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4 **Ontogenetic shifts and spatial associations in organ positions for snakes**
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27 23 Pages
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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.

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4 ***Keywords:*** Ontogeny; Organ position; Sexual dimorphism; Snakes
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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 semiaquatic snakes, phylogenetic analysis demonstrates that a strong phylogenetic signal underlies heart position within the body cavity (Gartner et al., 2010).

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4 Organ position may additionally exhibit intraspecific variation. Such variation may stem from
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6 differential growth of the body and organs with age, from sexual dimorphisms in body and organ
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8 sizes, from the positioning of an organ that is influenced by the placement of one or more other
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10 organs, and/or from local adaptations to regional selective forces (i.e., interpopulation variation)
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12 (Thorpe, 1989; Shine, 1994). Growth or position of internal structures (e.g., bone and organs) for
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14 snakes has been found to either change in equal proportions with increased body size (e.g.,
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16 isometrically), or vary in proportion with size (e.g., allometrically) (Bergman, 1956, 1962;
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18 Thorpe, 1975; Rossman, 1980; King et al., 1999)
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23 Across a diversity of snakes (viperids, colubrids, and elapids), several organs (e.g., heart, liver,
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25 pancreas, and spleen) are placed more anteriorly within the body cavity of females as compared
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27 to males (Bergman, 1956, 1961, 1962; Collins and Carpenter, 1969; Rossman et al., 1982;
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29 Thorpe, 1989; Nasoori et al., 2014). In contrast, as observed for the colubrids *Coluber radiatus*
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31 and *Natrix natrix*, the right kidney of female snakes is more distally placed compared to that of
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33 males (Bergman, 1961; Thorpe, 1989).
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38 An organ's position within the body cavity may also be dictated by the position of another organ
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40 with which it has a strong functional association. If selection has led to the adaptive positioning
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42 of an organ (e.g., the liver) that has a strong functional relationship with another organ (e.g., the
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44 heart), then the latter organ would likewise appear to be adaptively positioned. While modest
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46 attention has been directed to the variation in the placement of individual organs, the existence of
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48 correlated associations between organ positions has been largely unexplored. The occurrence of
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50 intraspecific variation and/or spatial associations in organ positions could confound existing
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52 hypotheses of the underlying source (e.g., phylogeny or habitat) responsible for interspecific
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54 variations in organ position.
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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 analyses 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 $\pm$ 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. constrictor*, *N. rhombifer*, *P. melanoleucus*, and *P. molurus*; and more distally for *A. piscivorus*, *C. cerastes*, and *C. hortulanus*. The start of the small intestine varied

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4 from 0.553 ± 0.002 of SVL for *N. rhombifer* to 0.680 ± 0.006 for *C. cerastes*. For *N. rhombifer* and
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7 *P. guttata*, the beginning of the small intestine was significantly ($P < 0.0058$) more anterior
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9 (relative to body size) compared to those of *A. piscivorus*, *C. cerastes*, *C. hortulanus*, and *L.*
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11 *getula*. The leading edge of the right kidney ranged from 0.696 ± 0.004 of SVL for *P. molurus* to
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14 0.832 ± 0.006 for *P. guttata*. Right kidney position for *P. molurus* did not vary from that of *B.*
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17 *constrictor* and *C. hortulanus*, but was significantly ($P < 0.004$) more anterior compared to the
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19 right kidney of the other seven species.
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24 3.2. Ontogenetic effects on organ position

