

How to assess musculature and performance in a constricting snake? A case study in the Colombian rainbow boa (*Epicrates cenchria maurus*)

Olivier Lourdais^{1,2,3*}, François Brischoux² and Laurent Barantin⁴

¹ School of Life Sciences, Arizona State University, 85287-4501 Tempe, AZ, U.S.A.

² Centre d'Etudes Biologiques de Chizé, CNRS, 79360, Villiers en Bois, France

³ Conseil Général des Deux Sèvres, rue de l'abreuvoir, 79000 Niort, France

⁴ Département de RMN, UFR de Médecine de TOURS, France

(Accepted 18 May 2004)

Abstract

The ability of a living organism to perform specific actions such as prey capture or predator avoidance is a critical feature that should affect individual fitness. Snakes have an elongate morphology that lacks the regional variation associated with girdles found in limbed vertebrates. In this context, this group offers interesting opportunities to study specific forms and functions. In this study, we examined musculature in a medium-sized boid snake, the Colombian rainbow boa *Epicrates cenchria maurus*, by combining two methods: magnetic resonance imaging (MRI) and calliper measurements after axial palpation. The MRI demonstrated an important development of epaxial musculature consistent with the predatory mode of constriction. We showed that MRI and palpation data were strongly correlated, indicating that musculature is an easily measurable phenotypic feature in the studied species. The calliper method was then applied in combination with assessments of physical performance (constriction and defence capabilities) in a group of snakes after a prolonged fast and again after a re-feeding period. A clear relationship was detected between dorsal musculature and both maximal traction and constriction forces. Re-feeding significantly increased dorsal musculature and traction capabilities. While musculature assessments are classically difficult to achieve among tetrapods, this study suggests that snakes are interesting models for examining variation in musculature and performance in natural and experimental conditions.

Key words: snake, musculature, magnetic resonance imaging, performance, *Epicrates cenchria maurus*

INTRODUCTION

Body size and morphology of living organisms are very variable across taxa (Darwin, 1859), and such diversity can be understood in the framework of variable selective pressures acting on phenotypic features. Notably, a functional linkage is classically recognized between morphological characters and performance abilities (Arnold, 1983; Jayne & Bennett, 1990; Meyers, 1992; Kearns *et al.*, 2002). An individual's morphology will determine its aptitude to perform specific functions and behaviours (e.g. prey capture or predator avoidance), and the success in conducting such activities will, therefore, influence individual survival, reproductive success, and growth (Arnold, 1983). Performance is thus considered as an intermediate 'link' between phenotypic features and fitness (Arnold, 1983). Because of its impact on fitness, studies on performance now occupy a central place in evolutionary biology. Recent studies clearly emphasize

the importance of ecologically relevant measurements of performance (Arnold, 1983). Notably, a precise understanding of the ecological context is required to examine the functional and evolutionary implications of phenotypic traits (Losos, 1990; Irschick & Losos, 1998).

Though critical, establishing morphology-performance correlates is often complicated in intact, living animals. For example, measurements of major morphological features, such as body musculature, involve complex techniques (Price *et al.*, 1960; Kearns, Keaver & Abe, 2002; Abe, Kearns & Fukunaga, 2003) and are generally difficult to obtain without dissecting dead specimens. Also, musculature and performance are generally difficult to correlate in vertebrates. Notably, a particular behaviour may involve a diversity of structures that cannot be assessed by a single measure. In this context, studies on morphology typically examine one aspect of a structural system, and thus generally provide only a partial understanding of a biological role (Cundall, 1987). In addition, diverse locomotor activities may require different specialized morphologies that impose functional tradeoffs. Such situations are probably widespread among living organisms, turning into adaptive compromises

*All correspondence to: Olivier Lourdais
E-mail: Olivier.lourdais@asu.edu

between conflicting optima (Shine & Shetty, 2001), rather than a single optimum for a defined activity.

Despite this apparent complexity, some groups may provide interesting opportunities to clarify the relationships among an organism's morphology, behaviour, and ecology (Cundall, 1987). Among tetrapods, snakes are characterized by an extremely elongated morphology (Coates & Ruta, 2000) made up of a large number of trunk vertebrae (up to 320, Hosffetter & Gasc, 1969) and an absence of legs. Consistent with this body shape is the longitudinal orientation of the trunk musculature that is not complicated by the regional variation associated with the girdles found in limbed vertebrates. Trunk cross-sections reveal the complex arrangement of the epaxial muscles, located beside the vertebrae, which are critical to the mechanics of locomotion (Jayne, 1982; Cundall, 1987). Epaxial muscles are particularly well-developed in constricting species, which use their body coils to suffocate prey before ingestion (Ruben, 1977; Moon, 2000). Constriction has been a key innovation in the evolution and radiation of snakes (Greene & Burghart, 1978). This predatory mode is a rule among snakes of the family Boidae, which are generally slow moving, sit-and-wait predators (Ruben, 1977; Ross & Marzec, 1990).

