Reply to "Heart Position in Snakes"

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ABSTRACT

In a previous paper comparing 155 species of snakes, we showed that the position of the heart relative to the head is statistically related to both habitat usage and phylogenetic position ("Phylogeny, ecology, and heart position in snakes," *Physiological and Biochemical Zoology* 83:43–54). More specifically, we found that, on average, arboreal snakes in our study had hearts placed more posteriorly than terrestrial species ($P < 0.0001$). In their response, Professors Lillywhite and Seymour express the concerns that readers "might be misled by this statement or conclude that gravity has no clear influence on heart position in snakes." We do not share these concerns, and we respond to all of the issues raised in their commentary. We look forward to new data on the positions of snake hearts and further analyses that seek to test adaptive hypotheses by rigorous phylogenetic approaches.

We appreciate the opportunity to reply to the response concerning our article "Phylogeny, Ecology, and Heart Position in Snakes" (Gartner et al. 2010). Professors Lillywhite and Seymour provide a short discourse on gravitational physiology in relation to heart position, and this material largely reiterates

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points made in our article and in their previous articles. They also take issue with several points in our article.

Lillywhite and Seymour (2011) state that "we are concerned that readers of the abstract, and indeed the whole article, might be misled by this statement or conclude that gravity has no clear influence on heart position in snakes." The statement in question from our abstract was, "arboreal snakes in our study tend to have hearts placed more posteriorly, opposite to the trend identified in previous studies." We do not share this concern; our article pointed out the possible importance of gravity in several places. For example, we say in the second sentence of the second paragraph of the "Introduction" that "gravity, in particular, may significantly affect the cardiovascular function of snakes, which are in essence long fluid-filled tubes (Lillywhite 1987)." Several other passages also implicate the importance of gravity.

Lillywhite and Seymour (2011) suggest that a flaw in our study was the use of snout-vent length (SVL) rather than total body length. This raises three issues. First, why did we choose a different measure than in the previous works by Lillywhite and Seymour (e.g., Seymour 1987)? Second, would the use of total body length have altered our original conclusions relating to relative heart position? Third, which measure of heart position is biologically most relevant?

We use SVL for multiple reasons. First, as argued below, we believe SVL is at least as relevant a measure as total body length—perhaps more relevant. Second, some of our available specimens had incomplete tails. Third, we were concerned about possible confounding effects of sexual size dimorphism in (relative) tail length. (Note that heart position scales isometrically with SVL in our sample of snakes; see Gartner et al. 2010, p. 48.) Sexual size dimorphism in snakes is well documented for both body size (Shine 1994) and relative tail length (Shine 1993; Sheehy 2006, p. 25 and references therein). As in Seymour (1987), our sample sizes for most species were small—of the 155 species represented in our data set, 129 were represented by a single individual (see online App. A in Gartner et al. 2010). Thus, we were concerned that (apparent) species differences in the relation between total length and heart position would be more confounded by the sex makeup of our sample compared with using SVL.

Lillywhite and Seymour's contention that we "dismiss differences in tail lengths as 'negligible from a hydrostatic standpoint' (p. 45) without justification" is misleading and confounds two different passages in our original article. The quote from our original article comes from a section (Gartner et al. 2010, p. 45) discussing the fact that we measured from the tip of the rostral scale, whereas they had measured from a point midway between the eyes. We do contend that differences in snake length caused by measurement from "between the eye"

(Seymour 1987) versus from the snout are indeed "negligible from a hydrostatic standpoint" (Gartner et al. 2010, p. 45).

In a separate part of our article (Gartner et al. 2010), we address the possible ramifications of our having measured SVL, whereas Lillywhite and Seymour's studies have generally measured total body length, including the tail. Figure 4 and the entirety of page 51 (including Tables 5 and 6) of our article constitute a section titled "Variation in Tail Length as a Possible Confounding Factor." We clearly state, "One possible explanation for this discrepancy [with results from Seymour 1987] is that arboreal snakes in our data set tend to have relatively long tails (H. B. Lillywhite, personal communication)" (Gartner et al. 2010, p. 51). We attempted to gauge the possible effect of tail length by reference to other data in the literature, and we clearly stated in our conclusions that "in these analyses, the partial regression coefficient for the arboreal dummy variable was always positive, thus again indicating that arboreal snakes have hearts placed more posteriorly (unlike in the analyses of Seymour 1987), although the effect was not statistically significant (two-tailed $P = 0.0846$ for the best-fitting RegOU model; see Table 5)" (Gartner et al. 2010, p. 51).