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26 For each of the ten species, the absolute position of each organ varied ($P < 0.0001$) as a function
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28 of SVL (Fig. 2). For the four organs and ten species, SVL explained on average $97.1 \pm 0.4\%$ of
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30 the variation in absolute organ position. For each species, the scaling slope of absolute organ
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32 position against SVL varied significantly ($P < 0.02$) among organs, increasing from heart to liver
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34 to small intestine to right kidney (Table 2). The only exception was a lack of significant
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36 difference between the scaling exponents of heart and liver position for *C. cerastes*.
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41 We also found that the relative position of the heart and liver of each species varied significantly
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43 ($P < 0.035$) as a function of SVL (Fig. 3 and Table 3). For all ten species, the relative positions
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45 of the heart and liver shifted anteriorly from neonate/juvenile individuals to adult snakes by an
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47 average of $18.1 \pm 2.7\%$ and $16.1 \pm 1.9\%$, respectively, of the neonate/juvenile fractional position.
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51 For example, relative heart position of juvenile *P. melanoleucus* (< 50 cm SVL) averaged
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53 0.249 ± 0.003 of SVL and decreased to 0.191 ± 0.004 among large adults (> 150 cm SVL) (Fig. 3).
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56 The relative beginning of the small intestine exhibited a similar negative ($P < 0.021$) relation
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58 with SVL for *C. cerastes*, *L. getula*, *M. flagellum*, *N. rhombifer*, *P. guttata*, *P. melanoleucus*, and
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4 *P. molurus* (Table 3). The most extreme shift was for *L. getula*; the anterior edge of the small
5 intestine was at 0.742 ± 0.010 of SVL for juveniles (< 35 cm SVL) and decreased to 0.641 ± 0.015
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7 for adults (> 80 cm SVL). For five species (*L. getula*, *M. flagellum*, *N. rhombifer*, *P.*
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9 *melanoleucus*, and *P. molurus*), the anterior edge of the right kidney varied significantly ($P <$
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11 0.018) with SVL, decreasing in relative position with increasing snake size (Fig. 3 and Table 3).
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17 *L. getula* also exhibited the largest change in relative position of the right kidney from
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19 0.905 ± 0.011 of SVL for juveniles to 0.840 ± 0.007 for adults.
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21 Individual slopes of relative position also differed between pairs of organs for *A. piscivorus*, *B.*
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23 *constrictor*, *L. getula*, *N. rhombifer*, *P. guttata*, and *P. melanoleucus*. A shared feature among
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25 each of these five species was that the slope for the relative position of the right kidney was
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27 significantly ($P < 0.03$) smaller than that of one or two of the other organs. A common trend
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29 among each of the ten species was larger slopes for the relative position of the liver and small
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31 intestine, and a smaller slope for the right kidney.
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38 3.3. Effects of sex

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40 When analyzed as either absolute (SVL as covariate) or relative position, there were significant
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42 ($P < 0.005$) differences between male and female *N. rhombifer* in the location of the anterior
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44 edge of the liver, small intestine, and right kidney. However, we found no difference in absolute
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46 or relative heart position between males and females. Male *N. rhombifer* consistently had more
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48 posteriorly placed livers and small intestines and more anteriorly placed right kidneys compared
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50 to females. For example, at a SVL of 80 cm, the anterior edges of the liver, small intestine, and
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52 right kidney (predicted by linear regression) were positioned at 0.302, 0.563, and 0.765 of SVL,
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55 respectively, for males compared to 0.293, 0.54, and 0.775 for females (Fig. 4A). In contrast,
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male and female *P. molurus* exhibited no sexual differences in the absolute or relative position of any of the four organs (Fig. 4B).

3.4. Spatial association of organs

The plotting of residuals (generated from regressions of absolute positions) identified significant ($P < 0.018$) positive spatial associations between heart and liver positions for *A. piscivorus*, *B. constrictor*, *C. cerastes*, *C. hortulanus*, *L. getula*, *M. flagellum*, and *N. rhombifer* (Fig. 5 and Table 4). However, this was not so for *P. guttata*, *P. melanoleucus*, and *P. molurus*. For each of the former species, individuals whose hearts were positioned anterior or posterior to the predicted position tended to have their liver similarly positioned. A spatial association between the heart and the small intestine or the right kidney was less common among species. Heart and small intestine position displayed a positive significant ($P < 0.028$) relationship for *C. cerastes*, *M. flagellum*, and *N. rhombifer* only. Heart and right kidney positions were positively ($P < 0.009$) associated for *C. hortulanus*, *M. flagellum*, and *N. rhombifer* (Fig. 5 and Table 4). We observed a positive relationship ($P < 0.047$) between residuals for liver and small intestine positions for *C. cerastes*, *L. getula*, *M. flagellum*, *N. rhombifer*, *P. melanoleucus*, and *P. molurus* (Fig. 5 and Table 4). Fewer species (*M. flagellum*, *N. rhombifer*, and *P. molurus*) demonstrated a positive relationship ($P < 0.004$) between liver and right kidney positions (Fig. 5 and Table 4). Residuals of small intestine and right kidney position were positively correlated ($P < 0.028$) for the snakes *B. constrictor*, *C. hortulanus*, *C. cerastes*, *M. flagellum*, *N. rhombifer*, and *P. molurus*. In one instance only was there a significant ($P < 0.046$) negative relationship between residuals: liver versus right kidney position for *P. guttata*.

