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UNIVERSITY OF CALIFORNIA
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Inter- and Intra-Specific Correlates of Habitat and Locomotion in Snakes

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Gabriel Emil Asher Gartner

August 2011

Dissertation Committee:

Dr. Theodore Garland, Jr., Chairperson

Dr. Mark A. Chappell

Dr. David N. Reznick

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2011

The Dissertation of Gabriel Emil Asher Gartner is approved:

Committee Chairperson

University of California, Riverside

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rather than to simply believe what I had read. Despite having read through “The Origin” several times, I never fully appreciated it until our readings during my first year in Riverside. It was certainly a highlight of my time at UCR and a course I hope to one day teach.

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For my mom and dad, and other unusual creatures I have known...

ABSTRACT OF THE DISSERTATION

Inter- and Intra-Specific Correlates of Habitat and Locomotion in Snakes

by

Gabriel Emil Asher Gartner

Doctor of Philosophy, Graduate Program in Evolution, Ecology and Organismal Biology
University of California, Riverside, August 2011
Dr. Theodore Garland, Jr., Chairperson

It is often thought that locomotion is the behavior that most shapes organismal form and function. Among tetrapods, differences in locomotor ability both within and among species are often thought to be the result of variation in the limbs and other aspects of the appendicular skeleton. Snakes are both elongate and completely without limbs. While this *bauplan* has served snakes well, it is nevertheless subject to constraint at several levels—particularly with reference to friction and the effects of gravity. Several functional hypotheses have been put forth attempting to relate variation in snake anatomy and physiology with movement through particular types of habitat, but none have been conducted in a rigorous phylogenetic context.

This dissertation addresses several of these hypotheses in an evolutionary context to ask whether variation in snake anatomy and physiology is attributable to adaptive

mechanisms, once we account statistically for phylogenetic history. It also examines variation in locomotor performance in the corn snake, *Pantherophis guttata*.

I first address a long-standing hypothesis that the heart position in arboreal snakes is an adaptive feature related to head-up postures during climbing. I use a phylogenetically diverse sample of snakes from several habitats to address the source of the variation in heart position. I found a trend opposite previous studies and found that phylogenetic effects (in the statistical sense) were equally important as ecological effects. I also present a response to criticism of this study in chapter two.

I then use a similar approach to that of chapter one to address whether variation in the musculature in snakes is associated with habitat. I use an information-theoretic approach to develop and compare models that incorporate morphological, behavioral, ecological, and phylogenetic variables. I found evidence that a model containing all independent variables best fit my data.

Finally, I examined variation in locomotor performance among individual corn snakes (*Pantherophis guttata*). Having found variation in speed, stamina, and maximal aerobic capacity, I found that variation in several novel, lower-level traits predicted this variation.

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Introduction

Locomotion—the ability of an animal to move through its environment—is among the behaviors most critical to the survival of an organism. Thus, the design of organisms is often shaped by evolution for locomotor ability that is at once adjustable, efficient, and reliable (Dickinson 2000). Among tetrapods locomotion requires the need to support the mass of the animal against the force of gravity; the mechanisms necessary to produce forward propulsive forces; sensory systems for steering and maneuvering; and a means of energy production to fuel the “locomotor apparatus.” For most terrestrial vertebrates, much of this is accomplished by variation and modification of the appendicular skeleton—particularly the limbs. Snakes, however, are elongate, limbless, and their musculature and internal anatomy modified to fit a tubular body. Yet snakes are able to negotiate and maneuver within a wide range of habitats with stunning ability (Greene 1997).

Snakes have a remarkably conserved body plan (Greene and McDiarmid 2005) and appear much less eco-morphologically diverse than lizards, the paraphyletic group from which they evolved (Huey et al. 1983; Pianka 1986; Bennett 1994; Pianka and Vitt 2003). Lizards have limbs with varied features, including toe pads for climbing and fringes for moving across sand and such features have been hypothesized to be adaptive for specific locomotor purposes. Limblessness and elongation have nonetheless proven to be a successful body-plan for snakes, which show a remarkable amount of functional and behavioral diversity despite certain morphological constraints (Uetz et al. 2007; Greene 1997). Further, snakes exhibit an extraordinary range of body sizes rivaled by

few vertebrates—from only a few centimeters in length to 10 meters (Lindell 1994; Greene 1997; Boback and Guyer 2003).

Snakes can be found on every continent except Antarctica and in nearly every conceivable habitat, limited only by extreme periods of prolonged cold. Concomitant with this radiation into diverse habitats, snakes have evolved numerous locomotor behaviors and, at least qualitatively, differences in locomotor performance that are associated with the differing needs to move through varied habitats in order to capture prey, elude or escape from predators, forage, and search for mates.

Variation in locomotor performance (or with morphology associated with locomotion) and how such variation relates to differences in behavior have been studied extensively in mammals (Garland et al. 1988; White et al. 2006) and lizards (Losos 1990a, 1990b, 1990c; Irschick et al. 1996; Elstrott and Irschick 2004; Vanhooydonck and Irschick 2006). Similar eco-morphological studies relating locomotor biology with differences in behavior and ecology are less common in snakes (Garland and Losos 1994). Further, such studies have been limited to comparisons of two or three widely divergent taxa (e.g., Ruben 1976), and thus are of limited utility (Garland and Adolph 1994), or have inadequately addressed the confounding effects of phylogenetic relatedness with the relationship between behavior and morphology (e.g., Seymour 1987).

Eco-morphological studies have traditionally relied on variation in morphology, and its putative link with variation in Darwinian fitness. However, as Arnold (1983) has pointed out, morphology, or other traits below the level of the whole-organism, may have

significant influence on fitness, but only to the extent that such morphological variation affects higher-level complex traits like performance. Thus, the morphology → performance → fitness paradigm (MPF) was developed by Arnold (1983) as a conceptual and applied model for studying adaptation. "Morphology," in the broadest sense, includes various sub-organismal characters, in addition to physiological and biochemical traits. The synergistic effects of sub-organismal traits, in turn, determine whole-animal performance abilities such as sprint speed, and performance abilities are more directly affected by natural and sexual selection than are lower-level traits (Oufiero and Garland 2007). The MPF paradigm was first proposed to study individual variation within populations (Arnold and Bennett 1988; Garland 1988), but has since been extended to the study of variation among populations or among species (Garland and Losos 1994). Interspecific variation at the phenotypic level can arise from various mechanisms. However, tight correlations between phenotype and the environment are typically seen as evidence of adaptation—differences among species caused by past natural selection that lead to increased organismal fitness (Harvey and Pagel 1991; Losos and Miles 1994; Rose and Lauder 1996; Larson and Losos 1996).

The MPF paradigm has been extraordinarily useful over the past 30 years in elucidating form-function relationships and in the development of the field of eco-morphology (Wainwright 1994; Kingsolver and Huey 2003). Numerous modifications have been made to the original paradigm, particularly with reference to behavior (Garland and Losos 1994). Behavior occupies a distinct level within the paradigm, acting as a filter between fitness and performance. Assuming intraspecific variation in

performance, it follows that selection will act most directly on the choices an animal makes—its behavior—rather than what it is capable of doing in terms of performance.

Perhaps most relevant to this dissertation are modifications of the MPF paradigm that reference variation in habitat types. Habitat may strongly influence the selective regime of an organism—broadly defined as the suite of biotic and abiotic features that combine to determine the expected action of natural selection (Baum and Larson 1991). Thus, variation in habitats is likely to have an evolutionary influence on an organism's behavior, as well as its functional and morphological capabilities (Figure 0.1).

In this dissertation, I examine variation in the locomotor biology of snakes both among species and within individuals. I use modern phylogenetic statistical methods to address several form-function hypotheses relating to locomotion through different types of habitats. First, I examine heart position in a phylogenetically diverse group of snakes. Previous hypotheses (Seymour 1987) have suggested that arboreal snakes have hearts closer to the head as an adaptation countering gravitational pressure gradients, but no falsifiable alternatives to this adaptive hypothesis have ever been tested. A response to criticism of aspects of this research is also presented. Second, I address several hypotheses relating to form-function trade-offs in the axial musculature in snakes. I take a model-building, information theoretic approach to address what factors—morphological, behavioral, ecological, or historical—best predict variation in the spinalis muscle in a broad, phylogenetically diverse group of snakes. Further, we discuss the implications of vertebral numbers on locomotor ability and flexibility. Third, I address the morphology → performance aspect of the MPF paradigm to ask whether variation in

locomotor ability in the corn snake, *Pantherophis guttata*, can be predicted by variation in lower-level traits related to morphology, physiology and biochemistry.

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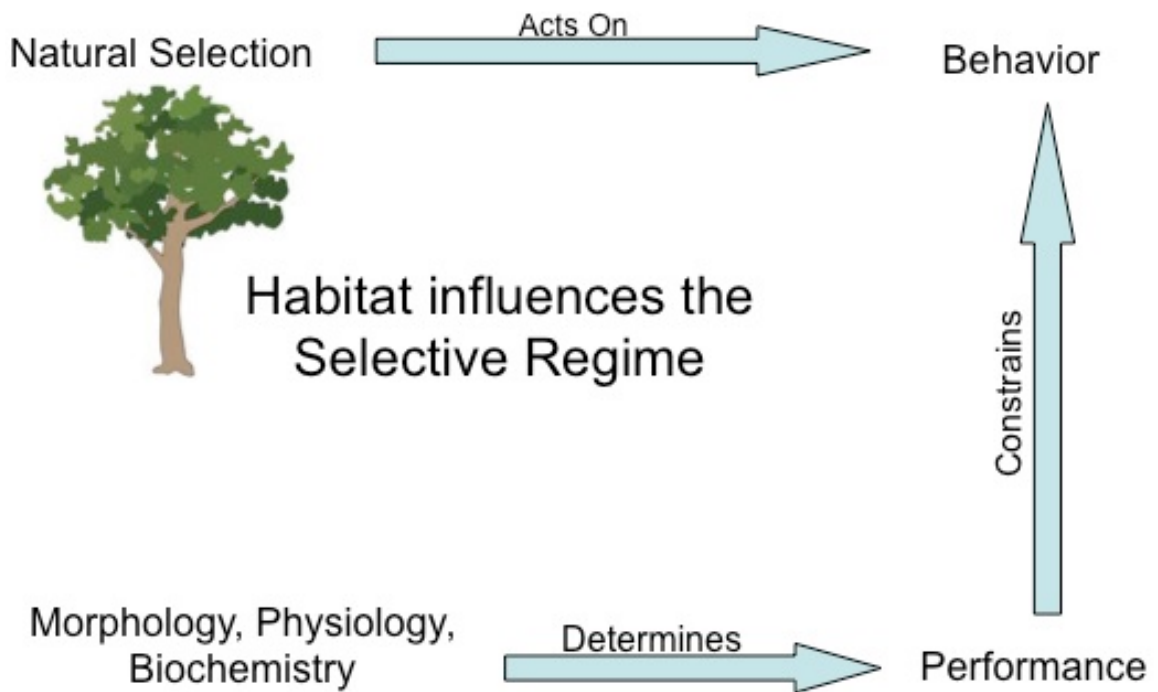
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Figure 0.1. Modified morphology → performance → fitness paradigm showing the relationship between habitat and behavior with morphology, performance, and fitness.

Modified from Garland and Carter (1994).



Chapter 1

Phylogeny, Ecology, and Heart Position in Snakes

ABSTRACT

The cardiovascular system of all animals is affected by gravitational pressure gradients, the intensity of which varies according to organismic features, behavior, and habitat occupied. A previous non-phylogenetic analysis of heart position in snakes—which often assume vertical postures—found the heart located 15-25% of total body length from the head in terrestrial and arboreal species, but 25-45% in aquatic species. It was hypothesized that a more anterior heart in arboreal species served to reduce the hydrostatic blood pressure when these animals adopt vertical postures during climbing, whereas an anterior heart position would not be needed in aquatic habitats where the effects of gravity are less pronounced. We analyzed a new data set of 155 species from five major families of Alethinophidia (one of the two major branches of snakes, the other being blind snakes, Scolecophidia) using both conventional and phylogenetically based statistical methods. General linear models regressing log snake heart position on log snout-vent length, as well as dummy variables coding for habitat and/or clade, were compared using likelihood ratio tests and the Akaike Information Criterion (AIC). Heart distance to the tip of the snout scaled isometrically with snout-vent length. In all instances, phylogenetic models that incorporated transformation of the branch lengths under an Ornstein-Uhlenbeck model of evolution (to mimic stabilizing selection) better fit the data as compared with their non-phylogenetic counterparts. 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. Our results suggest that overcoming gravitational pressure gradients in snakes most likely involves the combined action of several cardiovascular and behavioral adaptations, in addition to alterations in relative heart location.

Introduction

With the exception of body length, which varies by two orders of magnitude, the snake *bauplan* is conserved across all taxa (Greene and McDiarmid 2005). All snakes are limbless, elongate, and lack a pectoral girdle; their anatomy is elegantly modified to fit a tubular body plan (Greene 1997; Cohn and Tickle 1999). Although they might be viewed as occupying a relatively small region of “morphospace” with respect to basic body plan, snakes have radiated extensively in both number of species (>3,100 named: Uetz et al. 2007) and behavioral ecology. Even within the apparent restrictions associated with a limbless and elongate lifestyle, snakes have evolved to occupy almost all ecological niches, including fully aquatic and pelagic sea snakes, arboreal species that rarely if ever come to the forest floor, snakes that glide, and completely fossorial burrowers (Greene 1997; Martins et al. 2008).

Despite its evolutionary success, the snake body plan, is nevertheless subject to constraint at several levels. Gravity, in particular, may significantly affect the cardiovascular function of snakes, which are in essence, long fluid-filled tubes (Lillywhite 1987). In a vertical column of fluid (e.g., arteries and veins), gravity creates a vertical pressure gradient that increases with the height of the tube (i.e., ρgh ; where ρ is the density of blood, g is the acceleration due to gravity and h is the vertical height of the fluid column above or below a reference plane; in this case the heart). Gravitational pressure gradients have several physiological consequences. For example, increased gravitational pressure will distend the distal veins below the heart, and may cause significant blood pooling and increased plasma leakage into the surrounding tissues. In

addition, venous blood pooling will tend to reduce cardiac filling and cardiac output which, if not compensated by an appropriate baroreceptor response, can decrease arterial blood pressure and blood flow to the brain. Therefore, animals—particularly those that are upright or assume vertical postures—must find solutions to increased gravitational pressure gradients on the cardiovascular system. In the giraffe, for example, several mechanisms are well known to work together to prevent edema in the lower extremities, including thick and impermeable capillary basement membranes, arterial wall hypertrophy, and a prominent lymphatic system (Williamson et al 1971; Nilsson et al. 1988; Hargens 1991).

For at least two reasons, snakes are an interesting group in which to study potential cardiovascular adaptations that counter gravitational pressure gradients. First, some species are completely terrestrial and, therefore, rarely encounter changes in gravity, whereas arboreal species frequently assume vertical postures while climbing and aquatic species are less vulnerable to the effects of gravity. Second, the conservative body plan eliminates the confounding effects of appendages. The utility of snakes as models to study the effects of gravity and adaptations of the cardiovascular system to gravitational pressure gradients has been previously reported (Lillywhite 1987 1988; Seymour 1987). The most intriguing adaptive hypothesis to emerge from these studies is that heart position should correlate with behavioral ecology or habitat. Specifically, in non-aquatic snakes that frequently assume vertical postures, the height of the heart-head blood column should be reduced, thus reducing cardiac work. In contrast, aquatic species would have more centrally placed hearts due to the more dense and less gravity-stressing

nature of the medium they occupy (water). Finally, terrestrial species would have a heart position intermediate between aquatic and arboreal forms (Seymour and Lillywhite 1976; Lillywhite 1987 1988; Seymour 1987; Lillywhite and Henderson 1993; Lillywhite et al. 1996a). Although this general adaptive hypothesis seems reasonable, it must be compared with alternative explanations to describe the evolutionary origin and maintenance of traits (e.g., see Garland et al. 1993; Garland and Adolph 1994; Garland and Carter 1994; Rose and Lauder 1996; Clobert et al. 1998; Orzack and Sober 2001; Blomberg and Garland 2002; Garland et al. 2005).

In this paper, we analyze heart position in 155, primarily South American, snake species or subspecies, to investigate the generality of the heart position/habitat hypothesis using both conventional and phylogenetic statistical methods. We use ln likelihood ratio tests and the Akaike Information Criterion (AIC) to compare the fit of alternate models that account for phylogenetic and/or ecological effects (habitat) in predicting heart position of snakes.

Materials and Methods

Heart Position, Habitat, and Body Size

We gathered data on snake heart position in 155 taxa representing seven major families and subfamilies from both new material and museum specimens (Table 1.1; see Online Appendix 1.1 for data). Snakes came primarily from a data set collected in Brazil by P. R. Manzani and D. V. Andrade (Manzani 1995, n=120 species). The remaining species were measured by G. E. A Gartner (n=8 species) and S. M. Secor (n=27 species). We

used the mean snout-heart length when multiple individuals of a species were measured (see Online Appendix 1.1). In general, only adult snakes were used. Heart distance was measured by making a ventral incision from the neck until reaching the heart, then measuring the distance from the tip of the snout to the top of the atria. Snout-vent length (SVL) was measured from the tip of the rostral scale to the cloaca on the ventral side of the animal. Note that in Seymour (1987, p. 90) "the distances [were] measured between the head (eye), heart and tip of tail." This difference in measurements is, more likely than not, negligible from a hydrostatic standpoint, as it is unlikely that the evolution of the circulatory system is driven by such minimal differences in pressure.

Snakes were categorized with respect to general habitat usage using literature accounts—primarily field guides and various works on localized snake faunas (e.g., Wright and Wright 1957)—and the authors' own observations and experiences with many of the included species. *Fossorial* species actively burrow and are found underground or in litter and most possess obvious morphological adaptations for burrowing (e.g., *Typhlops*). *Semi-aquatic* species are commonly found in water where they often feed or flee from predators but frequently take to the shore to bask, reproduce, etc. (e.g., *Nerodia*). *Arboreal* species are often long and gracile in appearance and are most frequently encountered in trees or low-lying shrubs (e.g., *Boiga*, *Corallus*). *Terrestrial* species lack any obvious morphological adaptations to the terrestrial realm and thus cannot be easily classified into any of the other groups (e.g., most elapids, *Pituophis*, *Drymarchon*). When possible, habitat categories were chosen to reflect those of a previous study (Seymour 1987). Thus, the terrestrial group not only includes obviously

terrestrial animals found away from water or trees, but also those snakes occasionally found swimming or climbing (as most snakes appear to be able to swim and climb to some extent). Unlike in Seymour (1987), our data set included only one species that might be considered aquatic (*Micrurus surinamensis*), so it was coded as semi-aquatic for purposes of statistical analyses.

Phylogeny Construction

We constructed a composite tree using phylogenetic hypotheses from previously published studies. We began with higher-level relationships uniting the major lineages of snakes, and nested less inclusive groups (lower-level relationships) within this framework. Our initial intent was to use the best available phylogenetic estimate at each hierarchical level rather than combining a number of phylogenies for a particular group. Most published trees, however, contained only a few taxa of interest for any particular group, and so we were often forced to use multiple trees—each of which may have employed different characters and methods in their analyses—to place particular taxa into our tree.

When a number of trees were available for a given group, we followed the methods of de Queiroz and Rodríguez-Robles (2006): maximum likelihood trees were preferred over those obtained by other methods (e.g., parsimony), and strict consensus trees were used when available. If a lower-level group had multiple trees available to choose from, then we used the number of characters and the number of taxa to

differentiate among them. For instance, if two trees had similar numbers of taxa, we chose the tree with the greater number of characters (and vice versa).

In a few instances, the amount of clade support (i.e., bootstrap values) influenced our decision on tree selection. In those cases where no phylogenetic hypotheses could be found (particularly a problem for species-level relationships among the Xenodontinae) or where there was particularly weak nodal support, we collapsed clades to maintain a more conservative approach to our analysis.

Figure 1.1 shows the topology of the final tree and indicates the seven major clades identified in statistical analyses. Details of tree construction can be found in Appendix 1.2. For statistical analyses, branch lengths were set by the arbitrary method of Pagel (1992), as shown in Figure 1.1, using the PDTREE program. Appendix 1.3 presents the tree in a standard electronic format.

Statistical Analyses

Snout-vent length (SVL) and Snout-heart length (SHL) were \log_{10} transformed prior to analysis. To test and quantify phylogenetic signal, we used the methods of Blomberg et al. (2003).

We then described the simple allometry of snout-heart position in relation to SVL in the entire data set ($n = 155$) by use of ordinary least-squares (OLS) linear regression and reduced major axis (RMA), with both conventional (i.e., non-phylogenetic or assuming a star phylogeny) and phylogenetic versions, using the DOS PDTREE program (Garland et al. 1999; Garland and Ives 2000). PDTREE employs phylogenetically

independent contrasts, which yields estimates that are the same as from a phylogenetic generalized least-squares analysis (PGLS; Garland et al. 2005; Lavin et al. 2008). However, PDTREE provides certain statistics that are not currently available in most programs for PGLS. It is well known that OLS slopes will underestimate the true scaling relation when the independent variable (in this case, \log_{10} SVL) contains "measurement error," and if the amount of such error is not known then the RMA slope often gives a reasonable estimate (Rayner 1985; Warton et al. 2006; Ives et al. 2007). To obtain the likelihood of the alternative models, we used the Matlab Regressionv2.m program developed by A. R. Ives and T. Garland, Jr. (Lavin et al. 2008; for examples of applications, see Buchwalter et al. 2008; Jeffery et al. 2008; Warne and Charnov 2008; Huey et al. 2009; Swanson and Garland 2009).

Next, we examined the effects of body size (SVL), clade, and "ecology" using conventional multiple regressions with dummy variables for clade and habitat (i.e., analysis of covariance [ANCOVA] with parallel slopes). Formally, a "clade" is defined as a monophyletic group of organisms, including the ancestor and all descendant species. Practically, few if any comparative studies can include all members of a given clade, due to extinctions and/or inaccessibility of living representatives. In the present paper, we use "clade" to refer to all of the species included in the available data set that are members of a formal clade (e.g., Colubrinae, Viperidae, Elapidae). For analyses, Regressionv2.m automatically recoded clade as a series of six 0-1 dummy variables.

Our simplest model used only SVL as an independent variable, and hence was just a linear regression. Subsequent models added additional variables along with SVL

(e.g., SVL + Clade or SVL + Habitat). Our most complex or "full" model included SVL, clade, and habitat. Based on inspection of the partial regression coefficients for that most complex model (see Results), it was apparent that the major effect of habitat was that arboreal animals were different from all three other habitat types. Therefore, we ran one additional model that considered SVL, clade, and only arboreal animals as a distinct category.

All analyses were then repeated using PGLS ANCOVA models with the Regressionv2.m program. Finally, we implemented phylogenetic ANCOVAs with a branch-length transformation parameter based on the Ornstein-Uhlenbeck (OU) transform, termed RegOU (Lavin et al. 2008). The OU process has been suggested as a way to mimic the effects of stabilizing selection (e.g., see Felsenstein 1988; Garland et al. 1993; Blomberg et al. 2003; Halsey et al. 2006; Lavin et al. 2008; see also Martins and Hansen 1997). The statistical procedure begins with a user-specified phylogenetic tree (as shown in Figure 1.1), then moves the nodes of the tree up and down, thus simultaneously stretching and contracting the branch lengths above and below the nodes. Small values of the OU transformation parameter (d) yield trees that are more star-like (i.e., long terminal branches and short inter-node branches near the root), whereas values of d greater than unity yield trees that are even more hierarchical than the original tree. A d value of exactly unity yields the original tree. The regression model in question is fitted using the entire range of stretched and compressed trees. The tree that yields the lowest mean squared error (residual variance) has the highest likelihood, and is used to compute regression statistics. All of the multiple regression analyses were performed in

Matlab v7.0 using Regressionv2.m (Lavin et al. 2008). For the conventional (non-phylogenetic) multiple regressions, analyses were also run in SPSS for Windows version 11.5 as a verification, and results were identical to those of Regressionv2.m.

The fit of all alternate models considered was compared using the Akaike Information Criterion (AIC), computed in the smaller is better form:

$$\text{AIC} = (-2 * \ln \text{ML Likelihood}) + (2 * \# \text{ of parameters})$$

AIC is particularly well suited to situations such as this where numerous non-nested models are being compared (e.g., see Lavin et al. 2008). As a rule of thumb, models whose AIC is < 2 units larger can also be said to have substantial support, whereas a difference of 4-7 indicates considerably less support, and a difference >10 indicates essentially no support (Burnham and Anderson 2002, p. 70). Where one model was a nested subset of the other, we compared them by ln likelihood ratio (LR) tests, where twice the difference in ln likelihoods is assumed to be distributed asymptotically as a χ^2 distribution with degrees of freedom equal to the difference in the number of parameters in the two models. If an LR test and/or comparison of the AIC indicates that the phylogenetic version of the model is significantly better than the non-phylogenetic version, then one can conclude that "phylogenetic signal"—the tendency for similar species to resemble one another—(Blomberg and Garland 2002; Blomberg et al. 2003) is present in the residuals. From a more general perspective, if the clade variable is statistically significant in a model, then phylogenetic position is also important in

predicting the dependent variable (i.e., heart position in the present study), after controlling statistically for other independent variables in the model.

Results

Snout-vent length (log transformed) showed relatively low ($K = 0.265$; cf. Blomberg et al. 2003) but statistically significant ($P < 0.001$) phylogenetic signal. Once corrected for its association with \log_{10} SVL, \log_{10} heart position showed higher signal ($K = 0.580$, $P < 0.001$).

Among all taxa, heart position scales isometrically with body size (Table 1.2, Figure 1.2). The 95% confidence interval (0.931-1.124) about the ordinary least-squares (OLS) regression slope (1.027) includes unity, although, as must be the case, the RMA slope is higher (RMA slope = 1.193: $RMA = OLS/r$). The 95% confidence interval (0.856-0.995) about the phylogenetic least-squares regression (PGLS) slope (0.926) excludes unity, but again the RMA slope is higher (1.023). The phylogenetic regression with an OU transform (RegOU) is the best-fitting model (based on likelihood and AIC: Table 1.2), and has a slope of 0.937 with a 95% C.I. of 0.865-1.008.

Alternate models that include clade and/or habitat are presented in Table 1.3. Based on the AIC values (smaller is better), the relative fit of the models with various independent variables is the same for conventional OLS and RegOU models, improving in the following order: SVL (simple allometry), SVL + Habitat, SVL + Clade, SVL + Clade + Habitat, SVL + Clade + "Arboreal" (i.e., a single category of Habitat versus all others). However, for all models the RegOU versions (which contain one more

parameter) are significantly better than their OLS counterparts based on ln likelihood ratio (LR) tests, with the largest P value being 0.0041 for the model that includes SVL + Clade + Habitat. For both OLS and RegOU models, the best-fitting model, based on lowest AIC, includes SVL, Clade, and a single dummy variable for Arboreal snakes (rather than the set of three dummy variables to recognize all four habitat categories). This emphasizes that, for the present data set, the major habitat effect on snake heart position is that arboreal snakes have more posteriorly-positioned hearts (see partial regression coefficients for the full models in Table 1.4).

Table 1.3 also presents PGLS models, which incorporate the phylogeny with untransformed branch lengths, as shown in Figure 1. In all cases, these models are significantly worse than their RegOU counterparts (which contain one additional parameter), based on LR tests (largest $P = 0.0005$). Therefore, we defer further consideration of these models to the Discussion.

Discussion

We used a statistical approach that incorporates phylogenetic information to develop models that address whether ecological or historical factors (or a combination of the two) most affect relative heart position in snakes, and to test for the presence of phylogenetic signal in this trait. We determined which version of a given model, Ordinary Least-Squares (OLS) regression, Phylogenetic Generalized Least-Squares (PGLS) or phylogenetic Regression with an OU transformation (RegOU), better fit the data by use of ln likelihood ratio tests and by comparing AIC values.

Our general findings were that habitat, clade membership, and phylogenetic position within clades (and/or interclade relations) all accounted for some of the variation in relative heart position. The fact that the RegOU models fit better than the non-phylogenetic models even when clade is included as a factor (Table 1.3) means that the hierarchical structure within and/or among clades reflects some of the resemblance among related species (i.e., phylogenetic signal) in relative heart position. With respect to habitat, arboreal snakes had the most posteriorly placed hearts relative to snout-vent length; with respect to clade, the Viperidae had the most posterior hearts (Figure 1.3, Table 1.4).

In all cases, the RegOU versions of models performed significantly better (based on LR tests) than their OLS or PGLS counterparts. The relative fit of the PGLS models, as shown in Table 1.3, reveals an interesting situation. The PGLS models incorporate the phylogeny with branch lengths as shown in Figure 1.1, and do not allow any branch-length transform to improve the fit of the statistical model to the data. As compared with OLS models, their PGLS counterparts had much higher likelihoods when the clade variable was not in the model, but lower likelihoods when it was included. This reflects a "trade-off" in the sense that the phylogenetic signal (the tendency for related species to resemble each other: Blomberg and Garland 2002) present in the residuals can be apportioned either generally throughout the tree or among the specified clades, but not both given the branch lengths shown in Figure 1.1. However, when the branch lengths are allowed to vary to optimize fit in the RegOU models, the nodes are pulled towards the root (estimated d value ~ 0.2 , where 0 = a star and 1.0 = the original tree), thus making

the tree more star-like than shown in Figure 1.1, and models that include clade are much better (difference in AIC = 11.5 to 40.4) than those that do not. In these RegOU models that include clade, "phylogenetic signal" thus exists both among clades and among species within clades (or in the form of related clades resembling each other).

The importance of estimating a branch-length transformation parameter simultaneously with estimating parameters in a phylogenetic regression model was first emphasized by Grafen (1989; see review in Lavin et al. 2008). Similarly, Garland et al. (1992) emphasized the importance of various diagnostics and possible transformations of branch lengths when implementing phylogenetically independent contrasts (see also Díaz-Uriarte and Garland 1998). These points are now well-accepted in the comparative method literature (e.g., Martins and Hansen 1997; Freckleton et al. 2002; Halsey et al. 2006; Duncan et al. 2007), and the present study provides another clear example of how analyses can be improved by adding this flexibility. Moreover, debates about the importance of "ecology versus phylogeny" can be addressed statistically by comparison of a range of models in between a star and the original "starter" tree (e.g., Figure 1.1), while simultaneously testing the effects of including "ecological" (e.g., habitat) and/or "phylogenetic" (clade membership) variables in alternate models (see also Huey et al. 2009; Swanson and Garland 2009).

In our study, the single best predictor of heart position was body size, which is unsurprising given the large size range in the data set. After body size, clade membership alone (with SVL) appeared to be a significantly better predictor of heart position than habitat (with SVL) for both non-phylogenetic and RegOU models (AIC values of -241.3

versus -356.6 and -355.8 versus -367.3, respectively: Table 1.3). Moreover, those same AIC values indicate the clear superiority of the phylogenetic as compared with non-phylogenetic models. Thus, relative heart position of snakes varies in relation to both differences among the major branches of the phylogenetic tree and the detailed hierarchical structure of the tree. The best models, however, incorporated both Clade and Habitat on a hierarchical tree, and the single best model included Clade and only one aspect of habitat—inclusion in the arboreal category. It is clear that both phylogeny and habitat are important in predicting heart position. For instance, in viperids, Seymour (1987) noted that the heart as a group is generally shifted posteriorly (perhaps inherited from their common ancestor), but in arboreal members of the clade the heart is positioned more anteriorly than in terrestrial members, which Seymour interpreted as an adaptation.

Given their unusual heart positions, we also analyzed the viperids alone ($N = 29$ species), for which three habitat categories are represented (fossorial snakes are lacking). The ln likelihoods of the OLS, PGLS, and RegOU ANCOVA models were 45.0929, 44.8680, and 47.3945, respectively. Based on likelihood ratio tests, the RegOU model (estimated $d = 0.4630$) is significantly better than either the OLS ($P = 0.0319$) or PGLS model ($P = 0.0246$). The effect of habitat was not statistically significant in any of the models ($P > 0.5$ for the RegOU model).

For completeness, we also analyzed the Xenodontinae ($N = 51$) and Colubrinae ($N = 28$) alone. Results for Xenodontinae were similar to those for viperids, i.e., RegOU was the best-fitting model and habitat was not significant ($P > 0.5$ for the RegOU model). For Colubrinae, the conventional OLS model was best, and habitat was highly significant

($P < 0.0001$), with the effect attributable to arboreal species having hearts placed more posteriorly (partial regression coefficient = 0.1074, $P < 0.0001$). Thus, the habitat effect within Colubrinae alone is consistent with that shown in the overall analysis of all species (Table 1.4 and Figure 1.3).

Alternate Hypotheses for Variation in Heart Position

The anterior heart position of arboreal snakes is hypothesized to reduce the cardiac work required to “lift” the blood to the head when in the vertical orientation (Seymour 1987; Lillywhite 1988). Alternatively, Badeer (1998) hypothesized that heart position is “optimized” for cardiac filling pressures and is related to the compliance of the vessels above and below the heart. Briefly, this alternative view is based on the physical principles that determine the hydrostatic indifference point (HIP) in a vertically oriented vascular system (Wagner 1886; Clark et al. 1934; Gauer and Thron 1965). The HIP is a unique reference within the venous circulation where blood pressure is unaffected by vertical orientation (Gauer and Thron 1965; Buckner et al. 1999). 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 (Gauer and Thron 1965). The inferior location of the HIP results in a reduction in venous return and decreased cardiac filling pressure (Buckner et al. 1999; Jarvis et al. 2007). Conversely, reducing the compliance (stiffening) of the dependent vessels can raise the HIP above heart level (Gauer and Thron 1965) and increase cardiac filling pressures. The co-localization of the

HIP and heart ensures that cardiac filling remains relatively stable despite changes in vertical orientation (Buckner et al. 1999). Based on these fundamental hemodynamic principles, Badeer (1998) hypothesized that the position of the heart in snakes should be correlated with the HIP.