Lillywhite and Seymour claim that "throughout the article (at least four times), the text states that the posterior position of hearts in arboreal species is opposite to the results of Seymour (1987). In fact, Seymour (1987) explicitly concluded that heart position in arboreal snakes was not significantly different from terrestrial species, so the statistical results are identical between the two studies" (2011, p. 100). Seymour (1987, Table 1) reports mean values for relative heart position (% body length) of 17.4% for arboreal species versus 18.8% for terrestrial, a difference that was not statistically significant. A search of our published article reveals only a single instance in which we used the word "opposite." The exact passage, from the "Abstract," is as follows: "The best-fit model predicting snake heart position included aspects of both habitat and clade and indicated that arboreal snakes in our study tend to have hearts placed more posteriorly, opposite the trend identified in previous studies" (Gartner et al. 2010, p. 43). To the extent that our data revealed a statistically significant difference between arboreal and terrestrial species (when using SVL as the covariate: $P = 0.0001$ in Table 4) or a trend (when using an adjustment to approximate total length: $P = 0.0846$ for the best-fitting RegOU model in Table 5) that is in a direction different from that reported by Seymour (1987, Table 1), our use of the word "opposite" is appropriate.

Without citation, Lillywhite and Seymour assert that "the vent position has little cardiovascular significance" (2011, p. 99). This is an empirical question, and one can make a case that SVL is as relevant as total length in the present context. It is certainly true that the total length of a fluid-filled column will affect the pressure differential that develops between the two ends when the position of the column deviates from horizontal. However, the compliance of the column is also of key importance (especially with regard to cardiovascular function). If a column is entirely noncompliant, there will be no pooling of fluid at the lower end of the tilted column even though a

gravitational pressure gradient develops. In snakes that frequently assume a head-up semivertical posture (e.g., some "arboreal" species), it seems likely that past natural selection would have favored the evolution of reduced compliance in the tail vasculature, either through physical stiffening of the vessels and surrounding tissue or through increased autonomic response that serves to stiffen the vessels and reduce vascular compliance (e.g., Lillywhite 1996; Lillywhite and Seymour 2011). If such evolutionary adaptation has occurred, then tail length could essentially be "taken out of the equation" with respect to the problem of blood pooling when a snake assumes a head-up vertical position. Indeed, Lillywhite and Gallagher (1985) demonstrated that during head-up tilt, rat snakes (*Elaphe*) increase peripheral vascular resistance by active vasoconstriction in visceral organs, skeletal muscle, and skin of the lower portions of the body. Furthermore, Lillywhite (1985) empirically demonstrated that the shift in blood volume to the tails during head-up tilt in the semiarboreal *Pituophis melanoleucus* was substantially less than in the terrestrial viper *Crotalus viridis*. Lillywhite (1985, p. 763) suggests that if "similar levels of vasoconstriction occur in *Pituophis* [as compared with *Elaphe*], the resistance to flow in dependent vascular beds probably impedes postural edema in these species." Several other studies have either empirically demonstrated reduced compliance in climbing snakes (Lillywhite 1993, 11 species from 5 habits) or have suggested as much in review (Lillywhite and Henderson 1993; Lillywhite 1996; Badeer 1998 in reference to the formerly cited articles). This makes sense because the snake tail is a highly muscular structure (although this surely varies among species in relation to behavior and phylogeny), devoid of internal organs except for musk glands and the hemipenes in males. The tail is likely to be much less sensitive to changes in blood supply and with a much smaller blood supply per unit volume as compared with the body. The vent of a snake indicates the end of the body cavity, which contains the visceral organs. These organs (e.g., heart, lungs, kidneys) along with the brain have obvious vital importance and receive a great fraction of the total blood flow, at least under horizontal resting conditions (see Table 1 in Lillywhite and Gallagher 1985). The amount of blood in the internal organs, plus associated body musculature, will normally be far greater than in the tail, even for a snake with an exceptionally long tail.