4. Discussion

Variation in the relative position of internal organs among snake species is well documented (Bergman, 1961, 1962; Lillywhite, 1987; Seymour, 1987; Gartner et al., 2010; Lillywhite et al., 2012). The present study further supported these findings, and demonstrated that organ positions can vary intraspecifically as a function of size and sex, and can exhibit spatial associations. As expected, the distance from the snout to the anterior edge of each organ increased with growth. What may not be expected is that with growth, organs appear to move relatively anteriorly in the body cavity. The following discussion notes the interspecific differences in organ position among ten snake species and addresses the observed ontogenetic shifts in organ position, the effects of sex on organ position, and the extent to which organs demonstrate spatial associations.

4.1. Variation and shifting of organ position

It has been hypothesized that snake hearts are adaptively positioned as a function of habitat. It is postulated that hydrodynamic demands placed on the snake's cardiovascular system to maintain cranial pressure when vertically oriented (such as when a snake is climbing) have led to the selection of hearts to be placed more anteriorly in the body cavity for arboreal species (Seymour and Lillywhite, 1976; Lillywhite, 1987; Seymour, 1987; Lillywhite et al., 2012; however, see Badeer, 1998). This selective pressure is therefore relaxed for terrestrial and aquatic species, and thus their hearts are positioned more posteriorly within the body (Lillywhite, 1987; Seymour, 1987). Limited by a small number of species (ten), the majority of which are terrestrial, we were unable to adequately test this relationship. Our only arboreal species, *C. hortulanus*, possessed hearts positioned at 0.301 ± 0.004 of SVL (0.240 ± 0.003 of TL) in adults, similar to the position (0.252 of TL) reported by Lillywhite et al. (2012).

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4 An alternative explanation for heart position of snakes resides in their evolutionary history.

