey restraint time data. Mean values for restraint times within each prey category were ranked and non-parametric Tukey-type multiple comparisons were used to determine significant differences between prey categories.

To illustrate the variability of prey restraint behaviours across species, we calculated the percentages of non-modal states, V, for each taxon in each prey category. The value, V, is expressed as a decimal and is equal to 100% minus the modal character state in that prey category (Voris 1971). In this study, the prey restraint behaviour observed most frequently within a prey category was designated as the modal state. The modal state (i.e. Stereotypy) S, was calculated across all prey categories for each snake lineage.

We used the statistical program spss 12.0 (SPSS Inc., Chicago, IL, USA) to perform descriptive statistics and non-parametric tests. All tests were two-tailed. A Monte Carlo significance level was used to give precise estimates as small sample sizes were used in this study. Significance levels were set at p < 0.05.

Results

From July 2002 to September 2004, we recorded and analysed 96 feeding trials for *L. bicolor* (n = 12),

16 trials for *C. trivirgata* (n = 2), 16 trials for *C. bottae* (n = 2), 48 trials for *E. muelleri* (n = 6) and 40 trials for *B. constrictor* (n = 5). Overall feeding responses to prey category are summarized in Table 2 and the dominant behavioural states observed during each event in the predatory cycle are summarized in Table 3. We report the effects of prey size and status on the predatory cycle for the three boid snake lineages. The results are organized by dependent variables in the order in which they would appear in the predatory cycle. We then present the overall patterns of flexibility in prey restraint behaviour for the three snake lineages. The McNemar test did not detect differences in feeding responses between trials 1 and 2 for any of the predatory behaviours recorded (Table 2). Therefore, Pearson's chi-squared tests were performed with trial 1. To minimize our use of abbreviations, we refer to prey as either live or dead in the results, when snakes differed in their response to prey status and not prey size. When two or more prey categories that differ in both size and status are discussed, we use the following abbreviations for prey category: small alive (SA), large alive (LA), small dead (SD) and large dead (LD).

Capture position

Individuals of *L. bicolor* mostly captured live prey by the tail (70–75%) while dead prey were captured by the head (75%), indicating that capture position for *L. bicolor* was influenced by prey status rather than size (Pearson's chi-squared test: 12.36, p < 0.001). Erycine snakes and *B. constrictor* mostly captured prey by the head (89–100%) irrespective of size or

Table 3: Dominant behavioural states observed in the predatory cycle of *Loxocemus bicolor*, *Boa constrictor* and Erycine snakes across prey category

Behaviour	Behavioural states	Small live	Small dead	Large live	Large dead
Loxocemus bicolor					
Capture position	Anterior (A), middle (M), posterior (P)	P	A	P	A
Restraint behaviour	Simple-seizing (SS), loop (L), constriction (C)	L, C	SS, L[a]	L, C	SS, L, C[b]
Loop orientation	Horizontal (H), vertical (V), mixed (M)	H	H, V	H	H
Loop pattern	Lateral (L), ventral (V), ventral–lateral (V-L)	L	L	L	L
Ingestion condition	Alive (A), dead (D)	D	N/A	D	N/A
Swallowing position	Anterior (A), posterior (P)	A	A	A	A
Restraint time (mean + SE)	N/A	114.71 + 16.33	47.63 + 16.56	181.92 + 20.03	56.71 + 21.37
Boa constrictor					
Capture position	Anterior (A), middle (M), posterior (P)	A	A	A	A
Restraint behaviour	Simple-seizing (SS), loop (L), constriction (C)	C	C	C	C
Loop orientation	Horizontal (H), vertical (V), mixed (M)	H, V[a]	H	H	H
Loop pattern	Lateral (L), ventral (V), ventral–lateral (V-L)	V, V-L[a]	V	V, V-L[a]	V, V-L[a]
Ingestion condition	Alive (A), dead (D)	D	N/A	D	N/A
Swallowing position	Anterior (A), posterior (P)	A	A	A	A
Restraint time (mean + SE)	N/A	397.23 + 41.67	388.9 + 53.21	417 + 57.65	489 + 83.21
Eryx muelleri, *Charina trivirgata* and *C. bottae*					
Capture position	Anterior (A), middle (M), posterior (P)	A	A	A	A
Restraint behaviour	Simple-seizing (SS), loop (L), constriction (C)	C	SS, L, C[b]	C	SS, L, C[b]
Loop orientation	Horizontal (H), vertical (V), mixed (M)	H	H, V[a]	H	H
Loop pattern	Lateral (L), ventral (V), ventral–lateral (V-L)	L	L	L	L
Ingestion condition	Alive (A), dead (D)	D	N/A	D	N/A
Swallowing position	Anterior (A), posterior (P)	A	A	A	A
Restraint time (mean + SE)	N/A	144.75 + 11.62	42.55 + 18.29	163 + 10.31	41.8 + 13.22