Several studies have shown that arboreal snakes have less compliant caudal vessels, compared with other terrestrial and aquatic species. In these species, the integument is tightly coupled to the underlying tissues, which will help prevent venous pooling in the upright position (Lillywhite 1993, 1996; Lillywhite et al. 1996b). Interestingly, this “anti-gravity” feature of the integument is similar to the “skin and fascial anti-gravity suit” seen in the legs of giraffes (Hargens et al. 1987), thus providing an apparent example of convergent evolution in function. In snakes, a more posterior location of the heart will also act in preventing blood from pooling below the heart when adopting an upright position (see the preceding discussion about HIP). Given that both decreased compliance (Lillywhite 1993, 1996; Lillywhite et al. 1996b) and posterior heart location (present study) are prevalent among arboreal species, this seems to indicate venous pooling, and the associated risk of edema formation, as an important gravitational stressor. Thus, previous adaptive scenarios placing the heart of arboreal snakes closer to the head may have overweighted the importance of ensuring an adequate blood supply to the head in detriment of the importance of venous return from the regions below the heart. Obviously, ensuring an adequate blood flow — above and below the heart — are both important on the arterial and venous side, which is only made possible by the action of an orchestrated suite of cardiovascular and behavioral adaptations, rather than

alterations in heart location alone. This, combined with differences in the intensity of the gravitational stress imposed by different habitats, may help to explain the absence of any expected correlation between heart location and habitat for aquatic and terrestrial species. Future studies should address this question in addition to determining the compliance and the HIP of snakes from a variety of habitats, thus allowing direct tests of the hypothesis that heart position is correlated with HIP.

Tail Length

Seymour (1987) grouped snakes into six categories: Arboreal, Terrestrial, Semi-aquatic, Fossorial, and Viperidae, then analyzed heart position as a percent of total length (measured as eye to tail tip). Analysis of variance indicated highly significant group differences, with Arboreal having the most anteriorly placed hearts (Table 1.6). In contrast, we analyzed snout-heart length (SHL) by ANCOVA with snout-vent length (SVL) as the covariate and found that arboreal snakes have more posteriorly placed hearts (Figure 1.3, Table 1.4). One possible explanation for this discrepancy is that arboreal snakes in our data set tend to have relatively long tails (H. B. Lillywhite, pers. comm.). Indeed, some previous studies have found that arboreal snakes often do have relatively long tails (Goldsmith 1984; Guyer and Donnely 1990; Lillywhite and Henderson 1993).

In an attempt to test this proposition, we did the following. First, we analyzed a separate, published data set for 65 South American taxa (Martins and Oliveira 1998) that also measured SVL (as in our study) rather than total length, and also reported tail length

as a percent of total length. Tail length as a percentage of total length was given as a range of values, so we used the upper end of the range to compute absolute tail length for their data set. We then computed snout-vent length by subtraction. Figure 1.4 shows that, for their data set, arboreal snakes do indeed tend to have relatively long tails.

Conventional ANCOVA of their data yields the following equation:

$$\log_{10} \text{ total length} = 0.04634 + (1.01654 * \log_{10} \text{ snout-vent length}) + (0.06242 * \text{Arboreal})$$

where "Arboreal" is a dummy variable that is 1 for arboreal species and zero for all others. Second, we used that equation to compute a \log_{10} total length for all 155 species in our data set. Third, we analyzed the data with Regressionv2.m, mimicking the analyses presented in the bottom row of Table 1.3, but with log total length as the covariate rather than log snout-vent length. 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 (2-tailed $P = 0.0846$ for the best-fitting RegOU model; see Table 1.5).

Table 1.6 shows the mean values from Seymour (1987, his Table 1.1) and from our study when snakes are grouped according to his categories. As a percent of estimated (see above) total length, our data set does not indicate Arboreal species to have the most anteriorly placed hearts. Therefore, we conclude that the discrepancy between our

analyses and those of Seymour (1987) cannot be explained entirely by the difference in the measure of body size used.

Caveats and Conclusions

A limitation of any comparative study based on data sets comprised of faunal surveys or literature reviews is that the various species are not measured under “common garden” conditions (Garland and Adolph 1991, 1994; Garland et al. 2005). Hence, it is possible that some of the differences we have observed among habitats or among clades might be reduced in magnitude if all animals had been raised under identical conditions, or even housed under identical conditions for some weeks or months prior to measurement (see Garland and Adolph 1991, and references therein). On the other hand, it would not be possible to raise all species under identical conditions because, for example, they will not (voluntarily) eat exactly the same types of food. In addition, heart position can shift ontogenetically within a species (S. M. Secor, unpublished results), so variation in age among species in our sample would affect species differences to some extent.

Most of the species included here are represented by a single individual, which obviously introduces error when comparing species. To get some indication of how this “noise” may have influenced our comparison, in Figure 1.5 we show the relation between log snout-heart length and log snout-vent length for the eight species from the Secor sample that were represented by 10 or more non-juvenile individuals (total N = 150). As can be seen, the magnitude of the differences among some species in size-

relative heart position is much larger than the range of variation observed within species. In general, "noise" introduced by small sample sizes should tend to reduce statistical power to detect effects of habitat, clade, etc. Given the several statistically significant effects we have detected (Table 1.3), this sampling variation was not large enough to obscure major results. An improvement for future analyses would be to fit regression models that explicitly account for the magnitude of within-species variation (Ives et al. 2007).

An additional caveat is that the taxa used in this study were different than those used in previous studies. In particular, only 12 of the 155 taxa used in the present study were also used in Seymour (1987: see Table 1.7). Moreover, 51 of our species (33%) were South American xenodontines, a taxon lacking from Seymour's (1987) sample, whereas we lacked any fully aquatic snakes, such as hydrophiid sea snakes, which accounted for 17% of Seymour's sample. On the other hand, of the 14 families and subfamilies included in one or the other of the two studies, nine were included in both (Table 1.7).

A final limitation is that is very difficult to quantify and categorize behavior—especially in broadly defined regimes such as “habitat” (e.g., see Jayne 1982). Very few snakes live strictly in an arboreal regime or a terrestrial regime (sea snakes and blind snakes being notable exceptions), but instead tend to exist at the borders of any given behavioral spectrum (e.g., primarily terrestrial, but occasionally arboreal or primarily fossorial, but occasionally terrestrial). Thus, categorization of habitat becomes particularly difficult for animals that can be classified as semi-arboreal, or semi-fossorial.

For our purposes, any snake that frequently assumes vertical postures, even if commonly found in a terrestrial environment (e.g., *Pantherophis*) was considered arboreal, but one can see how tinkering with such a distinction could have important consequences for the results and conclusions of a study such as this. Note, however, that our data set does include some "strictly arboreal" species (e.g., *Corallus sp.*, *Atheris squamiger*, *Bothriopsis bilineata*, *Oxybelis sp.*, *Imantodes cenchoa*, etc.). Because we present all of the data analyzed here (see Appendix 1.1), it will be possible for future workers to try various recategorizations as field data become more available. In addition, future workers can incorporate additional ecological or behavioral predictors, such as diet (Hampton 2009).

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Table 1.1. Table of families or subfamilies included in the data set and their habitat distributions

	Xenodontinae	Dipsadinae	Natricinae	Colubrinae	Elapidae	Viperidae	Boidae	Total
Terrestrial	35	6	1	11	2	22	6	83
Fossorial	4	2	-	2	5	-	1	14
Arboreal	5	6	-	15	-	5	3	34
Semi-Aquatic	7	-	11	-	0	2	3	23
Aquatic	-	-	-	-	1*	-	-	1*
Total	51	14	12	28	8	29	13	155

* The aquatic *Micrurus surinamensis* was recoded as semi-aquatic for purposes of statistical analyses.

Table 1.2. Allometry of snout-heart length in relation to snout-vent length

Model	Slope	S.E.	Y-Intercept	S.E.	r²	ln maximum likelihood	AIC
Conventional, Least-Squares	1.027	0.0489	-0.729	0.1375	0.742	118.791	-231.582
Conventional, Reduced Major Axis	1.193		-1.191				
Phylogenetic, Least Squares	0.926	0.0351	-0.405	0.1204	0.820	168.798	-331.597
Phylogenetic, Reduced Major Axis	1.023		-0.679				
Phylogenetic, Regression with OU Transform (REML estimate of d = 0.7306)	0.937	0.0362	-0.447	0.1061	0.814	174.916	-341.832

Table 1.3. Table of alternate regression models for predicting heart position of snakes

	Conventional (OLS)		Phylogeny (PGLS)		Phylogeny with OU Transform (RegOU)					
Model	ln Maximum Likelihood	AIC	ln Maximum Likelihood	AIC	ln Maximum Likelihood	AIC	Mean Squared Error	Standard Error of the Estimate	R ² for Model	REML estimate of d (OU transform)
SVL (simple allometry)	118.791	-231.6	168.798	-331.6	174.916	-341.8	6.210E-03	0.07880	0.8140	0.7306
SVL + Habitat	126.673	-241.3	178.459	-344.9	184.909	-355.8	5.567E-03	0.07462	0.8367	0.7356
SVL + Clade	187.324	-356.6	170.298	-322.6	193.673	-367.3	5.114E-03	0.07151	0.8602	0.3024
SVL + Clade + Habitat	205.625	-387.3	180.134	-336.2	209.749	-393.5	4.234E-03	0.06507	0.8936	0.1975
SVL + Clade + "Arboreal"	204.655	-389.3	179.974	-339.9	209.075	-396.2	4.210E-03	0.06489	0.8923	0.2034

Note. "Conventional" (OLS) indicates ordinary least-squares (multiple) regression, which is mathematically equivalent to assuming a "star" phylogeny with no hierarchical structure. "Phylogeny" indicates generalized least-squares analysis (PGLS), which is mathematically equivalent to phylogenetically independent contrasts. "Phylogeny with OU Transform" (RegOU) is a regression model in which the residuals are modeled as an Ornstein-Uhlenbeck process. RegOU models contain one more parameter than their OLS or PGLS counterparts, so whether a RegOU model fits the data significantly ($P < 0.05$) better can be tested by comparing twice the difference in ln likelihoods with the value 3.841 (the 95th percentile of the distribution of χ^2 with 1 df). Similar ln likelihood ratio tests can be used to compare models within the OLS, PGLS or RegOU columns when one is a nested subset of the other (e.g., SVL + Habitat versus SVL, but not SVL + Habitat versus SVL + Clade). The AIC (see Methods) can be used to compare any models, with smaller (more negative) values indicating a better fit. As a rule of thumb, models whose AIC is < 2 units larger than the best model can also be said to have substantial support. See Methods and Lavin et al. (2008) for further explanation. For the models listed in the bottom row (SVL + Clade + "Arboreal"), the Arboreal variable was always highly significant (all $P < 0.0001$), and the partial regression coefficient was always positive, indicating that heart position is more posterior than for non-arboreal species.

Table 1.4. Full model including habitat and clade variables to predict \log_{10} snout-heart length (mm), analyzed by conventional multiple regression and phylogenetically with an OU transform

Variable	Conventional (OLS)					Phylogenetic with OU Transform (RegOU)				
	Coefficient	SE	F	Df	P	Coefficient	SE	F	Df	P
Y-intercept	-0.6465	0.0964	44.94			-0.5746	0.0968	35.26		
Log SVL (mm)	0.9694	0.0331	858.82	1,144	0.0039	0.9454	0.0333	806.77	1,144	<0.0001
Arboreal	0.0914	0.0148	38.14	1,144	<0.0001	0.0879	0.0155	32.06	1,144	<0.0001
Fossorial	0.0191	0.0217	0.77	1,144	0.3813	0.0199	0.0230	0.75	1,144	0.3879
Semi-Aquatic	0.0225	0.0196	1.31	1,144	0.2537	0.0156	0.0227	0.48	1,144	0.4895
Xenodontinae	-0.0049	0.0174	0.08	1,144	0.7789	-0.0094	0.0224	0.18	1,144	0.672
Dipsadinae	0.0691	0.0232	8.87	1,144	0.0034	0.0564	0.0307	3.37	1,144	0.0685
Natricinae	-0.0448	0.0297	2.28	1,144	0.1335	-0.0483	0.0366	1.74	1,144	0.1892
Boidae	0.1226	0.0235	27.14	1,144	<0.0001	0.1314	0.0303	18.81	1,144	<0.0001
Elapinae	0.0718	0.0301	5.70	1,144	0.0183	0.0666	0.0355	3.52	1,144	0.0627
Viperidae	0.2153	0.0187	132.27	1,144	<0.0001	0.2079	0.0229	82.49	1,144	<0.0001
Habitat			12.79	3,144	<0.0001			10.71	3,144	<0.0001
Clade			42.47	6,144	<0.0001			25.09	6,144	<0.0001

Note. For the Habitat variable, Terrestrial is arbitrarily chosen as the base group for comparison. For the Clade variable, Colubridae is arbitrarily chosen as the base group for comparison. Thus, all coefficients and significance levels for the individual dummy variables within these categorical variables are relative to those base groups. Overall tests for Habitat and Clade are at the bottom. For the OLS model, Rate of Evolution (MSE) = 0.004438, Standard Error of Estimate = 0.06620, Model R^2 = 0.9160, ln Maximum Likelihood of Model = 205.625, AIC = -387.250, AICc = -385.053. For the RegOU model, MSE = 0.004234, SEE = 0.06507, Model R^2 = 0.8936, REML estimate of OU transformation parameter (d) = 0.1975, ln Maximum Likelihood of Model = 209.749, AIC = -393.499, AICc = -390.917.

Table 1.5. Analysis of covariance of snake heart position with log₁₀ estimated total length (see text) as the covariate

	Conventional (OLS)		Phylogeny (PGLS)		Phylogeny with OU Transform (RegOU)					
Model	ln Maximum Likelihood	AIC	ln Maximum Likelihood	AIC	ln Maximum Likelihood	AIC	Mean Squared Error	Standard Error of the Estimate	R ² for Model	REML estimate of d (OU transform)
Total Length + Clade + "Arboreal"	204.655	-389.3	179.974	-339.9	209.075	-396.2	4.210E-03	0.06489	0.8923	0.2034
<i>P</i> for Clade	< 0.0001		0.7890		< 0.0001					
<i>P</i> for Arboreal	0.0575		0.3469		0.0846					

Note. See note to Table 1.3. The partial regression coefficient for the Arboreal dummy variable was always positive, indicating that heart position is more posterior than for non-arboreal species.

Table 1.6. Heart position as a percent of body length.

	Seymour 1987 % of Total Length		This study % of estimated Total Length		This study % of Snout- Vent Length	
	Mean	SD	Mean	SD	Mean	SD
Arboreal	17.4	2.2	17.9	4.1	25.6	5.8
Terrestrial	18.8	3.2	15.6	2.9	19.4	3.7
SemiAquatic	22.7	4.8	15.7	2.3	19.4	2.8
Fossorial	23.6	7.9	17.6	2.9	21.8	3.5
Viperidae	33.3	4.5	25.2	3.8	31.9	4.2
Aquatic	33.4	5.7				

Table 1.7. Unique and shared taxa between Seymour (1987) and this study. See Discussion.

Shared Taxa	Unique Taxa in This Study	Unique Taxa in Seymour (1987)	Shared Species
		Acrochordidae	
Boinae			<i>Agkistrodon piscivorus</i>
Colubrinae			<i>Arizona elegans</i>
Crotalinae			<i>Bothrops atrox</i>
	Dipsadinae		<i>Diadophis punctatus</i>
Elapinae			<i>Elaphe obsoleta</i>
Erycinae			<i>Lachesis muta</i>
		Homalopsinae	<i>Lampropeltis getulus</i>
		Hydrophiinae	<i>Masticophis flagellum</i>
Natricinae			<i>Nerodia sipedon</i>
Pythoninae			<i>Nerodia taxispilota</i>
Typhlopidae			<i>Pituophis melanoleucus</i>
Viperinae			<i>Thamnophis sirtalis</i>
	Xenodontinae		

Figure 1.1. Phylogeny used for statistical analyses, with Pagel's (1992) arbitrary branch lengths. See Appendix 1.2 for details of tree construction, and Appendix 1.3 for an electronic version.

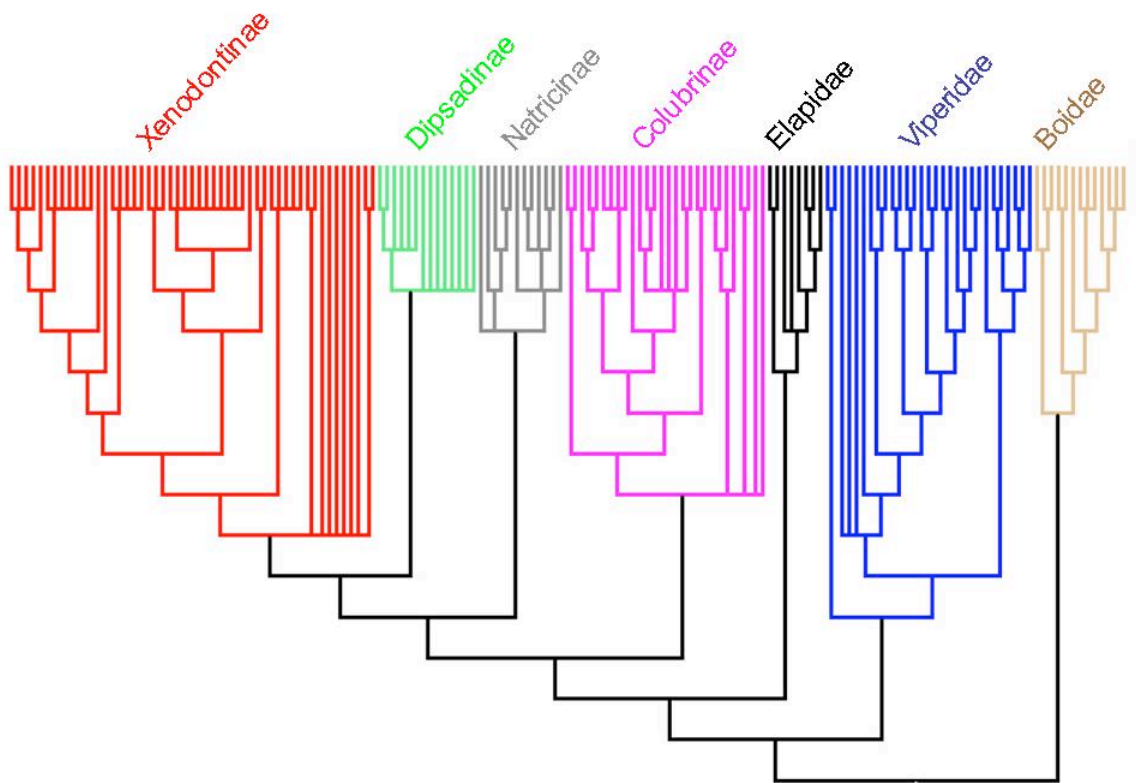


Figure 1.2. Figure 1.2 Isometry (slope not statistically different from 1.00) of snout-heart length in relation to snout-vent length for 155 species or subspecies of snakes. Line is conventional (non-phylogenetic) least-squares linear regression (slope = 1.027, S.E. = 0.0489; see Table 1.2 for this and alternatives).

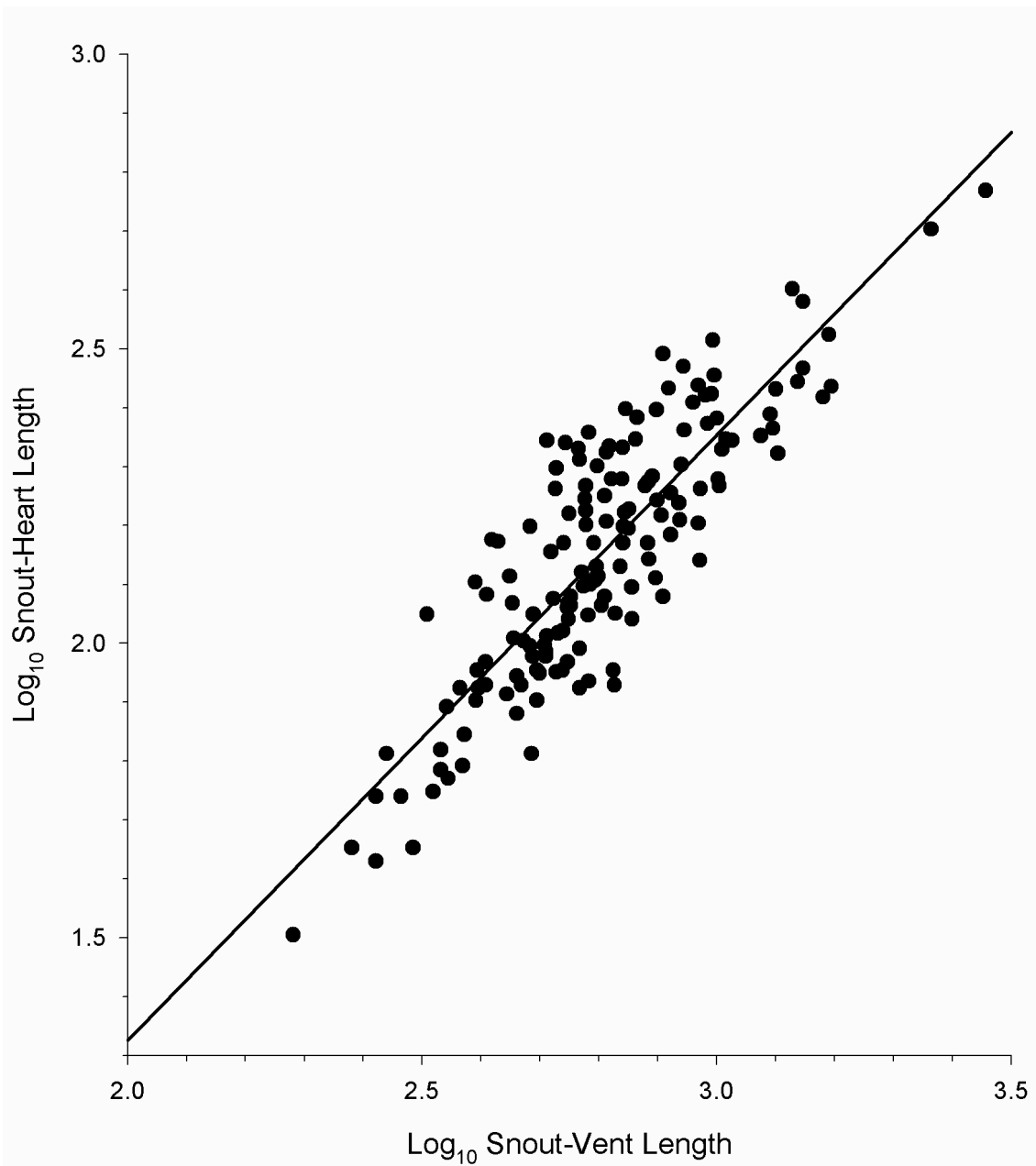


Figure 1.3. Snout-heart length in relation to snout-vent length for 155 taxa of snakes, indicating the clade that is most different (Viperidae) and the habitat classification (arboreal) that is most different from other snakes.

Figure 1.3.

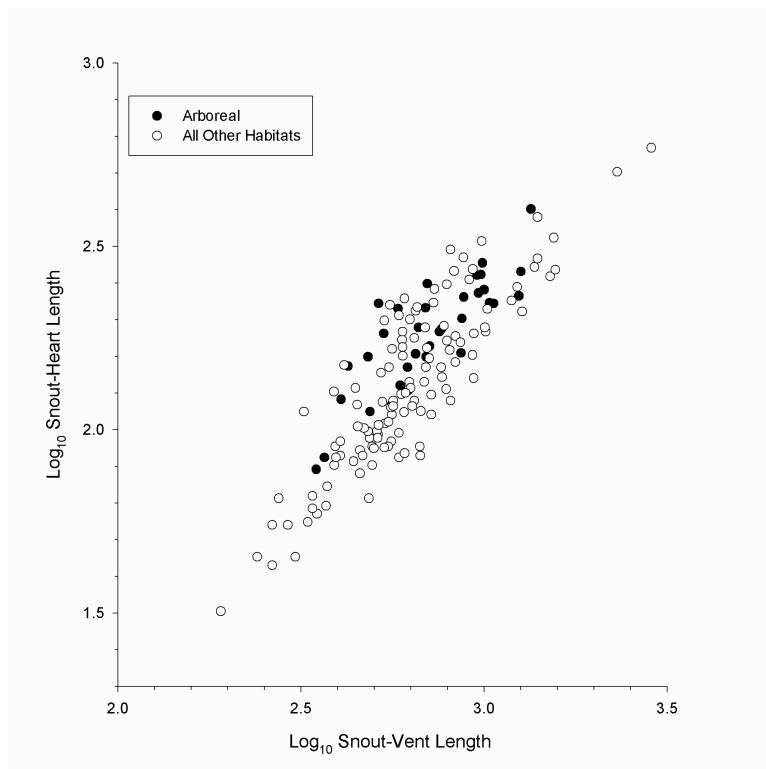
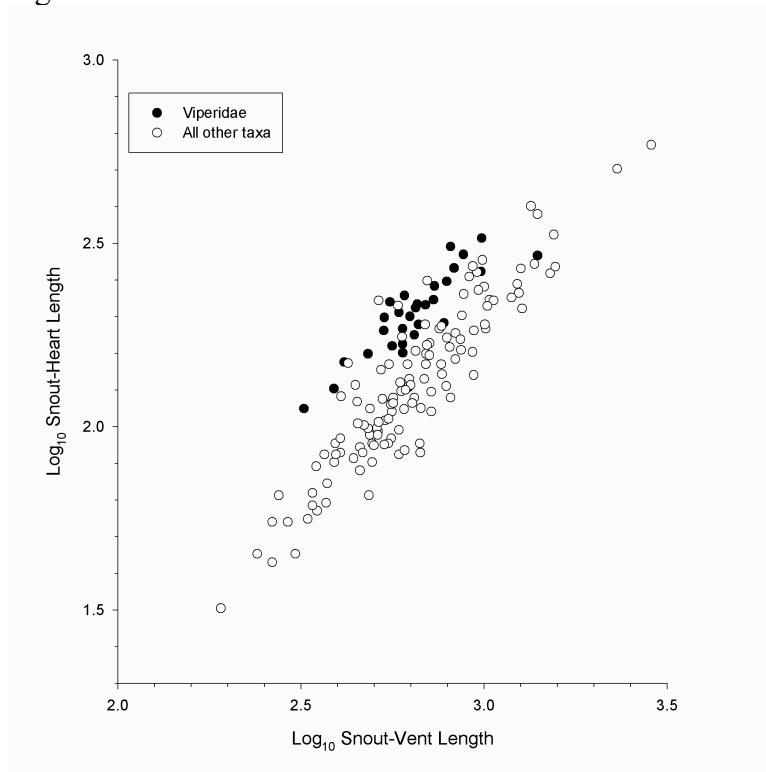


Figure 1.4. Tail length vs. snout-vent length from a published data set for 65 South American taxa (Martins and Oliveira 1998). Note that arboreal species generally have longer tails (see text).

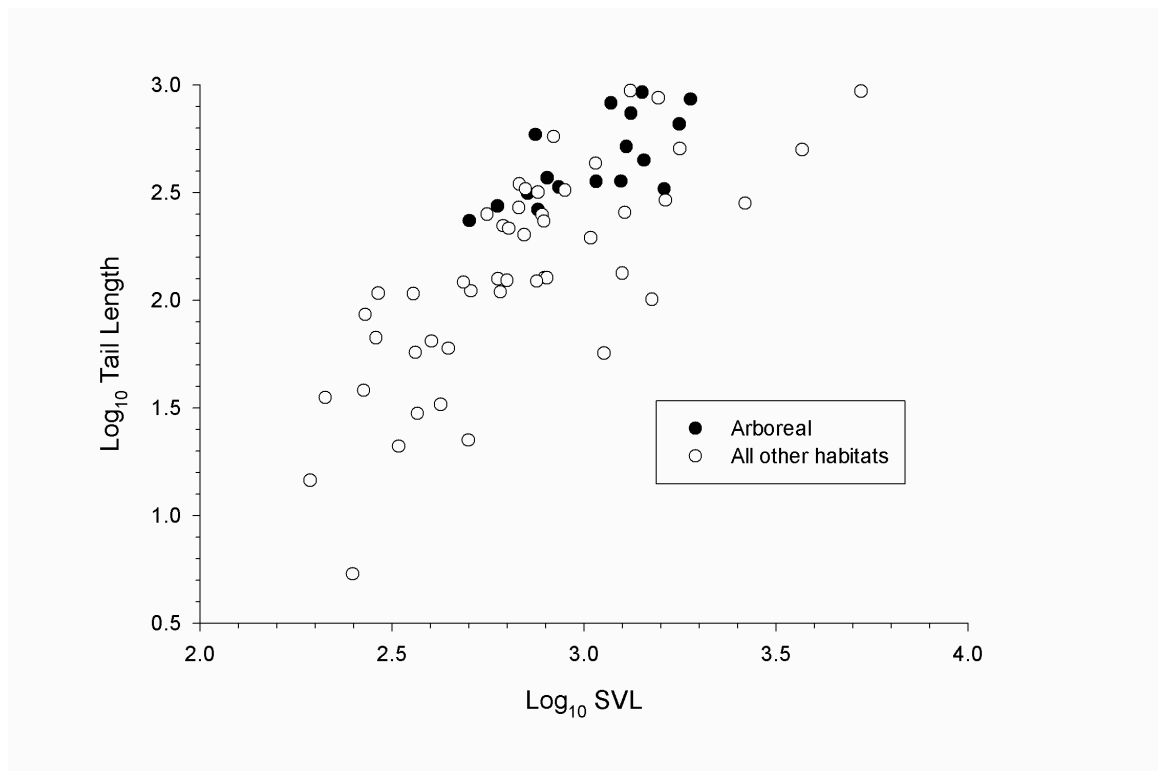
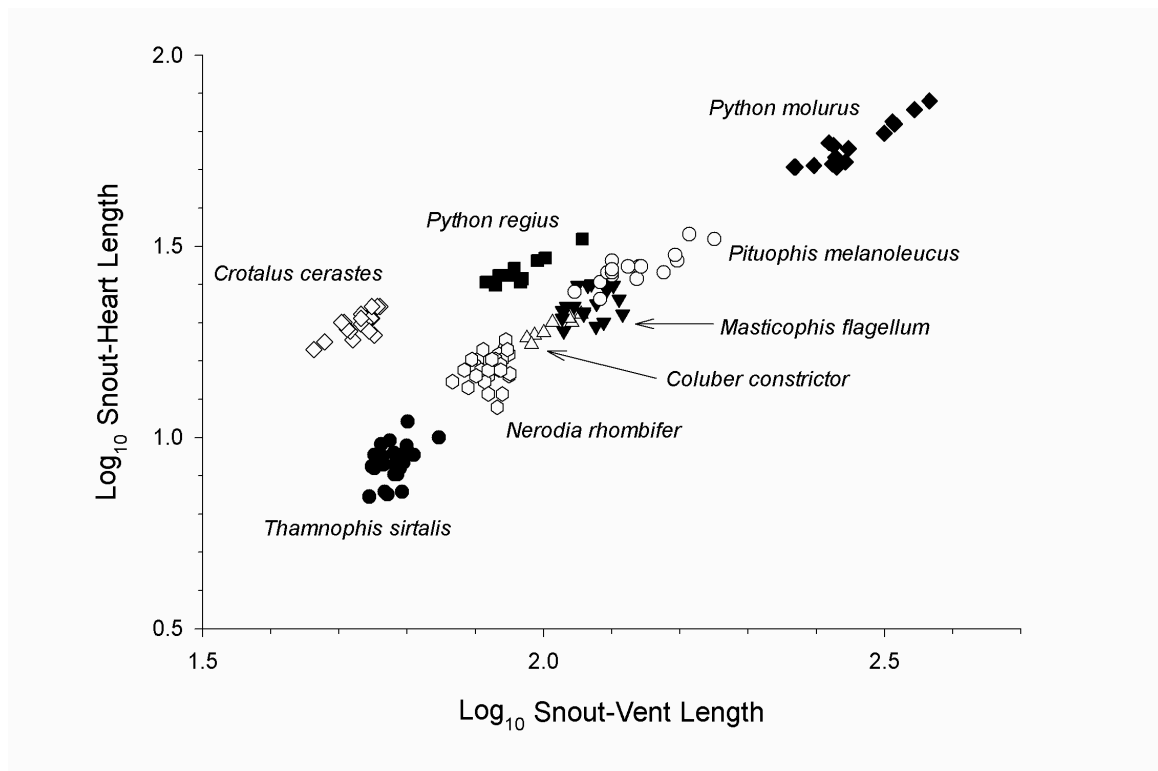


Figure 1.5. Illustration of the range of individual variation in snout-heart length in relation to snout-vent length for eight species in which we had at least 10 individual per species.



Appendices

Appendix 1.1. Microsoft Excel file of data

(Brazildata_2008_April_13_for_Online_Appendix_1.xls). Rows are arranged in phylogenetic order (starting from the left of the phylogeny, as shown in Figure 1.1 of the print version). **HP** and **LP** are heart and lung position, respectively, as a percentage of snout-vent length (**SVL**), **LVL** is the length of the vascularized lung tissue, **SHL** is absolute snout-to-heart length, and **SLL** is the absolute length from the snout to the beginning of the vascularized lung tissue. **LSVL** and **LSHL** are \log_{10} snout-vent and \log_{10} snout-heart lengths, respectively.

This file is available online at:

<http://www.jstor.org/stable/full/10.1086/648509#apa>

Appendix 1.2. The phylogenetic tree used for all analyses was constructed from published phylogenies for snakes using both high (e.g., family) and low (e.g., genus and species) level trees. Tree entry and manipulation were performed using Mesquite© (version 1.12; Maddison and Maddison 2006; <http://mesquiteproject.org>). All 155 species of snakes listed in the Excel© file database of Online Appendix 1.1 are represented in the tree. The final tree contained several polytomies for which we could not find published phylogenetic hypotheses or in which published phylogenies contained polytomies or weak nodal support.