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6 Gartner et al. (2010) examined heart position of fossorial, semi-aquatic, terrestrial, and arboreal
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8 snakes and found a strong phylogenetic signal of heart position as a function of SVL. That study
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10 found no evidence that arboreal snakes possessed more anteriorly placed hearts when compared
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12 with terrestrial species. Our modest number of species likewise did not allow us to test the
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14 strength of phylogeny in dictating organ position. However, we did find that heart positions for
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16 our five species of colubrids were clustered within 0.15–0.20 of SVL, whereas for the other five
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18 species (two boids, one pythonid, and two viperids), hearts were collectively more distal, 0.21–
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20 0.37 of SVL (Fig 1.)
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26 Besides the heart and lungs, there have been no adaptive or phylogenetic explanations for the
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28 position of other organs within the body cavity (Lillywhite et al., 2012). For most individuals of
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30 the present study (< 1.2 m SVL) the anterior edge of the liver was within 10 cm of the heart. This
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32 distance was notably short for *C. cerastes* and *A. piscivorus*, averaging only 1.9 ± 0.1 cm and
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34 3.2 ± 0.4 cm, respectively, between the leading edge of the heart and liver for adult snakes
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36 (54.2 ± 0.7 and 81.4 ± 2.9 cm SVL, respectively). The start of the liver for the five colubrids also
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38 tended to be more anterior in relative position (0.252–0.297 of SVL) as compared to that of the
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40 non-colubrid species (0.289–0.408 of SVL). A clear division between colubrids and non-
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42 colubrids was not evident for the start of the small intestine. Although *N. rhombifer* and *P.*
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44 *guttata* possessed the most anterior small intestine, and *A. piscivorus*, *C. hortulanus*, and *C.*
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46 *cerastes* the most distal, the next most distal small intestines belonged to the colubrids *M.*
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48 *flagellum* and *L. getula*. An explanation for their more distal small intestine compared to other
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50 colubrids and to *P. molurus* and *B. constrictor* may stem from the fact that *M. flagellum* and *L.*
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52 *getula* feed on other snakes (Wright and Wright, 1957; Greene, 1997). A relatively longer
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4 esophagus and stomach and thus a more distally positioned small intestine may be a structural
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6 adaptation for ingesting long prey items such as snakes (Jackson et al., 2004). The relative
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8 position of the right kidney likewise did not separate out between colubrids and non-colubrids.
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10 Interestingly, the two boas and the python possessed the most anteriorly positioned right kidney.
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12 All ten species shared a significant forward positioning of the heart and liver relative to body size
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14 as they grew. From neonate/juvenile to adult snakes, the heart and liver shifted forward by an
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16 average of 4.7 and 5.7 percentage points of SVL, respectively. There were no distinct differences
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18 in how much the heart and liver shifted forward between colubrid and non-colubrid snakes.
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20 Similarly, the degree of shifting did not appear to be significantly impacted by body shape, body
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22 length, or habitat (Table 1). Irrespective of their more middle and distal placement within the
23
24 body cavity, the small intestine and right kidney still exhibited forward movement relative to
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26 SVL with increased body size for half of the species (Table 3).
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28 For *N. rhombifer* and *P. molurus*, we further examined whether other organs (lung, stomach,
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30 spleen, gall bladder, pancreas, large intestine, and left kidney) likewise shifted forward relative
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32 to SVL with an increase in size. For both species, all seven of these organs experienced a
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34 significant ($P < 0.0004$) anterior shift of their relative position with increased SVL. As noted
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36 earlier for the four focal organs, slopes of relative position as a function of SVL differed among
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38 all eleven organs, with right and left kidneys exhibiting the smallest slopes, and organs in the
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40 middle region of the body (e.g., pancreas, small intestine, gall bladder, and spleen) possessing
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42 the greatest slopes.
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44 Bergman (1956, 1962) had documented the anterior movement of the relative position of the
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46 heart, liver, gall bladder, pancreas, spleen, gonads, and right and left kidneys with increasing
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48 body size for the elapid *Naja sputatrix* and the colubrid *Cerberus rynchops*. For female *N.*
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4 *sputatrix*, the heart and liver shifted anteriorly 2.5 and 4.5 percentage points as a function of
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6 SVL, respectively, from the neonate to the adult size class. For *C. rynchops*, both heart and liver
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8 shifted forward 2.0 percentage points as a function of SVL, respectively, from the juvenile to the
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10 adult age class. The addition of another family of snakes (Elapidae) that similarly experiences an
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12 anterior shifting of the relative position of organs with size, suggests that for snakes, an increase
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14 in body size is characterized by the moving forward of the relative position of organs. Bergman
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16 (1956, 1962) also demonstrated for these two species that the distance between the caudal end of
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18 organs and the vent increases with age.
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23 A potential adaptive explanation for these shifts in position is possible if it can be demonstrated
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25 that the forward movement of organs, relative to length, improves their physiological
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27 performance. The proposed adaptive advantage of an anteriorly placed heart for arboreal snakes
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29 can be rationalized from physiological and hydrostatic considerations. However, can the forward
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31 movement of other, and possibly all, organs be similarly rationalized? It is yet to be hypothesized
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33 and tested whether an advantage in organ performance can stem from a singular or collective
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35 shift in relative organ position with increased body size. Another question is whether the
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37 advantage resides with the movement of organs closer to the head, or further away from the vent
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39 or tail.
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48 *4.2. Sexual differences in organ position* 49