Dominance in a particular behavioural state is based on frequencies >70% except where noted.
[a]Dominance in a particular restraint behaviour is based on observations ≥50% within a prey category.
[b]Dominance in a particular restraint behaviour is based on observations ≥30% within a prey category.

status (Pearson's chi-squared tests: Erycines: 4.12, p < 0.661; *B. constrictor*: 8.46, p < 0.206).

Prey Restraint Method

Of the four possible prey restraint methods recorded during pilot observations, three were observed during this experiment: SS, loop (L) and coil (C). Although two behavioural states were possible for L and C: delayed loop (DL), and delayed coil (DC), DL and DC occurred at such low frequencies (<8%) that we did not separately analyse these behavioural states.

Frequencies for different prey restraint behaviours varied across the three snake lineages (Pearson's chi-squared tests: 18.273, p < 0.006; Fig. 2). Prey restraint behaviours for *L. bicolor* varied across prey category (Pearson's chi-squared test: 39.79, p < 0.001). *Loxocemus bicolor* mostly restrained live prey using L or C. Simple-seizing and L were reserved for SD prey while the behaviours SS, C/DC or L/DL were used to restrain LD prey. Individuals of *B. constrictor* did not vary prey restraint behaviours

across prey category. The five *B. constrictor* coiled around prey irrespective of prey size and status (Pearson's chi-squared test: 3.07, p < 0.380). Only in a single trial with SD prey did an individual *B. constrictor* employ SS. In this particular trial, the prey measured 40% of the snake's HW and did not exceed the length of the snake's mandible. Erycine snakes responded to prey status with different prey restraint behaviours, similar to those of *L. bicolor* (Pearson's chi-squared test: 24.204, p < 0.001). Erycines mostly coiled around live prey while SS, L or C were used to restrain dead prey.