The relationship between morphology and locomotion in snakes has attracted considerable scientific interest (Mosauer, 1932; Gasc, 1967, 1974; Jayne & Bennett, 1989, 1990; Walton, Jayne & Bennett, 1990; Shine & Shetty, 2001). A number of studies examined variation in trunk muscle configuration (i.e. variation in segmental length of epaxial muscles) in relation to locomotion or foraging mode across taxa (Mosauer, 1935; Ruben, 1977; Jayne, 1982, 1988a,b). Conversely, intraspecific variation in musculature and strength has attracted little interest despite its major ecological and evolutionary implications (Arnold, 1983). For example, to our knowledge, no study has attempted to assess variation in musculature and associated strength in live snakes. Because boid snakes use their enlarged musculature during vigorous prey constriction, they may provide unique models for examining the influence of musculature on physical performance.

In the present paper, our aim was first to test whether inter-individual variation in axial musculature was measurable in a medium-sized boid, the Colombian rainbow boa *Epicrates cenchria maurus*. We used magnetic resonance imaging (MRI), to examine the validity of calliper measurements of musculature width obtained on live animals. Then, we examined the relationship between our estimate of musculature and the strength deployed during major locomotor tasks and during prey constriction. Finally, we examined the influence of food intake on musculature and performance.

MATERIALS AND METHODS

Model species

Rainbow boas *Epicrates cenchria maurus* Gray 1849 are medium-sized (1500 mm snout–vent length, SVL),

non-venomous, constricting snakes from South and Central America (Ross & Marzec, 1990; Matz, 2001). Prey are first seized by the head and then constricted by several body coils. The snakes in our study were obtained in 2001 from a long-term captive colony. Snakes were maintained in the laboratory in separate cages (50 × 50 × 20 cm) that provided free access to a heat source (temperature range in the cage: 28 to 33 °C). Water was available *ad libitum* in a bowl, and snakes were fed rats (mean mass = 150 g) once per month except during experiments in which snakes were fasted (see below).

Variables examined

The aim of the study was to test the quality of several original estimators of musculature and physical performance that we developed to study female reproductive biology (Lourdais *et al.*, 2004). For this reason, the present analysis was concentrated on females. All measurements were obtained when snakes were non-reproductive. Our study was divided into three stages: musculature assessment; musculature and performance; influence of energy intake.

Musculature assessment

Among boid snakes, the epaxial musculature is highly developed, resulting in protruding ridges on each side of the vertebrae. Intraspecific variation in musculature should be characterized by variation in cross-sectional muscle area, reflecting differences in the number of myofibril proteins. The muscular ridges of epaxial muscles are often visually distinct, notably during intense contraction (prey constriction). Our observations suggest that they are also detectable by manual palpation of the dorsal region. By applying a slight pressure in this area with the forefinger and the thumb, it is possible to visualize and feel a clear bilateral demarcation in the epaxial muscles that is measurable with an electronic calliper (O. Lourdais, pers obs). In this context, a proper measurement of variation in the width of epaxial muscles may be crucial and may reflect differences in overall musculature and thus performance capabilities. Our aim was not to derive predictive equations regarding the relationship between MRI and calliper measurements of musculature, but rather to examine the extent to which calliper measurements might be used as a simple morphometric index of individual musculature. To do so, we combined complementary methods using a subgroup of 13 females in good body condition.

Magnetic resonance imaging First, we used MRI to provide descriptive information on epaxial musculature and to identify the specific muscles detected by palpation. For this purpose, we used an imaging spectrometer (Bruker Biospec 24/40) operating at 2.35 Tesla (i.e. proton frequency of 100 MHz). A Halderman–Grandt resonator (Ø12 cm) was used for emission and reception of the signal. After quick scans in 3 directions to verify



Fig. 1. Body cross-section of *Epicrates cenchria maurus* obtained by MRI. 1, spinalis–semispinalis; 2, longissimus dorsi; 3, iliocostalis; d, distance measured on each image that was compared to calliper-based epaxial widths. The white area indicates abdominal body fat.

positioning, images were acquired using a multislice/multiecho (MSME) sequence in T1 ponderation (which gives a precise view of the internal structures with optimal muscle/fat contrast, as seen in Fig. 1). Snakes were anaesthetized using isoflurane (Abbott Laboratories, Illinois, USA), which induced complete muscular relaxation. Snakes were then measured (SVL). Each individual was placed on a support made from a PVC tube (2 m) cut in half longitudinally. Capillary tubes filled with gadolinium (a detectable liquid used as a contrast agent in MRI) were glued to the support. The snake lying on the support was then introduced to the centre of the antenna and was maintained under anaesthesia during image acquisition. Cross-section images were obtained in 4 relative positions (20, 40, 60 and 80% of the snake's SVL) labelled with gadolinium. The procedure never exceeded 1 h, and recovery from anaesthesia was rapid (15–20 min) in each case.

The use of MRI provided detailed information on the major muscles located beside the vertebrae (Fig. 1). Importantly, it was possible to identify the muscles that accounted for the palpable protruding ridge. MRI revealed that this region consisted of 2 muscle series (spinalis–semispinalis and longissimus dorsi) that were particularly well-defined and measurable in width on the images (Fig. 1). For each segment we measured the width of epaxial muscles (mm, distance d; Fig. 1) along with the cross-sectional surface area (mm^2) of the 2 groups of epaxial muscles located in the area (groups 1 & 2; Fig. 1).