We used Vidal and Hedges's (2002a) molecular study as the basis of our higher levels relationships. The choice of this tree was arbitrary relative to Lee and Scanlon's (2002) tree, which used both morphological and molecular characters. The benefits of the Lee and Scanlon tree are generally higher bootstrap values, while the Vidal and Hedges tree used more taxa and many more characters.

The following list is constructed similar to that of de Querioz and Rodriguez-Robles (2006, app. B). Indentations are indicative of position in the phylogenetic hierarchy. For instance, the Xenodontine subfamily is indented relative to the Colubroidea because Xenodontinae is a subgroup of Colubroidea. Taxa indented to the same degree are in no way equivalent (e.g., sister taxa) and do not necessarily form a nested relationship.

Boinae. The relationships are from Burbrink (2005) figure 4. This tree uses a combined approach using Kluge's (1991, 1993) morphological data and newer sequences from they

cytochrome *b* gene. Burbrink's (2005) figure 3 (lacking molecular data) resolves the polytomy among the Anacondas (*Eunectes*) but we chose to maintain the polytomy for a more conservative approach to our analysis.

Erycinae. Relationships are from Burbrink (2005), though because we have only two taxa, they must be sister to one another..

Pythoninae. The relationships among the pythons comes from Kluge (1993).

Colubroidea (major groups). The relationships are from the combined C-mos, 12S and 16S rRNA, and ND4 tree of Vidal and Hedges (2002b); and the ML cyt *b*, 12S and 16S rRNA, and ND4 tree of Kelly et al. (2003). Both studies were used because the Kelly et al. study used the most informative characters while the Vidal and Hedges tree used the C-mos gene rather than the less phylogenetically informative cyt *b* (de Quieroz and Rodriguez-Robles 2006).

Crotalinae. Relationships within the rattlesnakes and Moccasins (*Crotalus* and *Agkistrodon*) follow Murphy et al. (2002). The South American palm vipers (*Bothrops*, *sensu lato*) were a particularly difficult group. Our tree is based primarily on that of Parkinson et al. (2002) though particular species may have come from other phylogenetic hypotheses (e.g., Crother et al. 1992; Werman 1992; Vidal et al. 1997; Parkinson 1999; Salomão et al. 1999; Wüster et al. 2002, 2005).

Viperinae. We have only two true vipers, thus they must be sister to one another. This is the sister group to the *Crotalinae*.

Elapidae. We have only two major groups of Elapids—the cobras (*Naja*) and the New World coral snakes (*Micrurus*). The relationships among the species of *Micrurus* come from Slowinski (1995) and Jorge Da Silva and Sites (2001).

Colubrinae. The relationships among this group were particularly difficult to resolve due to a lack of published phylogenetic hypotheses on the included taxa. We relied primarily on Creer (2001), but used Lopez and Maxon (1995) to distinguish the relationships between *Tantilla*, *Leptophis*, and *Chironius*.

Lamprodelphini. The relationships between the North American king snakes, gopher snakes and rat snakes came from Rodríguez-Robles and De Jesús-Escobar (1999, Figure 4).

Masticophis and Coluber. The relative relationships of these two genera, with respect to the rest of the Colubrinae are from Creer (2001) though relationships among these genera are from Nagy (2004).

Natricinae. The relationships among the water snakes and garter snakes come primarily from Alfaro and Arnold (2001, Figure 4) and de Quieroz et al. (2002). The placement of *Thamnophis sauritus* is from de Quieroz and Lawson (1994).

Xenodontinae. We primarily used the hypotheses from Vidal et al. (2000, Figure 2) over those of Cadle (1984a, 1984b). The former study contained more taxa and used individual characters rather than distances. We were forced to use the latter studies, however, for the placement of particular species or genera (e.g., *Waglerophis* from Cadle, 1984b).

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Appendix 1.3. Phylogenetic tree used for all analyses, as described in Appendix 1.2 and shown in Figure 1.1. This file (155P.BRK) was produced by the DOS PDTREE.EXE program (Garland et al. 1993, 1999), available on request from Theodore Garland, Jr. (<http://www.biology.ucr.edu/people/faculty/Garland/PDAP.html>). It is in the Newick Standard format (<http://evolution.genetics.washington.edu/phylip/newicktree.html>).

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((((((((((((((59: 1,60: 1,61: 1,62: 1): 1,91: 2): 1,(80: 1,81: 1,82: 1,83: 1,84: 1,85: 1,86: 1): 2):
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1,55: 1): 1,21: 2,22: 2,20: 2,23: 2): 1,24: 3,25: 3,af: 3,ag: 3,ah: 3,ap: 3,as: 3,aw: 3): 7): 1,(y9:
4,(y5: 3,(y8: 2,(y6: 1,y7: 1): 1): 1): 1,(x3: 3,((x2: 1,x5: 1): 1,x1: 2,(x6: 1,x4: 1): 1): 1,y4: 3): 1):
7): 1,(((x7: 1,x8: 1): 6,(((53: 2,(w3: 1,w9: 1): 1): 1,(w7: 1,w2: 1,w8: 1,x9: 1): 2): 2,(17: 4,((39:
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1,a6: 1): 1,a3: 2): 1): 1): 1): 8): 1,((d4: 1,d3: 1): 10,((ac: 9,ab: 9,c4: 9,(c7: 8,((b1: 2,(b8: 1,b6: 1):
1): 5,(((c6: 1,b7: 1): 1,(c1: 1,b9: 1): 1): 4,((a9: 2,(a8: 1,30: 1): 1): 3,(c2: 1,b5: 1): 3,(c5: 3,(b3:
2,(c3: 1,c8: 1): 1): 1): 1): 1): 1): 1): 1): 1,(d1: 4,(((w1: 1,e1: 1): 1,d9: 2): 1,((w5: 1,d6: 1): 1,c9:
2): 1): 1): 6): 1): 3): 1,((y1: 2,(y3: 1,y2: 1): 1): 4,((e4: 1,e6: 1): 4,(37: 4,((15: 1,16: 1,ad: 1): 2,(38:
2,(35: 1,34: 1,33: 1): 1): 1): 1): 1): 9): 0;
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Chapter 2

Response to Technical Comment on Heart Position in Snakes

Note: This paper is a rejoinder to *Heart Position in Snakes: Response to “Phylogeny, Ecology, and Heart Position in Snakes”* (Lillywhite and Seymour, 2011 PBZ 84(1): 99-101 available online at <http://www.jstor.org/stable/10.1086/658082>

We appreciate the opportunity to respond to the Technical Comment concerning our paper “Phylogeny, ecology, and heart position in snakes” (Physiol Biochem Zool 83: 43-54). Professors Lillywhite and Seymour provide a short discourse on gravitational physiology in relation to heart position, and this material largely reiterates points made in our paper, and that they have made previously in other papers. They also take issue with several points in our paper.

Lillywhite and Seymour (2011) state that, "We are concerned that readers of the abstract, and indeed the whole paper, might be misled by this statement or conclude that gravity has no clear influence on heart position in snakes." We do not share this concern; our paper pointed out the possible importance of gravity in several places. For example, we begin the second paragraph of the Introduction with "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 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 snout-vent length for multiple reasons. First, as argued below, we believe SVL is an equally if not more-relevant measure than total length. 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 Appendix A that accompanies our original paper). 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 as 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 paper. The quote from our original

paper comes from a section (page 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 original paper, we address the possible ramifications of our having measured snout-vent length, whereas Lillywhite and Seymour’s studies have generally measured total body length, including the tail. Figure 1.4 and the entirety of page 51 (including Tables 1.5 and 1.6) of our paper constitute a section titled "Variation in Tail Length as a Possible Confounding Factor." We clearly state that, "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)." 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 (p. 51) 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 1.5).”

Lillywhite and Seymour (2011, p. 100) 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 from 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.”

Seymour (1987, his 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 paper 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." 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 different direction to that reported in Seymour (1987, his Table 1), our use of the word opposite is appropriate.

Without citation, Lillywhite and Seymour (2011) assert that, "The vent position has little cardiovascular significance." This is an empirical question, and one can make a case that snout-vent length 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 non-compliant, 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, semi-vertical 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 semi arboreal *Pituophis melanoleucus* was substantially less than in the terrestrial viper, *Crotalus viridis*. Lillywhite (1985) suggests that if "similar levels of vasoconstriction occur in *Pituophis* [as compared to *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 papers). 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 non-horizontal 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, i.e., 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 towards the head, rather than towards 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

non-arboreal species (specifically Lillywhite 1987, 1988; Seymour 1987). Badeer (1998, p. 403) 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." He went on (p. 404) 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."

Lillywhite and Seymour (2011) state: "the paper [Gartner et al. 2010] incorrectly implies 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 (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: "The Gartner et al. paper invokes Badeer's (1998) proposal that, to maintain cardiac filling, the HIP should match heart position, and implies 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" position of arboreal snakes. Rather, 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, with an emphasis on elementary principles in physics. Thus, his hypothesis can potentially be used to explain snake heart position much more generally, 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 (p. 100) “The importance of heart position with respect to gravity becomes most dramatic when one considers aquatic snakes in contrast with arboreal or terrestrial snakes.” 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 paper, but only in as much 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” (p. 50); this should have contrasted sub-aquatic and terrestrial animals, as those were two of the habitats included in our study. We agree with their statement that semi-aquatic species more than likely resemble terrestrial taxa in their baroregulatory physiology and morphology (Lillywhite and Seymour 2011). However, we explicitly

stated (p. 45) that, “When possible, habitat categories were chosen to reflect those of a previous study (Seymour 1987).” 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 (p. 52) 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.”

The authors find it “puzzling” as to why we didn’t perform phylogenetically informed analyses on Seymour’s (1987) data. As stated on page 44, “... we analyze[d] 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 upon 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 paper. 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 paper 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 non-phylogenetic regression statistics with which the allometric scaling exponent (0.05) was barely different from zero -- and it was not statistically different from zero in birds. As has been shown many times, phylogenetic analyses often yield important differences in estimates of scaling exponents as compared with non-phylogenetic 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 paper. We agree completely that the cardiovascular system of snakes most certainly *is* affected by gravity. Our paper 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 (non-phylogenetic) statistical analyses can be misleading (e.g., see Garland et al. 1993; Garland et al. 2005; Revell et al. 2007; Lavin et al. 2008; references therein).

Our original paper 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 (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 (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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Chapter 3

Ecological and phylogenetic variability in the spinalis muscle of snakes

Abstract

A substantial amount of functional variation in tetrapods clearly stems from modifications and variation in limb structure. Among limbless tetrapods, however, the basis of functional diversity is not so well understood. Among snakes, several studies have examined variation in the axial musculature and its putative relationship to locomotor function. A previous, non-phylogenetic analysis of axial muscle lengths found that arboreal species had longer muscle segment lengths relative to terrestrial snakes; a separate study suggested a functional trade-off between locomotor and constriction ability. We examined muscle length of 134 species from 13 major clades of snakes using both conventional and phylogenetic statistics. We first use an information-theoretic approach to compare models predicting muscle length from independent variables coding for constriction, habitat, clade, and \log_{10} body vertebrae. A model based on a star phylogeny that contained all independent variables (including clade) had the greatest support based on AICc and likelihood ratio tests. Partial regression coefficients indicate that arboreal animals do have increased muscle lengths, while constricting animals tend to have reduced muscle lengths, presumably to increase flexibility.

Introduction

Snakes are the most successful group of limbless terrestrial vertebrates (>3,100 names species; Uetz et al. 2007). Elongation has generally come about through an increase in the numbers of trunk vertebrae (Greene 1997). The combination of limblessness and elongation appears to be a versatile overall body plan, as snakes have radiated into nearly every conceivable niche. Adaptive changes to gross patterns of morphological and physiological variation are often thought to accompany transitions to new habitats (Brischoux et al. 2011). For instance, many aquatic animals display traits related to an aquatic selective regime (marine mammals: Castellini et al. 2010; aquatic snakes; Brischoux et al. 2011) while arboreal animals may show a separate set of traits associated with living in trees (snakes: Lillywhite and Henderson 1993). Unlike other tetrapods, snakes rely entirely on their axial structures for locomotion. Thus, their ability to move effectively and/or efficiently (e.g., see Garland et al. 1988; Lauder 1991; Irschick and Garland 2001) through different habitats most likely stems from modifications of segmentation in the axial skeleton and its associated musculature (e.g., variation in vertebral numbers [Lindell 1994, 1996]), body proportions or stoutness [Gasc 1976], body length [Lindell 1996], body mass [Shine 1986], and musculature [Jayne 1982]).

Despite extensive variation in their axial morphology, snake locomotion may nonetheless be subject to various "constraints" because it is likely—in the absence of limbs—that the performance of several key functions may be interrelated if they share a common mechanistic basis (e.g., the axial skeleton and associated muscles).

Limblessness may not constrain evolution in burrowing or marine snakes, but may be particularly relevant in arboreal and scansorial snakes whose bodies are often suspended between branches a considerable distance apart. An increase in the effectiveness of the axial musculature to allow for cantilevering across gaps is likely to be under strong selection in arboreal snakes, but may also be constrained because large increases in muscle mass would increase the gravitational force exerted on snakes while moving through branches (Jayne and Riley 2007). A second consideration is that many snakes use constriction to subdue prey. Constriction requires considerable axial flexibility in conjunction with force production strong enough to subdue and kill prey (Greene and Burghardt 1978). The ability for a particular region of the trunk to produce constrictive forces is proportional to the cross sectional area of the muscle in the constrictive coil (Moon and Mehta 2007) and the cross-sectional area within a coil will vary with the diameter of the snake (Moon and Candy 1997). All else being equal, more muscular and thicker snakes will be better constrictors. Finally, in addition to mass considerations and the production of constrictive forces, constriction requires the ability to form tight coils and therefore may require increased flexibility (Jayne 1982; Lindell 1994).

At least two previous studies have examined whether variation in the axial musculature and associated vertebrae is associated with habitat use or predatory mode. Ruben (1977), in a two-species comparison (Garland and Adolph 1994), contrasted the rapid and active foraging of the coachwhip, *Masticophis flagellum* (a non-constrictor) with the ambush strategy of the rosy boa, *Lichanura roseofusca* [sic] (a powerful constrictor) and suggested that the longer major axial muscle units (in particular, long

tendons) in the coachwhip allow for its rapid movement without a concomitant increase in body mass. Alternatively the shorter muscle units in the rosy boa allowed for strong constriction but may increase the number of static points of support used during locomotion, resulting in an increased lateral resistance to forward undulatory movements. Ruben (1977) concluded that constriction and rapid locomotor speed might be mutually exclusive in snakes.

Jayne (1982) found evidence for increased numbers of segments (vertebrae) in constrictors and suggested that this increases flexibility while coiling. In addition, he argued that movement through particular habitats may strongly influence locomotor morphology. For example, arboreal snakes generally had increased segmental lengths of their axial musculature, as a result of increased tendon lengths, whereas many aquatic specialists had relatively short tendons in their axial muscle segments. Finally, his taxonomically broad sample consisting of 107 snakes from 11 families (85 genera and 94 species) allowed him to conclude that phylogenetic relatedness may account for some of the variation in muscle lengths among species of snakes.

In the present study, we expand on Jayne (1982), particularly with reference to potential historical influences on the relative length of the axial musculature in snakes. We ask first whether statistical models that incorporate aspects of phylogeny, ecology or behavior (or some combination thereof) best fit our data. Second, we examine the generality of the relationship between habitat and muscle length. Third, we address the hypothesis that constriction is associated with short segments of the axial musculature. Associations with phylogenetic position are addressed by comparing the fit of models

that assume a tree with no hierarchical structure (a “star”) versus a hierarchical tree and alterations of that tree under an Ornstein-Uhlenbeck (OU) model of residual muscle length evolution (see Methods). We additionally allow for phylogenetic effects in our statistical models by coding the major branches of our tree as separate levels in a categorical variable, “clade” (see Table 3.1). We also compare the fit of models that include a separate categorical variable that codes for some of the variation in “ecology”—namely habitat use (Table 3.1).

We compare nested models using likelihood ratio tests (LRTs) and compare among all models using the Akaike Information Criterion and associated statistics—allowing for the simultaneous consideration of the statistical effects of habitat and phylogeny on the length of the axial musculature in snakes. Our overall approach is becoming widely used in comparative biology and allows for the simultaneous consideration of ecological and historical effects on trait evolution (e.g., Huey et al. 2009; Swanson and Garland 2009; Gartner et al. 2010).

Materials and Methods

Muscle Length, Vertebral Number, and Habitat

We gathered anatomical data on the number of vertebrae spanned by a single, mid-body segment of the spinalis muscle and associated tendons in addition to vertebral numbers for 134 species representing 13 major clades (families or subfamilies) from both published data and new measurements of museum specimens (Table 3.1). The spinalis is one of the three largest epaxial muscles of snakes (Ruben, 1977), and it contributes to

lateral or dorsal flexion of the vertebral column, depending on the mode of locomotion (Gasc 1974, 1981; Jayne 1988a 1988b; Moon 1991). The axial musculature of snakes has been described in detail elsewhere (summarized in Jayne 1982 and references within) and will not be repeated here. Data on muscle spans and vertebral numbers came primarily from a previously published study by Jayne (1982, N=98). The remaining data (N=36) were collected by G.E.A.G and B.C.J. New taxa were selected to fill in phylogenetic lineages or habitat types poorly represented in the previous study.

We counted vertebral numbers in the body and tail by proxy from the number of ventral scales of the specimen (Alexandar and Gans 1966; Kerfoot 1970), a trait that is known to be heritable within snake populations (Dohm and Garland 1993). For those few species where vertebral numbers cannot be estimated from ventral scale counts, we used average values from the literature (e.g., Voris 1975 for most Hydrophiid sea snakes) to estimate vertebral numbers.

A low-power dissecting scope was used to examine the musculature of all but the largest specimens. A skin-deep, mid-dorsal incision was made at mid-body and the skin reflected laterally above and below the cut to expose segments of the spinalis muscle. Fascia was carefully removed and the tendons of the M. semispinalis-spinalis anterior to the segment of interest were cut. Cutting these anterior tendons was necessary to facilitate separating the anterior tendons of adjacent muscle segments, which were often twisted together in a cord-like fashion. The axial musculature of snakes is arranged in serially repeating units with more posterior segments lying superficially to those immediately in front of it. Thus, to expose the segment of interest, the anterior tendons

of more superficial and posterior segments were cut near the muscle tissue and reflected anteriorly and pinned at its origin on the vertebrae. In this manner, we could directly count the number of vertebrae spanned by a single muscle segment by counting the number of pins between the origin and insertion of the segment of interest (each pin corresponding to one vertebra). Once the segment of interest was revealed, we counted the number of vertebrae involved in the origin (if more than one) and the numbers of vertebrae spanned by the posterior tendon, muscle tissue, anterior tendon, and the number of vertebrae spanned by the entire segment. We use "length" here to indicate the number of vertebrae spanned by a given region of the muscle. Thus, "spinalis length" and "span" are used interchangeably.

General habitat usage (see also Gartner et al. 2010; Brischoux et al. 2011) was categorized using literature accounts—primarily field guides and works on local snakes faunas (e.g., Wright and Wright 1957) as well as the authors' own observation and experiences with many of the taxa included in this study (e.g., BCJ with Homalopsinae). The following habitat categories were used:

Burrowing/Fossorial: Fossorial taxa actively burrow and are found primarily underground or in leaf litter. Most possess obvious behavioral and external morphological adaptations for burrowing (e.g., reduced eyes, underslung jaw, etc.).

Arboreal: Arboreal or scansorial taxa are those that freely climb or are otherwise frequently encountered in trees or low-lying shrubs (e.g., *Boiga*, *Corralus*).

Aquatic: Fully aquatic species that live in fresh water.

Marine: Marine species live almost exclusively at sea and rarely, if ever, come to land.

Semi-marine: Species that are primarily marine, but are obligated to come ashore (e.g., *Laticauda*).

Terrestrial: Terrestrial species lack any obvious morphological or behavioral adaptations to the terrestrial realm and thus cannot be easily classified into any of the other groups (e.g., many elapids, *Pituophis*). The terrestrial group therefore includes not only obviously terrestrial species, but also animals that may, on occasion, be found swimming or climbing (as most snakes appear to be able to climb or swim to some extent). In addition, some species occur in multiple habitats (e.g., *Notechis scutatus*, Bonnet et al. 2002 in semi-aquatic or xeric habitats). In such situations, we selected the most typical habitat for a particular species or, if specified, the population or habitat of the specimen used in the analyses.

Phylogeny Construction

We constructed a composite tree (Figure 3.1, see Appendix A), which consists of phylogenetic hypotheses from previously published studies. We first constructed trees of higher-level relationships uniting the major groups of snakes within our study. Next, we nested less inclusive (lower-level) relationships within this framework until we could no longer resolve relationships (usually among species within a particular genus). Ideally, we wanted to use the best available phylogenetic estimate at each hierarchical level within the tree rather than combining a number of phylogenies together for a particular group. Most published phylogenies, however, contained only a few taxa of interest for

any given group. Therefore, we frequently were forced to use multiple estimated trees—each of which may have employed differing methods and characters in their analyses—to place taxa into our tree.

We followed the general methods of de Queiroz and Rodríguez-Robles (2006) for those instances where a number of trees were available for any particular group: maximum likelihood trees were preferred over those obtained by other methods (e.g. parsimony) and strict consensus trees were used when available. When lower-level groups had multiple trees to choose from, we used the number of characters and the number of taxa to differentiate between them. For example, if two trees had similar numbers of taxa, then we chose the tree with the greater number of characters (and vice-versa).

In a few instances, the amount of clade support (e.g., bootstrap values) influenced our decision on tree selection and construction. In those cases where no phylogenetic hypotheses could be found, or where there was particularly weak nodal support, we collapsed clades to maintain a more conservative approach to our analysis. The final tree contained several soft polytomies for which we could not find published phylogenetic hypotheses or in which published phylogenies contained polytomies or weak nodal support. For simplicity, we did not subtract any degrees of freedom (see Garland and Díaz-Uriarte 1999) when calculating partial F tests (see below).

Statistical Analyses

Total numbers of body vertebrae and the vertebrae spanned by a segment of the spinalis muscle (the muscle tissue and both anterior and posterior tendons) were \log_{10} transformed prior to analyses. The effects of \log_{10} total body vertebrae, clade, habitat, and constriction (whether animals use constricting coils to subdue and kill prey) were tested using conventional (non-phylogenetic) multiple regressions (Ordinary-Least Squares [OLS] regressions), with the latter three variables coded as a series of dummy variables (equivalent to analysis of covariance [ANCOVA] with parallel slopes). It is unlikely that a comparative study can include all members of a given clade *sensu stricto*; thus, we define a “clade” to refer to all taxa in our data set that represent members of a formal taxonomic group (family or subfamily) or are otherwise distinct branches on our tree (e.g., all vipers in our data set are represented as one “clade” despite the family being only partially represented in Figure 3.1). All models were estimated using the Matlab Regressionv2.m program (Lavin et al. 2008; for further examples of applications, see Buchwalter et al. 2008; Jeffery et al. 2008; Warne and Charnov 2008; Huey et al. 2009; Swanson and Garland 2009; Gartner et al 2010). We estimated models with all 16 possible combinations of our four predictor variables (Table 3.2). We then repeated the analysis using phylogenetic models incorporating a branch-length transformation based upon an Ornstein Uhlenbeck (OU) model of evolution for residual muscle length variation (henceforth, RegOU)—a process suggested as a way to mimic the effects of stabilizing selection (see Gartner et al. 2010 and references within). In total, we

compared 32 models—16 using conventional ordinary least squares (OLS or a “star” phylogeny) and 16 transformed “RegOU” models.

The fit of alternate models was compared using AICc (Akaikes’s Information Criterion [AIC] with a second order correction for small sample sizes; Burnham and Anderson 2002). Smaller values of AICc indicate a better fit of the model to the data:

$$AICc = (-2 \times \ln \text{maximum likelihood}) + (2 \times \text{no. parameters} \times n/(n - \text{no. parameters} - 1))$$

In general, the use of AICc is suggested when the ratio $n/K < 40$ (where K = number of parameters in the global or full model [Burnham and Anderson 2002, 2004]). However, because AICc converges to AIC as n gets large, AICc can be employed regardless of sample size. As a general rule, smaller $\Delta AICc$ values indicate better-supported models; however, no general heuristic or statistical test using solely $\Delta AICc$ scores separates well-supported models from poor models (Burnham and Anderson 2002; Burnham et al. 2011). Thus, as an additional qualitative measure of model fit, we present the probability of each model—the Akiake weight—given by w_i , where the probability of a given model is equal to the likelihood of model i divided by the sum of the likelihoods across all models. We also present evidence ratios (ER), which are the ratios of likelihoods of any two models i and j (larger values for evidence ratios are indicative of a less-supported model—Burnham et al. 2011). All models in the Results section are compared relative to our top model (i.e., $\Delta AICc = 0$, $ER = 1$)

Natural log maximum likelihood ratios tests (LRTs) were used to compare phylogenetic models with their non-phylogenetic counterparts, where twice the difference in ln likelihoods is assumed to be distributed asymptotically as a χ^2 distribution with degrees of freedom equal to the difference in the number of parameters between the two models (df = 1 for all LRTs used in this study). When phylogenetic versions of models fit the data better than conventional, non-phylogenetic versions (a significant LRT), then “phylogenetic signal”—the tendency for related species to resemble one another (Blomberg and Garland 2002; Blomberg et al. 2003)—is present in the residuals of the dependent variable in the non-phylogenetic model. However, in a more general sense, phylogenetic signal is also indicated by OLS models that include a statistically significant variable representing clade (e.g., see Gartner et al. 2010).

Results

Based on AICc scores, three models predicting total muscle length appear to be better supported than others (using an arbitrary cutoff of $\Delta\text{AICc} < 2$, see Burnham and Anderson 2002). However, in conjunction with model likelihood scores, probabilities and evidence ratios (Table 3.2), only two of the three previously mentioned models seem well supported. Importantly, all of these models include both the habitat and clade variables. The full, non-phylogenetic model (OLS with numbers of \log_{10} total body vertebrae, constriction, habitat, and clade as independent variables) was best ($\Delta\text{AICc} = 0$, $w_i = .2947$, ER = 1.00; Table 3.2), while a non-phylogenetic model with \log_{10} total body vertebrae, habitat, and clade also had substantial support ($\Delta\text{AICc} = 0.1990$, $w_i = 0.2668$,

ER = 1.10). The RegOU versions of the same models (a full model and the full model omitting constriction) had weaker support ($\Delta\text{AICc} = 2.714$, $w_i = 0.0759$, ER = 3.88 and $\Delta\text{AICc} = 2.573$, $w_i = 0.0814$, ER = 3.62 respectively) and small estimated d values, indicating that the phylogeny describing residual covariance was nearly a star. All other models had substantially less support based upon ΔAICc scores, model likelihoods, model probabilities, and evidence ratios.

Likelihood Ratio Tests (LRTs) across our top two regression models (comparing OLS and RegOU versions with the same independent variables) were consistent across all comparisons that showed support based on ΔAICc (see above). As expected, the RegOU log likelihood score was always higher than its OLS counterpart due to the additional OU transformation parameter, d . In all instances, however, the RegOU model did not fit the data significantly better than did the OLS model ($\chi^2 \lll 3.841$ for all comparisons; Table 3.2—results of likelihood ratio tests not shown). The above results indicate two features with regards to phylogenetic signal. First, the strong support of models that include our “clade” variable indicate that phylogenetic signal is present among the major branches (the clades) of our tree. Thus, grade shifts—shifts in the mean value of our dependent variable—have occurred among the major families of snakes. However, no significant phylogenetic signal remains in the residuals of our dependent variable within the major branches (clades) or in the nodes that link them, as indicated by the non-significant LRTs comparing OLS and RegOU models with the same independent variables.

Our single best model was a full, non-phylogenetic model (containing all independent variables) and this model was used for our parameter estimates (shown also are results of a full RegOU model, Table 3.3). All significance tests are two-tailed. Numbers of body vertebrae were significant in both non-phylogenetic and phylogenetic models ($F_{OLS \text{ df } 1, 114} = 14.04, P = 0.027$ and $F_{RegOU \text{ df } 1, 114} = 13.42, P = 0.024$ respectively). Clade, and particularly habitat were both highly significant predictors of \log_{10} total muscle length ($F_{Clade \text{ df } 12, 114} = 4.48, P = <<0.001$ and $F_{Habitat \text{ df } 5, 114} = 25.22, P = <<0.001$ for an OLS model; $F_{Clade \text{ df } 12, 114} = 4.19, P = <<0.001$ and $F_{Habitat \text{ df } 5, 114} = 23.34, P = <<0.001$ for the RegOU model). There was no significant effect of constriction after controlling for effects of habitat, clade, and numbers of body vertebrae.

Partial F tests indicate that after controlling for effects of both numbers of body vertebrae, constriction, and habitat, the Typhlopidae, Pythonidae, Tropicophiidae and Boidae have significantly shorter spinalis segment lengths (Table 3.3 and Figure 3.2B), although the Typhlopidae and Tropicophiidae are both represented by only a single specimen. Likewise, after controlling for effects of clade, constriction, and numbers of body vertebrae, aquatic, burrowing, and marine snakes have significantly reduced spinalis segment lengths, while arboreal species have muscles that span significantly more vertebrae than other habitat groups (Table 3.3 and Figure 3.2A).

Discussion

We addressed two principle themes in this paper. First, we used a phylogenetically informed, information-theoretical approach to develop and compare models that examine

whether ecological factors, historical factors, or some combination therein most affect the relative span of the spinalis muscle in snakes while simultaneously testing for the presence of phylogenetic signal in this trait. Second, we addressed functional hypotheses relating habitat structure with variation in the relative length of a spinalis segment—in particular, that arboreal snakes have longer segmental lengths and that constrictors have shorter segmental lengths, presumably to increase flexibility.

We found that the number of body vertebrae, habitat (as classified in Methods), and clade membership all accounted for substantial variation in the relative distance spanned by the spinalis muscle. OLS models that include habitat and clade as variables were always higher than their RegOU counterpart and each of the top eight models contain both habitat and clade (Table 3.2). Constriction is included in our best performing model, but is absent from most other models with substantial support (Table 3.2). When clade or habitat is removed from the top models, model-fit drops precipitously ($\Delta AICc = 19.818$ and 77.627 ; evidence ratios = $ER = 2.01 \times 10^4$ and 7.19×10^{16} , respectively, Table 3.2). Akaike weights (w_i) reflect this dichotomy as well (Table 3.2). No model—given the data—has a probability greater than thirty percent, but the top two models have probabilities nearly twice that of the third-best model ($w_i = 0.2947$, 0.2668 , and 0.1547 for the top three models, respectively).

The top models show the utility of using “clade” as a predictor variable while simultaneously indicating an improved fit of non-phylogenetic models over RegOU models. Taken together, these models demonstrate that phylogenetic signal is present among the specified clades within our tree (Figure 3.1), but not necessarily in the

underlying hierarchical structure connecting clades to one another, or among species within clades. A lack of phylogenetic signal may suggest that other non-random processes, such as natural selection (Blomberg et al. 2003; Revell et al. 2008) primarily drive the evolution of the trait (or traits) in question (Smith et al. 2011) and several evolutionary processes may lead to weak relationships between phylogenetic relatedness and phenotypic similarity (Hansen and Martins 1996). The strong clade effect in our study, however, shows conclusively that both phylogenetic history, in addition to other potential adaptive processes (e.g., selection to particular habitats), shapes variation in the spinalis muscle of snakes.

After controlling for effects of habitat, clade, and constriction we found a significant positive effect of \log_{10} body vertebrae on spinalis length in both non-phylogenetic and phylogenetic full models ($\beta_{\text{OLS df } 1, 114} = 0.2055, F = 5.00$, and $\beta_{\text{RegOU df } 1, 114} = 0.2123, F = 5.25$). Numbers of body vertebrae are an important component of the axial musculoskeletal system in snakes and are the functional unit across which the axial muscles function (as opposed to body length). Snakes show pleomerism (Lindell 1994)—the tendency for increased segmentation with increased body length. Thus, for our functional hypotheses of variation in spinalis length to be valid, they must be testable and falsifiable (Larson and Losos 1996) relative to a null model where spinalis length simply increases as a function of increasing vertebral numbers or body length. Because body length and numbers of body vertebrae are correlated, however, only one of those two variables could be incorporated into our models as a covariate (due to problems with collinearity). We chose to use body vertebrae because the number of vertebrae is

constant throughout ontogeny, whereas body length increases throughout the life of the snake.

We found strong support for longer spinalis muscle lengths in arboreal animals in both non-phylogenetic and phylogenetic full models (Table 3.2, Table 3.3: $\beta_{\text{OLS df 1, 114}} = 0.1583$, $F = 35.43$ and $\beta_{\text{RegOU df 1, 114}} = 0.1526$, $F = 32.12$, respectively). This result is in agreement with the previous, non-phylogenetic, analyses of spinalis length (Jayne 1982). Arboreal animals frequently support their body weight between suspended objects (such as branches in trees). Morphologically, the ability to cantilever across gaps is best accomplished by increasing the length of the anterior tendons of the semispinalis-spinalis (Jayne and Riley 2007). Not surprisingly, the increased span of the semispinalis-spinalis muscle in arboreal snakes is due primarily to an increase in the anterior tendon length (Ruben 1977; Jayne 1982). As it lengthens, the anterior tendon increases the lever arm through which the muscle works, which in turn, reduces the possibility of catastrophic buckling due to increased torque while bridging larger gaps in trees (Jayne and Riley 2007).

Interestingly, all other habitat categories, with the exception of semi-marine snakes, showed significantly shorter muscle lengths than our generalist colubrid snake (see Methods, Table 3.3). Active burrowing through heavy mud or packed soil may require substantial force production from the epaxial musculature, and some squamates produce very high forces while moving through soil (Navas et al. 2004). However, the contractile tissue length of burrowers is nearly equal that of terrestrial animals (Gartner and Jayne, unpublished data). Nevertheless, it would not be surprising to find strong

burrowers with short contractile lengths, as shorter contractile lengths may indicate a force-velocity trade-off in the spinalis muscle. Shorter muscle segments may allow for increased loads at the expense of reduced contractile velocity (Wilson and Lichtwark 2011). Thus, shorter segments could result in a more favorable strain regime for enhancing force production on the concave side of the body. More work, however, is needed in understanding the relationship between force production and burrowing in snakes, as we still lack sufficient understanding of how the vertebrae move and what the lines of action of the muscles are—both of which are needed to fully understand the issue of lever arm mechanics and force production in burrowing snakes.