Loop Orientation

Loop orientation varied across species (Pearson's chi-squared test: 32.75, p < 0.001). Individuals of *L. bicolor* used mostly horizontal loops while coiling or looping around mice with the exception of those in the SD prey category. Small dead prey were restrained with horizontal or vertical loops. Individuals of *B. constrictor* wound horizontal loops around all prey with the exception of small live mice which
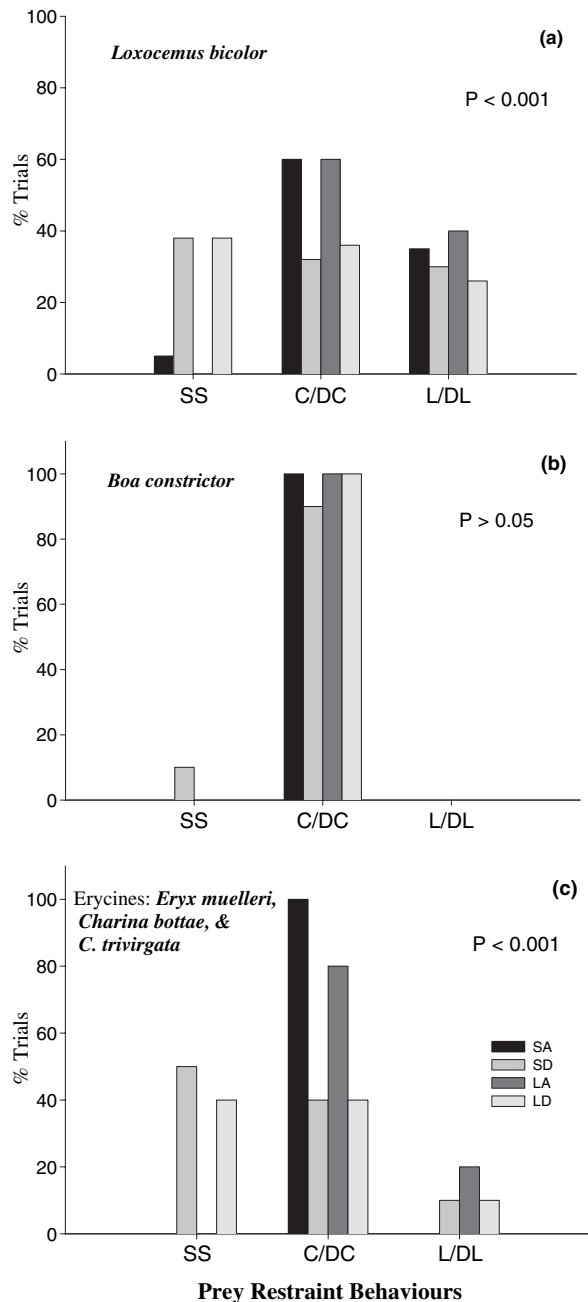
Fig. 2: Percentage of trials in which prey from each of the four prey categories were restrained using SS, C/DC, L/DL for the three lineages examined: (a) *Loxocemus bicolor*, (b) *Boa constrictor* and (c) Erycine snakes. Abbreviations for restraint behaviours are: SS, simple seizing; C, coil; DC, delayed constriction; L, loop; DL, delayed loop. Abbreviations for prey categories are: SA, small live; SD, small dead; LA, large alive; LD, large dead. p-Values indicate significant differences in prey restraint response across prey categories.

were restrained using horizontal (50%) or vertical (50%) loops. Erycine snakes mostly applied horizontal loops around prey. In trials with SD prey,

Erycines applied either horizontal or vertical loops to restrain prey (60%).

Loop Application Pattern

Three different loop application patterns were observed: lateral, ventral and a combination of ventral–lateral bending. In trials where L/DL and C/DC were used to restrain prey, *L. bicolor* and Erycine snakes bent laterally around prey. Individuals of *B. constrictor* used ventral bends and ventral–lateral bends when applying loops around prey.

Prey Ingestion Condition

Prey items were dead prior to ingestion for majority of live prey trials. *Loxocemus bicolor* and *B. constrictor* immobilized and killed live prey prior to ingestion. In only one of 80 Erycine feeding trials was prey still alive prior to swallowing.

Prey Swallowing Position

In 98% of feeding trials, snakes consumed prey by the head (Pearson's chi-squared test: 2.98, $p < 0.001$). There were no significant differences observed in swallowing position between snake lineages (Pearson's chi-squared test: 0.79, $p < 0.83$) and across prey categories (Pearson's chi-squared test: 0.81, $p < 0.67$).

Prey Restraint Time

Prey restraint times demonstrated a non-normal distribution. Deviation from normality resulted from trials in which snakes immediately swallowed prey after SS. In these specific cases, prey restraint times were close to 0 (ranging from 3 to 12 s). Therefore, only prey restraint times for the behaviours C/DC and L/DL were used in the following analysis. Snakes differed significantly in mean prey restraint times across prey categories (Kruskall–Wallis tests – SA: $H_2 = 14.23$, $p < 0.001$; SD: $H_2 = 12.16$, $p < 0.002$; LA: $H_2 = 11.82$, $p < 0.003$; LD: $H_2 = 11.84$, $p < 0.003$; Table 3). Mean prey restraint times did not differ across prey categories for *B. constrictor* (Kruskall–Wallis test: $H_3 = 3.8$, $p = 0.284$), whereas mean restraint times significantly differed across prey categories for *L. bicolor* (Kruskall–Wallis test: $H_3 = 20.19$, $p < 0.001$) and Erycine snakes (Kruskall–Wallis test: $H_3 = 20.15$, $p < 0.001$). *Loxocemus bicolor* and Erycine snakes took a longer time to restrain active prey compared to dead prey within each size category.