The iliocostalis muscle (group 3, Fig. 1) was lying out of the palpated area. It was often less clearly defined on the MRI, and cross-section values were not available for this group. We also measured the total musculoskeletal surface area using the method described by Moon & Candy (1997). For each body section we measured the total cross-sectional area (TA, mm^2) defined by the outline of the body wall. Then we measured the inner circle defined by the ribs and corresponding to the coelomic cross-sectional area (CA). The difference between TA and CA corresponds to a cross-sectional area of muscular and skeletal tissue (musculoskeletal surface area, MSA). All measurements were conducted using Cyberview 2.5 software (Cervus International, 1994).

Calliper measurements After imaging, the width of epaxial muscles was measured at the same four equidistant points as the MRI using a calliper (Mitutuyo, Japan) and in 2 situations:

- (1) on anaesthetized snakes shortly after imaging to provide muscular measurements on fully relaxed animals;
- (2) on alert snakes 2 days after imaging. This procedure required 2 operators, 1 firmly holding the snake and the other conducting the measurements when the snake appeared relaxed. Calliper measurements were obtained in triplicate and were always collected by the same individual (O. L.).

Musculature and performance

Boid snakes rely on muscular contractions to generate the force needed to subdue and kill prey by constriction, as well as to escape from predators (Cundall, 1987). We used the calliper method to examine how musculature correlates with physical performance. First, we measured the width of epaxial muscles in a group of snakes ($n = 21$) that had faced an extended fasting period (>12 weeks). At that time, 10 females were post-reproductive, and we therefore expected to find considerable variation in musculature within the group. We measured muscular strength in 2 contexts designed to mimic biologically crucial situations. As no standard methods were previously available, we developed a set of complementary techniques described below.

Escape from a predator These snakes react to handling, especially attempts to stretch out their bodies, by vigorous and extended body contraction. We quantified this behaviour using a dynamometer. The snake was maintained on the ground as follows. One operator immobilized the first section of the body while the other maintained the snake in a stretched position by firmly holding the tail with one hand. A dynamometer was then attached to the wrist of the second operator and adjusted to maintain only slight traction (0–5 newtons). Then, the operator simply released his traction force, allowing his arm to be displaced laterally by the traction force of the snake while maintaining a grip on the snake's tail (Fig. 2). The whole body contraction was stimulated by gentle palpation of the back and resulted

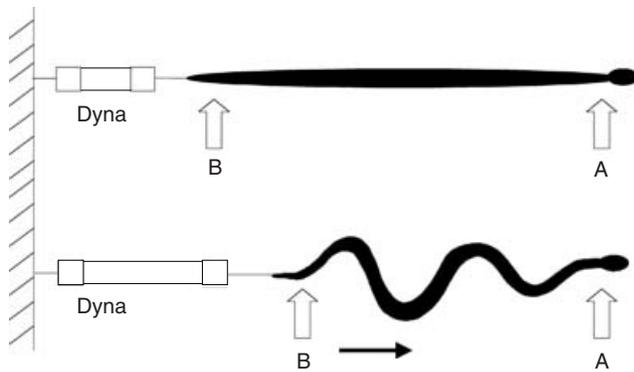


Fig. 2. Schematic representation of the method used to quantify traction force. The snake was maintained on the ground as: one operator (A) immobilized the first section of the body while the other (B) maintained the snake in a stretched position. A dynamometer (Dyna) was attached to the wrist of operator B and adjusted to maintain only slight traction (0–5 newtons). Then, operator B simply released his traction force allowing his arm to be displaced laterally by the traction force of the snake while maintaining a grip on the snake's tail.

in a displacement of a cursor on the dynamometer. The highest value obtained during 5 min of stimulation was recorded. We acknowledge that such a method may introduce operator bias. However, the strength deployed by the snake during traction was considerable (>3 kg) which made it impossible to affix a harness directly to the snake without injury and, therefore, minimized the effect of any unintentional traction from the operator's wrist (<500 g). To maintain consistency, the same operator (O. L.) performed all traction measurements.

Prey handling We measured the intensity of muscular contractions during prey constriction using a compressible lure (a 10 cm long by 2 cm diameter water-filled rubber tube covered with mouse-scented cloth; Lourdais *et al.*, 2004). One end of the lure was filled with cotton to mimic the head of a rat. The opposite end was connected to an open titration column via a rigid plastic tube (5 mm diameter). Upon presentation, the lure was rapidly grasped by the snake and then wiggled for 10 s by the operator to mimic a typical prey response and encourage normal coiling by the snake. The displacements of the water

column were videotaped so that maximal displacement could be measured (cm) subsequently. The linear response of the transducer was controlled by manual pressure to record the maximal possible value of water displacement. Constriction forces recorded were much lower, never reaching one-third of the total displacement obtained manually.

Influence of energy intake

Finally, we examined the influence of energy intake on musculature and performance. Musculature profiles and traction capabilities were re-measured (as in above section) on the 21 snakes after a 6-month feeding period. During this period, 400 ± 50 g of rats (approximately 4 meals) were given to each snake. Measurements were carried out approximately 2 weeks after the last meal, and no food was present in the stomachs.

Statistics

All statistics were performed with Statistica 6.0. Variation in MSA and combined area of the 2 epaxial muscles among body sections were investigated using mixed-model ANOVA. Body section was treated as a fixed factor and individual identity as a random factor. The measurements of epaxial muscle width obtained from MRI and the calliper method were compared using paired *t*-tests. Changes in epaxial muscle width over the feeding period were examined using a repeated measures ANOVA procedure with epaxial width as the dependent variable, body section number as a factor, and individual identity as a random factor. Finally, changes in SVL, body mass, and traction force over this period were examined using paired *t*-tests.