The greatly reduced spinalis spans in fully marine snakes are most likely attributable to fact that they are rarely, if ever, subjected to the effects of gravity. In addition, it is possible that short segments are an ancestral condition inherited from a mud-burrowing ancestor (i.e., the Homalopsines—B.C. Jayne, pers. comm.). From a strictly mechanical standpoint, they are unlikely ever to need to suspend their bodies against gravity. In the absence of gravitational considerations, other factors relating to locomotor performance may have a greater influence on muscle spans.

The semi-marine *Laticaudid* sea snakes forage at sea and are often found foraging on reefs during the day. However, they are partly terrestrial and can be found in rocky crevices, sometimes long distances from the sea (need refs.). In addition, unlike other sea snakes, they deposit their eggs on land (Cogger 1994). At least one study has shown a trade-off between locomotor performance on land and in the water in amphibious semi-marine sea snake taxa that vary in their degree of “terrestrialness” (Shine et al. 2003).

Thus, semi-marine snakes may represent a compromise between selection for terrestrial locomotion under a gravitational regime, and a marine existence, where the influence of gravity is greatly reduced.

Among-clade patterns of spinalis variation were particularly interesting. The sole scolecophidian in our study, *Rhinotyphlops schlegeli brevis*, has highly reduced segmental lengths in addition to some striking qualitative differences in muscle shape (Jayne 1982). However, given only one scolecophidian in our study, caution should be taken when drawing conclusions regarding their segmental lengths. Nevertheless, it is likely representative of the clade as a whole, as many scolecophidians are morphologically and behaviorally similar.

Among all other clades, only the basal macrostomates (Boidae, Pythonidae, and *Tropidophis*) show reduced segmental lengths as compared our generalist terrestrial colubrid. These animals are generally stout and heavy bodied and are all powerful constrictors (Greene 1997). Although arboreal animals in this clade are certainly more attenuate than terrestrial members of the same clade, they are still relatively heavy-bodied compared with most other clades of snakes (the Vipers being a notable exception) other snakes. The lone arboreal member of the Boidae (*Corralus caninus*) in our analysis had far and away the lowest spinalis length among all arboreal snakes in our study (spinalis length = 12 vertebrae spanned versus 22 vertebrae spanned for the next lowest arboreal snake, the stout bodied viper *Atheris squamigera*). Previous studies have shown that even thin-bodied arboreal snakes are at the physiological limits of their epaxial musculature while bridging gaps (Jayne and Riley 2007). Thus, heavy-bodied boas and

pythons may demonstrate that an upper-limit to size exists above which “adaptive” arboreal musculature simply cannot compensate mechanically for increased size in larger and heavy-bodied snakes.

The Tropidophiidae are morphologically and behaviorally similar to both boas and pythons, but their phylogenetic position has been highly debated (see Appendix B). Interestingly, morphological studies place them within the Macrostromata (as in Figure 3.1), while molecular studies tend to place them at the base of the alethinophidian tree. The reduced muscle length and unusual? muscle morphology (Jayne 1982) in this group appears homologous with those of the boas and pythons, and would thus tend to support their placement among the Macrostromata—if not, then the degree of convergence in several complex morphological traits between *Tropidophis* and other basal macrostromatans is striking.

Several studies have demonstrated that constrictors have significantly increased numbers of body vertebrae relative to body lengths, and it has been suggested that this indicates selection for increased flexibility (Jayne 1982; Lindell 1994). Other studies have shown that non-constrictors have more elongated vertebrae relative to constrictors (Pough and Groves 1983). We predicted that constricting animals would show reduced spinalis lengths, presumably to increase axial flexibility while coiling around prey. We found no statistically significant effect of constriction on spinalis span in either non-phylogenetic or phylogenetic models ($\beta_{\text{OLS df 1, 114}} = 0.0474$, $F = 2.59$, and $\beta_{\text{RegOU df 1, 114}} = .0304$, $F = 2.00$), though a trend exists in that constrictors do have shorter segments than non-constrictors.

The response to selection for increased flexibility could occur in either of two mutually exclusive ways; increase the number of body segments for a given length of axial musculature (i.e., keep segmental length constant while increasing the number of vertebrae spanned by the muscle) or reduce the segmental span of the axial musculature responsible for axial flexion. Increased segmentation (and by proxy, body length [Lindell 1994]) may be the result of selection favoring increased flexibility. Interestingly, among snakes that appear most specialized for an arboreal lifestyle (*Imantodes*, *Thelotornis*, *Ahaetulla*) almost none are constrictors. It is true that increased vertebrae would increase lateral flexion, however, we do not know if flexibility is limiting in these animals. However, increased segmentation could also likely be the result of natural or sexual selection on other aspects of morphology or life histories (Shine 2001). For instance, within a population of garter snakes, Arnold and Bennett (1988) found a relationship between an increased body length, reduced numbers of vertebral abnormalities, and increased numbers of tail vertebrae with maximum sprint speed and a measure of endurance capacity. Selection favoring increased fecundity could also lead to increased numbers of body vertebrae, and larger female snakes produce larger clutches intraspecifically (Shine 1994).

Caveats and Conclusions

Any comparative study that relies on data gleaned primarily from literature reviews or from surveys of preserved specimens faces limitations. First is we are limited to a degree by the available taxa. Although we were careful to select taxa that

represented a broad range of snake diversity, we nonetheless have sampled only a fraction of snake species (134 of approximately 3,100 species [Uetz et al. 2007]). Further, certain clades are much more well represented than others in our tree (in particular the Homalopsines and Hydrophiid sea snakes).

It is also difficult to quantify and categorize behavioral ecology, particularly in such broadly defined regimens as habitat. This is also an issue even with such behaviors as constricting versus non-constricting. Rarely are snakes habitat specialists—sea snakes and blind snakes being notable exceptions. Instead, most facultatively use environments, moving between one habitat type or another opportunistically (Lillywhite and Henderson 1993). Modifying how we classified habitat types could lead to different conclusions. For instance, we classified marine snakes separately from aquatic snakes (freshwater). We chose this classification arbitrarily over an *a priori* hypothesis of no functional or morphological difference in the axial musculature of swimming snakes (a classification that would group marine and aquatic snakes together).

Constriction is also confounded with clade—that is, there is multicollinearity between at least two of our categorical variables—particularly among the pythons, boas, and *Tropidophis*. There is no simple solution to the issue of collinearity, particularly when dealing with categorical variables. One can do nothing, remove one of the correlated variables, or create a new composite variable. Composite variables are likely only to be useful with continuous data. In our data set, removal of constriction from the model leads to poor model fit (Table 3.2), while not changing the significance of the

partial regression coefficients of the other variables in the model (results not shown). We have therefore left all variables in our full model (Table 3.3).

Problems due to a lack of “common garden” conditions (Garland and Adolph 1991, 1994; Garland et al. 2005) are unlikely to be an issue because spinalis length (in terms of segments spanned) does not vary ontogenetically within an individual, and varies very little within a species (Jayne 1982). This last point is important because, in our study, most species are represented by only one specimen. If intraspecific variation in muscle span were abundant, then our small sample size per species could introduce significant error into our data when comparing among species, with potentially important statistical consequences (Ives et al. 2007).

In summary, we found that models that employ aspects of morphology, ecology, phylogenetic history, and behavior best reflect variation in the length of the spinalis muscle among 134 species of snakes. We found strong support for the hypothesis that arboreal snakes should have increased muscle lengths relative to other habitat types. We found that constricting behavior in snakes, although likely associated with selection for increased segmentation and flexibility, did not lead to statistically shortened axial musculature. Among the basal Macrostromata, we found several clades that demonstrated significantly reduced segmental lengths. Although we studied only one aspect of axial morphology, additional studies may help elucidate whether variation in the axial morphology of snakes may represent a key innovation in snake evolution—allowing terrestrial snakes to move into a previously unoccupied arboreal niche—in much the same way that an expanded diet via increased cranial kinesis and streptostyly allowed for

the expansive radiation of Colubroidea during Miocene (Greene 1997; Cundall and Greene 2000).

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Table 3.1. Coding of clades and habitats used for statistical analyses. Numbers in parentheses refer to the number of constricting taxa in each category.

	Aquatic	Arboreal	Burrowing	Marine	Semi-Marine	Terrestrial	Total
Acrochordidae	1 (1)	0	0	0	0	0	1
<i>Asthenodipsas</i>	0	0	0	0	0	1	1
Lamprophiidae	0	0	6 (1)	0	0	1 (1)	7
Boidae	0	1 (1)	1(1)	0	0	7 (7)	9
Colubrinae	1	7 (1)	5 (2)	0	0	15 (11)	28
Viperidae	0	3	0	0	0	11	14
<i>Cylindrophis</i>	0	0	1 (1)	0	0	0	1
Elapidae	0	1	7	11	2	21	42
Homalopsidae	16	0	0	1	0	0	17
Pythonidae	0	0	2 (2)	0	0	3 (3)	5
Typhlopidae	0	0	1	0	0	0	1
<i>Tropidophis</i>	0	0	0	0	0	1 (1)	1
Dipsadinae	1	2	1	0	0	3 (1)	7
Total	19	14	24	12	2	63	134

Table 3.2. Alternate regression models predicting total muscle length (muscle segment plus anterior and posterior tendons) among species of snakes. Models are listed in ascending order of ΔAICc (the best model is at the top).

NOTE: REML d = OU transformation parameter; $\ln\text{ML}$ = maximum log likelihood; ΔAIC = Difference in model AICc score from the “top” model; l_i = model likelihood; w_i = Akiake weighted probability; ER = Evidence Ratio (see Methods). “OU” indicates a regression model in which the residuals are modeled as an Ornstein-Uhlenbeck process (which can be viewed as mimicking stabilizing selection: Lavin et al. 2008). All other models are ordinary least squares “OLS” multiple regressions, which are mathematically equivalent to analysis on a “star” phylogeny with no hierarchical structure (Blomberg et al., 2003; Lavin et al. 2008). OU models contain one more parameter than their OLS counterparts, so model fit can be tested using likelihood ratio tests (twice the difference in log likelihoods is compared with a critical value of 3.841, which is the ninety-fifth percentile of the χ^2 distribution with 1 df). AICc values in conjunction with model likelihood scores, Akiake weights, and Evidence Ratios can be used to compare between all models, with smaller (more negative) AICc values indicating a better fit of a given model to the data. Likelihood Ratio Tests indicate no statistical difference between OLS and RegOU versions of the top two models ($F = 0.152$ for the Full Model and $F = 0.442$ for a model omitting Constriction).

Table 3.2.

Model	REML d	r^2	lnML	AICc	Δ AICc	l_i	w_i	ER
Constriction + Habitat + Clade + Log ₁₀ Body Vertebrae		0.747	159.727	-269.203	0.000	1.0000	0.2947	1.00E+00
Habitat + Clade + Log ₁₀ Body Vertebrae		0.741	158.219	-269.004	0.199	0.9053	0.2668	1.10E+00
Habitat + Clade		0.733	156.290	-267.914	1.289	0.5249	0.1547	1.91E+00
Habitat + Clade + Log ₁₀ Body Vertebrae (OU)	0.0812	0.717	158.440	-266.630	2.573	0.2762	0.0814	3.62E+00
Constriction + Habitat + Clade + Log ₁₀ Body Vertebrae (OU)	0.0155	0.728	159.803	-266.489	2.714	0.2574	0.0759	3.88E+00
Constriction + Habitat + Clade		0.736	156.851	-266.268	2.935	0.2305	0.0679	4.34E+00
Habitat + Clade (OU)	0.0179	0.712	156.363	-265.292	3.911	0.1415	0.0417	7.07E+00
Constriction + Habitat + Clade (OU)	0.0138	0.717	156.871	-263.492	5.711	0.0575	0.0170	1.74E+01
Constriction + Habitat + Log ₁₀ Body Vertebrae (OU)	0.1642	0.543	135.587	-249.385	19.818	0.0000	0.0000	2.01E+04
Constriction + Habitat + Log ₁₀ Body Vertebrae		0.627	133.832	-248.213	20.990	0.0000	0.0000	3.61E+04
Constriction + Habitat (OU)	0.1660	0.525	132.999	-246.547	22.656	0.0000	0.0000	8.31E+04
Constriction + Habitat		0.610	130.843	-244.535	24.668	0.0000	0.0000	2.27E+05
Habitat (OU)	0.4002	0.400	126.974	-236.796	32.407	0.0000	0.0000	1.09E+07
Habitat + Log ₁₀ Body Vertebrae (OU)	0.4540	0.399	128.121	-236.791	32.412	0.0000	0.0000	1.09E+07
Habitat		0.519	116.688	-218.487	50.716	0.0000	0.0000	1.03E+11
Habitat + Log ₁₀ Body Vertebrae		0.519	116.752	-216.353	52.850	0.0000	0.0000	2.99E+11
Clade + Log ₁₀ Body Vertebrae (OU)	0.4526	0.241	114.113	-191.576	77.627	0.0000	0.0000	7.19E+16
Log ₁₀ Body Vertebrae (OU)	0.6284	0.064	99.700	-191.091	78.112	0.0000	0.0000	9.16E+16
Constriction + Log ₁₀ Body Vertebrae (OU)	0.5834	0.076	100.621	-190.774	78.429	0.0000	0.0000	1.07E+17
Constriction + Clade + Log ₁₀ Body Vertebrae (OU)	0.4352	0.249	114.777	-190.279	78.924	0.0000	0.0000	1.37E+17
No Independent Variables (OU)	0.6180	0.000	95.290	-184.395	84.808	0.0000	0.0000	2.61E+18
Constriction + Clade + Log ₁₀ Body Vertebrae		0.467	109.816	-182.983	86.220	0.0000	0.0000	5.28E+18
Constriction (OU)	0.6015	0.003	95.497	-182.684	86.519	0.0000	0.0000	6.13E+18
Clade (OU)	0.5322	0.130	106.316	-178.564	90.639	0.0000	0.0000	4.81E+19
Clade + Log ₁₀ Body Vertebrae		0.431	105.541	-177.013	92.190	0.0000	0.0000	1.04E+20
Constriction + Clade (OU)	0.5457	0.127	106.323	-175.997	93.206	0.0000	0.0000	1.74E+20
Clade		0.327	94.248	-156.966	112.237	0.0000	0.0000	2.35E+24
Constriction + Clade		0.336	95.171	-156.275	112.928	0.0000	0.0000	3.33E+24
Constriction + Log ₁₀ Body Vertebrae		0.116	75.982	-143.653	125.550	0.0000	0.0000	1.83E+27
Log ₁₀ Body Vertebrae		0.038	70.275	-134.365	134.838	0.0000	0.0000	1.90E+29
Constriction		0.036	70.189	-134.192	135.011	0.0000	0.0000	2.08E+29
No Independent Variables		0.000	67.710	-131.328	137.875	0.0000	0.0000	8.69E+29

Table 3.3. Partial regression coefficients and F tests for the best model to predict \log_{10} number of vertebrae spanned by one segment (muscle and anterior and posterior tendons) of the spinalis muscle, which includes all independent variables considered (\log_{10} total number of body vertebrae, habitat, clade and constriction), analyzed by conventional (OLS) multiple regression and phylogenetically with an OU transform (see Table 3.2 for model comparisons). All tests are two tailed. Variables significant in both models are highlighted in bold. \log_{10} body vertebrae was a highly significant predictor under both evolutionary models. Colubrinae and Terrestrial categories were chosen as base groups for comparison among all other levels of “clade” and “habitat,” respectively. These were chosen because a terrestrial colubrid snake best represents the “general snake condition”—one without any obvious structural or behavioral modifications for a terrestrial lifestyle (see descriptions in Methods).

Table 3.3.

	OLS					OU				
Variable	Coefficient	SE	F	df	P	Coefficient	SE	F	df	P
y -intercept	0.8098	0.2161				0.7980	0.2178			
Log₁₀ body vertebrae	0.2055	0.0919	5.00	1, 114	0.027	0.2123	0.0927	5.25	1, 114	0.024
Typhlopidae	-0.2279	0.0842	7.34	1, 114	0.008	-0.2285	0.0851	7.22	1, 114	0.008
Cylindrophiiidae	-0.0398	0.0845	0.22	1, 114	0.640	-0.0448	0.0854	0.28	1, 114	0.598
Pythonidae	-0.1470	0.0414	12.58	1, 114	<<0.001	-0.1533	0.0446	11.81	1, 114	<<0.001
Tropidophiidae	-0.2719	0.0826	10.82	1, 114	0.001	-0.2752	0.0834	10.89	1, 114	<<0.001
Boidae	-0.1876	0.0332	31.94	1, 114	<<0.001	-0.1889	0.0352	28.71	1, 114	<<0.001
Acrochordidae	-0.0911	0.0960	0.90	1, 114	0.345	-0.0928	0.0965	0.92	1, 114	0.340
Pareatidae	-0.0114	0.0834	0.02	1, 114	0.888	-0.0102	0.0842	0.01	1, 114	0.921
Viperidae	-0.0275	0.0311	0.78	1, 114	0.379	-0.0240	0.0330	0.53	1, 114	0.468
Homalopsidae	-0.0228	0.0523	0.19	1, 114	0.664	-0.0190	0.0525	0.13	1, 114	0.719
Dipsadinae	0.0311	0.0354	0.77	1, 114	0.382	0.0393	0.0380	1.07	1, 114	0.303
Elapidae	0.0027	0.0274	0.01	1, 114	0.921	0.0011	0.0289	0.00*	1, 114	0.999
Lamprophiidae	-0.0338	0.0380	0.79	1, 114	0.376	-0.0359	0.0409	0.77	1, 114	0.382
Aquatic	-0.1521	0.0507	9.00	1, 114	0.003	-0.1541	0.0500	9.49	1, 114	0.003
Arboreal	0.1583	0.0266	35.43	1, 114	<<0.001	0.1526	0.0269	32.12	1, 114	<<0.001
Burrowing	-0.0923	0.0229	16.24	1, 114	<<0.001	-0.0908	0.0235	14.92	1, 114	<<0.001
Marine	-0.2584	0.0297	75.83	1, 114	<<0.001	-0.2566	0.0308	69.32	1, 114	<<0.001
Semi-Marine	-0.0868	0.0585	2.20	1, 114	0.141	-0.0849	0.0636	1.78	1, 114	0.185
Constrictors	-0.0474	0.0294	2.59	1, 114	0.110	0.0430	0.0304	2.00	1, 114	0.160
Clade			4.48	12, 114	<<0.001			4.19	12, 114	<<0.001
Habitat			25.22	5, 114	<<0.001			23.34	5, 114	<<0.001

* $F < 0.01$

Figure 3.1. Phylogeny of all 134 taxa used in the analyses, with Pagel's (1992) arbitrary branch lengths. See Appendix A for details of tree construction and Appendix B for an electronic version of the tree.

Figure 3.1. Phylogeny of all 134 taxa

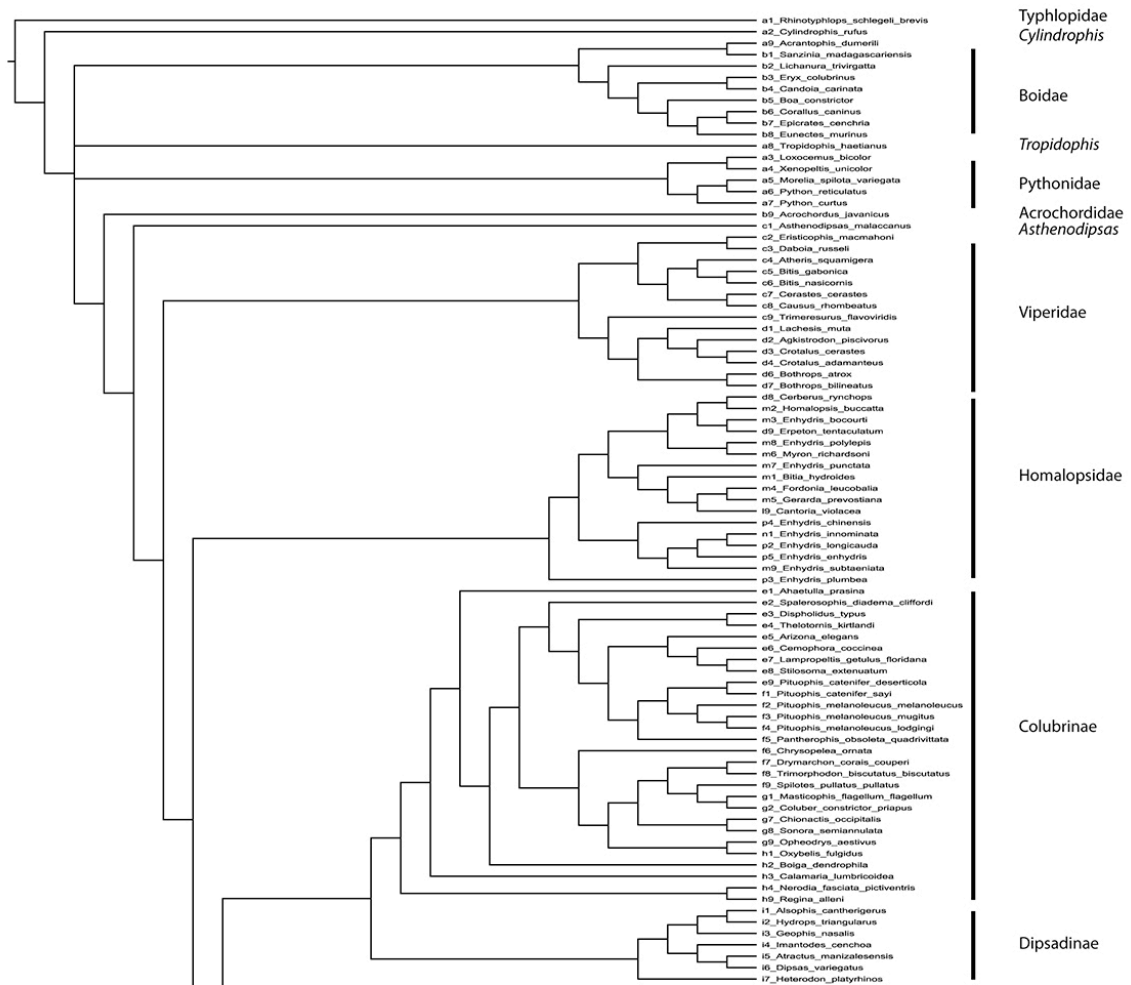


Figure 3.1 continued from previous page

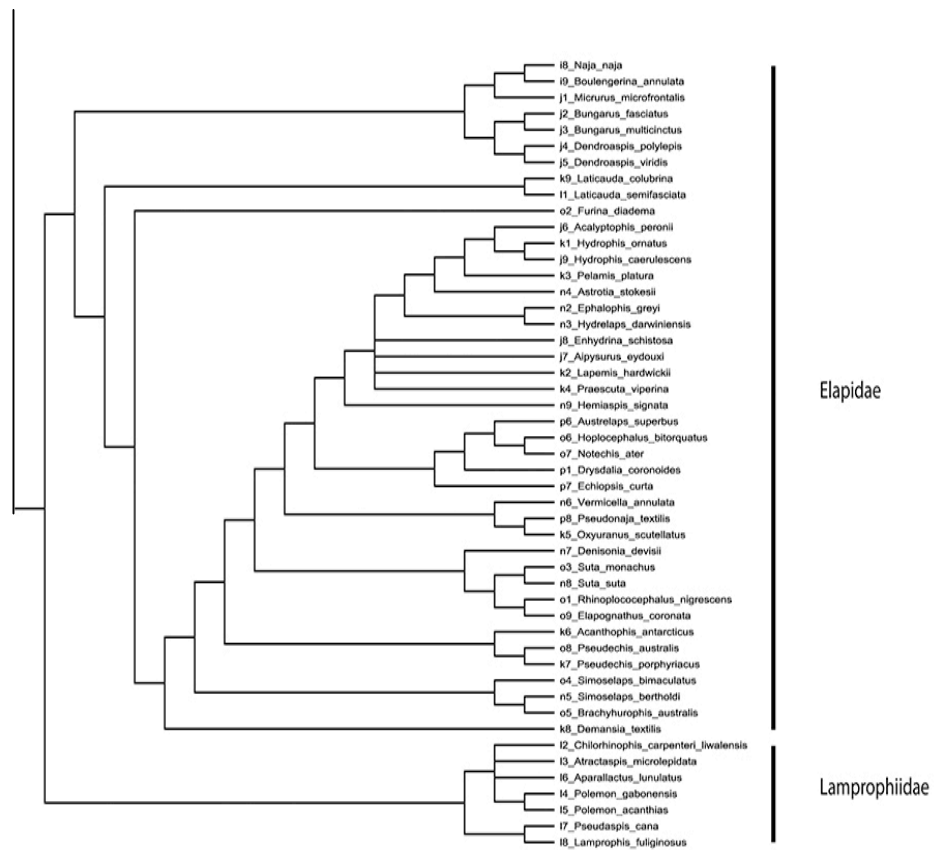


Figure 3.2. Log-log plots of vertebrae spanned by one segment of the spinalis muscle (including both anterior and posterior tendons) in relation to total numbers of body vertebrae for 134 snake taxa, indicating (A) the habitat that is most different (arboreal) and (B) clades that are most different (Pythonidae, Tropidophiidae, and Boidae) from other snakes. See Table 2 for complete results of multiple regression.

Figure 3.2A.

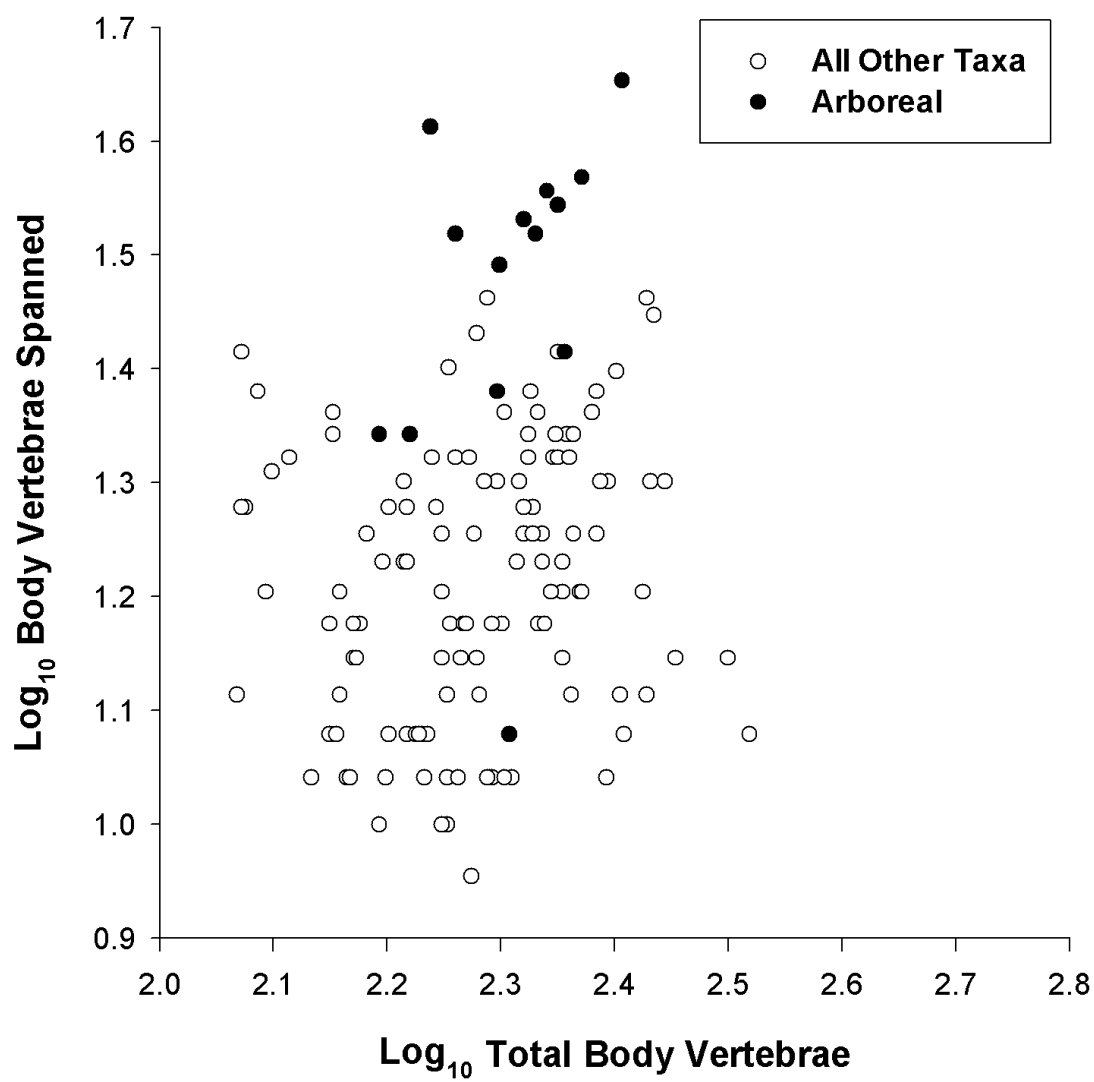
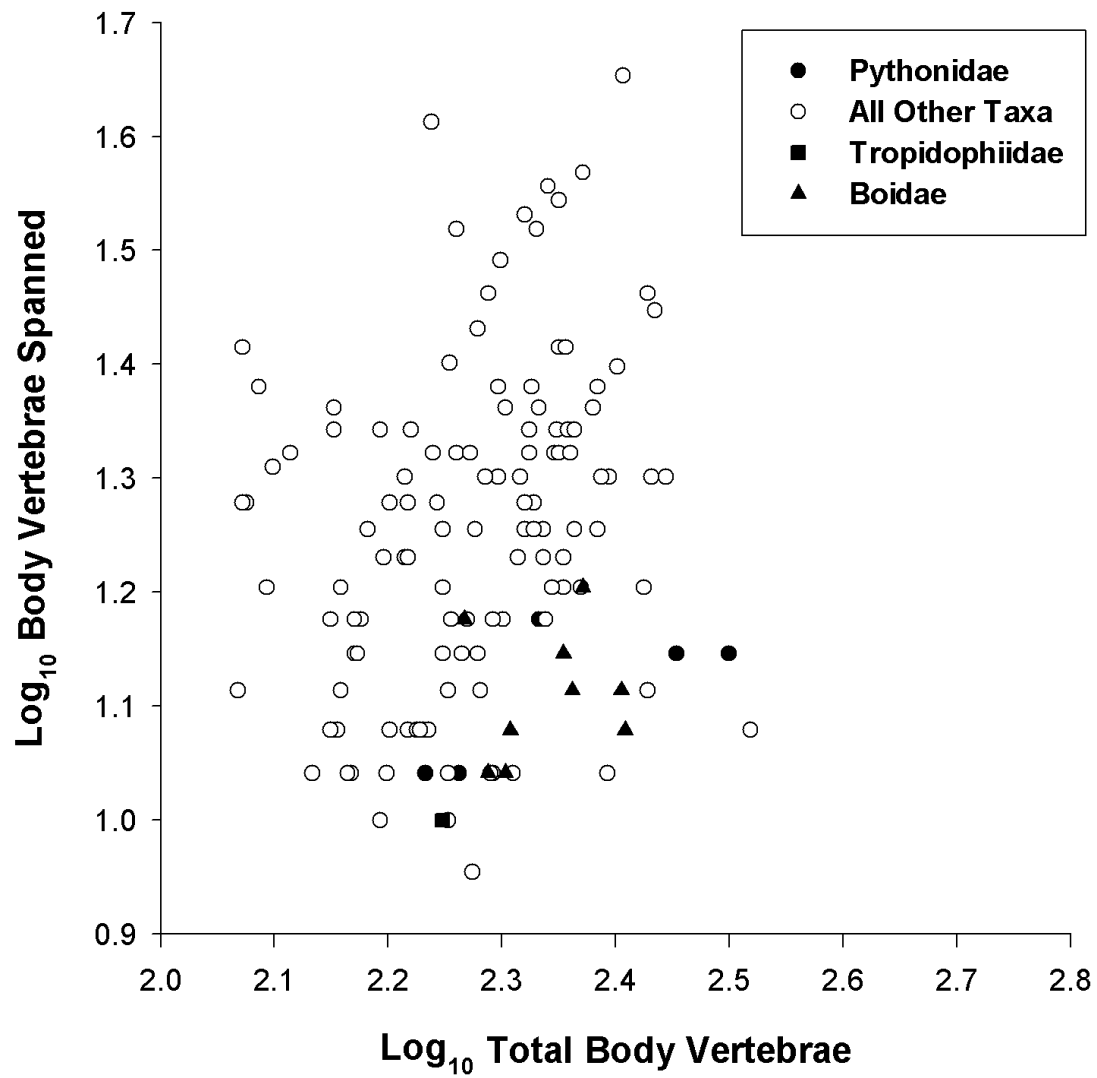


Figure 3.2B.



Appendices

Appendix 3.1: The phylogenetic tree used for all analyses was constructed from published trees for snakes using both high (e.g. family) and low (e.g. genus and species) level trees. Trees were constructed and manipulated in Mesquite[®] (version 1.12; Maddison and Maddison 2006; <http://mesquiteproject.org>). All 134 species of snakes are represented in the final tree. The final tree contained several polytomies for which we could not find published phylogenetic hypotheses or in which published phylogenies contained polytomies or weak nodal support. The current taxonomy of species was checked using the TIGR Reptile Database (Uetz et al. 2007).

Our understanding of higher-level snake relationships has drastically improved in the last few years. Nevertheless, there is still significant disagreement between studies using primarily molecular characters versus those using morphological characters—particularly among the relationship between basal alethinophidians and the placement and monophyly of the Tropidophiids (Vidal et al. 2007a).