Even if snake tails contain relatively little blood and the compliance of their blood vessels is low (in arboreal species), we may still find pressure differences between the head and tail when a snake adopts a nonhorizontal body posture. This brings us to the "hydrostatic indifferent point" (HIP), a unique reference position within the venous circulation where blood pressure is unaffected by vertical orientation, that is, a type of "balance point" (see Gartner et al. 2010 and references therein). As we discussed, the HIP will obviously be strongly influenced by the distribution of vessel compliance along the snake's body and tail. A coincident position for the HIP and heart ensures that venous pressure and hence cardiac filling remains relatively unaffected when vertical orientation changes (Buckner et al. 1999). Based on these hemodynamic principles, Badeer (1998)

hypothesized that the position of the heart in snakes should be related to the HIP (assuming that, in general, snakes have hearts positioned toward the head rather than toward the tail or vent). Badeer (1998) was motivated by Lillywhite and Seymour's claim that arboreal snakes have relatively anteriorly positioned hearts as compared with nonarboreal species (specifically Lillywhite 1987, 1988; Seymour 1987). Badeer (1998) stated that "there is ample evidence that the gravitational pressure in the arteries going to the head is counterbalanced (neutralized) by the gravitational pressure of the blood in the veins going down to the heart. Hence, the heart does not do extra work, so another explanation must be sought" (p. 403). He went on to propose that "the position of the heart may be related to the location of the HIP and its effect on the filling pressure of the heart" (Badeer 1998, p. 404).

Lillywhite and Seymour (2011) state "they [Gartner et al. (2010)] incorrectly imply that the HIP is a constant in a species." This is not so. Nowhere did we imply that HIP is a constant. Rather, we stated (Gartner et al. 2010, p. 50) that "HIP is determined by in vivo compliance of the veins above and below the heart. In the upright position, if the dependent veins (vessels below the heart) are highly compliant relative to the vessels above the heart, then the venous HIP will shift below heart level. ... Conversely, reducing the compliance (stiffening) of the dependent vessels can raise the HIP above heart level ... and increase cardiac-filling pressures."

Lillywhite and Seymour (2011) continue: "Gartner et al. (2010) invoke Badeer's (1998) proposal that to maintain cardiac filling, the HIP should match heart position, and they imply that this is a possible explanation for the 'posterior' heart position of arboreal snakes. Ironically, Badeer was trying to explain the anterior position of the heart in arboreal snakes that we know have more anteriorly located HIPs." This statement is misleading because Badeer (1998) was not foremost trying to explain the putatively "anterior" heart position of arboreal snakes. Rather, with an emphasis on elementary principles in physics, he was trying to provide a generalized model that considered the role of compliance in addition to other hemodynamic factors that would be expected to affect cardiovascular physiology. Thus, his hypothesis can potentially be used to explain snake heart position much more generally and not only the possibility that arboreal snakes have relatively anteriorly placed hearts. His point was that heart position should match the HIP because of its effect on the filling pressure of the heart. In turn, he argued that the HIP is affected by many factors, not just the length of the snake.

Beyond differences in which measure of body size was used, Lillywhite and Seymour (2011) take issue with our method of sampling and our classification of habitat categories—specifically, that we should have included fully aquatic snakes. They contend that "the importance of heart position with respect to gravity becomes most dramatic when one considers aquatic snakes in contrast to arboreal or terrestrial snakes" (Lillywhite and Seymour 2011, p. 100). This may be true, but it is not germane to our result that arboreal snakes have more posteriorly positioned hearts than terrestrial snakes in our data set.

We do contrast aquatic and terrestrial species several times in our article, but only inasmuch as it was necessary to discuss previous work on the implications of gravity on the cardiovascular system. We committed a typographic error when we stated that there was an "absence of any expected correlation between heart location and habitat for aquatic and terrestrial species" (Gartner et al. 2010, p. 50); this should have contrasted subaquatic and terrestrial animals, as those were two of the habitats included in our study. We agree with their statement that semiaquatic species more than likely resemble terrestrial taxa in their baroregulatory physiology and morphology (Lillywhite and Seymour 2011). However, we explicitly stated, "When possible, habitat categories were chosen to reflect those of a previous study (Seymour 1987)" (Gartner et al. 2010, p. 45). In two separate instances—in the "Methods" and in the "Discussion"—we state that unlike in Seymour (1987), our data set does not include aquatic taxa. This is particularly relevant to the "Discussion," where we stated that this could be another source of difference between the two studies: "An additional caveat is that the taxa used in this study were different than those used in previous studies. ... We lacked any fully aquatic snakes, such as hydrophiid sea snakes, which accounted for 17% of Seymour's [1987] sample" (Gartner et al. 2010, p. 52).