RESULTS

Musculature assessment

We detected significant variation in MSA among body sections (Table 1), with the highest values observed

Table 1. Variation in musculature surface area (mm^2) among the fourbody sections of 13 *Epicrates cenchria maurus*. We considered (a) the musculoskeletal surface area (total cross-sectional surface area of muscular and skeletal tissues) and (b) the epaxial surface area (combined bilateral surface of the two muscle groups spinalis–semispinalis and *longissimus dorsi*). In both cases body section (SECTION) was a fixed factor while individual identity (IDENTITY) was a random factor

| | Effect | df | MS effect | MS error | <i>F</i> | <i>P</i> -value |
|----------------------------------|--------|----|-----------|----------|----------|-----------------|
| (a) Musculoskeletal surface area | | | | | | |
| 1 SECTION | Fixed | 3 | 25720.35 | 1050.46 | 24.48 | < 0.0001 |
| 2 IDENTITY | Random | 12 | 9986.47 | 963.57 | 10.36 | < 0.0001 |
| 1 * 2 | Random | 36 | 963.57 | | | |
| (b) Epaxial surface area | | | | | | |
| 1 SECTION | Fixed | 3 | 888.74 | 19.38 | 45.84 | < 0.0001 |
| 2 IDENTITY | Random | 12 | 199.74 | 17.63 | 11.32 | < 0.0001 |
| 1 * 2 | Random | 36 | 17.63 | | | |

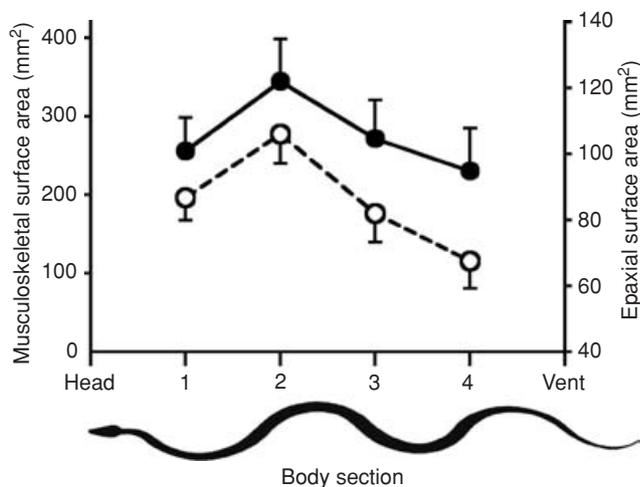


Fig. 3. Variation in musculature surface area (mm^2) obtained by MRI among the four body sections of 13 *Epicrates cenchria maurus*. The musculoskeletal surface area (black circles, left axis) corresponds to the total cross-sectional surface area of muscular and skeletal tissues. Epaxial surface area (open circles, right axis) corresponds to the combined bilateral surface of the two muscle groups (spinalis–semispinalis and longissimus dorsi). Error bars represent standard deviation.

in section 2 (Fig. 2). This analysis also revealed a significant effect of individual identity on musculature, suggesting variation in this trait among individuals in the group (Table 1). Similar variation was detected when considering the combined area of the two epaxial muscles (semispinalis–spinalis and longissimus dorsi) lying between the ridges detectable by palpation (Table 1 & Fig. 3). The sectional surface areas of the two groups were significantly correlated ($r^2 = 0.61$, $n = 52$, $P < 0.0001$), and the combined bilateral surface area of those muscles accounted for most of the variance in overall musculature obtained from MSA ($r^2 = 0.73$, $n = 52$, $P < 0.0001$). The variation in surface area of the two muscles was tightly linked to the epaxial width obtained from imaging ($r^2 = 0.69$, $n = 52$, $P < 0.0001$). Consequently, the width of epaxial muscles was a significant correlate of MSA ($r^2 = 0.73$, $n = 52$, $P < 0.0001$).

The three consecutive calliper measurements of epaxial width obtained on anaesthetized or alert snakes were highly repeatable (mean coefficient of variation = 1.4% and 1.2%, respectively). The widths of epaxial muscles obtained from MRI were significantly greater than those obtained by direct palpation either in anaesthetized snakes (paired t -test: $t_{(1,51)} = 22.32$, $P < 0.0001$) or alert snakes (paired t -test: $t_{(1,51)} = 32.59$, $P < 0.0001$). Interestingly, measurements during relaxation were lower than those from alert snakes (paired t -test: $t_{(1,51)} = 7.18$, $P < 0.0001$). However, data obtained from the two methods were highly correlated ($r^2 = 0.77$, $n = 52$, $P < 0.0001$). We found a significant correlation between MRI data and calliper measurements on alert snakes ($r^2 = 0.71$, $n = 52$, $P < 0.0001$). The correlation between MRI and calliper measurements was well illustrated when pooling each