For our purposes, we have used Lee et al.'s (2007, Figure 6) study combining morphological characters with both nuclear and mitochondrial DNA. This study is congruent with most molecular studies (e.g., Slowinski and Lawson 2002; Vidal and Hedges 2002; Wilcox et al. 2002; Lawson et al. 2004; Vidal and Hedges 2004; Vidal et al. 2007a) in its basal placement of scolecophidians within extant snakes and of the monophyly of the Alethinophidia, Caenophidia and Colubroidea. These molecular studies, however, place Tropidophiids near the base of the alethinophidians, whereas Lee et al. (2007) nests them within the Macrostromata, basal to the Caenophidia. The

placement of *Tropidophus* in Lee et al. (2007) retains a more conventional view of snake evolution—that of increasing gape size and elaboration of the feeding apparatus, and a trend towards a progressively more terrestrial lifestyle (Greene 1997; Cundall and Greene 2000). In a more recent multilocus study, Wiens et al. (2008) were unable to completely resolve the pythons, boas and *Tropidophis*. To maintain a more conservative approach we treat these 3 clades as a polytomy in our analysis.

The relationship among the Caenophidia was determined using the hypothesis of Vidal et al. (2007b), which uses seven nuclear protein-coding genes (though only 24 taxa). This study—which generally has strong nodal support—was chosen for the large number of genes used [e.g. Heise et al. 1995 (two mitochondrial genes); Vidal and Hedges 2002 (1 mitochondrial gene, 3 nuclear genes); Vidal and Hedges 2004 (two nuclear genes); Lawson et al. 2005 (one nuclear and one mitochondrial gene)] and for its focus on nuclear protein-coding genes, which have generally proven to yield more consistent results in previous studies of higher level squamate phylogeny (Townsend et al. 2004; Vidal and Hedges 2004; Vidal and Hedges 2005). Several troublesome taxa, most notably the Preatids (e.g., *Asthenodipsas*) were placed using the hypothesis of Zaher et al. [(2009) 1 nuclear and 2 mitochondrial genes, but 132 taxa]. This study has strong support for placing *Acrochordus*, Xenodermatids and Preatids as successive ougroups to the Coluboridea (*sensu* Lawson et al. 2005). Previous studies showed weak nodal support for Preatids as the sister group to the Viperidae or as an unresolved group placed at the base of the Colubroidea (e.g., Wüster et al. 2008). For our purposes,

Caenophidia is defined as the Colubroidea and its sister groups, the Achrochordids, Xenodermatids, and Preatids..

The following list is constructed such that indentations are indicative of position in the phylogenetic hierarchy. For instance, subfamilies such as Colubrinae or Dipsadinae would be indented relative to the Colubroidea because both of these subfamilies are subgroups of the more inclusive Colubroidea. Taxa that are indented to the same degree are in no way equivalent (e.g. sister taxa) and do not necessarily form a nested relationship (de Queiroz and Rodríguez-Robles 2006; Gartner et al. 2010).

Pythonidae: We follow the phylogenetic hypothesis of Rawlings' et al. (2008, Figure 3), molecular study over Kluge's (1993) morphological study for its generally better nodal support and larger number of characters used. The pythons form a well-supported clade, sister to a group that includes *Loxocemus* and *Xenopeltis*. Rawlings et al. (2008) uncovered a well-supported Afro-Asian clade, including *Python curtus*, and a well supported Indo-Australo clade that is sister to an Australo-Papuan clade (which includes *P. reticulatus* and *M. spilota* respectively).

Boidae: We use the hypothesis of Noonan and Chippendale (2006) for the relationship among the Boids. This tree uses many more genes (5 nuclear genes and 1 mitochondrial genes) and more characters than Burbrink's (2005) combined morphology/DNA tree, though the latter uses more taxa. Both trees are generally well resolved and demonstrate the paraphyly of the Boinae with respect to the Erycines (who are nested within the Boinae). Both studies show instead that the evolutionary relationships among the Boids are better reflected by current geographic distribution than by the morphological

characters traditionally used to elucidate Boid relationships (Burbrink 2005; Noonan and Chippendale 2006). We maintain use of the subfamily names Erycinae (the Afro-Indian sand boas), and the Boinae (all non Erycine Boids) for convention.

Colubroidea

Viperidae (including subfamilies Viperinae and Crotalinae): We used the hypothesis of Wüster et al. (2008) for elucidating the relationships among the Viperids. This study was preferable to others (e.g., Lenk et al. 2001; Parkinson et al. 2002) for several reasons. First, it uses a large number of taxa and several mitochondrial markers in its analysis. Second, it treats the group as a whole, whereas previous studies focus on either the Crotalinae (where a monophyletic consensus is generally agreed upon) or on the Viperinae, where there is significant disagreement between molecular and morphological data (Lenk, et al. 2001) or in some cases, weak evidence of monophyly (Cadle 1992). Last, it tests the relationship of two presumed primitive taxa, *Azemiops* and *Causus* to other Viperid genera. In the case of the latter, it was found to nest within the Viperinae—thus we must call into question those studies using *Causus* as the outgroup to other Viperids in their analyses. The sister group of the Vipers in our tree is *Asthenodipsas*, in the Colubrid subfamily Pareatinae. This placement—though poorly supported—comes from separate bayesian and likelihood models (Lawson et al. 2005). When analyses are combined, the Pareatinae are placed in an unresolved position outside of the remaining Colubridae, Elapidae, and Atractaspididae.

Elapidae: The elapids are broken into an Afro-Asian-American clade and an Australo-Melanesian clade (Keogh 1998). The relationships between the cobras,

mambas, kraits and coral snakes were derived from Keogh (1998). The Australo-Melanesian taxa in our data set consist of viviparous Hydrophiid sea-snakes and terrestrial, oviparous Australian Hydrophiids. Many Hydrophiid sea snake taxa are polyphyletic (Lukoschek and Keogh 2006). We therefore were unable to assume the monophyly of genera for which we had some taxa, but not others, available in a phylogeny. To maintain a more conservative approach, we collapsed all Hydrophiid viviparous sea-snakes to a soft polytomy. The relationships between the remaining terrestrial members of the Australo-Melanesian clade were resolved using the hypothesis of Sanders et al. (2008).

Homalopsidae: The relationship among the Homalopsinae comes from Alfaro et al. (2008).

Natricinae: We have only two species of Natricines, thus they must be sister to one another.

Xenodontinae and Dipsadinae: We primarily used the hypothesis of Vidal et al. (2000). This is the largest, most comprehensive study of the Xenodontines to date. We used Pinou et al. (2004) for the placement of *Dipsas* and Crother (1999) for the placement of *Geophis* within the group.

Colubrinae: The majority of the relationships among the Colubrinae come from the hypotheses of Lawson et al. (2005, Figure 1) and Creer (2001, Figure 2.1 A and B).

Dispholidini: The placement of *Dispholidus typus* comes from Fry et al. (2008) and Broadley and Wallach (2002). Both show the close affinity of *Dispholidus* to *Thrasops* and *Thelotornis*.

Sonorini: We have only two members of the tribe Sonorini (Greene 1997). We have placed them as sister taxa, though Creer (2001) presents evidence that the tribe may be paraphyletic.

***Masticophis* and *Coluber*:** Nearly all phylogenies show the sister relationship between *Masticophis* and *Coluber* (Creer 2001 Figure 2.4; Nagy et al. 2004). The several specimens of *Coluber* were reduced to a single branch and mean character values used in the analysis.

Lampropeltini: The phylogenetic relationships among the new world Lampropeltines (*Arizona*, *Cemophora*, *Pantherophis*, *Lampropeltis*, *Pituophis* and *Stilosoma*) come from the hypotheses of Rodríguez-Robles and De Jesús-Escobar (1999, 2000).

Lamprophiidae: The relationships among the Lamprophiids come from the hypothesis of Nagy et al. (2005). The two species of *Polemon* must be sister to one another. However, we do not have a hypothesis for the placement of *Chilorhinophis*, we have collapsed the clade to a soft polytomy.

Boodontinae: We have only two species of Boodontids, *Pseudaspis* and *Lamprophis*. Though Lawson et al. (2005) presents evidence for the paraphyly of the group, he has both of our representative species as sister to one another.

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Appendix 3.2.

File of Phylogenetic tree. This file (J134P2.txt) of the phylogenetic tree (described in Appendix A, and shown in Figure 1) was produced in Mesquite (Version 2.72 Maddison and Maddison, 2006; <http://mesquiteproject.org>). It is in the Newick Standard File Format.

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Chapter 4

Morphological and Physiological Correlates of Individual Variation in Locomotor Performance in the Corn Snake (*Pantherophis guttata*).

Abstract

We examined individual variation in locomotor performance, metabolic rate, and several sub-organismal traits in wild-caught adult corn snakes, *Pantherophis guttata*. We studied relationships between standard metabolic rate [SMR], maximum oxygen consumption (VO_{2max}), sprint speed, treadmill endurance, and whether variation in these whole-organism traits was related to lower-level traits; ventricle and liver mass, snout-vent length (SVL), total length, tail length, number of body, tail and total vertebrae, the product of tail and body vertebrae (vertebrae product), resting and post-endurance concentrations of blood glucose and lactate, myosin heavy chain isoforms, hematocrit (HCT), and citrate synthase and lactate dehydrogenase activity in the heart, muscle, and liver. Sprint speed, endurance and VO_{2max} were repeatable ($P < 0.05$). Most morphological and performance traits as well as SMR were correlated with body mass or snout-vent length, while biochemical traits were not. Sprint speed and endurance, as well as several morphological traits differed between the sexes. Individual (residual) variation in sprint speed, endurance, and VO_{2max} were uncorrelated. Sprint speed was positively correlated with numbers of body vertebrae. VO_{2max} was significantly correlated only with variation in a myosin heavy chain isoform. Two variables—post endurance glucose and resting lactate—best predicted endurance.

Introduction

It is often presumed that form facilitates function in animals (Gans 1974, 1988; Bennett 1987a), and this may be particularly true for locomotor biology (Dickinson et al. 2000). Differences in body size, shape, and composition, in conjunction with limb design and proportions, are largely responsible for the variation in locomotor ability among different types of animals. Traditionally, comparative studies of form-function relationships have focused on interspecific, or even interfamilial comparisons, likely because differences among species or higher taxa are large (Bennett 1987b).

Studies of individual variation are less common. Leaving aside studies of human beings (e.g., Eisenmann et al. 2004, 2009), such studies have been used to examine variation in a broad suite of traits, including personality (fish: Dingemanse et al. 2009; mammals: Réale et al. 2010), metabolism (crustaceans: Baldwin et al. 1999; lizards: Garland 1984; Pough and Andrews 1984; Garland and Else 1987; amphibians: Gomes et al. 2004; mammals: Hayes and Jenkins 1997), diet (birds: Pryce 1987; mammals: Lyons 1989) and many others. From an evolutionary standpoint, the utility of studying individual variation or intra-population variability is clear, given that among-species variation is derived, in part, from adaptive shifts in response to natural selection within populations.

Among squamates, lizards have typically served as model organisms in studies of individual variability in locomotor performance and metabolism (Garland 1984; Garland and Else 1987; Bennett 1987b; Bennett and Huey 1990; Huey et al. 1990; Garland 1993; Calsbeek and Irschick 2007). Studies of individual variation in snakes are less common

(Garland 1994; Garland and Losos 1994), and more rare still are studies of snakes that have attempted to link morphology and performance—termed the “performance gradient” (Arnold 1983). All of those studies have used juvenile garter snakes in the genus *Thamnophis*. This work has shown that variation in metabolic rates and several measures of locomotor performance, including maximal sprint speed and endurance capacity are variable, repeatable, and heritable (Garland 1988; Arnold and Bennett 1988; Jayne and Bennett 1989; Jayne and Bennett 1990a, 1990b; Garland 1990; Brodie 1989, 1992; Brodie and Garland 1993; Brodie and Russell 1999).

Snakes show remarkable ecological and behavioral variation (Greene 1997). The absence of limbs requires that such variation stem from modifications of the axial musculoskeletal system (e.g., body vertebrae: Lindell 1994; body length: Boback and Guyer 2003; Lindell 1996; relative tail length: Sheehy 2006; musculature: Jayne 1982). Such reliance on the trunk, however, potentially imposes functional constraints on locomotion and other behaviors, particularly those that share a common mechanistic basis. Therefore, we investigated whether morphological correlates of locomotor ability in non-natricine snakes (the natricines include *Thamnophis*) would differ with those of another, ecologically and behaviorally distinct species.

We used adult corn snakes, *Pantherophis guttata* to ask two questions. First, how much variation exists in three ecologically relevant measures of locomotor performance and metabolism in *P. guttata*, and is it comparable to the amount seen in other studies? Second, what is the relationship between individual variation in metabolic rates, performance, morphology, physiology, and biochemistry?

Our study is novel in several ways. All previous studies examining morphological correlates of individual variation in locomotor performance in snakes have been conducted on garter snakes (genus *Thamnophis*). Corn snakes and other rat snakes (genus *Pantherophis*) are very different from garter snakes in terms of their diet, prey capture mode, habitat utilization, and other aspects of their life history, ecology and behavior (Wright and Wright 1957). Further, our use of adult animals differs from all previous studies of individual variation in snakes. Finally, previous studies of individual variation in snakes have correlated measures of locomotor performance or metabolism with only a few sub-organismal traits simultaneously.

Here, we examine the broadest data set to date, including measures of metabolism and locomotor performance, in addition to several morphological, physiological, and biochemical measures (sub-organismal traits). The general mechanisms of different types of snake locomotion are understood at a basic functional level (e.g., Gans 1962; Jayne 1986, 1988a 1988b; Moon and Gans 1988; Moon 1999). However, our understanding of how variation in underlying traits correlates with variation in locomotor performance remains poor. Nevertheless, all of our measured traits have been suggested to be important functional elements involved in snake locomotion or in vertebrate locomotion in general.

Materials and Methods

Animal Care and Husbandry

Thirty adult corn snakes, *Pantherophis guttata*, were collected from a single location in Palm Beach County, Florida by Glades Herp Farms (Bushnell, Florida) and shipped to Riverside, CA. Both sexes were represented (Males: N = 17, Females: N = 12), but sex was not determined until the end of the study when it was verified post-mortem. All snakes appeared healthy and were non-reproductive.

Animals were maintained at ~25 °C with a 12Light:12Dark photoperiod. Snakes were kept individually in rack-style caging (Model # V-35 Rack, Vision Products, Canoga Park, CA) in plastic tubs measuring 51 cm long x 38 cm wide x 13.5 cm tall. A base of shredded aspen shavings was used as substrate, with a piece of newsprint for cover. Water was provided *ad libitum*. Maintenance and experiments were approved under a UC Riverside Animal Care and Use Protocol (AUP Protocol 20070044).

Snakes were fasted for two weeks to ensure they were post-absorptive prior to the beginning of experiments because food in the gut may reduce certain measures of performance (Garland and Arnold 1983, Secor and Diamond 1997; Zaidan III and Beaupre 2003, Sievert et al. 2005).

Testing Schedule

Whole-organism measures were conducted between August and November of 2009 in the sequence shown in Figure 4.1. The time between any two measures was the same for all animals (plus or minus one day). Prior to all measurements, animals were warmed to 30

°C for a minimum of four hours (Chappell and Ellis 1987; Walton et al. 1990; see also Bennett and Dawson 1976). Body temperature was measured after each performance trial with a vinyl-covered thermocouple (calibrated against a mercury thermometer in water) inserted approximately 3 cm into the cloaca. Mass was measured $\pm .001$ g prior to every measure. All animals were offered food (laboratory mice) after SMR trials and after $VO_{2\max}$ trials (though not all snakes ate). In addition to parametric data, performance trials were assigned a subjective “quality” score from 0-5, with zero indicating a complete failure to perform and 5 indicating a trial with no logistical or behavioral problems. Following endurance trials, animals were euthanized by rapid decapitation and several morphological and biochemical measures assessed (Table 4.1).

Standard Metabolic Rate

SMR was measured using positive-pressure open-flow respirometry (Lighton 2008) with an Apple Macintosh computer (Cupertino, CA) equipped with National Instruments A-D converters (Austin, TX) and LabHelper software (<http://www.warthog.ucr.edu>).

Measurements were made overnight (1800-0800 h local time). Corn snakes may be active at almost any hour of day in the late summer and early fall (G.E.A.G, pers. obs.).

We chose evening hours because snakes were least likely to be disturbed during this period. Sealed metabolic chambers—lucite boxes or glass jars of varying sizes depending on the animal—were placed inside larger environmental chambers maintained at 30 °C. Airflow was maintained at approximately 150 ml/min, 180 ml/min or 320 ml/min $\pm 1\%$ depending on the size of the metabolic chamber, using Porter Instruments

mass flow controllers (Hatfield PA, USA) located upstream (Figure 4.2A). For the largest chamber, a 100 ml/min sub-sample of excurrent gas was dried with magnesium percholorate and redirected to a carbon dioxide analyzer (CA-2A, Sable Systems, Henderson, NV; Li-Cor model LI-6251, Lincoln, NE) before being dried and scrubbed of carbon dioxide with ascarite and directed to an oxygen analyzer (Oxzilla, Sable Systems; Applied Electrochemistry oxygen sensor N-37 M and oxygen analyzer S-3A, Sunnyvale, CA). Three-minute reference readings were obtained automatically every 45 min with a solenoid-manifold system operated by an Apple Macintosh computer. The software computed SMR as the lowest rate averaged over a continuous 1 hr period during which oxygen or carbon dioxide consumption were low and stable.

$VCO_{2\text{standard}}$ was calculated as:

$$VCO_{2\text{standard}} = STP * FR((FeCO_2 - FiCO_2) - FeCO_2 * (FiO_2 - FeO_2)) / (1 - FeCO_2)$$

Where T = ambient temp °K/273.2, P = ambient pressure in Torr/760, FR = flow rate in ml/min and Fi and Fe are incurrent and excurrent fractional concentrations respectively for either oxygen or carbon dioxide (<http://www.warthog.ucr.edu>). $VO_{2\text{standard}}$ was calculated as:

$$VO_{2\text{standard}} = STP * (FiO_2 - FeO_2) * FR / (1 - FeO_2)$$

Periods of activity—identifiable by large and irregular increases in oxygen consumption—were avoided when computing SMR.

Sprint Speed

Sprint speed was measured using previously described techniques (Garland 1988). From a stationary position, snakes were “chased” along a 6.0 m, photocell-timed racetrack (12 sets of photocells evenly spaced every half meter; Figure 4.3) to elicit maximum sprint speed. The racetrack was lined with artificial plastic grass “turf” (Sierra Pro synthetic turf, Tiger Express Landscape, Anaheim, CA). The track was maintained at the ambient room temperature (~28 °C). Animals were held in cloth sacks in a large, sealed environmental chamber at least four hours prior to trials to allow body temperature to reach 30 °C. Snakes were removed from their sacks and placed just behind the first photocell. Sprinting was induced by vigorously tapping the tail tip and the area immediately behind the animal. Each animal was chased, picked up and returned to the start, then chased again in quick succession. The number of times an animal reversed direction or became defensive—stopping and facing the experimenter and striking vigorously—was noted (e.g., Brodie 1989, 1992). In total, there were four sprints per snake, per day; a back-to-back trial in the morning and another in the afternoon. Snakes were allowed to rest for four hours between morning and afternoon trials. This process was repeated on two consecutive days followed by two full days of rest, and then repeated on two additional consecutive days. In total, 16 sprints were conducted for each snake.

For each trial, the fastest 1.0 m interval (3 consecutive photocells) was calculated. The single fastest speed for each of the four days was used for calculating repeatability.

Maximum Oxygen Consumption (VO_{2max})

We recorded maximum oxygen consumption during forced exercise (VO_{2max}) with negative-pressure open-flow respirometry (Figure 4.2B) with the same equipment used for SMR. Airflow was generated with a pump immediately downstream of the animal and maintained at 500 ml/min \pm 1% using mass flow controllers (Porter Instruments, Hatfield, PA) located downstream from the pump (Figure 4.2B). Excurrent air was subsampled at approximately 100 ml/min.

Trials were performed on a custom-built motorized treadmill (Figure 4.4A; Bonine and Garland 1999). The belt surface of the tread (working dimensions 163 cm X 20 cm), was rubberized cloth bordered by 2.5 cm melamine boards to prevent the tails of locomoting animals from moving under the belt surface. Two 100-watt incandescent bulbs 43 cm above the tread were adjusted with a rheostat to maintain a temperature of 30 °C.

Animals were warmed to 30 °C for a minimum four hours prior to the start of a trial, then quickly fitted with a small, tight-fitting mask held in place by a soft piece of foam at the posterior-most portion of the jaw. Several masks were used, depending on the size of the animal. Small-diameter Tygon® tubing (Saint-Gobain Performance Plastics, Garden Grove, CA) was attached to the top front of the mask and held above the treadmill so that the snake could move freely about the apparatus. Animals were placed on the stationary treadmill belt, which was immediately started and held at approximately 0.1 km/hr until steady-state locomotion was achieved. Speed was increased incrementally until further increases in speed elicited no further increase in VO_2 . After

the belt was stopped, snakes usually remained motionless and breathed heavily for several minutes. VCO_{2max} was calculated as:

$$VCO_{2max} = STP * FR * ((FeCO_2 - FiCO_2) + FiCO_2 * (FiO_2 - FeO_2)) / (1 + FiCO_2)$$

VO_{2max} was calculated as:

$$VO_{2max} = STP * ((FiO_2 - FeO_2) * FR + VCO_2 * (FeO_2 - FiO_2)) / (1 - FiO_2)$$

We used the highest 60 seconds of VO_2 and the corresponding VCO_2 for analyses.

Treadmill Endurance

Endurance was measured on the same treadmill used for VO_{2max} measurements, but with 10 cm x 5 cm X 2.5 cm high pieces of artificial turf glued to the belt for traction (Figure 4.4B).

The goal was to measure endurance supported primarily by aerobic metabolism. We tested snakes at 0.6 km/hr (see Walton et al. 1990); all individuals crawled readily at this speed-until they neared exhaustion.

Snakes were preheated to 30 °C and weighed, placed on the moving tread, then induced to crawl by tapping with a gloved finger at the base of the tread or on the tail tip. As snakes tired, tapping frequency increased to approximately 5 taps/sec (Garland 1988). Eventually animals no longer maintained tread speed and fell off the back of the belt, whereupon they were immediately placed back on the tread and induced to crawl. We defined exhaustion as the fifth time that an animal fell off the back of the tread. For animals that were reluctant to crawl (either falling off of the tread before any signs of exhaustion or becoming defensive), additional motivation (through tapping of the tail)

often resulted in several more minutes of movement. In general, snakes were exhausted to the point of having reduced muscle tone (i.e, lack of a righting response or failure to flee or become defensive when stimulated). Animals were tested on two consecutive days to assess repeatability.

Following exhaustion on the second day, ~ 0.1 cc of blood was immediately removed from the caudal vein and assessed for blood glucose and lactate levels (measured in duplicate as mmol/L; Contour blood glucose test strips and Ascensia contour diabetes meter kit-blood glucose monitoring system, American Diabetes Wholesale, Pompano Beach, FL; Accutrend lactate analyzer and BM lactate test strips, Lactate.com). Both measures were taken again 1.5 hours after the initial measure.

Morphological, Physiological, and Biochemical Traits

Approximately one week after endurance trials, snakes were warmed to 30 °C, weighed, and euthanized. We recorded the time between when an animal was first picked up and when a blood sample was taken or an organ was removed and frozen. Arterial blood was collected, and measures of resting blood glucose and lactate levels were obtained as described above. Hematocrit (HCT) was measured in triplicate using heparinized microhematocrit tubes following the methods of Garland and Bennett (1990).

Following blood collection, organs were exposed through a ventral incision. The heart was removed and the ventricles cut free from the atria, blotted dry, weighed \pm 0.001g, and flash-frozen (liquid nitrogen). The liver was removed, blotted, and weighed similarly. A medial portion was cut away and flash-frozen. Finally, a portion of the

major epaxial muscles (semispinalis-spinalis, longissimus dorsi, and iliocostalis) was removed from mid-body and flash-frozen.

After organ removal, total length and tail length were measured ± 1 mm and snout-vent length (SVL) was measured by subtraction. Vertebrae were counted from numbers of ventral scales (each ventral scale indicates one vertebra [Alexandar and Gans 1966; Kerfoot 1970]). Carcasses were stored in plastic bags in a -20 °C freezer.

Citrate Synthase and Lactate Dehydrogenase

Citrate synthase (CS) and lactate dehydrogenase (LDH) activities were assayed in the ventricle, liver, and epaxial muscles, following previously published methods (Somero and Childress 1980; Bennett et al. 1982; Garland 1984). We used kinetic assays, measuring the maximum velocity of the reaction at 30 °C (V_{\max}) with a thermostatted SPECTRAmax Plus microplate spectrophotometer (Molecular Devices Corporation, Sunnyvale, CA)

Samples were homogenized in a buffer solution (50mM Imidazole, 2mM EDTA [Ethylenediaminetetraacetic acid], pH 6.6 on ice) equal to one part tissue in nine parts buffer using a tissue-terror (Biospec Products, Bartlesville, OK). Chilled homogenates were centrifuged (Allied Fisher Scientific micro-centrifuge model 235C) for 10 minutes at 12,400 rpm at 1.5 °C. Following centrifugation, 1 ml of supernatant was removed and used for measurements.

Serial dilutions were used to determine the proper concentration of substrate to homogenate. Forty microplate wells were run concurrently for both CS and LDH assays.

Each sample was measured in triplicate with its own control with 10 samples measured simultaneously. Samples for each run were chosen at random. Citrate synthase activity was measured at 412 nm using 10 μ l of supernatant with 100 μ l of assay mixture (0.8 μ l of 400mM MgCl₂, 8.0 μ l of 2.0mM DTNB, 10 μ l of 1.6mM acetyl CoA, and 81.2 μ l of 80mM Tris Buffer (pH 8.0 at 20 °C); all chemicals were mixed in 80mM Tris buffer. The reaction was initiated by adding 50 μ l of 1.6mM oxaloacetate to each well (control wells received an additional 50 μ l of 80mM Tris buffer). This resulted in a final concentration of 2.0mM MgCl₂, 0.1mM DTNB, 0.1mM acetyl CoA, and 80mM Tris. Absorbance was recorded at 30 °C. The spectrophotometer measured the rate of reduction of DTNB (from colorless to yellow) by cycling through reading 8 wells at a time for 10 minutes, with an 8 second interval between each. V_{\max} was obtained as the slope of time vs. optical density for the steepest 20 points.

For LDH assays, ten μ l of diluted supernatant was added to 100 μ l of assay mixture (15 μ l 3.2mM NADH, 32 μ l 500mM KCL [dissolved in 50mM Imidazole, pH 7.0 at 20 °C] and 53 μ l 50mM Imidazole). The reaction was initiated with 50 μ l of pyruvate resulting in 160 μ l of solution with a final concentration in each well of 0.32mM NADH, 100mM KCL, 50mM Imidazole and 1.0mM pyruvate. The oxidation of NADH to NAD⁺ (from blue to clear) was measured at 30 °C and 340 nm. Each assay was measured at 5-second intervals for 7 minutes for a total of 85 readings. V_{\max} was obtained from the 20 steepest points during the linear portion of the reaction.

We obtained the mean and coefficient of variation (CV) for the 3 replicates of each sample. If the CV was greater than 10%, we removed the replicate that was furthest

from the mean and recalculated (the mean) based upon the new sample. For each mean, we then subtracted the control value. We used the absolute value of LDH V_{\max} because the slope of time versus optical density is negative for LDH.

Myosin Heavy Chain Isoforms

Myosin heavy chain (MHC) isoform analysis was conducted using a modified version of a previously published extraction method (Talmedge and Roy 1993; Bryan Rourke pers. comm.). A known mass of muscle was homogenized using a tight-fitting, glass-glass homogenizer with a 19:1 ratio of homogenization buffer (250mM sucrose, 100mM KCL, 5mM EDTA, 1 mM Tris-HCL, pH 6.8 at 4 °C) to muscle. 250 μ l of buffer was added to the homogenate and spun at 1,000g for 10 minutes in a temperature-controlled microfuge. The supernatant was discarded and the pellet saved. 1 ml of a second solution (10 mM Tris-HCL pH 7.4, 2 mM EDTA and 175 mM KCL—adjusted to pH 6.8 when cold) was added to the pellet, vortexed vigorously, and spun for 5 minutes at 1,000g. The supernatant was removed and 1 ml of a third solution (10 mM Tris-HCL pH 7.4 and 150 mM KCL, adjusted to pH 7.0 when cold) was added, vigorously vortexed, and spun for 5 minutes at 1,000g. The supernatant was again discarded and 250ml of solution three added. This final protein homogenate (relatively pure myofibrils) was diluted to 1 mg/ml in a storage solution (50% glycerol, 50mM Na₄p207, 2.5mM EGTA, 1mM β mercaptoethanol; pH 8.8), vortexed well, and stored immediately at -20 °C for later MHC gels.

Gels were run using the CBS vertical gel electrophoresis apparatus (plates measuring ~ 22 cm x 16.5 cm). The separating gel consisted of 30% glycerol, 8% total acrylamide (2% Bis), 0.2M Tris Base (pH 8.8), 0.1M glycine, 0.4% SDS, 0.1% APS, and 0.05% TEMED. After preparation, the separating gel was left to set, and homogenate samples were prepped in sample buffer (0.35µl sample buffer to 0.2µl sample—buffer consisted of 100mM Tris-Base [pH 6.8], 5% glycerol, 4% SDS, 0.05% Bromophenol Blue, 5% βmercaptoethanol). These samples were heated to 100 °C for 2-3 minutes and then removed for loading into the stacking gel. Stacking gels (prepared at least 1 hour after pouring separating gels) consisted of 30% glycerol, 4% total Acrylamide (2% Bis), 70mM Tris HCL (pH 6.8), 0.4mM EDTA, 0.4% SDS, 0.1% APS and 0.05% TEMED. The upper and lower buffer tanks were filled with cold running buffer (100mM Tris Base, 150 mM glycine, 0.1 % SDS in milliQ water). Running buffer in the lower reservoir was diluted 1:1 with cold water. 7.5µl of prepared homogenate was loaded into the prepared wells. The entire apparatus was placed in an incubator at 4 °C with a constant voltage of 275 volts and run for 23 hours.

Gels were stained using a Silver Stain Plus Kit (Biorad, Hercules, CA) and photographed with a digital camera (Canon USA, Irvine, CA) and saved as JPEG files (Figure 4.5). Images were inverted and converted to 16 bit gray-scale using PhotoImpact Pro (Nova Development). The relative density of individual bands was determined with densitometry using ImageQuant® software (Molecular Dynamics, Sunnyvale CA). Data points are the area under a curve of optical density for a given band (e.g., if there are two bands, there are two peaks; larger values indicate darker bands with more protein).

Statistics

We only used performance trials scored as 3, 4 or 5 on our “quality” scale. We excluded any animal that had lost 20% or more of its original body mass, as first measured for SMR (defined as the cutoff for starvation in snakes, McCue 2006, 2007). For example, if an animal had lost 21% of its body mass at the time endurance was measured, endurance and all subsequent measures were removed from the data set for that animal. Four animals were excluded from measures of endurance due to mass loss and an additional three animals were removed from biochemical and physiological measurements. Morphological measurements, such as “length” and vertebral numbers, were retained as these are not affected by body mass or body condition.

Body size may affect most if not all traits included in this study. We therefore first examined allometric equations by \log_{10} transforming all traits and regressing them on either \log_{10} body mass (M) or \log_{10} snout-vent length (SVL). We thus obtained, for each trait, an equation of the form:

$$\log_{10} Y = \log_{10} a + b \log_{10} M \text{ (or SVL)}$$

or in arithmetic form:

$$Y = aM^b$$

“**a**” is the Y-intercept or position of the line, and **b** is the slope of the log-log relationship, i.e., the allometric scaling exponent. We substituted SVL for M in several instances because several traits (e.g., tail length, numbers of body and tail vertebrae) are likely to scale with SVL in addition to mass (Polly et al. 2001). In those instances where the allometric scaling exponent differs significantly from zero, allometric equations are

given. For statistically non-significant relationships with M or SVL, only the range, mean, and Y-intercept are given.

Variability was assessed by comparing the standard deviations (SD) of residuals from the allometric equations for all traits regardless of their dependence on mass. The SD of a natural log-transformed data set is roughly equivalent to the coefficient of variation of the untransformed data set (Garland 1984). Thus, by multiplying the SD of residuals for each allometric equation by 2.3026 (to convert to base e), we can compare variability among traits. Note that comparisons of standard errors of the estimates from allometric equations give similar results, but CVs are more useful for comparison among previously published values (Garland 1984).

Residuals were calculated from ANCOVAs including size (either body mass or SVL) and sex. For resting glucose and lactate levels, residuals were calculated with “bleed delay” as an additional covariate. Traits were \log_{10} transformed only when doing so improved linearity of the bivariate relation and normality of the residuals. These residuals were used for bivariate correlations and multiple regressions.

We used the residuals (described above) to assess to what extent variation in locomotor performance and metabolism (SMR) is related to variation in sub-organismal traits. Regression models predicting locomotor performance and metabolic rate were constructed from those traits showing significant ($P < 0.05$) pairwise correlations with the dependent variable (performance or metabolic rate). We used backwards stepwise multiple regression, rather than running all possible models, for metabolic or

performance measures that showed significant bivariate correlations with more than one other trait, thereby reducing the total number of models run.

Results

Repeatability

Sprint speed was significantly repeatable between morning and afternoon trials within all four days ($r = 0.395$, $N = 28$; $r = 0.627$, $N = 24$; $r = 0.514$, $N = 26$; and $r = 0.608$, $N = 26$ for days 1-4, respectively, $P < 0.05$ for all correlations). Sprint speed was also repeatable across consecutive days ($r = 0.515$, $N = 27$ for maximum sprint speed on days one and two; $r = 0.737$, $N = 27$ for maximum sprint speed on days 3 and 4, $P < 0.05$ for all correlations). There was no significant difference in maximum sprint speed across all four days (2-way ANOVA with Trial and Individual as fixed effects with no interaction, $F_{\text{trial}} = 2.913$, $df = 3, 111$, $P = 0.095$).

