Zoology 118 (2015) 403–412

Contents lists available at ScienceDirect

Zoology

journal homepage: www.elsevier.com/locate/zool

Ontogenetic shifts and spatial associations in organ positions for snakes

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ARTICLE INFO

Article history:
Received 13 March 2015
Received in revised form 31 July 2015
Accepted 19 August 2015
Available online 21 August 2015

Keywords:
Ontogeny
Organ position
Sexual dimorphism
Snakes

ABSTRACT

Snakes possess an elongated body form and serial placement of organs which provides the opportunity to explore historic and adaptive mechanisms of organ position. We examined the influence of body size and sex on the position of, and spatial associations between, the heart, liver, small intestine, and right kidney for ten phylogenetically diverse species of snakes that vary in body shape and habitat. Snake snout–vent length explained much of the variation in the position of these four organs. For all ten species, the position of the heart and liver relative to snout–vent length decreased as a function of size. As body size increased from neonate to adult, these two organs shifted anteriorly an average of 4.7% and 5.7% of snout–vent length, respectively. Similarly, the small intestine and right kidney shifted anteriorly with an increase in snout–vent length for seven and five of the species, respectively. The absolute and relative positioning of these organs did not differ between male and female Burmese pythons (*Python molurus*). However, for diamondback water snakes (*Nerodia rhombifer*), the liver and small intestine were more anteriorly positioned in females as compared to males, whereas the right kidney was positioned more anteriorly for males. Correlations of residuals of organ position (deviation from predicted position) demonstrated significant spatial associations between organs for nine of the ten species. For seven species, individuals with hearts more anterior (or posterior) than predicted also tended to possess livers that were similarly anteriorly (or posteriorly) placed. Positive associations between liver and small intestine positions and between small intestine and right kidney positions were observed for six species, while spatial associations between the heart and small intestine, heart and right kidney, and liver and right kidney were observed in three or four species. This study demonstrates that size, sex, and spatial associations may have potential interacting effects when testing evolutionary scenarios for the position of snake organs.

1. Introduction

Snakes present a unique opportunity to study organ position because of their distinctive body plan and ecological diversification. Due to their elongated body shape, major organs tend to be long and slender (e.g., lung, liver, stomach, and kidneys), and arranged sequentially in the body cavity. For snakes, there is a characteristic pattern of organ placement within the body: anteriorly are the heart, liver, and vascular lung; the mid body houses the saccular lung, stomach, pancreas, spleen, gall bladder, gonads, and start of the small intestine; and distally are the large intestine and kidneys. While this pattern of organ topography is fairly consistent among snakes, there are notable interspecific differences in the relative positioning of organs within the body cavity and hence in their spacing. For example, the anterior edge of the heart varies in position from 14% of total body length (from the snout) for *Coluber constrictor* to 43% of body length for *Acrochordus granulatus* (Lillywhite et al., 2012).

Interspecific variation in heart position among snakes has been explained as a function of habitat and of phylogeny. Since arboreal snakes are frequently oriented vertically (i.e., climbing), it is proposed that their hearts are adaptively positioned more anteriorly in the body cavity in order to maintain adequate hydrostatic blood pressure to the head (Lillywhite, 1987; Seymour, 1987; Lillywhite et al., 2012). Terrestrial snakes are typically horizontal, thus selection has favored heart position to be slightly more distal. For aquatic species, a more centrally located heart is considered to be hydrostatically advantageous for body circulation (Lillywhite, 1987; Seymour, 1987; Lillywhite et al., 2012). Phylogeny has also been shown to be an important determinant of heart position for snakes. For a diverse set of arboreal, terrestrial, fossorial, and semi-aquatic snakes, phylogenetic analysis demonstrates that a strong

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http://dx.doi.org/10.1016/j.zool.2015.08.002
0944-2006/

phylogenetic signal underlies heart position within the body cavity (Gartner et al., 2010).

Organ position may additionally exhibit intraspecific variation. Such variation may stem from differential growth of the body and organs with age, from sexual dimorphisms in body and organ sizes, from the positioning of an organ that is influenced by the placement of one or more other organs, and/or from local adaptations to regional selective forces (i.e., interpopulation variation) (Thorpe, 1989; Shine, 1994). Growth or position of internal structures (e.g., bone and organs) for snakes has been found to either change in equal proportions with increased body size (e.g., isometrically), or vary in proportion with size (e.g., allometrically) (Bergman, 1956, 1962; Thorpe, 1975; Rossman, 1980; King et al., 1999).

Across a diversity of snakes (viperids, colubrids, and elapids), several organs (e.g., heart, liver, pancreas, and spleen) are placed more anteriorly within the body cavity of females as compared to males (Bergman, 1956, 1961, 1962; Collins and Carpenter, 1969; Rossman et al., 1982; Thorpe, 1989; Nasoori et al., 2014). In contrast, as observed for the colubrids *Coluber radiatus* and *Natrix natrix*, the right kidney of female snakes is more distally placed compared to that of males (Bergman, 1961; Thorpe, 1989).