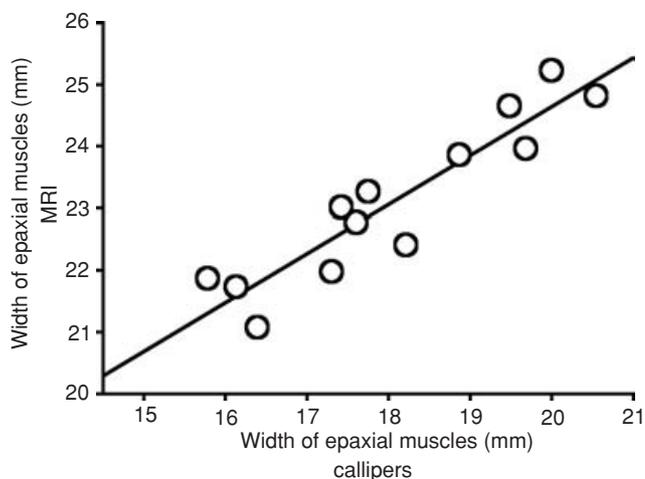


Fig. 4. Relationship between mean epaxial muscle widths measured by callipers on alert snakes and by using MRI images.

individual body section and considering mean epaxial muscle width ($r^2 = 0.85$, $n = 52$, $P < 0.0001$; Fig. 4). As a consequence, we found a strong correlation between the width of epaxial muscles from palpation measurements (alert snakes) and epaxial surface area ($r^2 = 0.68$, $n = 52$, $P < 0.0001$) or MSA ($r^2 = 0.60$, $n = 52$, $P < 0.0001$).

Musculature and physical performance

Body size (SVL) can be a major correlate of absolute musculature and performance, so it is crucial to consider this factor. Body size variation was low in our sample group (mean SVL = 122.12 ± 6 cm, coefficient of variation = 4%) and at the time of testing, we found no relationship between body size and musculature for each of the four segments considered (all P -values > 0.2). In the following analysis, measurements of epaxial muscle width were not adjusted by body size.

The three maximal traction measurements recorded during the 5 min of stimulation were highly repeatable (coefficient of variation $< 7.5\%$). We detected a clear relationship between the mean maximal traction force and the width of epaxial muscles for each body section (see Table 2). However, most of the variance observed in traction force was explained when considering segments 3 and 4 (Table 2 & Fig. 5). No influence of body size was found (Table 2). Data obtained on constriction patterns indicated a clear change in constriction force throughout the constriction episode (see Fig. 6). After biting, the snake rapidly wrapped two to four coils (approximately one-third of the body) around the lure. The tail and posterior body were often used as a ‘support’ by pushing against the side of the testing box. A peak in displaced water was observed after the bite, while wiggling the lure (step 1). After a rapid decrease (step 2), a prolonged plateau was then observed (step 3). Finally, the relaxation of body coils was associated with a rapid drop in pressure (step 4). Constriction and traction maxima were strongly correlated

Table 2. Correlates of physical performance (traction and constriction maxima) measured after an extended fasting period. We considered separately the width of epaxial muscles in the four body segments (S1 to S4 starting from the head). SVL, snout-vent length

| Variable | Traction force ($n = 21$) | | | Constriction maxima ($n = 21$) | | |
|----------|--------------------------------|------|--------------------|-------------------------------------|-------|---------------|
| | r^2 | F | P | r^2 | F | P |
| S 1 | 0.19 | 4.8 | 0.039 | 0.20 | 4.8 | 0.037 |
| S 2 | 0.45 | 16.9 | 0.005 | 0.42 | 13.71 | 0.001 |
| S 3 | 0.72 | 51.4 | < 0.0001 | 0.48 | 17.61 | 0.0004 |
| S 4 | 0.79 | 75.7 | < 0.0001 | 0.44 | 15.16 | 0.0009 |
| SVL | 0.005 | 0.10 | 0.75 | 0.04 | 0.81 | 0.37 |

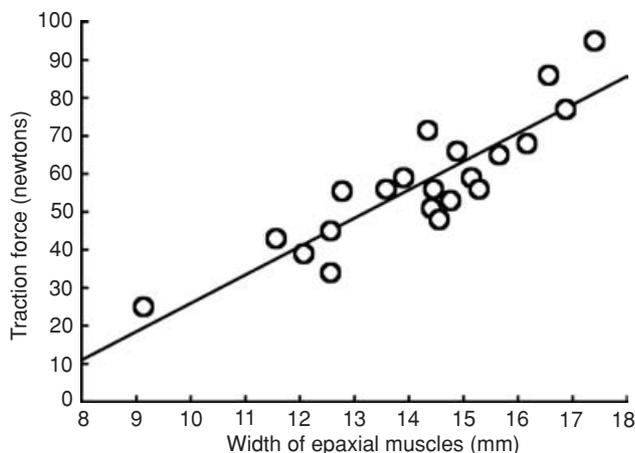


Fig. 5. Relationship between traction maxima and the width of epaxial muscles of body section 3 measured by callipers on alert snakes.

($r^2 = 0.56$, $n = 21$, $P < 0.0002$). Consistently, we found a significant correlation between constriction maxima and epaxial muscle width (Table 2), but there was no influence of body size (Table 2). The width of epaxial muscles measured in segment 3 accounted for 48% of the variance observed in constriction maxima. No relationship was found between epaxial muscle width and constriction duration (all P -values > 0.11).