Maximum oxygen consumption was highly repeatable between the first and second trials ($r = 0.889$, $N = 28$, $P < 0.001$; Figure 4.7a). Mean values from the second trial showed a small (~9.5%) but significant increase (two-tailed paired sample t-test, $t = 2.489$, $df = 27$, $P = 0.019$). Endurance was also highly repeatable across the first and second trial ($r = 0.922$, $N = 23$, $P < 0.001$). There was a large (~18.6%) increase in endurance between the first and second trials (two-tailed paired sample t-test, $t = -2.911$, $df = 22$, $P = 0.008$; Figure 4.6b).

Allometry

Standard and maximal rates of oxygen scaled as $M^{0.530 \pm 0.241}$ and $M^{0.842 \pm 0.183}$ (scaling exponent \pm 95% Confidence Interval) respectively (Figure 4.7 A and E). Endurance scaled with both body mass and SVL ($M^{0.908 \pm 0.732}$ and $SVL^{2.068 \pm 2.153}$, respectively; Figure 4.7 I and J). Sprint speed was unrelated to body mass or SVL (Table 4.1, Figure 4.7 C and D).

Ventricle and liver mass scaled isometrically with body mass ($M^{0.930 \pm 0.109}$ and $M^{0.757 \pm 0.175}$ respectively). Overall, longer animals (total length) appeared less stout (had a higher length to girth ratio, assessed qualitatively) than shorter animals ($M^{0.235 \pm 0.051}$) Tail length scaled isometrically with body length ($SVL^{0.942 \pm 0.364}$). There was no effect of size (SVL) on any measure of vertebral count (body vertebrae, tail vertebrae, or total vertebrae) nor were there any significant effect of body mass on any of the biochemical traits measured.

Post-endurance measures of glucose and lactate did not scale significantly with body mass ($r^2 = 0.138$, $P = 0.081$, $r^2 = 0.158$, $P = 0.060$, respectively). Post-endurance glucose was significantly lower than glucose measured 1.5 hours after endurance trials (mean = 30.6 mmol/L versus mean = 36.7 mmol/L, respectively, paired t-test $P < 0.001$). As expected, post-endurance lactate was significantly higher immediately after endurance trials than 1.5 hours after trial (mean = 9.614 mmol/L versus mean = 5.421 mmol/L, paired t-test $P < 0.001$). Both measures of post-endurance glucose were higher than resting values, but this may be due to the decline in body condition between these measurements (see Discussion). Resting values of lactate were less than lactate measured

1.5 hours after endurance trials (1.602 mmol/L versus 5.492 mmol/L, respectively, paired sample t-test, $P < 0.001$).

Sex Differences

There were pronounced differences between the sexes for several traits (Table 4.2).

Males had significantly higher sprint speeds and treadmill endurance than females. In addition, with body mass as a covariate, males had shorter snout-vent lengths and fewer body vertebrae, while having longer tails and increased numbers of tail vertebrae. There was no difference in total length after accounting for both sex and body mass, nor were there sex differences in body mass after accounting for total length (results not shown).

Among biochemical traits, males had increased levels of liver LDH.

Correlations Among Characters

Correlations among characters were made using residuals from ANCOVAs including size, sex, and bleed delay time (for resting glucose and lactate).

Sprint speed was not correlated with endurance or metabolic rate. There was a significant negative correlation between endurance and SMR (Figure 4.8A; $r = -0.463$, $P = 0.03$, $N = 22$). Treadmill endurance also showed a significant negative correlation with post-endurance glucose ($r = -0.559$, $P = 0.006$, $N = 23$, Figure 4.8H).

Neither ventricle nor liver mass were correlated with any other character, although the relationship between ventricle mass and ventricle CS approached significance ($r = -0.443$, $P = 0.0503$, $N = 20$).

Hematocrit was correlated with treadmill endurance (Figure 4.8B; $r = 0.479$, $P = 0.033$, $N = 20$), liver lactate dehydrogenase [LDH] $r = 0.603$, $P = 0.005$, $N = 20$), and \log_{10} snout-vent length ([SVL] $r = -0.492$, $P = 0.028$, $N = 20$).

Resting lactate and glucose were positively correlated ($r = 0.759$, $P < 0.001$, $N = 20$), and both were negatively correlated with treadmill endurance (Figure 4.8D and 4.5C; $r = -0.546$, $P = 0.013$, $N = 20$ and $r = -0.605$, $P = 0.005$, $N = 20$ respectively). Resting glucose was correlated with liver CS ($r = -0.488$, $P = 0.029$, $N = 20$). Among enzymes, ventricle CS and LDH were positively correlated ($r = 0.519$, $P = 0.019$, $N = 20$), as were muscle CS and LDH ($r = 0.737$, $P < 0.001$, $N = 19$).

As expected, there were several significant relationships among mass- and sex-independent length and vertebral numbers. \log_{10} SVL was strongly correlated with \log_{10} total length ($r = 0.966$, $P < 0.001$, $N = 26$). Total length was not correlated with any other linear measure. Tail length was significantly positively correlated with tail vertebrae ($r = 0.550$, $P = 0.004$, $N = 26$), total vertebrae ($r = 0.440$, $P = 0.024$, $N = 26$) and the vertebrae product—the product of tail and body vertebrae ($r = 0.564$, $P = 0.003$, $N = 26$). Tail vertebrae were correlated with body and total vertebrae, as well as the vertebrae product (Table 4.3). Tail vertebrae were also correlated with treadmill endurance (Figure 4.8F; $r = 0.560$, $P = 0.008$, $N = 21$). Body vertebrae were highly correlated with both total vertebrae and the vertebrae product. In addition, body vertebrae were positively correlated with sprint speed (Figure 4.8G; $r = 0.482$, $P = 0.009$, $N = 28$). The vertebrae product was positively correlated with all measures of vertebral

numbers (Table 4.3), and was positively correlated with treadmill endurance (Figure 4.8E; $r = 0.459$, $P = 0.037$, $N = 21$).

Predicting Performance from Sub-Organismal Traits

Sprint speed was related only to numbers of body vertebrae (Table 4.3), but the regression ($P = 0.009$) explained almost a quarter of the variation ($r^2 = 0.232$).

Maximum oxygen consumption ($VO_{2\max}$) was related only to myosin heavy chain (MHC) band 1 (Table 4.3, $P = 0.022$), which explained 1/3 of the variation ($r^2 = 0.343$).

Treadmill endurance was correlated with several traits (Table 4.3), including SMR, tail vertebrae, vertebrae product, resting lactate and glucose levels, post-endurance glucose, and hematocrit. A backwards stepwise multiple regression (Table 4.5, $F_{df\ 2,14} = 13.253$, $P = 0.001$) explained 65% of the variation in treadmill endurance (Table 4.5, $r^2 = 0.654$) and included post-endurance glucose and resting lactate ($\beta_{\text{standardized}} = -0.619$, $P = 0.002$ and $\beta_{\text{standardized}} = -0.620$, $P = 0.002$, respectively).

Standard metabolic rate was negatively correlated with treadmill endurance, but was not correlated with any other traits.

Discussion

Allometry

Several performance and morphological traits scaled significantly with body mass or SVL, but there was significant scaling for any biochemical measures. In the lizard *Amphibolourus* [*Ctenophorus*] *nuchalis*, CS, PK, and LDH activity scaled with heart,

liver, and thigh muscle mass (except CS in the heart [Garland and Else 1987]). In *Ctenosaura similis* (Garland 1984), only three of eleven measured enzyme-tissue combinations showed significant scaling with body mass—two of which (LDH in the liver and PK in the heart) showed negative allometric relationships and the third (LDH in thigh muscle) showed a positive relationship. A decrease in CS activity with increasing size in our corn snakes may be expected, given the significant decrease in both standard and maximal rates of oxygen consumption with size (Table 4.1). Given these findings—among several species of lizard and snake, we conclude that relationships between body mass and aerobic and glycolytic enzyme activity in squamates may vary among species, tissues, and enzymes.

Longer snakes weighed less for a given length than shorter snakes (SVL scales as $M^{0.251 \pm 0.062}$ [$r^2 = 0.718$, CV 7.7%]) and had shorter tails, although differences in tail length may be due to sexual size dimorphism between males and females. Why might longer snakes be thinner than shorter snakes? Differences in body condition are one possibility. However, larger snakes maintained body condition during starvation better than smaller snakes. An alternate possibility is that snakes change body geometry during maturation. Anecdotal evidence from several species (e.g., *Python reticulatus* G.E.A.G. pers. obs.) suggests that snakes grow long early in adulthood, and fat later. Speculatively, if this occurs in *P. guttata*, it may explain several of the above scaling relationships.

The 95% confidence interval for our scaling exponent for SMR does not appear to be significantly different than that previously reported for corn snakes at 25 °C ($M^{0.70}$,

Smith 1976), or for that predicted for intraspecific scaling by Heusner (1982, $M^{0.67}$), though differences in SMR that may be expected if snakes are exposed to chronic stress or lack of food (Beaupre 1993).

Both VO_{2max} and VCO_{2max} showed substantial variation after correcting for body mass (Table 4.1) but body mass accounted for the majority of variation in both traits. Garland and Bennett (1990) report mass scaling of $M^{1.110 \pm 0.129}$ for VO_{2max} at 30 °C in newborn *Thamnophis sirtalis*, substantially higher than in our corn snakes ($M^{0.842}$). There are no published values of VCO_{2max} in snakes, but it would be expected that it would scale in a similar fashion to VO_{2max} .

CV's for mass-corrected and SVL-corrected sprint speed were higher than that reported in a previous study on neonate *Thamnophis sirtalis* (Garland 1988), but lower than reported by Arnold and Bennett (1988) in neonate *Thamnophis radix*. We found no mass dependent relationship with sprint speed, in contrast to Garland's (1988) finding of a significant positive relationship of sprint speed with body mass. In agreement with Garland (1988), Arnold and Bennett (1988) found a significant but weak correlation between body mass and sprint speed in a study juvenile *Thamnophis radix* but cautioned that experimental designs with fewer individuals may fail to uncover real, but weak correlations.

The mass scaling of endurance in *P. guttata* is considerably less than that reported by Garland (1988) for neonate garter snakes ($M^{1.798}$). However, in agreement with Garland (1988) and several studies on lizards (Garland 1984; Garland and Else 1987),

there is a stronger mass dependent relationship with endurance than sprint speed for corn snakes.

Sex Differences

We found differences among the sexes for several traits in our study (Table 4.2) and, as expected, the relative variation in those traits decreased after accounting for sex differences (contrast CV's in Tables 4.1 and 4.2). The most pronounced differences were body proportions. Sexual dimorphism is common in snakes and can include differences in body size, shape, coloration, diet, habitat use, and other aspects of ecology and behavior (Shine 1991, 1993 [and references within], 1994, 2001; Shine et al. 1996, 1998). Dimorphism among the rat snakes (genus *Elaphe* and *Pantherophis*) is generally restricted to body size (Schulz 1996), with males being larger in some species and females larger in others. However, among close relatives of *P. guttata*, there is anecdotal evidence of size dimorphism in only one species (*Elaphe [Pantherophis] obsoleta* [Schulz 1996]) where females tend to be larger. Females may have evolved larger body lengths (SVL) as a result of selection for female fecundity and evidence suggests that larger females tend to have more offspring (Shine 2001). Our data are in agreement with at least one other study on the black rat snake (*Elaphe [Pantherophis] obsoleta* [Schulz 1996]). In addition, males of most species of rat snakes have proportionally longer tails than females and in general, “females have a higher number of ventrals, but a lower number of subcaudals” (Schulz 1996). Thus, our findings of sex differences among body and vertebral numbers are in agreement with data on the genus as a whole.

Although not as extensively studied as morphological and dietary dimorphism, sex differences in activity patterns have been observed, though no general pattern exists (Gregory et al. 1987; Macartney et al. 1988). Among species most closely related to *P. guttata*, the evidence is equivocal—males have larger home ranges in *Elaphe (Pantherophis) obsoleta* (Fitch, 1963a) and *Coluber constrictor* (Fitch 1958, 1963b), while females have larger home ranges in *Masticophis taeniatus* and *Pituophis melanoleucus* (Parker and Brown, 1980). Male *P. guttata* were more frequently encountered away from refugia in the spring and summer in South Florida, while females were generally more cryptic, remaining hidden (G. E. A. Gartner, pers. obs.). This coincides with the period when males are seeking mates (Gregory et al. 1987; Macartney et al. 1988). Differences among the sexes in activity patterns may be reflected in differences in performance such as endurance and sprint speed. It seems unlikely, even in a wide ranging and active snake, that animals of either sex will need to move consistently at a high crawl rate that approaches their maximal aerobic speed. However, increased activity also subjects snakes to increased risks of predation (Greene 1997). Though speculative, selection may act to increase either endurance or VO_{2max} in response to increased predatory encounters—particularly if increased endurance or VO_{2max} allow for a more vigorous and prolonged struggle. While there was no difference in our study between the sexes in VO_{2max} , males did have greater endurance, which is consistent with the behavior of free-living *P. guttata*.

Performance and Sub-Organismal Traits

Given the substantial variation observed in whole-animal performance, a necessary next step is to ask whether differences are related to variation in other traits (Garland 1984; Garland and Else 1987). Residual measures of length, in addition to measures of segmentation (vertebral numbers), were generally highly correlated with one another after accounting for differences in size and sex. A weak, but significant interspecific relationship has been demonstrated between body size and body vertebrae in snakes (Lindell 1994). In addition, changes in the body vertebrae are rarely decoupled from associated changes in tail vertebrae (Polly et al. 2001). In our study, tail, body, and total vertebrae in addition to the vertebrae product were all positively correlated with one another, indicating that intraspecific increases or decreases in vertebral numbers in the trunk region are closely associated with increases or decreases in the tail vertebrae (or vice versa; Table 4.3).

Enzyme activities were not correlated with performance, but CS and LDH were positively correlated in ventricle and epaxial muscles. One other study explored intraspecific relationships between enzyme activities and performance in snakes, but no relationships were found (Garland et al. 1990). Ruben (1976a, 1976b) looked at aerobic and anaerobic metabolism during activity, in three species of snakes although the data set was small both in terms of numbers of species ($N = 3$) and number individuals ($N = 4$ per species). Anaerobic enzyme activity levels were highly correlated with oxygen consumption and lactate levels (though Ruben made no direct measurements of aerobic enzyme activity). A highly active species (*Coluber*) had higher levels of both

phosphofructokinase and LDH (both measures of glycolytic activity) relative to the prairie rattlesnake (*Crotalus viridis*), a less active animal, which had still higher levels over an even more sedentary snake, the rosy boa (*Lichanura*). Among snakes, aerobic and anaerobic capacities may be strongly correlated with one another (Ruben 1976b), particularly because highly active snakes, such as *Masticophis*, need both high endurance to pursue prey, and high glycolytic activity during intense activity (e.g, subduing prey and escaping from predators). Our results with corn snakes show similar patterns intraspecifically, as resting glucose and lactate were highly correlated with one another, as were muscle CS and LDH. Intraspecific differences are less likely to be explained by ecology or behavior than interspecific variation (Garland et al 1987). Thus, further investigation is needed to explain the basis of functional differences within populations of corn snakes.

The biochemical profile of a snake changes with starvation, particularly glucose (which decreases considerably) and fatty acid composition (McCue 2006, 2007). This is evident in our data. Post-endurance measures of glucose were *higher* than levels measured several weeks later during dissections. Because of the relationship of glucose levels with dietary state and body condition, we were hesitant to make further comparisons among performance measures because observed concentrations of glucose (and lactate) may not reflect physiological conditions during performance measurements. Regardless, glucose was not correlated with any measure of performance other than endurance.

Among our measures of performance, only SMR and endurance were correlated. SMR did not correlate with any other measure of performance or lower-level trait. VO_{2max} for snakes shows substantial interspecific variation (e.g., Ruben 1976b, Walton et al. 1990; Secor et al. 1992, McCue and Lillywhite 2002). Studies from lizards suggest that the relationship between VO_{2max} and other measures of performance is equivocal. Garland and Else (1987) found no relationship between VO_{2max} and endurance in *Amphibolurus* and a significant relationship in another lizard, *Ctenosaura* (Garland 1984). Garland and Bennett (1990) found a weak, but significant positive correlation between VO_{2max} and treadmill endurance in juvenile garter snakes (*Thamnophis*). We found no relationship between VO_{2max} and other character except myosin heavy chain band 1. Wilkinson et al. (1991) isolated three MHC isoforms from the intercostals muscles of *Thamnophis sirtalis* and found these to be associated with three separate fiber types. Fast-twitch fibers, which presumably would be associated with high contractile velocities and rapid fatigue, were of high molecular weight. However, our heaviest bands (MHC Band 1) were positively correlated with VO_{2max} . It is likely that fiber type distribution in snake muscles is heterogeneous and that the epaxial musculature shows a separate MHC profile from the intercostal musculature measured by Wilkinson et al. (1991). However, the relationship between VO_{2max} and MHC band 1 needs further study.

We found no correlation between hematocrit and VO_{2max} . In two studies on lizards, VO_{2max} was positively correlated with hematocrit, but not with hemoglobin or heart mass [Garland 1984; Garland and Else 1987]).

Sprint speed is the most commonly studied performance trait in snakes, in part because it is easy to measure, but also because of its assumed ecological relevance for escaping from predators (Arnold and Bennett 1988). In addition, sprint speed was correlated with one-year juvenile survivability in *Thamnophis sirtalis* (Jayne and Bennett 1990a). We found no significant relationships between sprint speed and any other measure of performance. Garland (1988) and others have suggested an unavoidable trade-off between sprint speed and endurance due to physiological and morphological considerations (e.g., muscle fiber types, separate biochemical profiles, etc.). However, the opposite trend has been observed in snakes (Ford and Shuttlesworth 1986 in juvenile *T. marcianus*; Garland 1988; Arnold and Bennett 1988; and Jayne and Bennett 1990b in *Thamnophis sirtalis*; Brodie 1989, 1992, 1993). We found no correlation between sprint speed and endurance. Our lack of a significant correlation may relate to the fact that we used adult snakes. In the study of *T. sirtalis* by Arnold and Bennett (1988), many residual correlations with sprint speed were weak, and thus comparisons in older snakes may not reveal significant differences, particularly because selection has had time to remove poor performers from the population thereby reducing variation. The few interspecific comparisons in snakes suggest that positive correlations between speed and endurance may be the rule, rather than the exception. In one of the earliest studies on locomotor performance in snakes, Mosauer (1935) found that maximum speeds, and speeds used for “prowling” were highly correlated, but his results are ambiguous because he failed to control for body size, temperature, and other critical variables. Ruben (1976a, 1976b) showed that aerobic and anaerobic capacities were correlated with one another

and with ecology among three species of snakes (highly active, moderately active, and rather sedentary) but his data set was small and he did not account for phylogenetic history.

In our study, sprint speed was significantly correlated only with numbers of body vertebrae, which are highly heritable (Dohm and Garland 1993). The functional relationship between numbers of vertebrae and locomotion are poorly understood (Jayne 1982; Arnold and Bennett 1988; Kelly et al. 1997; Moon 1999), but may involve the flexibility and stiffness of the vertebral column. Ruben (1977) suggested that increased numbers of vertebrae should be costly because they decrease locomotor speeds due to increased lateral resistance to forward undulatory movements. This hypothesis is supported by at least one other study. Jayne (1986) found a non-constricting colubrid snake, *Nerodia fasciata*, crawled faster than a constricting snake, *Elaphe obsoleta*, that had 50% more vertebrae in the body and tail (though see Garland and Adolph 1994 for caveats to two-species comparative studies). Others have pointed out that because snakes push off from static objects in the environment, performance should vary based upon the distribution of such “push-points.” Increased flexibility should be beneficial (opposite the suggestion of Ruben 1977) to lateral undulation when such push points are far apart (or non-existent) because snakes must form increasingly large bends to find static contact points (Gray and Lissman 1950; Gasc and Gans 1990; Kelly et al. 1997).

In our study, lateral undulation during tests of sprint speed was performed without static push-points, and our finding of positive correlations between numbers of body vertebrae and sprint speed is consistent with the 'push-point' hypothesis.

In contrast, Arnold and Bennett (1988) found no correlations between body and tail vertebrae and sprint speed. Using curvilinear regression, they did find a significant quadratic effect of the product of tail and body vertebrae on the regression coefficient for sprint speed suggesting that disproportionate numbers of either could reduce performance. In our study, no traits other than residual numbers of body vertebrae showed significant correlations with residual sprint speed, so only simple linear regressions were performed.

We found no relationship between the vertebrae product and sprint speed, similar to Kelly et al. (1997) who measured locomotor performance relative to the number of static push points available. Our results were opposite to the findings of Arnold and Bennett (1988). Similarly, Jayne and Bennett (1989) found size-corrected sprint performance to be related to a quadratic effect of tail length; snake with intermediate tail lengths performed best, while snakes with tails that were either too long, or too short did not perform as well (Jayne and Bennett 1989, their Figure 4). We found no effect of tail length or vertebrae on sprint speed. Of course, other variables unaccounted for in our study can affect sprint speed, such as substrates, coefficients of friction and the properties of skin (Hu et al. 2009).

Treadmill endurance is heritable (Garland 1988; Jayne and Bennett 1990b) and correlated with one-year survivability in juvenile *Thamnophis* (Jayne and Bennett 1990a). In our corn snakes, both post-endurance glucose and resting lactate were negatively correlated with endurance. This would indicate that individuals with high endurance have low post-endurance glucose, and individuals with low endurance have high resting

lactate, which might constrain prolonged aerobic activity. It has long been known that lactic acid (lactate and associated proton) does build up in isolated muscle fibers, contracted until fatigued (Hill 1932). However, more recently, studies have shown several beneficial effects of lactate during exercise in humans and rats (Brooks 1991, 2001; Nielsen et al. 2001). Thus, whether lactate is directly causative of fatigue in our snakes or simply correlates with reduced endurance, is unknown.

Garland (1990) found a weak positive correlation between-treadmill endurance and VO_{2max} in juvenile *T. sirtalis*. Measures of blood oxygen carrying capacity have generally been associated with variation in VO_{2max} but not necessarily endurance. We found no correlation of HCT with VO_{2max} , but a positive correlation with endurance. Hematocrit generally increases after training in mammals, but not in lizards (Garland et al. 1987 and references within; O'Connor et al. 2011). Since snakes are phylogenetically close to lizards, it is possible their hematocrit also does not respond to training.

All our animals, regardless of size, were tested at the same speed, and the relationship between endurance and other traits may be affected by the speed at which animals are tested. Among lizard species of equal size run at a 1 km/hr, endurance can vary by an order of magnitude (Garland 1984, 1993; Garland and Else 1987). Endurance is likely to vary greatly among species of snakes as well—although possibly not to the extent seen in lizards (Secor and Nagy 1994). Given the wide variation of endurance in our study, it is possible that some animals were run above their maximal aerobic speed, while others were tested at or below this value. Thus, the physiological response and

other factors leading to exhaustion and fatigue in these animals would be expected to differ (Garland and Else 1987).

Caveats and Conclusions

Two points of caution must be noted. First, we used wild-caught adult snakes, while previous studies used neonates or juveniles that had not experienced postnatal selection. It has been suggested that many of the correlations found in juveniles may not be apparent in a sample exposed to selection (Arnold and Bennett 1988). Further, correlation coefficients and regression slopes for allometric equations are dependent on the range of body mass observed, and non-significant correlations may be likely if the mass range of the sample is small, though in our study we had a nearly six-fold range in mass (Garland and Else 1987).

Second, the body condition of our animals changed substantially during the course of our study. It is likely that substantial biochemical changes may have taken place (McCue 2007), so caution is warranted for comparisons across the duration of our study (e.g., SMR and biochemical data).

With those caveats in mind, Can we predict whole-animal performance from sub-organismal traits in *P. guttata*? For SMR the answer is no. However, several other performance indices were predictable from suborganismal traits. For VO_{2max} , a myosin heavy chain isoform explained 35 % of the variation. Numbers of body vertebrae explained 23% of variation in sprint speed, and resting lactate and post endurance blood glucose levels explained 65% of variation in treadmill endurance.

Although studies of selection in wild snakes are exceedingly rare (Jayne and Bennett, 1990a; Brodie 1992), our measures of performance are probably ecologically relevant. Burst speed over one meter is intuitively related to escaping from predators. Endurance is also likely ecologically relevant, as corn snakes may move long distances in search of food and mates. The most common predators of corn snakes are likely birds, carnivorous mammals, and other snakes. Thus, the ability to maintain a vigorous struggle in the event of capture is likely critically important as well.

We found several sub-organismal traits that correlated with whole-animal performance in corn snakes, but given the results of similar studies, and the wide morphological, behavioral, and ecological variation among snakes (Greene 1997), it seems almost certain that predictors of performance will vary among taxa. Future studies should address form and functional variation with respect to specific hypotheses regarding types of locomotor behavior (e.g., burrowing or climbing) and habitat composition (e.g., Kelly et al. 1997). In addition, continued studies of the biology of snakes and selection in the wild may yield important information on whether certain measures of performance are more important for survival and reproduction than others.

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Table 4.1. Allometric equations with \log_{10} body mass or SVL (where indicated) for all characters measured in *Pantherophis guttata*. Equations are in the form of **character = a(body size)^b** where **a** is the intercept and **b** is the allometric scaling coefficient. Significant relationships include both slope and intercept, as well as the model R^2 and standard error of the estimate. Non-significant relationships show only univariate descriptive statistics. $CV = 2.3026 \times SD$ of residuals from allometric equations with either body mass or SVL as a covariate. This value approximates the coefficient of variation from untransformed data (see Garland 1984 and Methods for further description). These equations do not account for differences between the sexes or preservation time (see Table 4.2). Mean values and range are for untransformed values.

Table 4.1.

Character	N	Mean	Range	a \pm 95% CI	b \pm 95% CI	R ² %	SEE	CV % [*]
<i>Whole-Organism</i>								
SMR ml O ₂ /min	28	0.137	0.047—0.226	-2.066 \pm 0.535	0.530 \pm 0.241	43.9	0.114	25.7
Sprint m/sec	29	0.914	0.53—1.45	0.074 \pm 0.451				23.8
Sprint (SVL) m/sec	28	0.914	0.53—1.45	-0.087 \pm 1.656				23.0
VO _{2max} ml O ₂ /min	24	2.153	0.82—3.79	-1.573 \pm 0.408	0.842 \pm 0.183	80.5	0.085	19.2
VCO _{2max} ml CO ₂ /min	24	2.117	0.67—3.91	-1.653 \pm 0.600	0.870 \pm 0.269	67.1	0.125	28.2
Endurance minutes	23	44.265	7.45—120.02	-0.458 \pm 0.691	0.908 \pm 0.732	24.0	0.304	68.4
Endurance (SVL) minutes	23	44.265	7.45—120.02	-4.542 \pm 6.319	2.068 \pm 2.153	16.0	0.320	72.0
<i>Morphology</i>								
Ventricle Mass g	21	0.273	0.07—0.53	-2.633 \pm 0.24	0.930 \pm 0.109	94.3	0.044	9.9
Liver Mass g	21	2.184	0.90—4.03	-1.343 \pm 0.384	0.757 \pm 0.175	80.2	0.071	15.9
Snout-Vent Length mm	28	853.89	633—1168	2.391 \pm 0.132	0.251 \pm 0.062	71.8	0.034	7.7
Total Length mm	26	980.73	722—1247	2.488 \pm 0.101	0.235 \pm 0.051	78.2	0.027	6.1
Tail Length (SVL) mm	26	132.42	89—173	-0.639 \pm 1.065	0.942 \pm 0.364	54.3	0.051	11.5
Body Vertebrae (SVL)	28	224.32	201—243	2.174 \pm 0.343				4.3
Total Vertebrae (SVL)	26	277.23	245—298	2.089 \pm 0.400				4.3
Tail Vertebrae (SVL)	26	53.23	38—64	0.565 \pm 1.193				12.9
<i>Biochemistry</i>								
Resting Lactate mmol/L	21	1.637	0.7—2.9	0.426 \pm 1.001				41.4
Resting Glucose mmol/L	21	23.48	11—41	2.102 \pm 1.041				43.0
HCT %	20	20.75	11—28	1.007 \pm 0.678				26.2
Muscle LDH V _{max}	19	33.619	2.46—68.16	1.660 \pm 1.37				54.0
Muscle CS V _{max}	20	55.052	16.95—93.61	2.019 \pm 1.200				44.4
Liver LDH V _{max}	21	41.613	16.55—98.26	2.000 \pm 1.054				43.6
Liver CS V _{max}	21	53.765	20.43—106.16	1.971 \pm 0.938				38.8
Ventricle LDH V _{max}	20	67.048	36.60—108.83	2.158 \pm 0.675				27.7
Ventricle CS V _{max}	20	82.391	44.75—110.88	1.954 \pm 0.650				26.7
Myosin Heavy Chain Band I	17	76.393	56.38—85.97	1.945 \pm 0.271				9.9

Table 4.2. Sexual variation in whole-organism performance and sub-organismal traits in *Pantherophis guttata*. Only those traits with significant differences between the sexes are shown. Asterisks (*) indicate non-significant independent variables in ANCOVAs.

Resting lactate shows no significant differences between sexes, but a significant effect of bleed delay time (how long after euthanasia the sample was taken). Values were log transformed only when doing so improved linearity of the bivariate relationship and/or normality of residuals (see Methods). Note: $\alpha = 2.3026 \times \text{SD of the residuals from allometric } (\log_{10}\text{-}\log_{10}) \text{ versions of the below equations (see Methods).}$

Table 4.2.

Variable (N)	=	Intercept	Predictors and Covariates	r^2	CV% ^a
Whole-Organism					
Sprint Speed (29)	=	0.880	- 0.001 (body mass)* + 0.235 (if male)	0.321	20.2
Sprint Speed (29)	=	0.783	-7.0 x 10 ⁻⁶ (SVL)* + 0.215 (if male)	0.263	19.8
Log ₁₀ Endurance (23)	=	0.972	+ 0.002 (body mass) + 0.293 (if male)	0.398	60.4
Log ₁₀ Endurance (23)	=	0.336	+ 0.001 (SVL) + 0.348 (if male)	0.392	60.2
Morphology					
Log ₁₀ Snout-Vent Length (28)	=	2.398	+ 0.255 (log ₁₀ body mass) - 0.026 (if male)	0.771	7.1
Tail Length (26)	=	-12.112	+ 0.156 (SVL) + 20.481 (if male)	0.734	8.8
Body Vertebrae (28)	=	223.248	+ 0.009 (SVL)* - 11.726 (if male)	0.397	3.4
Tail Vertebrae (26)	=	25.796	+ 0.028 (SVL) + 6.521 (if male)	0.343	11.5
Biochemistry					
Resting Lactate (21)	=	4.69 x 10 ⁻⁴	+ 0.025 (body mass)* + 0.144 (if male)* + 2.033 (bleed delay)	0.271	35.8
Liver LDH V _{max} (21)	=	61.740	- 13.626 (body mass)* + 16.946 (if male)	0.210	38.2

Note: as indicated in the table, in most cases, SVL and body mass were not log-transformed.

Table 4.3. Pearson product-moment correlation coefficients (r) of residual trait values obtained from multiple regressions on either body mass or SVL with sex and preservation time (for resting lactate and glucose). Values in bold indicate two-tailed significance ($P < 0.05$).

Table 4.3.

[illegible]

Table 4.3 Continued.

	SMR	VO _{2max}	Sprint Speed	log ₁₀ Endurance	log ₁₀ Vent. Mass	log ₁₀ Liver Mass	log ₁₀ SVL	log ₁₀ Total Length	log ₁₀ Tail Length	Tail Vertebrae	Body Vertebrae	Total Vertebrae	Vertebrae product
Resting Lactate	0.070	0.380	0.101	-0.546	0.295	-0.138	0.078	-0.125	-0.098	-0.268	0.010	-0.089	-0.183
Resting Glucose	0.250	0.103	0.070	-0.605	0.148	0.007	0.215	-0.142	-0.164	-0.303	0.002	-0.123	-0.232
Hematocrit	-0.281	0.205	0.002	0.479	0.104	0.009	-0.492	-0.365	0.293	-0.075	-0.270	-0.234	-0.164
MHC Band 1	0.303	0.586	-0.010	-0.028	-0.443	-0.380	0.309	0.249	-0.122	0.070	0.024	0.050	0.040
Ventricle CS	0.316	-0.123	-0.383	-0.040	-0.443	-0.142	0.154	-0.033	-0.073	0.187	0.037	0.130	0.137
Ventricle LDH	-0.030	0.138	-0.060	0.270	-0.378	-0.251	0.173	0.041	0.158	0.333	0.189	0.254	0.289
Liver CS	0.023	-0.190	-0.387	0.134	0.415	0.207	-0.174	0.128	0.036	-0.108	-0.281	-0.220	-0.155
Liver LDH	-0.359	0.133	0.281	0.220	0.143	-0.307	-0.393	-0.384	0.007	-0.256	-0.291	-0.304	-0.293
Muscle CS	-0.039	0.102	0.026	0.020	-0.125	0.195	-0.498	-0.241	0.442	0.196	0.100	0.172	0.182
Muscle LDH	0.133	0.208	0.088	-0.059	-0.185	-0.141	-0.199	-0.005	0.120	0.120	0.001	0.048	0.089

[illegible]

Table 4.4. Pearson product-moment correlation coefficients among residual enzyme activities. Values above the diagonal are correlations among residual enzyme values, while those below the diagonal are two-tailed *p* values and sample size, respectively. Correlations significant at the 0.05 level are in bold.