50 Sexual size dimorphism is pervasive among the major clades of snakes, with females growing to
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52 larger sizes for boids, pythonids, and natricine snakes, and males becoming larger for many
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54 viperids, elapids, and colubrids (Shine, 1994). Differences in adult body size may also translate
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56 to sexual differences in relative organ position. Alternatively, organ position may also be
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4 impacted by the positioning and size of reproductive structures and developing eggs and
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6 embryos. For seven phylogenetically diverse species – *A. piscivorus* (Viperidae), *Bungarus*
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8 *candidus* (Elapidae), *C. rynchops* (Colubridae), *Coluber radiatus* (Colubridae), *Gongylosoma*
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10 *balioderius* (Colubridae), *Macrovipera lebetina* (Viperidae), and *Naja oxiana* (Elapidae) –
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12 females possessed hearts that are more anteriorly placed compared to those of males (Bergman,
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14 1956, 1961, 1962, 1963; Collins and Carpenter, 1969; Nasoori et al., 2014). In contrast, we
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16 found no sexual differences in heart position for either *N. rhombifer* or *P. molurus*. We did find
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18 sexual differences in the position of the other three organs for *N. rhombifer*; however, not so for
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20 *P. molurus*. As previously noted for *A. piscivorus*, *B. candidus*, *C. rynchops*, *C. radiatus* and *G.*
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22 *balioderius*, the liver, pancreas, and spleen are likewise more anteriorly placed for females as
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24 compared to males (Bergman, 1956, 1961, 1962; Collins and Carpenter, 1969). The forward shift
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26 of these organs in females can be explained by the need for increased space immediately
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28 posterior to them for developing embryos and eggs (Collins and Carpenter, 1969). In contrast,
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30 kidneys tend to be more anteriorly placed in males compared to females, as noted for *C.*
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32 *radiatus*, *G. balioderius*, *N. sputatrix*, and *N. rhombifer* (Bergman, 1961, 1962, 1963, present
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34 study). Kidneys of male snakes tend to be longer than those of females, which may explain, in
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36 part, why the leading edge of males' kidneys are more anterior compared to those of females
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38 (Bergman, 1956, 1961, 1962, 1963; Collins and Carpenter, 1969).
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50 4.3. Spatial association of organs 51

52 We identified a significant spatial association between at least one pair of organs for each of the
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54 ten species. However, for *P. guttata*, the single significant correlation of residuals (liver–right
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56 kidney) had a negative relationship. For the other nine species, the position of one organ relative
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4 to its expected location (distance from snout) was matched by the relative positioning of at least
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6 one other organ. This matching was exemplified by *M. flagellum* and *N. rhombifer* (Fig. 5),
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8 where both species exhibited positive associations for all six possible pairings of organs (Table
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10 4). In short, if one organ (e.g., the heart) was anterior to its predicted position in an individual
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12 snake, then it was likely that the other three organs (liver, small intestine, and right kidney) were
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14 also anterior to their predicted locations for that snake. The same was also true if one organ was
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16 more distally placed; in this case the other three were more distally placed as well. For *M.*
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18 *flagellum* and *N. rhombifer*, organs tend to develop at set distances from each other regardless of
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20 the variation in how they are originally positioned within the body cavity. However, most snakes
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22 exhibited significant associations for three or fewer pairs of organs (Table 4). Thus, establishing
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24 a set distance between organs from the onset of development is not a shared feature among
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26 snakes.
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33 One element of these spatial relationships that stands out is the greater incidence of significant
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35 associations between organs that are closer together in sequence within the body cavity.
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38 Associations between the heart and liver, liver and small intestine, and small intestine and right
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40 kidney were significant for seven, six, and six species, respectively (Table 4). Significant
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42 associations between heart and small intestine, heart and right kidney, and liver and right kidney
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44 only occurred for three, three, and four species, respectively (Table 4). When considering
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46 absolute (or relative) distances between organs, the heart and liver are the closest together and
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48 exhibit the greatest incidence of associations (seven of the ten species). Organs were not
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50 significantly more spatially associated for colubrid versus non-colubrid snakes (Table 4).
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58 *4.4. Shifting organs*