An organ's position within the body cavity may also be dictated by the position of another organ with which it has a strong functional association. If selection has led to the adaptive positioning of an organ (e.g., the liver) that has a strong functional relationship with another organ (e.g., the heart), then the latter organ would likewise appear to be adaptively positioned. While modest attention has been directed to the variation in the placement of individual organs, the existence of correlated associations between organ positions has been largely unexplored. The occurrence of intraspecific variation and/or spatial associations in organ positions could confound existing hypotheses of the underlying source (e.g., phylogeny or habitat) responsible for interspecific variations in organ position.

We drew from this discussion the following three questions: (1) Does relative organ position vary ontogenetically? (2) Are there sexual differences in organ position? (3) Are there distinct spatial associations between organs? We addressed these questions by examining intraspecific and intersexual variations in organ positions and the spatial correlation between organs for ten species of snakes. We focused our attention on the position of four major organs: the heart, liver, small intestine, and right kidney. These organs were selected because they span the placement of organs within the body cavity (anterior to posterior) and are fairly independent of each other functionally and structurally. The snakes used in the present study exhibited a variety of body plans and habitats and represent four families: Boidae, Pythonidae, Viperidae, and Colubridae. Across these taxa, we shall show that organ positions do in fact shift anteriorly (relative to body size) with an increase in body size, that organ position can, but does not necessarily vary as a function of sex, and that organs do exhibit significant spatial associations.

2. Materials and methods

2.1. Data collection

The ten species used in the present study are taxonomically diverse and vary in body size, body shape, and habitat (Table 1). For each species, the minimum sample size was 20 individuals that spanned at the minimum a 3-fold range in snout–vent length (SVL). The majority of specimens used in the present study were either freshly killed or were thawed after frozen storage. We also included measurements of organ positions taken from museum specimens for five species (*Agkistrodon piscivorus*, *Boa constrictor*, *Masticophis flagellum*, *Pantherophis guttata*, and *Pituophis melanoleucus*). For each individual we measured SVL and total length (TL), and after making a mid-ventral incision, we measured the distance from the tip of the snout to the anterior edge of the heart, liver, small intestine, and right kidney.

2.2. Data analysis

We used SVL as our reference body length rather than TL because tail length, a portion of TL, can vary significantly between male and female snakes (Klauber, 1943; Clark, 1966; King, 1989), and all of our species sets were of mixed sex. For example, within our data set for *N. rhombifer*, female and male snakes of equivalent SVL of 80 cm possessed TL averaging 101 cm and 106 cm, respectively. We quantified relative organ position (expressed as a fraction) as the distance from the snout to the leading edge of the organ divided by SVL (distance/SVL).

To explore interspecific variation in organ positions among these species, we limited our analysis to those individuals (2–229/species) that we identified as sexually mature adults. This adult data set included individuals that were on average in the upper 35% (8–52%/species) of SVL for each species. We used analysis of covariance (ANCOVA with SVL as the covariate) and analysis of variance (ANOVA), respectively, on absolute and relative positional data to identify interspecific variation in organ position. We followed these analyses with post-hoc Tukey pairwise comparisons to determine the extent to which the investigated species differed in organ position.

To examine ontogenetic variation in organ position for each species, we plotted absolute and relative position of each of the four organs as a function of SVL. We subjected the data to linear regression analyses to identify whether the position of each organ, absolute or relative, varied significantly as a function of SVL. We compared regression slopes to determine whether organs varied intraspecifically in how they scale as a function of body size.

To investigate sexual differences in organ position, we compared absolute and relative positioning of each organ between male and female *N. rhombifer* and *P. molurus*, the two species with the largest sample sizes. These data sets included 312 male and 137 female *N. rhombifer* and 108 male and 126 female *P. molurus*. We used ANCOVA (SVL as a covariate) to test for the effects of sex on organ position.

To explore for significant spatial association between pairs of organs for each species of snake, we generated individual residuals (observed minus predicted) for each organ from the linear regressions of absolute position versus SVL. We plotted residuals for each possible pair of organs. For each residual plot, we used a correlation analysis to determine whether each organ pair demonstrated a significant association. Throughout this paper we report results as means ± 1 SE, and designate statistical significance as $P < 0.05$.

3. Results

3.1. Interspecific variation of organ position

Data from adult snakes revealed significant ($P < 0.0001$) variation in the absolute (SVL as a covariate) and relative position of each organ among the ten species (Fig. 1). Heart position ranged from 0.153 ± 0.004 of SVL for *L. getula* to 0.375 ± 0.003 for *C. cerastes*. Relative heart position for each of *A. piscivorus*, *B. constrictor*, *C. cerastes*, *C. hortulanus*, and *L. getula* was statistically distinct ($P < 0.045$) from that of the other nine species. The position of the liver ranged from 0.252 ± 0.005 for *L. getula* to 0.408 ± 0.004 for *C. cerastes*. Relative liver position was statistically divided into three groups; more anteriorly for *L. getula*, *M. flagellum*, and *P. guttata*; mid-range for *B.*