Flexible Prey Restraint Behaviours

The proportion of non-modal (V) and modal (S) states across prey categories are shown in Table 4. Overall, *L. bicolor* and Erycines exhibited considerable behavioural flexibility, as indicated by their average stereotypy coefficients (*L. bicolor*: 0.56, Erycines: 0.64), while *B. constrictor* exhibited a high stereotypy value (0.99). *Loxocemus bicolor* and Erycines exhibited the greatest flexibility with small live prey and the least flexibility with large live prey. *Boa constrictor* exhibited almost no variation across prey categories, indicated by non-modal states (V) of zero, and a V of 0.4 in the SA prey category.

Discussion

Effects of Prey Size and Status

Our results reveal interspecific variation in prey restraint behaviour between five macrostomate snake species. Five of the seven behavioural measures for *L. bicolor* and Erycine snakes were flexible and varied with respect to prey size and status (Table 2). *Loxocemus bicolor* and Erycines employed loop or constriction behaviours to restrain active prey. Dead prey, irrespective of size, were restrained using any one of the three restraint methods: SS, loop and constriction. *Loxocemus bicolor* and Erycines used one side of their body to apply loops laterally around prey. *Boa constrictor* revealed little flexibility in predatory behaviour across prey categories and only restrained prey using a coil. Coiling was achieved by either ventral bending or ventral–lateral bending around prey. Thus, not only did these snake species exhibit interspecific differences in context-dependent flexible prey restraint behaviour, but marked differences were observed in their coil application patterns. Although our sample sizes were small for some species and different age classes were used in this study (Erycine snakes), snakes were

consistent across trials and strong trends were observed. Therefore, the behavioural responses observed in this experiment are an example of flexibility in feeding behaviour rather than differences in snake age class.

Although there is considerable dietary overlap between the five snake species examined, prey size and status affected the predatory behaviour for only *L. bicolor* and Erycines. Our observations support earlier claims that *B. constrictor* has a very consistent feeding strategy (Greene 1977; Willard 1977; Milostan 1989). Differences in the feeding responses of closely related predators that are known to consume similarly bulky prey suggests that other factors may shape flexibility in snake prey restraint behaviours. Our results along with additional behavioural observations enable us to offer alternative hypotheses for the interspecific differences observed in prey restraint behaviour for early macrostomate snake lineages.

Our observation that the semi-fossorial snakes, *L. bicolor, E. muelleri, C. trivirgata* and *C. bottae* exhibit context-dependent flexible prey restraint behaviours suggests that semi-fossorial habits may select for behavioural flexibility in boas and pythons. This idea is supported by additional feeding observations for two semi-fossorial macrostomate snakes, *Xenopeltis unicolor* and *Calabaria reinhardtii*. Both *X. unicolor* and *C. reinhardtii* exhibit variable prey restraint behaviours when feeding on live and dead mice of different sizes in the laboratory (R. S. Mehta, unpubl. data). *Xenopeltis unicolor* and *C. reinhardtii* also apply loops laterally around prey. These observations and empirical studies support the idea that context-dependent flexible prey restraint behaviours may be particularly useful for snakes inhabiting or hunting in subterranean or leaf litter environments and further suggest that lateral bending may also be associated with this flexibility.

Flexibility in prey restraint behaviour may increase capture success when the predator is in a

Table 4: Summary of the contextual flexibility in prey restraint behaviours across the four prey categories: SA, small live; SD, small dead; LA, large live; LD, large dead

Species	Proportion of non-modal states (V)				Average stereotypy (S)	Genera comparison coefficient of similarity (CS)[a]
	SA	SD	LA	LD		
Loxocemus bicolor (A)	0.38	0.33	0.58	0.46	0.56	A & B (0.28) A & C (0.62)
Boa constrictor (B)	0.00	0.04	0.00	0.00	0.99	B & A (0.28) B & C (0.33)
Eryx muelleri, Charina trivirgata and *Charina bottae* (C)	0.34	0.38	0.40	0.33	0.65	C & A (0.68) C & B (0.33)