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4 We asked earlier whether an anterior shift in organ positions with size is adaptive by maintaining
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6 or enhancing physiological performance as the snake increases in size. If all studied species
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8 became more arboreal with size, then we could enlist the rationale of Seymour and Lillywhite to
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10 explain the observed anterior shifting of the heart (Lillywhite, 1987; Seymour, 1987; Lillywhite
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12 et al., 2012). However, five of the species (*A. piscivorus*, *C. cerastes*, *L. getula*, *N. rhombifer*,
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14 and *P. melanoleucus*) seldom venture far off the ground (two of them are semi-aquatic), *B.*
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16 *constrictor* and *P. molurus* become less arboreal with size, and *M. flagellum* limits its arboreal
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18 foraging to shrubs and small trees. Only *C. hortulanus* and *P. guttata* retain arboreal habits
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20 (although less so for *P. guttata*) as adults. Without a functional explanation that fits for all
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22 species and organs, we need to consider alternative mechanisms.
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28 Snake organs are housed within a cavity constructed of serial repeats of a vertebra, two ribs, and
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30 inter-connecting muscles. If, during embryogenesis, each organ develops within a set span of
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32 body segments, then each may remain structurally linked to those segments throughout growth.
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34 Hence, we can hypothesize that it is the developmental program of the snake's body that is
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36 responsible for the ontogenetic shifting in organ position. One possible explanation is the
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38 differentiated growth of body segments. For this developmental scenario, more distal regions of
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40 the body grow at a faster rate than more anterior segments. Thus, segments and their aligned
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42 organs, especially those more anterior in the body, will become relatively closer to the head as
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44 the snake grows. The advantage of a developmental hypothesis that explains the shift in relative
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46 organ position is that it is more parsimonious (applicable to multiple organs) and does not
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48 require an adaptive explanation for each organ.
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53 Testing the validity of this developmental hypothesis would require the following three elements:
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56 (i) assuming (or verifying) that each ventral scale is associated with a body segment (i.e., a
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vertebra); (ii) determining the body segment(s) aligned with each organ (e.g., its anterior edge); and (iii) measuring the size (e.g., length) of vertebrae at regular intervals from head to vent. To verify the matching of ventral scales with vertebrae, the number of ventral scales (head to vent) is compared to the number of vertebrae between the head and vent (Alexander and Gans, 1966). Determining the body segment aligned with each organ can be accomplished by dissection and noting the ventral scale number (counting from the head) that is in line with the anterior edge of each organ (Garrigues, 1962). Vertebrae number and size (e.g., length, width, mass, etc.) can be determined from the prepared skeletons of sampled snakes. A developmental hypothesis would be supported by the findings that each ventral scale is associated with a vertebra, that each organ is aligned with a set of body segments regardless of body size, and that there is differential growth of body segments as demonstrated by vertebrae growing at a faster rate in the distal portion of the body compared to the anterior region. However, finding a disassociation between organ position and vertebra number with growth (i.e., organs shift forward independent of body segment growth) and/or the lack of any differential growth of body segments would draw us to conclude that other (possibly functional) mechanisms are responsible for the observed shift in relative organ position.

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Figure legends

Fig. 1. Illustrated positions of the anterior edge of the heart, liver, small intestine, and right kidney for ten species of snakes. Organ positions are depicted relative to snout–vent length and were determined for adult individuals of each species.

Fig. 2. Distance of the anterior edge of the heart (●), liver (○), small intestine (▼), and right kidney (▽) from the snout as a function of snout–vent length (SVL) for ten species of snakes. For each organ, SVL explained more than 95% of the size-based variation in organ position. For each species, slopes of organ position differed significantly between organs.

Fig. 3. Relative position (distance from snout/snout–vent length) of the anterior edge of the heart (●), liver (○), small intestine (▼), and right kidney (▽) as a function of snout–vent length for ten species of snakes. For each species and for two to four organs, there was a significant negative relationship between relative organ position and SVL. In these cases, an increase in body length generated a forward movement in relative position.

Fig. 4. Absolute (top row) and relative (bottom row) position of the heart, liver, small intestine, and right kidney as a function of snout–vent length for male (○) and female (●) Burmese python (*Python molurus*) and diamondback water snake (*Nerodia rhombifer*). Male and female snakes shared similar increases in absolute organ position and decreases in relative position with size. However, for *N. rhombifer*, the livers and small intestines of males were positioned more posteriorly than those of females and the right kidneys of males were more anterior than for females.

Fig. 5. Residual plots of pairs of organs for the Burmese python (*Python molurus*) and the diamondback water snake (*Nerodia rhombifer*) to illustrate the spatial association between organs for two of the ten species of snakes. Residuals for the heart, liver, small intestine, and right kidney were obtained from regressions of the distance from the snout to the anterior edge of each organ as a function of snout–vent length. Significant correlations of residuals were found between the liver and small intestine, liver and right kidney, and small intestine and right kidney for *P. molurus* and between all pairs of organs for *N. rhombifer*.