	Liver		Ventricle		Muscle	
	CS	LDH	CS	LDH	CS	LDH
Liver CS		0.297	0.170	-0.088	0.054	-0.072
Liver LDH	0.192 21		-0.264	-0.286	0.011	-0.240
Ventricle CS	0.472 20	0.260 20		0.519	0.110	0.220
Ventricle LDH	0.713 20	0.221 20	0.019 20		0.008	0.340
Muscle CS	0.823 20	0.965 20	0.653 19	0.975 19		0.737
Muscle LDH	0.769 19	0.322 19	0.380 18	0.167 18	<<0.001 19	

Table 4.5. Regression analysis predicting maximum sprint speed and maximum oxygen consumption from sub-organismal traits showing significant correlations with residual sprint speed (top [from Table 4.3]) and a backwards stepwise multiple regression predicting residual \log_{10} treadmill endurance from residual sub-organismal traits (bottom). Values based on residuals from allometric equations regressing characters on body mass (or SVL), sex, and in some instances bleed delay time (see Methods).

Sprint Speed					
Variable	Unstandardized Coefficient	Std. Error	Standardized Coefficient	t	P
Y-Intercept	-0.009	0.030		-0.296	0.770
Body Vertebrae	0.011	0.004	0.482	2.803	0.009
Model df = 1, 26	$r^2 = 0.232$	$F = 7.859$	$p = 0.009$		
Log₁₀ Treadmill Endurance					
Variable	Unstandardized Coefficient	Std. Error	Standardized Coefficient	t	P
Y-Intercept	-0.022	0.043			
Post End. Glucose	-0.018	0.005	-0.619	-3.903	0.002
Resting Lactate	-0.300	0.077	-0.620	-3.895	0.002
Model df = 2, 14	$r^2 = 0.654$	$F = 13.253$	$p = 0.001$		

VO _{2max}					
Variable	Unstandardized Coefficient	Std. Error	Standardized Coefficient	t	P
Y-Intercept	0.059	0.092		0.642	0.532
MHC Band 1	0.035	0.013	0.586	2.606	0.022
Model df = 1, 13	$r^2 = 0.343$	$F = 6.793$	$p = 0.022$		

Table 4.5 Continued

Figure 4.1. Timeline and ordering of experiments.

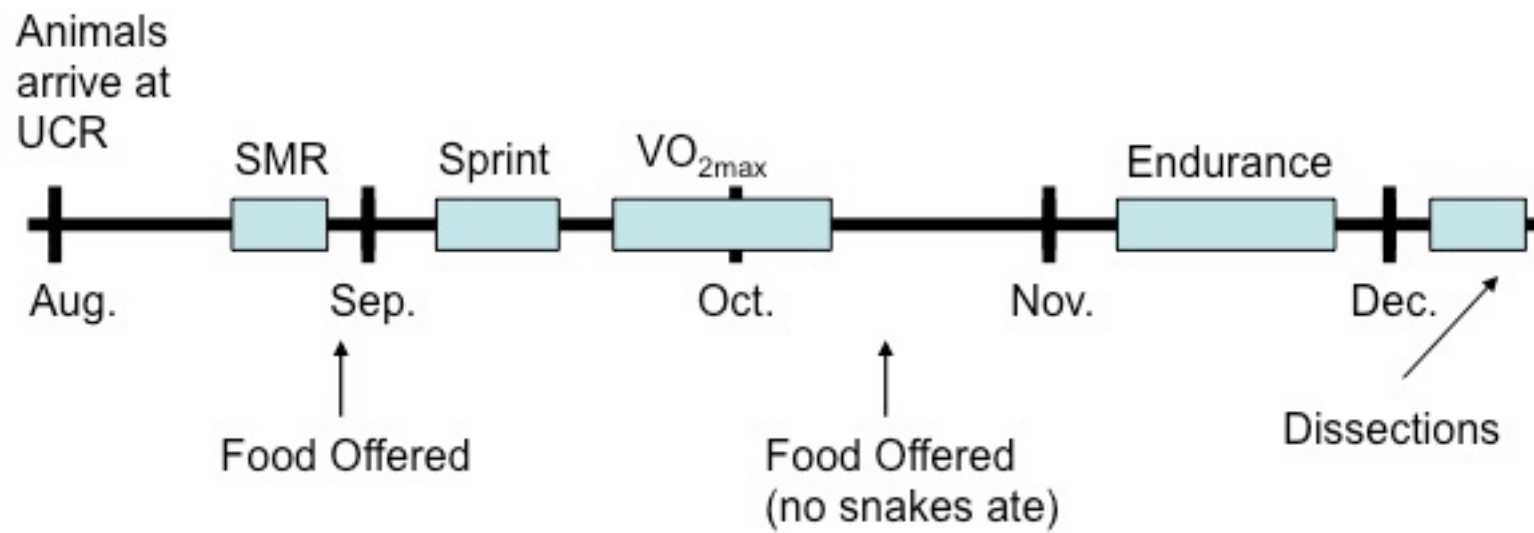


Figure 4.2. Direction of flow and arrangement for metabolic measurements for (A) SMR and (B) maximum oxygen consumption.

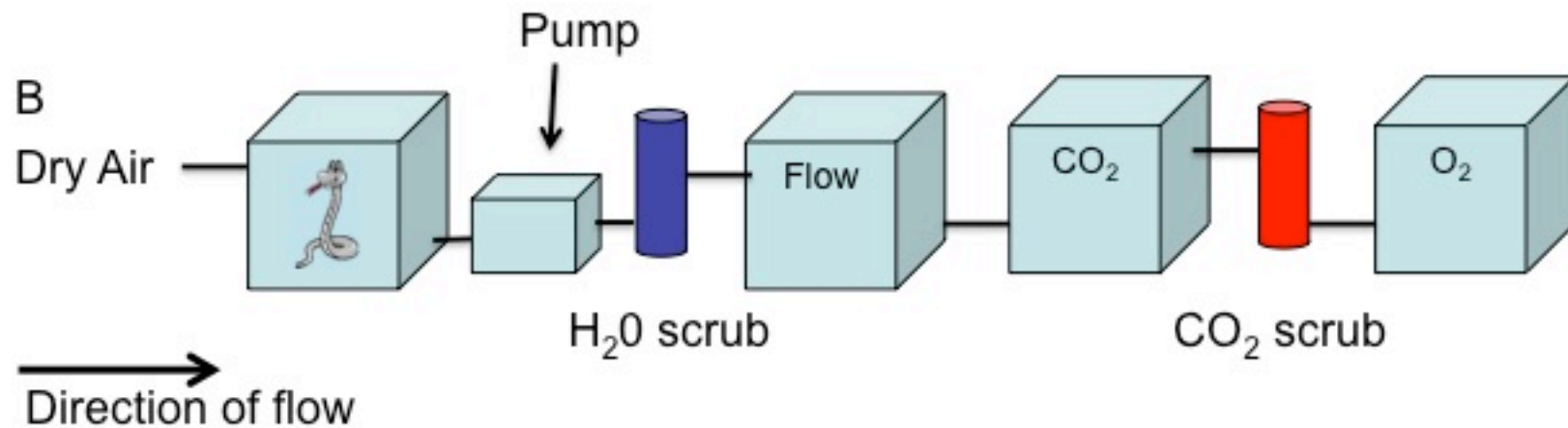
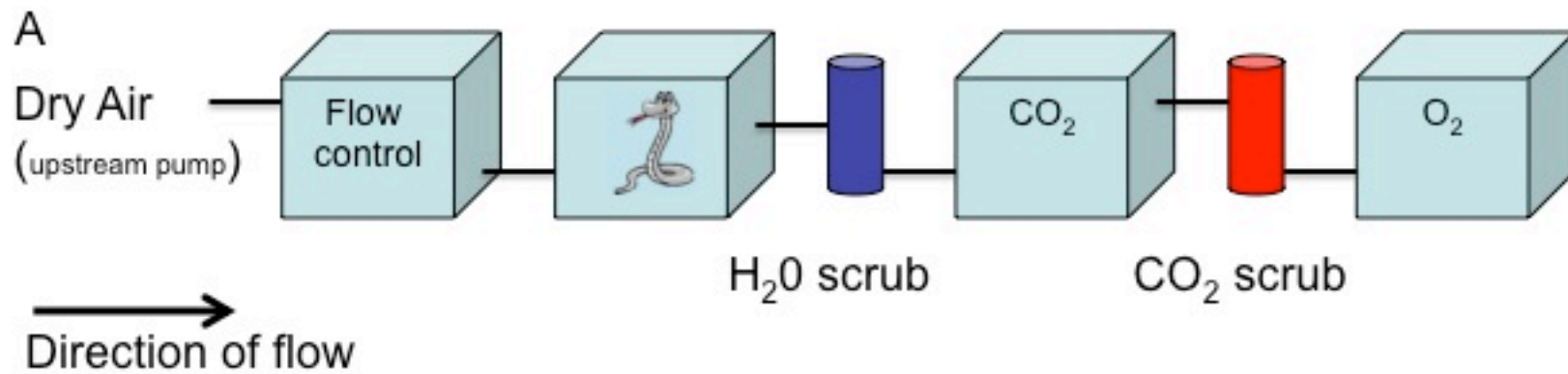


Figure 4.3. Photocell-lined racetrack used for sprint speed measurements.



Figure 4.4. Experimental set-up used for maximum oxygen consumption (A) and for treadmill endurance (B).



Figure 4.5. Hi resolution (300 DPI) JPEG images of an inverted myosin heavy chain gel. Proteins are separated by molecular weight with heavier proteins at the top of the image. These images were inverted and converted to 16 bit gray-scale using the program PhotoImpact Pro (Nova Development). The darker band along the top is MHC band 1 used in the analysis, the smaller band immediately below MHC band 1 is MHC band 2.

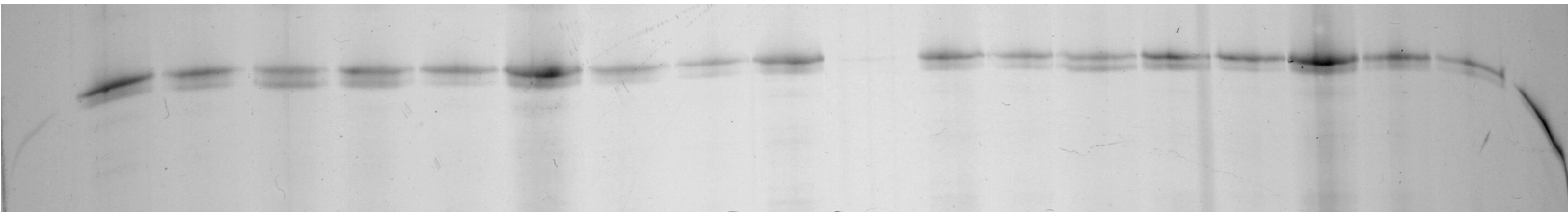


Figure 4.6. Repeatability between 1st and 2nd trials for (a) VO₂max and (b) Treadmill

Endurance

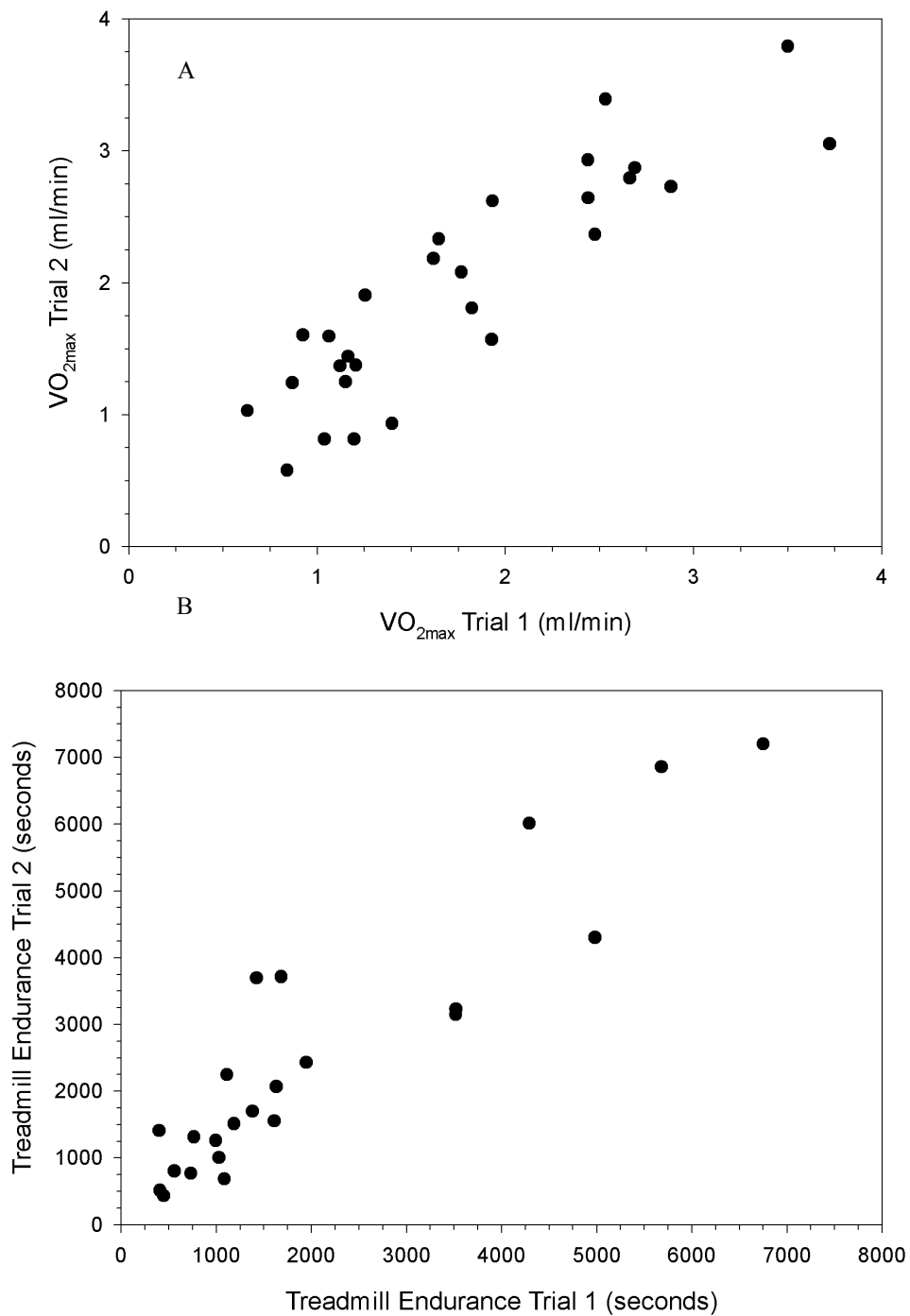


Figure 4.7. Allometric relationships between metabolism (SMR), performance traits, and sub-organismal traits with body mass or snout-vent length. Plots of raw data are on the left and \log_{10} transformed data are to the right. Filled circles represent females, while open circles represent males. Allometric scaling coefficients for statistically significant relationships can be found in Table 4.1.

Figure 4.7.

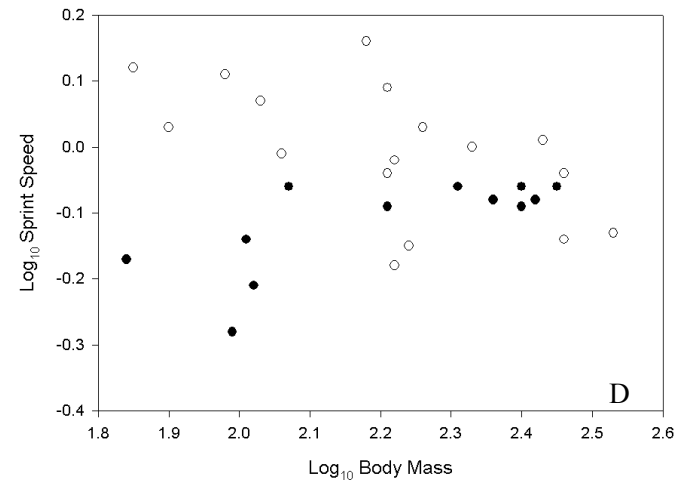
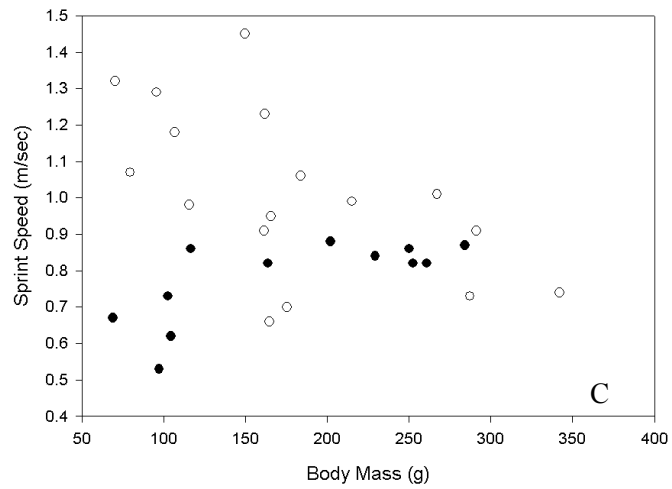
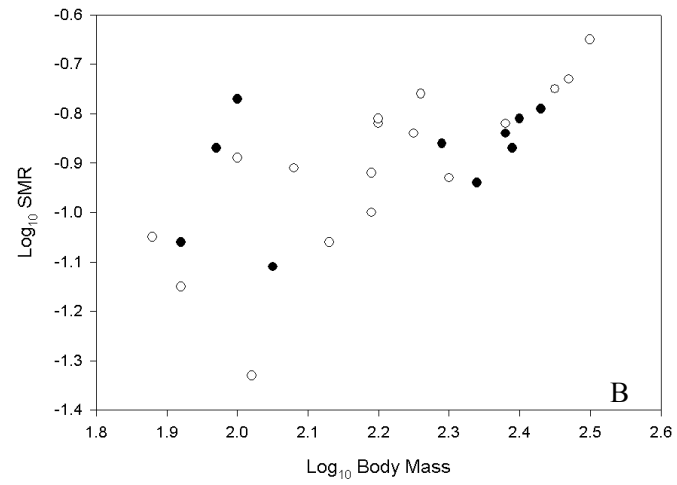
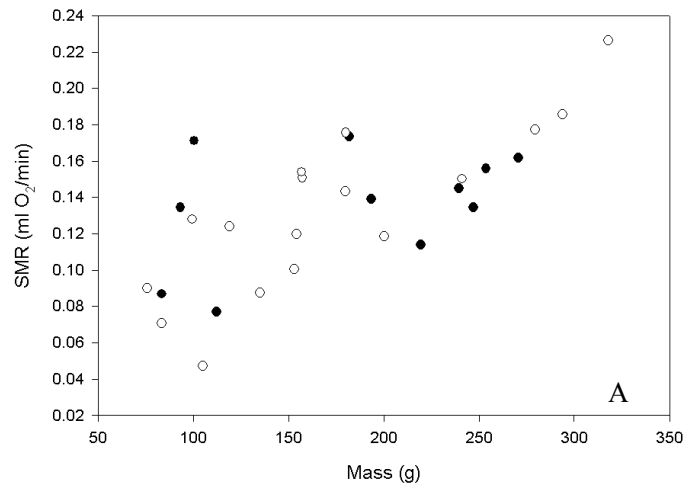


Figure 4.7 Continued.

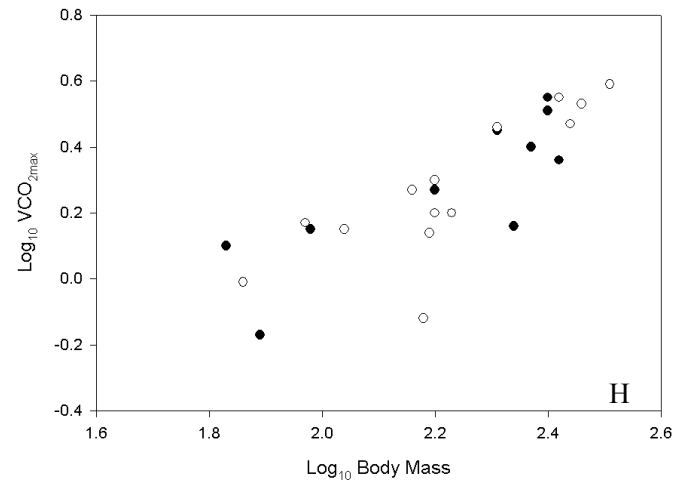
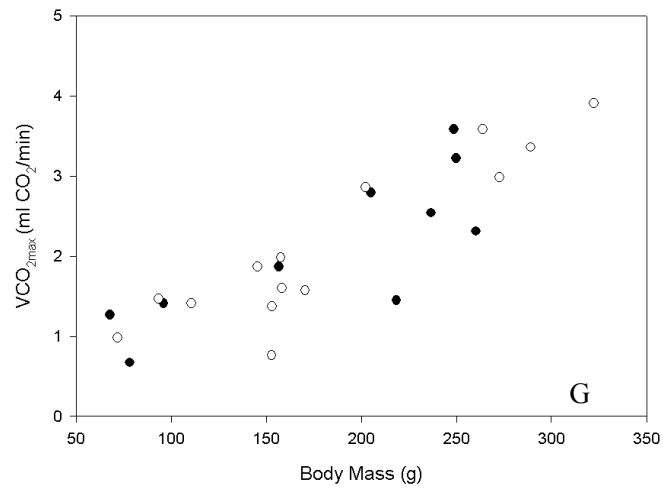
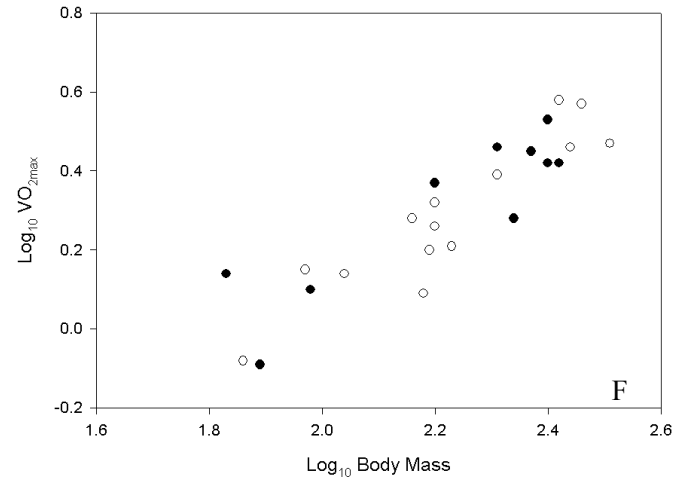
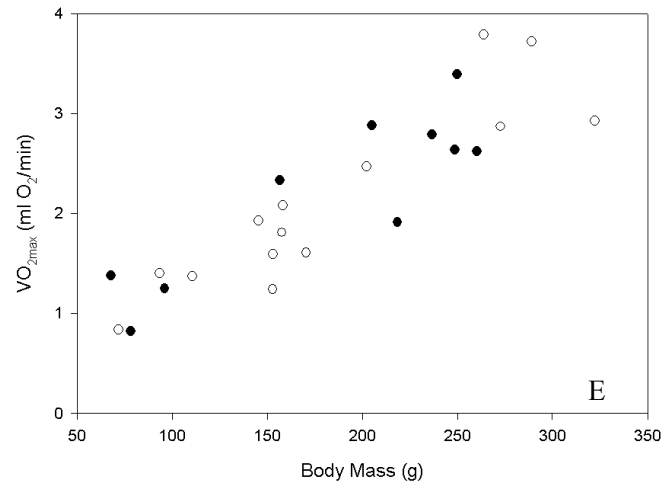


Figure 4.7 Continued

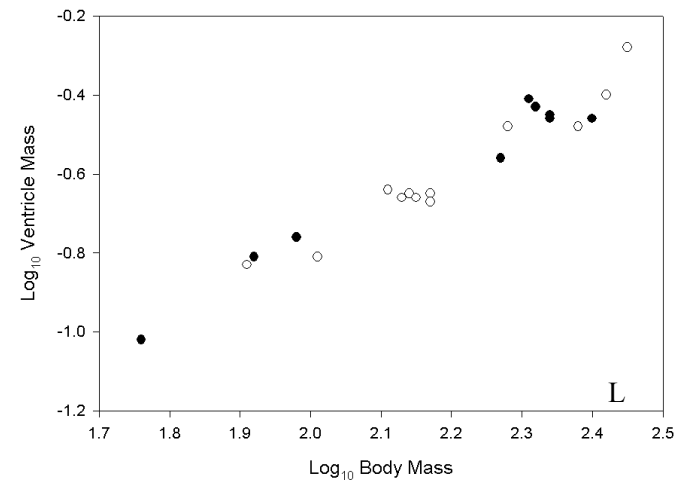
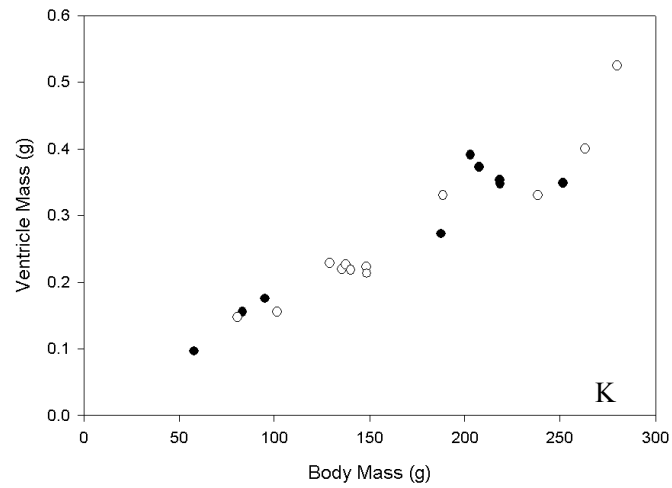
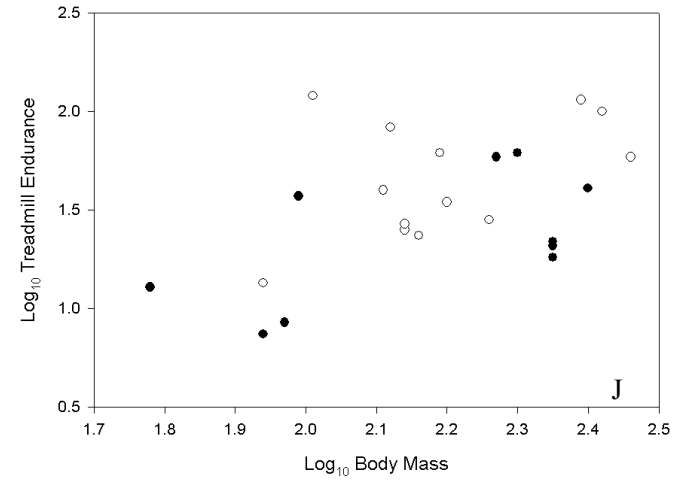
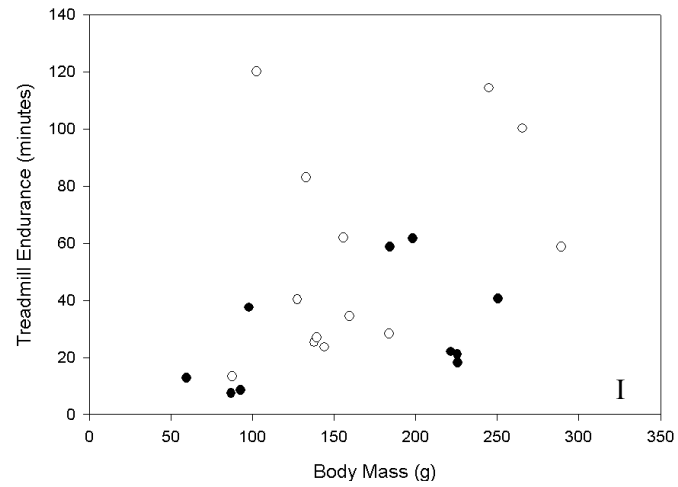


Figure 4.7 Continued

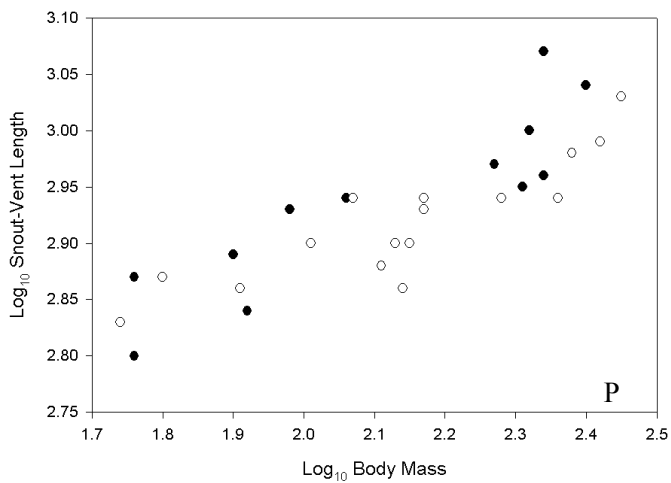
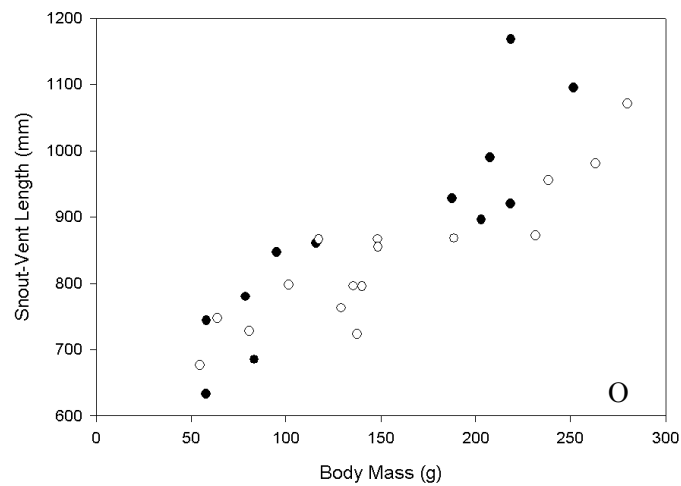
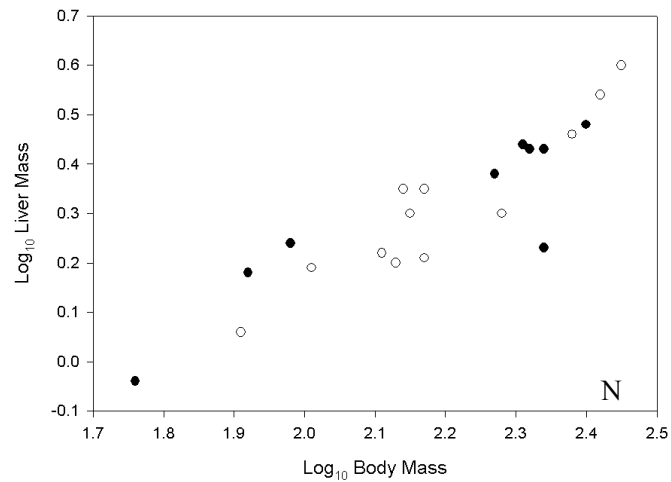
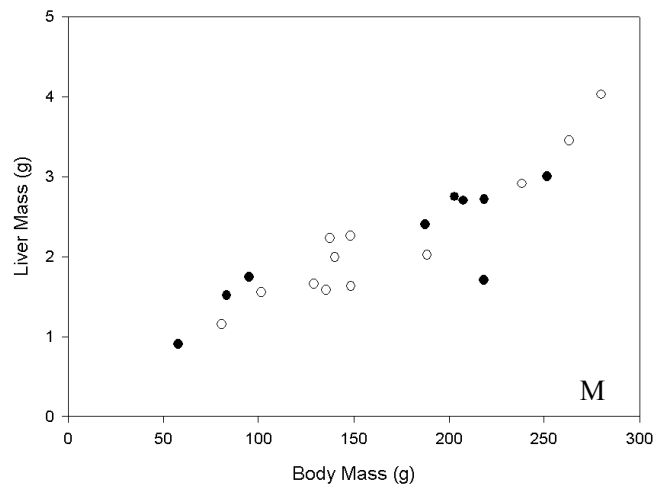


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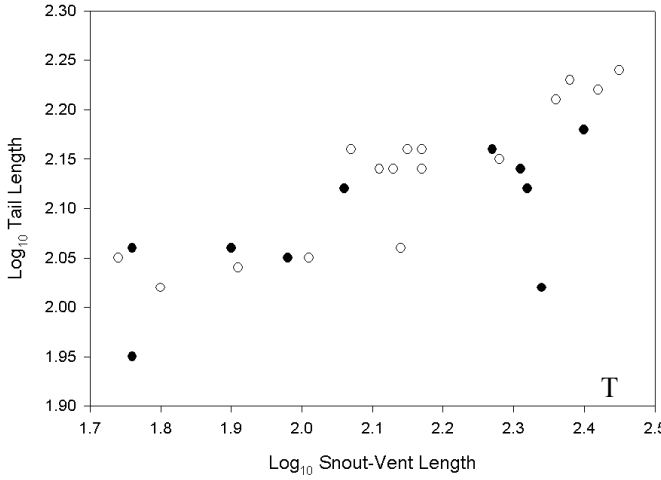
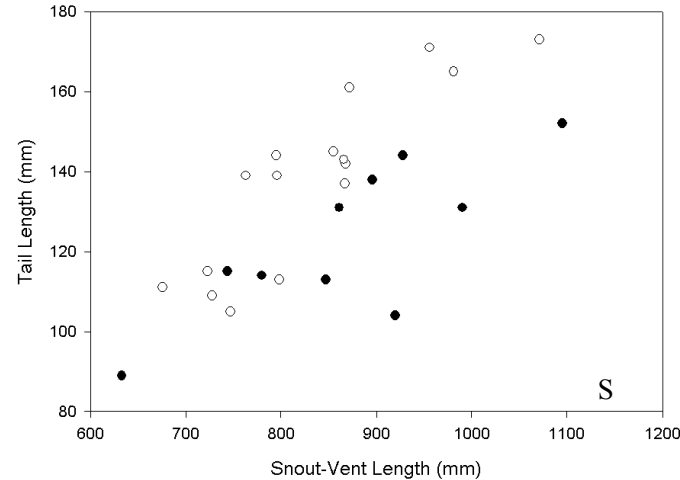
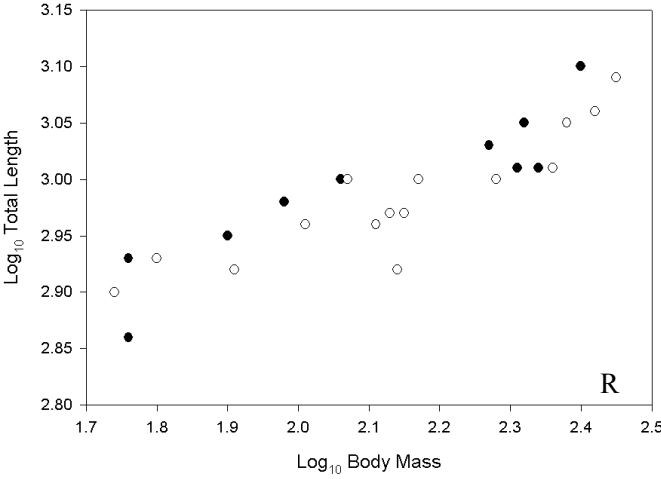
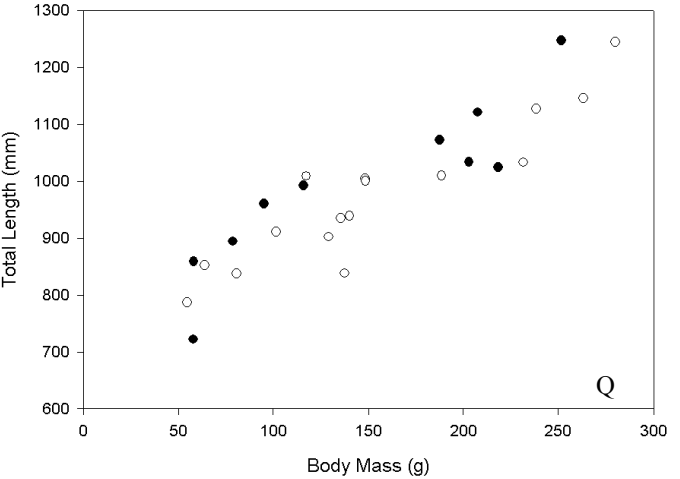