Influence of energy intake

Food intake resulted in a significant increase in body mass (paired t -test: $t_{(1,20)} = 9.6$, $P < 0.0001$, mean values 890.4 ± 167 g vs 1078.9 ± 152 g) while no significant growth in SVL occurred (paired t -test: $t_{(1,20)} = -0.94$, $P < 0.35$, mean values 122.73 ± 6 cm vs 122.21 ± 6 cm). Individual muscle profiles were strongly affected, with a distinct increase in the width of epaxial muscles for each segment (repeated measures ANOVA, time effect: $F_{(1,20)} = 70.49$; $P < 0.0001$). Interestingly, we found significant variation in the width of epaxial muscles change across sections (ANOVA, $F_{(3,60)} = 11.48$; $P < 0.0001$), with the highest increase observed in

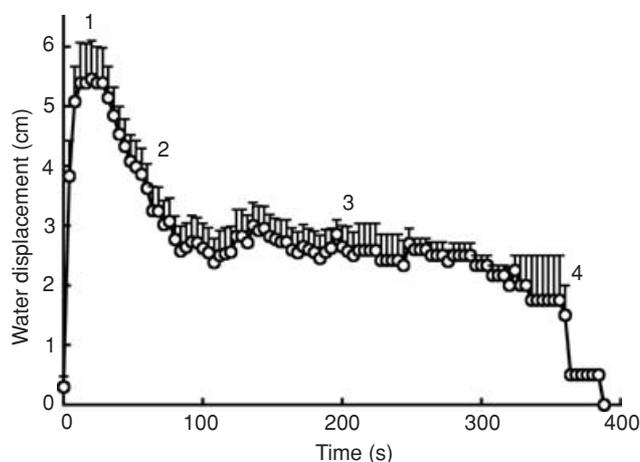


Fig. 6. Constriction patterns (cm of water displaced) measured in 21 female *Epicrates cenchria maurus*. The lure was wiggled for 10 s after being seized by the snake. The time interval between values is four seconds. Error bars represent standard error. 1, peak; 2, decrease; 3, plateau; 4, end.

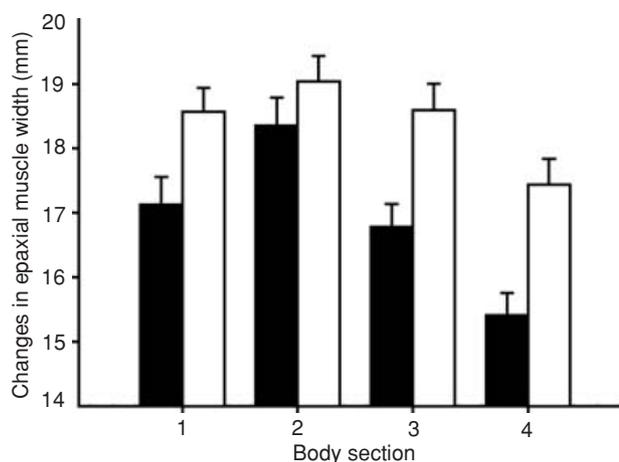


Fig. 7. Changes in the width of epaxial muscles in the four body sections over a 6-month feeding period. Black bars: initial conditions; white bars: after the 6 months of feeding. Error bars represent standard error.

the posterior region, see Fig. 7). Consistent with this result was an increase in traction performance (mean values: 78.39 ± 20 vs 69.38 ± 10 newtons, paired t -test: $t_{(1,20)} = 10.61$, $P < 0.01$). Interestingly, we detected a significant linear relationship between the change in traction force and the change in epaxial muscle width of the posterior region ($r^2 = 0.23$, $n = 21$, $P < 0.0001$, and $r^2 = 0.29$, $n = 21$, $P < 0.01$ respectively for sections 3 and 4).

DISCUSSION

The use of MRI as an original and non-invasive technique (Abe *et al.*, 2003) provided detailed information on the

internal morphology and musculature of *E. c. maurus*. First, MRI indicated that epaxial musculature was well-developed, with three muscle groups in particular showing a large cross-sectional area (Fig. 1). This descriptive result probably reflects a high demand for muscular force (Moon & Candy, 1997), and is consistent with the predatory mode of constriction. MRI data indicated that musculature was significantly variable across body sections, being most developed in the second section, which is a region directly used during prey seizing and constriction (Greene & Burghardt, 1978; Moon, 2000).

We found that variation in MSA was associated with significant variation in the combined surface area of two major epaxial muscles, the semispinalis–spinalis (Fig. 1). The width of epaxial muscles (Fig. 1) was a significant correlate of the combined surface area of these two muscles groups and MSA. Variation in overall musculature was thus tightly linked to variation in the epaxial width measured. This result reflects the significant dorso-ventral flattening of epaxial muscles that was illustrated in Fig. 1, and supports the use of epaxial width as a reliable index of musculature.

Manual palpation of the dorsal region discriminated the distinct bilateral ridge, and calliper measurements of anaesthetized snake trunk musculature were strongly correlated with MRI data. For instance, more than 75% of the variance observed in MRI measurements was explained by calliper data. Direct calliper measurements impose a significant squeezing of dorsal muscles, and therefore muscle width values obtained with callipers were consistently lower than values from MRI data. Interestingly, calliper measurements conducted on alert snakes were significantly lower than those obtained during anaesthesia. Nevertheless, data obtained by MRI and callipers were highly correlated, suggesting that the bias introduced was very limited and consistent. As a consequence, we found a significant correlation between epaxial width values obtained from calliper measurements and MSA values from MRI. Overall, our results clearly suggest that major components of the large axial musculature of constricting snakes can be measured in width using callipers and used as an estimate of overall musculature.