Table 1. Snake species used in the present study with pertinent information.

Species	Family	N	SVL range (cm)	Age range	Length/Body type	Habitat
<i>Agkistrodon piscivorus</i>	Viperidae	21	20.0 – 87.5	N – A	Medium/Heavy	Semi-aquatic
<i>Boa constrictor</i>	Boidae	23	45.3 – 141	N – A	Long/Heavy	Terrestrial
<i>Corallus hortulanus</i>	Boidae	23	47.0 – 161	J – A	Long/Slender	Arboreal
<i>Crotalus cerastes</i>	Viperidae	46	18.7 – 63.1	N – A	Short/Medium	Terrestrial
<i>Lampropeltis getula</i>	Colubridae	28	24.0 – 111	N – A	Medium/Medium	Terrestrial
<i>Masticophis flagellum</i>	Colubridae	45	30.5 – 131	J – A	Long/Slender	Terrestrial
<i>Nerodia rhombifer</i>	Colubridae	434	18.2 – 117	N – A	Medium/Medium	Semi-aquatic
<i>Pantherophis guttata</i>	Colubridae	20	31.1 – 122	J – A	Medium/Medium	Terrestrial
<i>Pituophis melanoleucus</i>	Colubridae	30	31.7 – 178	N – A	Long/Medium	Terrestrial
<i>Python molurus</i>	Pythonidae	295	46.5 – 368	N – A	Long/Heavy	Terrestrial

Age range: N – neonate; J – juvenile; A – adult

Table 2. Regression equations for the absolute position (distance from snout) of the anterior edge of the heart, liver, small intestine, and right kidney as a function of snout–vent length. All equations are statistically significant (P 's < 0.0001).

Species	Heart	Liver	Small intestine	Right kidney
<i>Agkistrodon piscivorus</i>	$0.310x + 2.23$	$0.344x + 2.81$	$0.647x + 1.26$	$0.820x + 0.10$
<i>Boa constrictor</i>	$0.256x + 1.25$	$0.301x + 2.79$	$0.601x + 1.44$	$0.727x - 1.63$
<i>Corallus hortulanus</i>	$0.288x + 1.76$	$0.356x + 3.63$	$0.650x + 1.67$	$0.736x + 1.23$
<i>Crotalus cerastes</i>	$0.360x + 0.66$	$0.385x + 1.21$	$0.647x + 1.67$	$0.806x + 0.35$
<i>Lampropeltis getula</i>	$0.138x + 1.73$	$0.223x + 2.79$	$0.599x + 4.23$	$0.814x + 2.35$
<i>Masticophis flagellum</i>	$0.174x + 2.23$	$0.260x + 1.09$	$0.586x + 2.22$	$0.790x + 1.41$
<i>Nerodia rhombifer</i>	$0.167x + 1.68$	$0.274x + 1.95$	$0.512x + 3.42$	$0.757x + 0.80$
<i>Pantherophis guttata</i>	$0.178x + 2.26$	$0.232x + 4.42$	$0.548x + 2.91$	$0.830x + 0.29$
<i>Pituophis melanoleucus</i>	$0.177x + 3.37$	$0.266x + 3.62$	$0.570x + 4.80$	$0.783x + 1.93$
<i>Python molurus</i>	$0.198x + 4.21$	$0.270x + 4.30$	$0.589x + 4.56$	$0.689x + 3.15$

Table 3. Regression equations for the relative position (position/snout–vent length) of the anterior edge of the heart, liver, small intestine, and right kidney as a function of snout–vent length.