The authors find it "puzzling" as to why we did not perform phylogenetically informed analyses on Seymour's (1987) data. As stated on page 44 (Gartner et al. 2010), "we analyze heart position in 155, primarily South American, snake species or subspecies to investigate the generality of the heart position/habitat hypothesis." Therefore, we required a new and independent data set. Although Professor Seymour had graciously provided us with his data on request, and Professor Lillywhite cautioned us with respect to possible effects of tail length, we did not see any nonproblematic way to combine the data sets, given their different measures of body size.

In closing, let us reiterate the purpose of our original article. Our goal was to "investigate the generality of the heart position/habitat hypothesis" (Gartner et al. 2010, p. 44). Our motivation was twofold. First, Seymour (1987) himself suggested that factors other than gravity might affect heart position in snakes and concluded that "there remains great potential for further work ... especially if the phylogenetic component of variability could be reduced" (Seymour 1987, p. 106). Second, the claim that arboreal or scansorial snakes have anterior heart positions as an adaptation to counter the effects of gravitational pressure gradients is pervasive in the literature (e.g., Lillywhite 1987, 1988, 1996, 2005; Lillywhite and Henderson 1993; Lillywhite and Donald 1994; Seymour and Arndt 2004), including the medical literature (e.g., Rowell 1993; Lackner and DiZio 2006), despite the fact that falsifiable alternatives have not been tested.

The goal of our original article was not to discuss in exhaustive detail the functional consequences of snake heart position. As noted by Lillywhite and Seymour (2011), we could have cited other mechanistic studies, such as Seymour and Arndt (2004), but we are cautious about such two-species comparisons for reasons detailed many years ago in this journal (Garland and Adolph 1994). We could also have mentioned

"that central arterial pressure increases in proportion to head-to-heart distance in mammals in general" (Seymour and Blaylock 2000), but that study used nonphylogenetic regression statistics with which the allometric scaling exponent (0.05) was barely different from 0, and it was not statistically different from 0 in birds. As has been shown many times, phylogenetic analyses often yield important differences in estimates of scaling exponents as compared with nonphylogenetic estimates (e.g., White et al. 2009; references in Garland et al. 2005).

We also did not intend to imply that gravity is entirely unimportant for snake heart position or more generally for snake circulatory systems. We made no such claims in our article. We agree completely that the cardiovascular system of snakes most certainly is affected by gravity. Our article addressed the "adaptive" heart position/habitat hypothesis simultaneously with one falsifiable alternative—historical contingency—specifically, that the interspecific correlation between habitat and heart position may reflect a correlation between phylogenetic position and heart position. When an interspecific pattern appears to support an adaptive hypothesis—but "ecology" is highly confounded with phylogenetic position—conventional (nonphylogenetic) statistical analyses can be misleading (e.g., see Garland et al. 1993, 2005; Revell et al. 2007; Lavin et al. 2008; references therein).

Our original article shows that modern phylogenetic statistical methods can begin to tease apart the relative magnitudes of the relations of a trait with both ecology and phylogeny and do so in a way that is unbiased by our preconceived notions regarding the likelihood of finding support for adaptive hypotheses (Gartner et al. 2010). Indeed, we found that both "ecology" (habitat) and phylogenetic position are significant (simultaneous) predictors of snake heart position (Gartner et al. 2010, Table 4). In our analyses, statistical models that incorporated phylogenetic information in a flexible fashion (RegOU models of Lavin et al. 2008) always fit the data much better than those that did not (Gartner et al. 2010, Table 3). We view these results as very encouraging. We certainly did not intend for the analysis of snake heart position to become a contentious issue, and we welcome future cooperation in addition to any new analysis that seeks to test adaptive hypotheses through a rigorous phylogenetic approach.

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