[a]Higher CS values indicate greater similarity in prey restraint behaviour.

confined space and does not have room to form a coil. A study examining the feeding behaviour of gopher snakes (*Pituophis ruthveni*), revealed that gopher snakes pinioned gophers (*Geomys breviceps*) in burrow systems but coiled around prey during open situations (e.g. laboratory arena; Rudolph et al. 2002). Gopher snakes (*Pituophis melanoleucus*) are also known to use lateral bends when applying loops around prey (Moon 2000). The observation that a colubrid snake that hunts in burrow systems exhibits flexible prey restraint behaviour suggests that subterranean environments may select for behavioural flexibility across disparate snake taxa but this idea necessitates further examination. In similar stimulus control studies, non-fossorial colubrid taxa are also known to exhibit flexibility in their prey restraint behaviours (Mori 1991, 1994; Mehta 2003). Thus, other variables may affect flexible prey restraint behaviour particularly in colubrid snakes where constriction is thought to have re-evolved independently multiple times (Greene 1994). Aside from prey restraint behaviour, many other important survival behaviours are limited to areas along a snake's trunk. Diverse behaviour patterns that require the snake's axial skeleton such as locomotion, reproduction and swallowing may result in competing demands (Ruben 1977) which may manifest in interspecific differences in prey restraint flexibility.

Why Might Snakes Exhibit two Different Loop Application Strategies?

In this study, snakes that exhibited more than one prey restraint behaviour bent their bodies laterally to wrap around prey. Lateral bending also appears to permit 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. 3). The ability to apply loops laterally with either the anterior or posterior portion of the body may increase hunting success in confined spaces, especially when relying on tactile cues to locate prey. 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 behaviour support this idea (Hopley 1882). Using the posterior portion of the trunk to subdue prey also frees up the anterior portion of the trunk, thus allowing the snake to remain vigilant while in a prey

(a) Anterior Coil

(b) Posterior Coil

Fig. 3: Coiling behaviours observed for *Loxocemus bicolor*. Coiling with the anterior portion of the body (a) and coiling with the posterior portion of the body (b).

restraint posture (Mehta 2003). Increased vigilance may be especially important for hatchlings, neonates and many smaller snakes that either rely on rapid escape or more static cryptic postures to evade predators.

The ventral loop application patterns exhibited by *B. constrictor*, has been associated with strike kinematics (Greene and Burghardt 1978; Cundall & Greene 2000). During one type of striking known as the MAN strike, the snake' mandibles contact the prey first. During a MAN strike, momentum from mandibular movement may facilitate ventral flexion of the snake's head to initiate the first coil. Although booid striking kinematics are variable (see Cundall & Deufel 1999), striking prey ultimately provides an anchor point on the prey surface from which constriction coils may be formed. The bodies of small prey that remain out of the snake's jaws are much harder to compress in a coil and can retaliate against the predator. Mandibular striking which leads to rapid formation of the first coil and a ventral bending loop application strategy observed in many booid

snakes may be a behavioural adaptation related to consuming larger prey. However, this idea needs to be tested within a phylogenetic framework.

Our study systematically reveals that prey status and size influences one axis of behavioural diversification across snake lineages. Our data reveal that flexibility in prey restraint behaviour varies interspecifically across early macrostomate snake taxa, and we suggest that physiological and ecological mechanisms may underlie this variation. Previous studies have documented flexible prey restraint patterns deployed by neonate and adult colubroid snakes (Greene 1977; De Queiroz 1984; Milostan 1989; Mori 1991, 1993a,b, 1994, 1995; Rodriguez-Robles & Leal 1993; De Queiroz and Groen 2001; Mehta 2003). Whether all or only some of these colubrids also apply loops laterally around prey necessitates re-examination. Future studies mapping the evolution of flexible prey restraint repertoires and loop application strategies may provide valuable insight into trophic diversity and resource use in snakes.

Acknowledgements

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