Species	Heart	Liver	Small intestine	Right kidney
<i>Agkistrodon piscivorus</i>	$-0.0009x + 0.404^{***}$	$-0.0010x + 0.460^{***}$	$-0.0005x + 0.700^{NS}$	$-0.0001x + 0.824^{NS}$
<i>Boa constrictor</i>	$-0.0002x + 0.290^*$	$-0.0004x + 0.372^*$	$-0.0002x + 0.634^{NS}$	$0.0002x + 0.686^{NS}$
<i>Corallus hortulanus</i>	$-0.0003x + 0.336^*$	$-0.0005x + 0.444^*$	$-0.0002x + 0.687^{NS}$	$-0.0002x + 0.768^{NS}$
<i>Crotalus cerastes</i>	$-0.0006x + 0.404^*$	$-0.0008x + 0.452^*$	$-0.0013x + 0.750^*$	$-0.0005x + 0.840^{NS}$
<i>Lampropeltis getula</i>	$-0.0006x + 0.210^{***}$	$-0.0010x + 0.343^{***}$	$-0.0015x + 0.781^{***}$	$-0.0009x + 0.921^{**}$
<i>Masticophis flagellum</i>	$-0.0004x + 0.238^{***}$	$-0.0004x + 0.308^*$	$-0.0006x + 0.668^*$	$-0.0003x + 0.836^*$
<i>Nerodia rhombifer</i>	$-0.0007x + 0.242^{***}$	$-0.0007x + 0.356^{***}$	$-0.0011x + 0.646^{***}$	$-0.0004x + 0.802^{***}$
<i>Pantherophis guttata</i>	$-0.0005x + 0.248^{***}$	$-0.0010x + 0.373^{***}$	$-0.0008x + 0.652^*$	$-0.0002x + 0.853^{NS}$
<i>Pituophis melanoleucus</i>	$-0.0005x + 0.265^{***}$	$-0.0004x + 0.353^{***}$	$-0.0005x + 0.679^{***}$	$-0.0003x + 0.833^*$
<i>Python molurus</i>	$-0.0003x + 0.274^{***}$	$-0.0002x + 0.340^{***}$	$-0.0002x + 0.664^{***}$	$-0.0002x + 0.740^{***}$

*, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$; NS, not significant

Table 4. Correlation coefficients (r) resulting from correlation analyses of residuals of pairs of organs. Residuals were generated from regression analyses of absolute position of organs as a function of snout–vent length.

Species	H – L	H – SI	H – RK	L – SI	L – RK	SI – RK
<i>Agkistrodon piscivorus</i>	(+)0.719**	(+)0.268 ^{NS}	(–)0.251 ^{NS}	(+)0.332 ^{NS}	(–)0.354 ^{NS}	(+)0.021 ^{NS}
<i>Boa constrictor</i>	(+)0.490*	(+)0.108 ^{NS}	(+)0.204 ^{NS}	(+)0.120 ^{NS}	(+)0.366 ^{NS}	(+)0.569*
<i>Corallus hortulanus</i>	(+)0.659**	(+)0.034 ^{NS}	(+)0.548*	(–)0.113 ^{NS}	(+)0.167 ^{NS}	(+)0.660**
<i>Crotalus cerastes</i>	(+)0.691***	(+)0.325*	(+)0.261 ^{NS}	(+)0.295*	(+)0.229 ^{NS}	(+)0.325*
<i>Lampropeltis getula</i>	(+)0.840***	(+)0.277 ^{NS}	(+)0.117 ^{NS}	(+)0.521*	(+)0.164 ^{NS}	(+)0.214 ^{NS}
<i>Masticophis flagellum</i>	(+)0.619***	(+)0.558***	(+)0.386*	(+)0.472*	(+)0.502**	(+)0.335*
<i>Nerodia rhombifer</i>	(+)0.601***	(+)0.415***	(+)0.135*	(+)0.505***	(+)0.139*	(+)0.131*
<i>Pantherophis guttata</i>	(+)0.185 ^{NS}	(+)0.080 ^{NS}	(–)0.068 ^{NS}	(–)0.033 ^{NS}	(–)0.452*	(+)0.089 ^{NS}
<i>Pituophis melanoleucus</i>	(–)0.004 ^{NS}	(+)0.026 ^{NS}	(+)0.329 ^{NS}	(+)0.558*	(+)0.180 ^{NS}	(+)0.215 ^{NS}
<i>Python molurus</i>	(–)0.057 ^{NS}	(–)0.067 ^{NS}	(+)0.012 ^{NS}	(+)0.438***	(+)0.232***	(+)0.454***

Organs: H, heart; L, liver; SI, small intestine; RK, right kidney

Direction of correlation: +, positive slope; –, negative slope.

*, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$; NS, not significant

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5