In this study, we also considered different indicators of locomotor performance that may possibly be affected by axial musculature. First, we estimated individual traction force using a dynamometer. This technique has been previously used in snakes to quantify individual responses to manipulation (Schwaner & Sarre, 1988). Maximal traction strength was highly repeatable and showed a low coefficient of variation. We found a close relationship between epaxial width and traction force that was most notable in the posterior sections (3 and 4), which were the least muscular. This result suggests that traction force involves a whole body effort. In this context, the weakest region (i.e. posterior sections) in the serially arranged force generator will impose the limit on the force that can be generated by the whole structure. We also assessed constriction capabilities using an original technique based on a compressible lure. This method

provided detailed information on constriction patterns, with a notably distinct peak in pressure during and shortly after prey agitation. Though it was not possible to control for inter-individual differences in coiling posture (i.e. position of body coils, number of coils), a significant relationship was found between axial muscle width and constriction maxima. Consistent with this was a significant correlation detected between constriction and traction maxima. Interestingly, posterior sections were also significantly related to constriction while only the anterior part of the body is involved in coiling around the prey. This result may only reflect cross-correlation in musculature values among body sections. Alternatively, it may suggest that constriction also involves a whole body effort as well. For instance, after seizing the prey the snakes typically moved the posterior parts of their bodies to find support against the side of the testing box. Such behaviour may be functionally important and may influence the overall strength deployed during a constriction bout. Further study is necessary to examine the validity of this statement.

Variation in musculature and performance may be linked to variation in body size, and the above results may simply reflect the fact that bigger snakes are more muscular and stronger. Importantly, this hypothesis was not supported here: variation in body size was low in our sample, while musculature was variable across individuals. In addition, we found no relationship between body size and musculature for each of the four body sections. Thus variation detected in performance can be directly linked to variation in musculature rather than to individual differences in size. Importantly, defence and prey capture are major ecological tasks that directly affect individual survival in natural conditions (Arnold, 1983). In this context, a proper identification of the functional factors accounting for inter-individual variation in physical performance is crucial. Our study suggests that epaxial muscles act as a major determinant of strength intensity deployed during those behaviours.

Finally, we examined the impact of energy intake on musculature and performance. Importantly, this feeding period occurred after an extended fast, and several individuals were post-reproductive and showed visibly reduced musculature. After the 6 months of feeding, we detected a clear increase in body mass associated with an increase in the width of epaxial muscles for each of the four sections. Although the snakes probably gained fat as well during this time period, the epaxial muscles measured are located dorsally and therefore would not be affected by an increase in abdominal diameter. Muscle gain was higher in the posterior area, suggesting that the fasting period preceding the food intake period had induced localized protein use in the posterior area. This hypothesis was also supported by a significant correlation between changes in traction force and muscle width gain in sections 3 and 4. Hence, we show that our estimates of musculature profiles and physical performance were affected by food intake and permitted detection of structural and functional restoration of musculature after an extended fasting period.

In conclusion, our results clearly demonstrate the value of the different estimators developed in this study. First, the use of MRI validated the use of calliper-derived dorsal musculature measurements. The role of epaxial muscles in constriction has attracted considerable interest (Ruben, 1977; Jayne, 1982; Moon, 2000). Our original approach was based on considering inter-individual variation in the width of epaxial muscles. As expected, we found a clear relationship between musculature and the strength deployed during major behavioural tasks, such as defence and prey constriction. Finally, we show that the estimators of musculature and performance reacted consistently to a major variable – food intake. Mass gain was associated with a significant increase in musculature and performance. The close correlation between musculature measures, traction and constriction forces supported the reliability of those estimators. In a pilot study we have successfully used those variables to examine the impact of pregnancy on musculature and performance (Lourdais *et al.*, 2004). It would now be valuable to apply the estimators developed here in both natural and standardized conditions. Importantly, in this study we used MRI to test the validity of a simple morphometric variable as an index of overall musculature. More detailed approaches focusing on the muscular structural system are still needed. For instance, we may expect that the muscles located furthest from the mid-sagittal axis, such as iliocostalis (not measured here), should have greater mechanical advantage than those located close to the axis (semispinalis–spinalis). In this context the use of MRI with high-resolution imaging should permit assessment of the size of each epaxial muscle group and clarify their relative contributions during specific behavioural tasks.

Acknowledgements

We thank Gwénaél Beauplet, Dale DeNardo and Emily Taylor for helpful comments on the manuscript. We are grateful to Gilbert Matz for providing us the snake colony. Financial support was provided by the Conseil Général des Deux-Sèvres, the CEBC-CNRS. Finally, my bike and its flat tire were useful in providing raw material for building the constriction system.

REFERENCES

- Abe, T., Kearns, C. F. & Fukunaga, T. (2003). Sex differences in whole body skeletal muscle mass measured by magnetic resonance imaging and its distribution in young Japanese adults. *Br. J. Sports Med.* **37**: 436–440.
- Arnold, S. J. (1983). Morphology, performances and Fitness. *Am. Zool.* **23**: 347–361.
- Coates, M. & Ruta, M. (2000). Nice snake, shame about the legs. *Trends Ecol. Evolut.* **15**: 503–507.
- Cundall, D. (1987). Functional morphology. In *Snakes: ecology and evolutionary biology*: 106–140. Seigel, R. A., Collins, J. T. & Novak, S. S. (Eds). New York: Macmillan.
- Darwin, C. (1859). *On the origin of species by means of natural selection or the preservation favoured races in struggle for life*. London: John Murray.
- Gasc, J. P. (1967). Introduction à l'étude de la musculature axiale des squamates serpentiformes. *Mém. Mus. Natl. Hist Nat. (Paris)* **48**: 69–124.
- Gasc, J. P. (1974). L'interprétation fonctionnelle de l'appareil musculo-squelettique de l'axe vertébral chez les serpents (Reptilia). *Mém. Mus. Natl. Hist Nat. (Paris)* **83**: 1–182.
- Greene, H. W. & Burghardt, G. M. (1978). Behavior and phylogeny: constriction in ancient and modern snakes. *Sciences* **200**: 74–77.
- Hosffetter, R. & Gasc, J. P. (1969). Vertebrates and ribs of modern reptiles. In *Biology of the Reptilia*: 201–310. Gans, C., Parsons, T. S. & Bellairs, A. d'A. (Eds). New York: Academic Press.
- Irschick, D. J. & Losos, J. B. (1998). A comparative analysis of the ecological significance of maximal locomotor performance in Caribbean Anolis lizards. *Evolution* **52**: 19–226.
- Jayne, B. C. (1982). Comparative morphology of the semispinalis–spinalis muscle of snakes and correlation with locomotion and constriction. *J. Morphol.* **172**: 83–96.
- Jayne, B. C. (1988a). Muscular mechanism of snake locomotion: an electromyographic study of the sidewinding and concertina modes of *Crotalus cerastes*, *Nerodia fasciata* and *Elaphe obsoleta*. *J. Exp. Biol.* **140**: 1–33.
- Jayne, B. C. (1988b). Muscular mechanism of snake locomotion: an electromyographic study of lateral undulation of the Florida banded water snake (*Nerodia fasciata*) and the yellow rat snake (*Elaphe obsoleta*). *J. Morphol.* **197**: 159–181.
- Jayne, B. C. & Bennett, A. F. (1989). The effect of tail morphology on locomotor performance of snakes: a comparison of experimental and correlative methods. *J. Exp. Zool.* **252**: 126–133.
- Jayne, B. C. & Bennett, A. F. (1990). Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution* **44**: 1204–1229.
- Kearns, C. F., Keever, McK. H. & Abe, T. (2002). Overview of horse body composition and muscle architecture: implications for performance. *Vet. J.* **164**: 224–234.
- Kearns, C. F., Keever, McK. H., Kumagai, K. & Abe, T. (2002). Fat-free mass is related to one-mile race performance in elite standardbred horses. *Vet. J.* **163**: 260–266.
- Losos, J. B. (1990). The evolution of form and function: morphology and locomotor performance in West-Indian anolis lizards. *Evolution* **44**: 1189–1203.
- Lourdais, O., Brischoux, F., DeNardo, D. & Shine, R. (2004). Protein catabolism in pregnant snakes (*Epicrates cenchria maurus*, Boidae) compromises musculature and performance after reproduction. *J. Comp. Physiol. B.* **174**: 383–391.
- Matz, G. (2001). Biologie de la reproduction de *Epicrates maurus* GRAY, 1849. *Situla* **3**: 10–15.
- Meyers, R. A. (1992). Morphology of the shoulder musculature of the American kestrel, *Falco sparverius* (aves), with implication for gliding flight. *Zoomorphology* **112**: 91–103.
- Moon, B. R. (2000). The mechanics and muscular control of constriction in gopher snakes (*Pituophis melanoleucus*) and a king snake (*Lampropeltis getula*). *J. Zool. (Lond.)* **252**: 83–98.
- Moon, B. R. & Candy, T. (1997). Coelomic and muscular cross-sectional areas in three families of snakes. *J. Herpetol.* **31**: 37–44.
- Mosauer, W. (1932). On the locomotion in snakes. *Science* **76**: 583–585.
- Mosauer, W. (1935). The myology of the trunk region of snakes and its significance for ophidian taxonomy and phylogeny. *Univ. Calif. Publ. Biol. Sci.* **1**: 81–120.
- Price, J. F., Pearson, A. M., Pfost, H. B. & Deans R. J. (1960). Application of ultrasonic reflection techniques in evaluating fatness and leanness in pigs. *J. Anim. Sci.* **19**: 381–387.
- Ross, R. A. & Marzec, G. (1990). *The reproductive husbandry of pythons and boas*. Stanford, CA: Institute for Herpetological Research.

- Ruben, J. A. (1977). Morphological correlates of predatory modes in the coachwhip (*Masticophis flagellum*) and Rosy boa (*Lichanura roseofusca*). *Herpetologica* **33**: 1–6.
- Schwaner, T. D. & Sarre, S. D. (1988). Body size of tiger snakes in southern Australia, with particular reference to *Notechis ater serventyi* (Elapidae) on Chappell Island. *J. Herpetol.* **22**: 24–33.
- Shine, R. & Shetty, S. (2001). Moving in two worlds: aquatic and terrestrial locomotion in sea snakes (*Laticauda colubrina*, Laticaudidae). *J. Evolut. Biol.* **14**: 338–346.
- Walton, M., Jayne, B. C. & Bennett, A. F. (1990). The energetic cost of limbless locomotion. *Science* **249**: 524–527.