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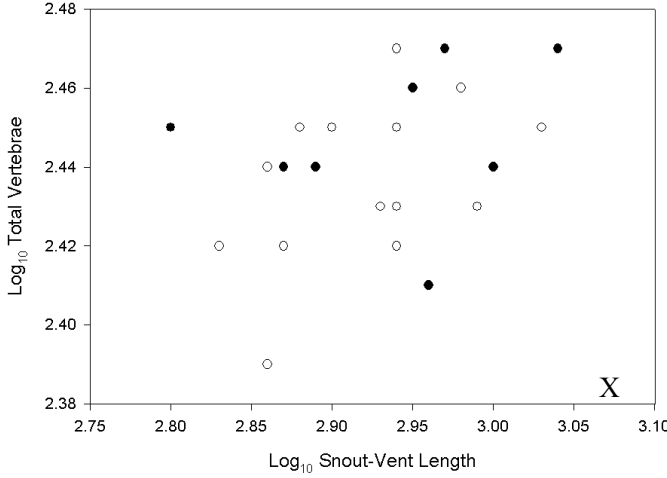
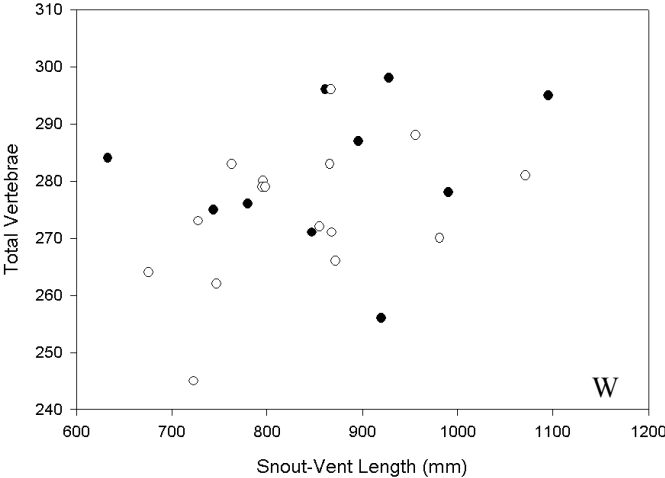
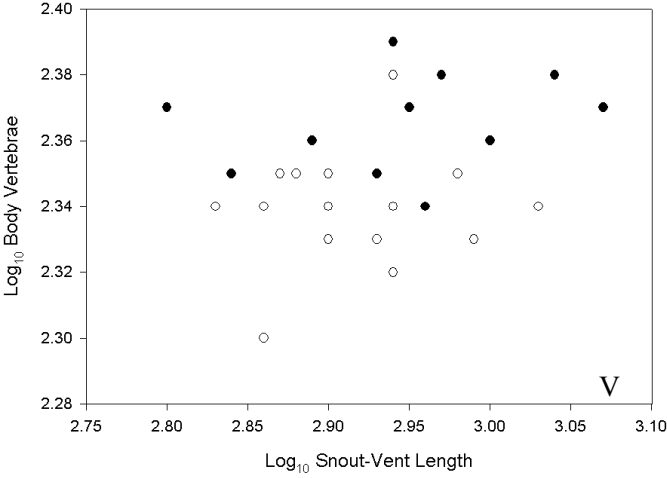
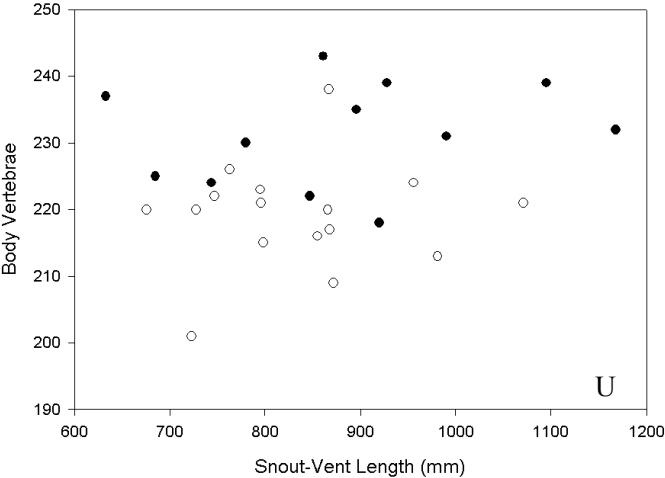


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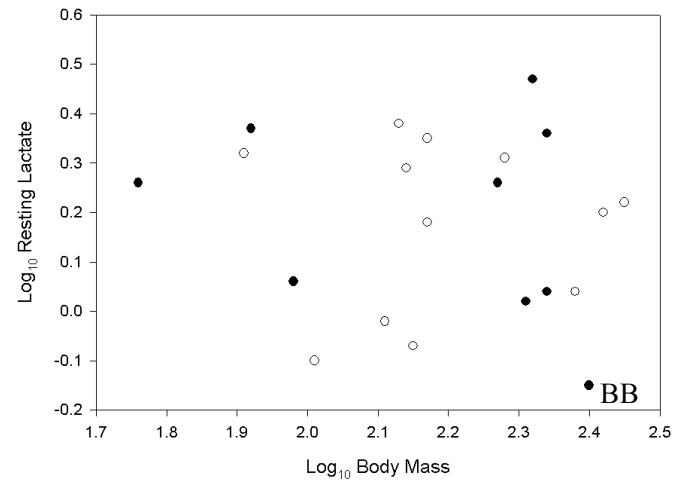
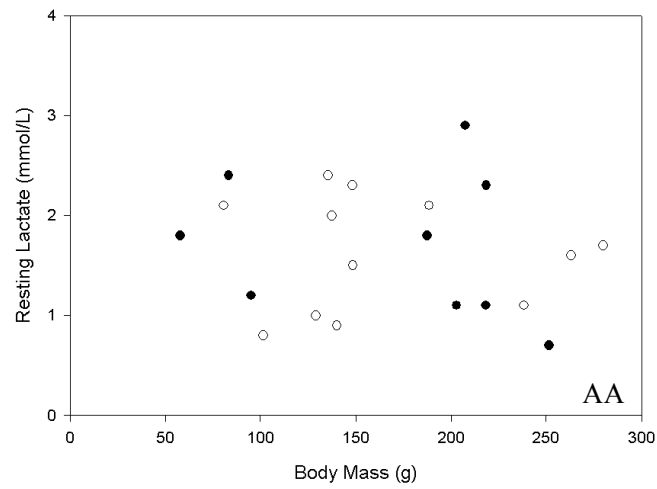
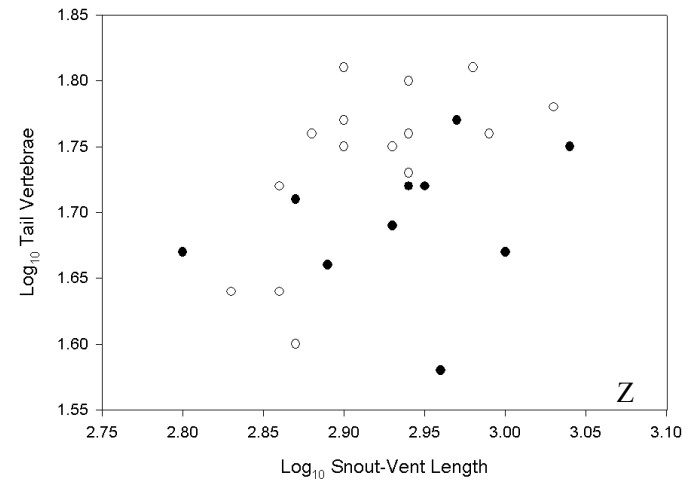
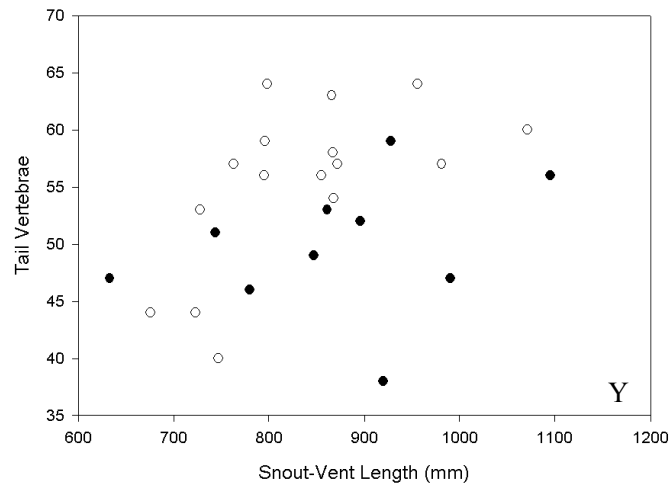


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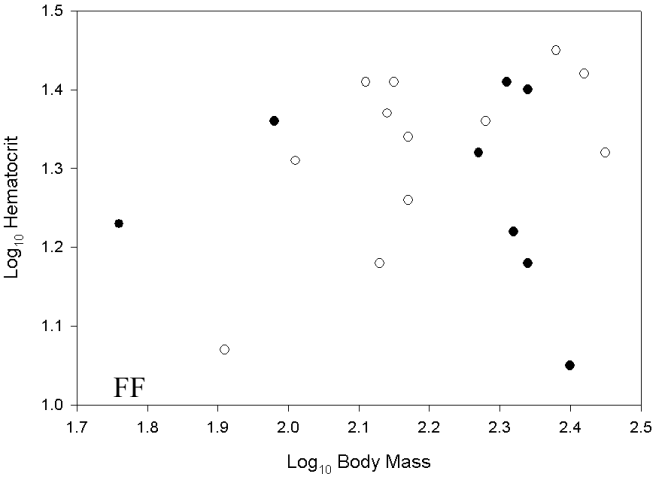
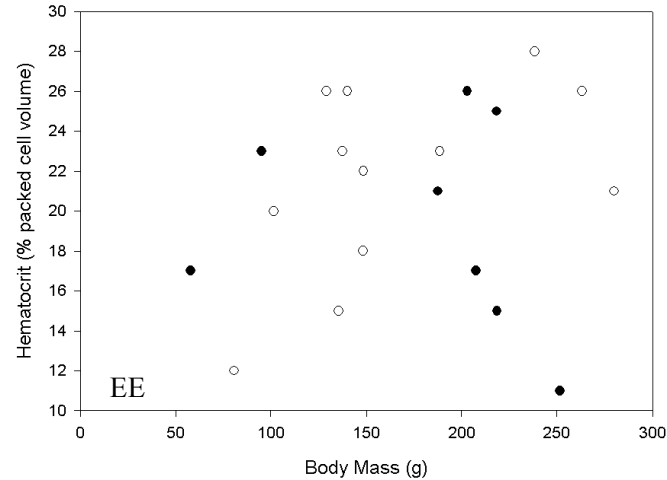
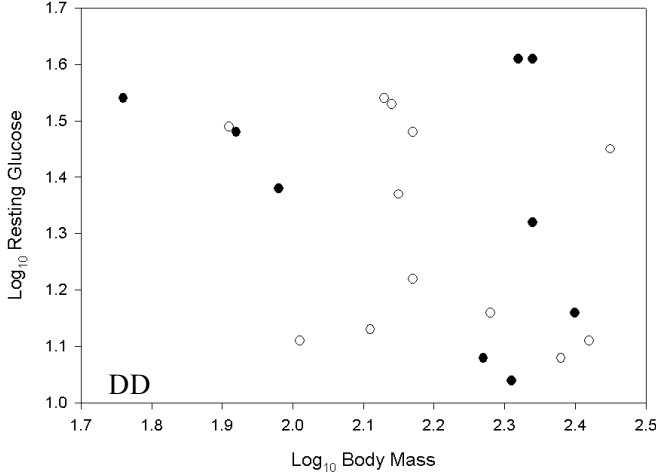
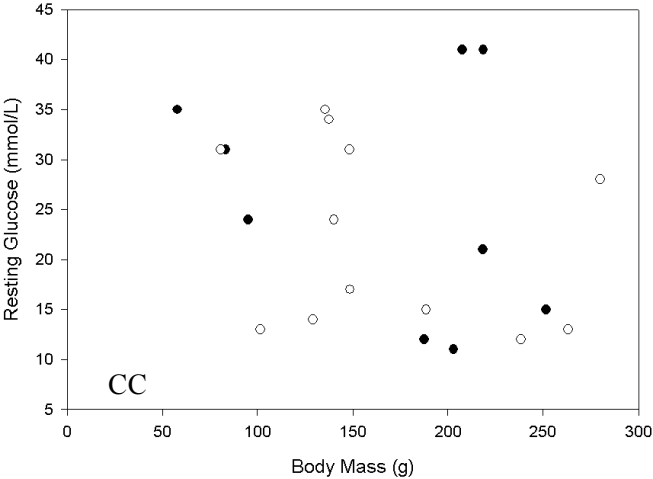


Figure 4.7 Continued

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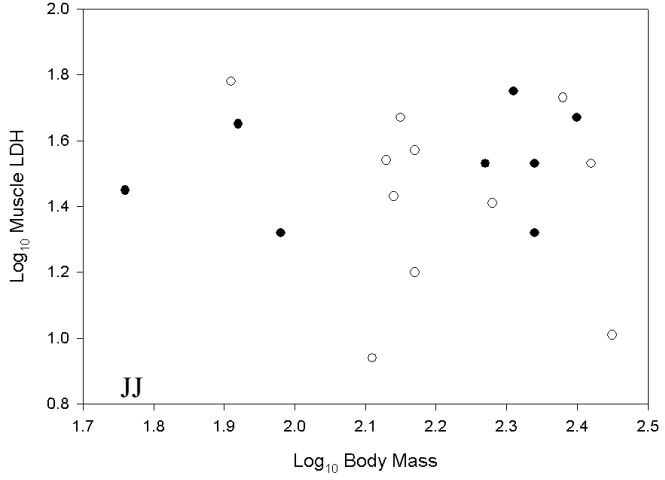
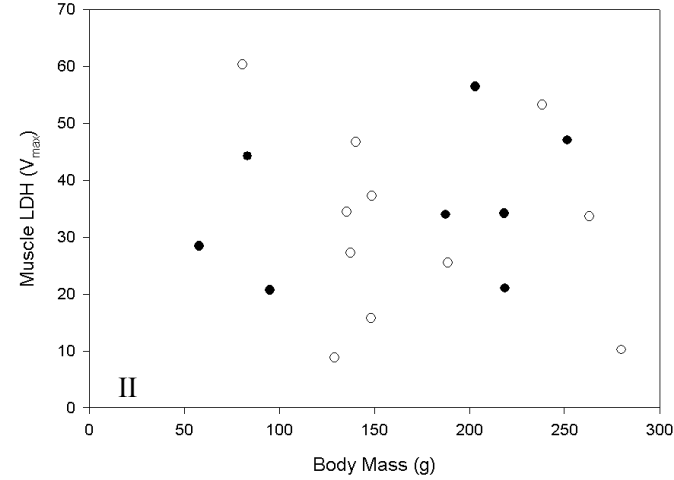
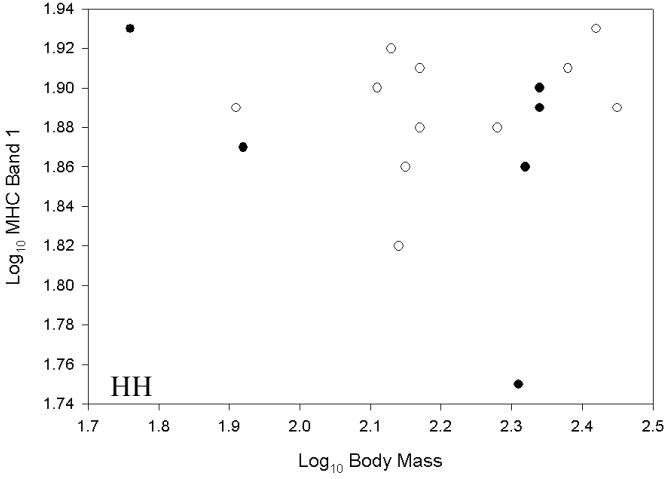
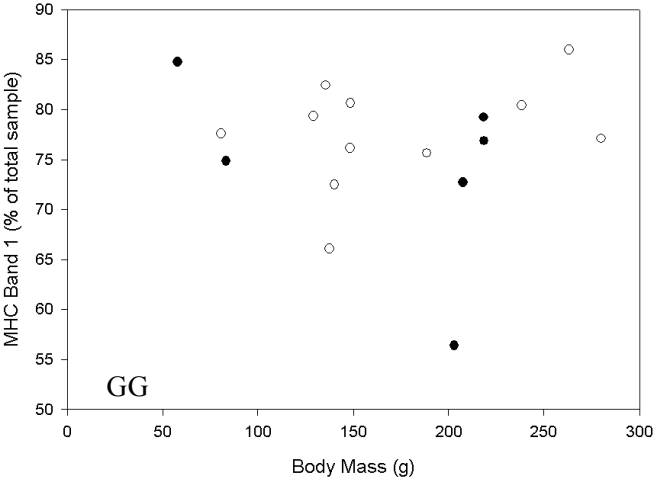


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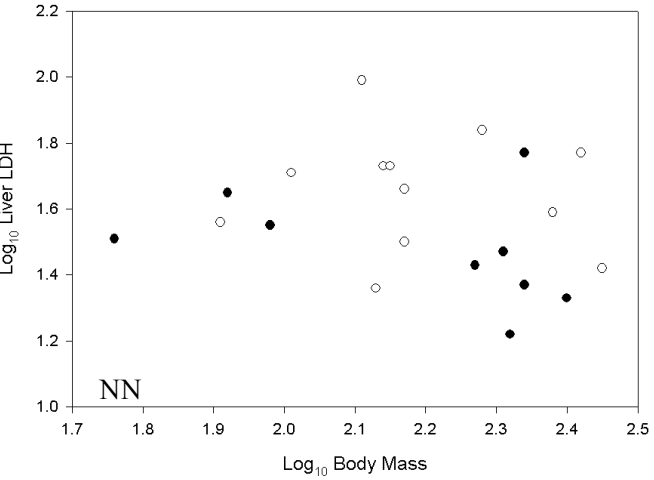
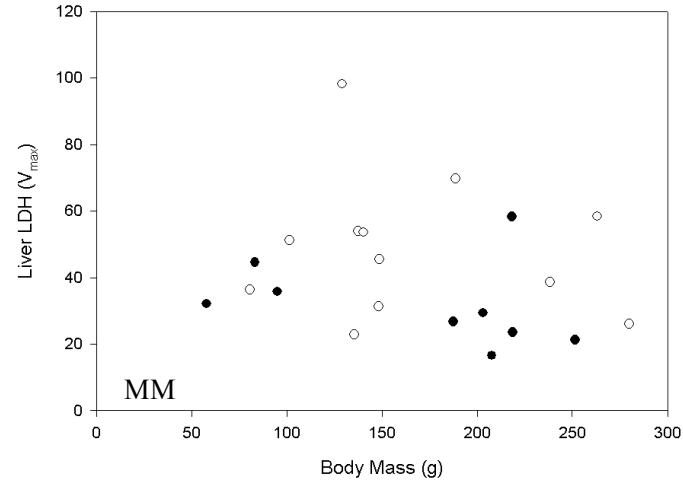
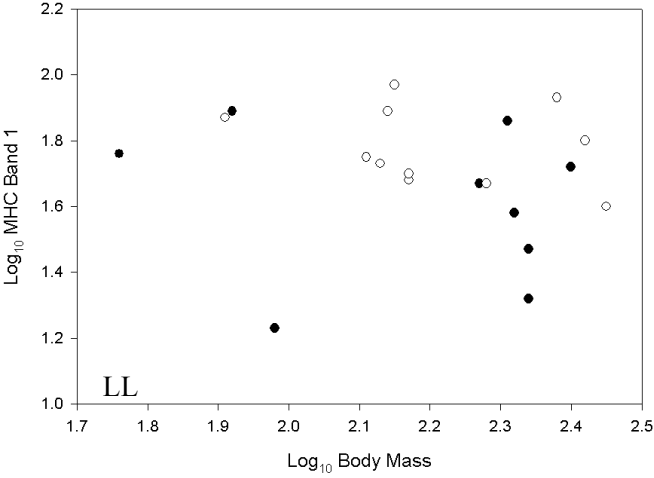
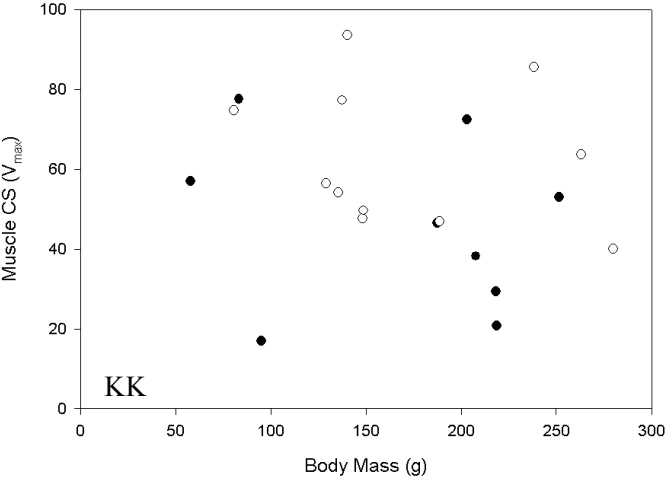


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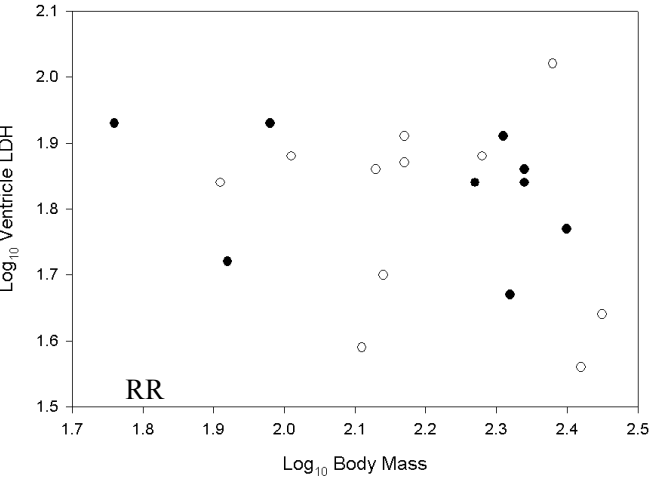
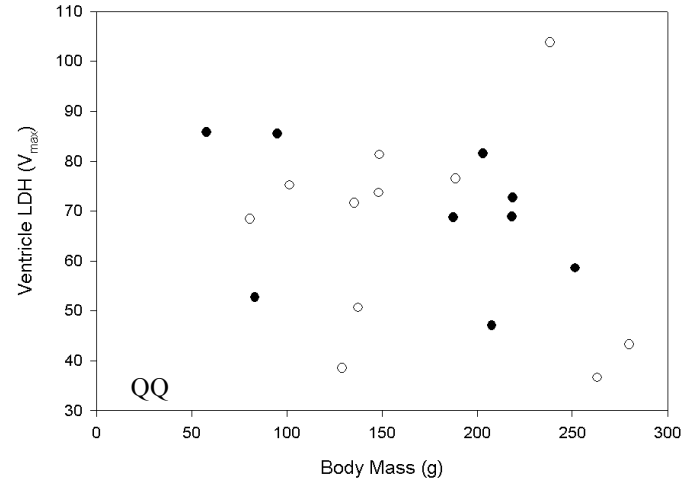
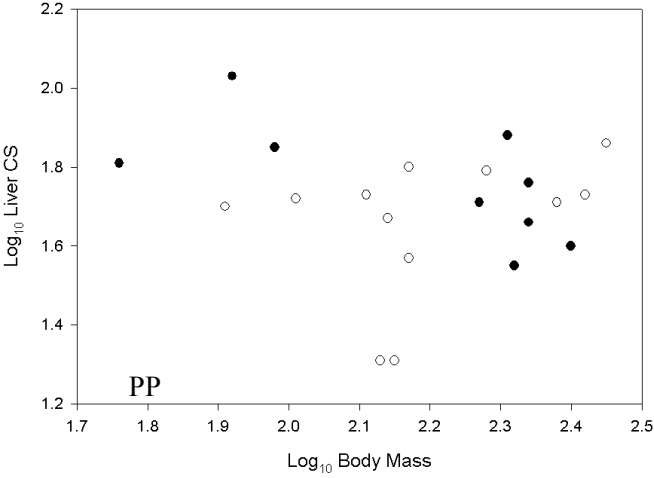
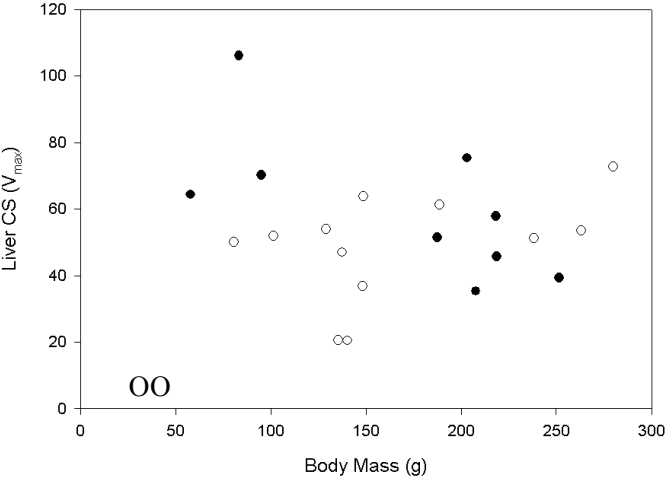


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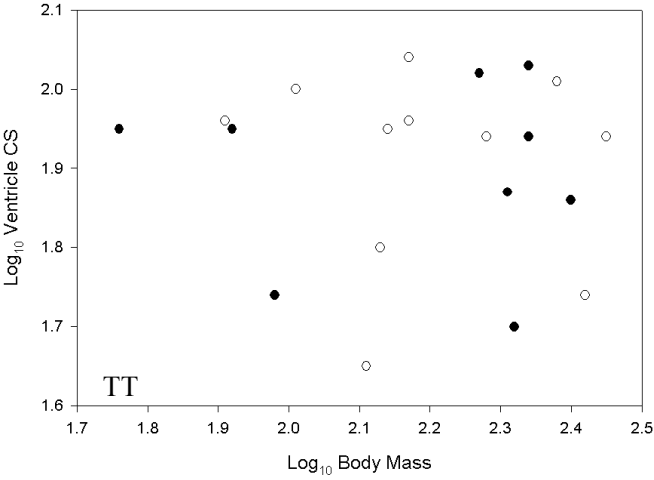
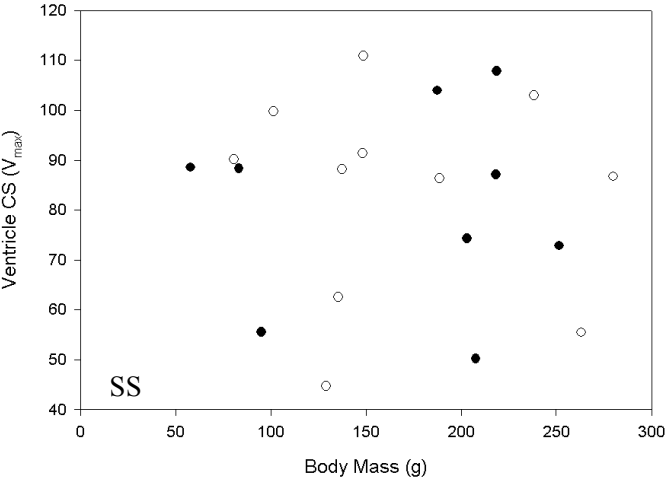


Figure 4.8. Graphs of residual whole-organism performance measures with residual sub-organismal characters. Residuals are from multiple regressions predicting whole-organism performance from body mass (or SVL), sex, and preservation time (for resting lactate and glucose [see Methods and Table 4.2]). Only statistically significant (2-tailed $P < 0.05$) correlations between measures of locomotor performance and other characters are shown; non-significant correlations (from Table 4.3) are not shown. No sub-organismal traits showed significant correlations with $VO_{2\max}$.

Figure 4.8.

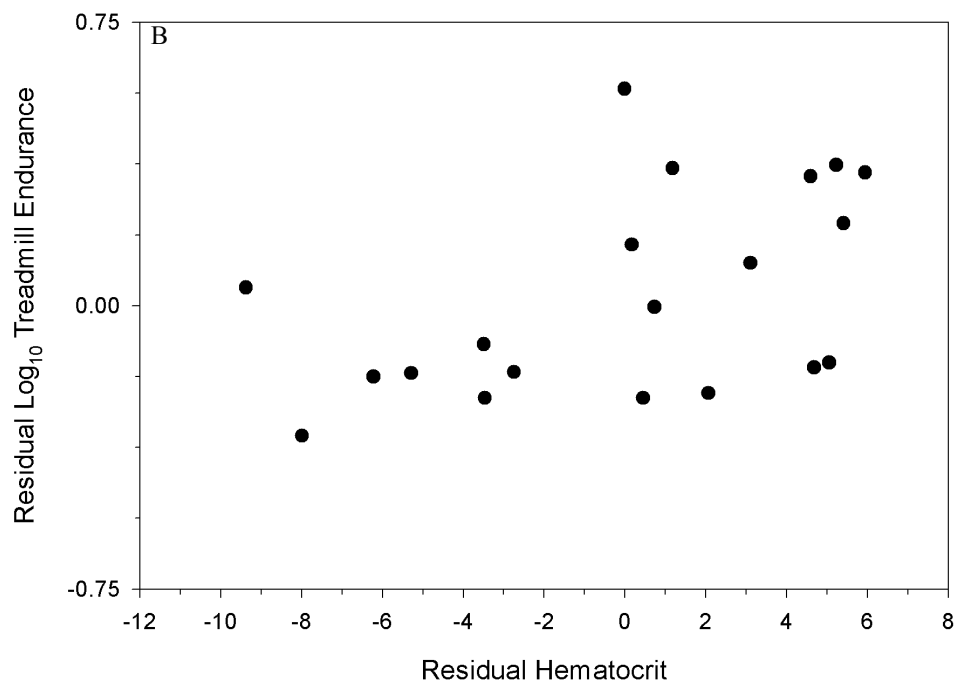
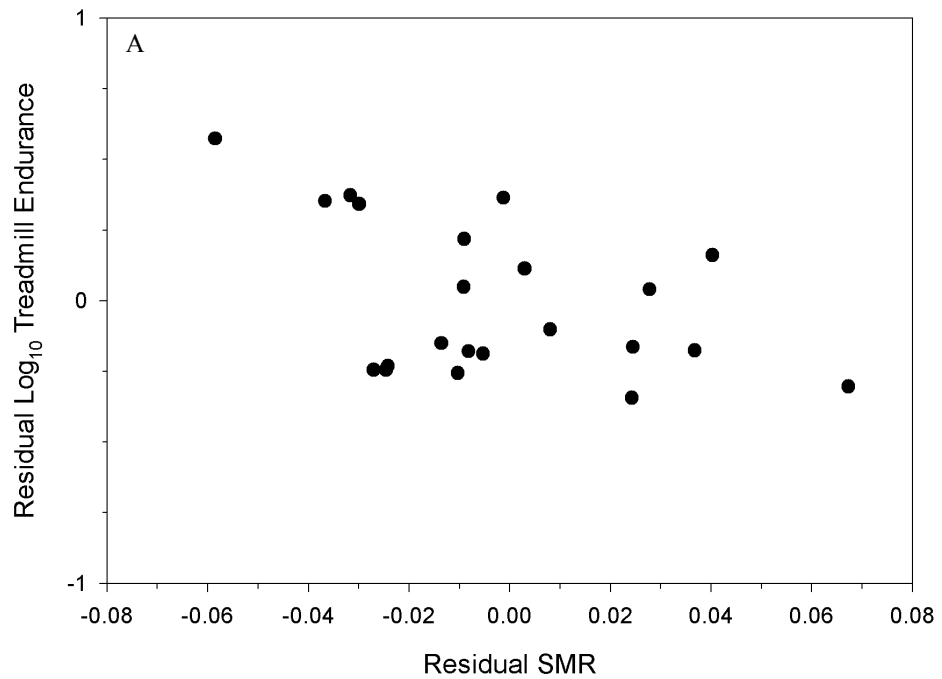


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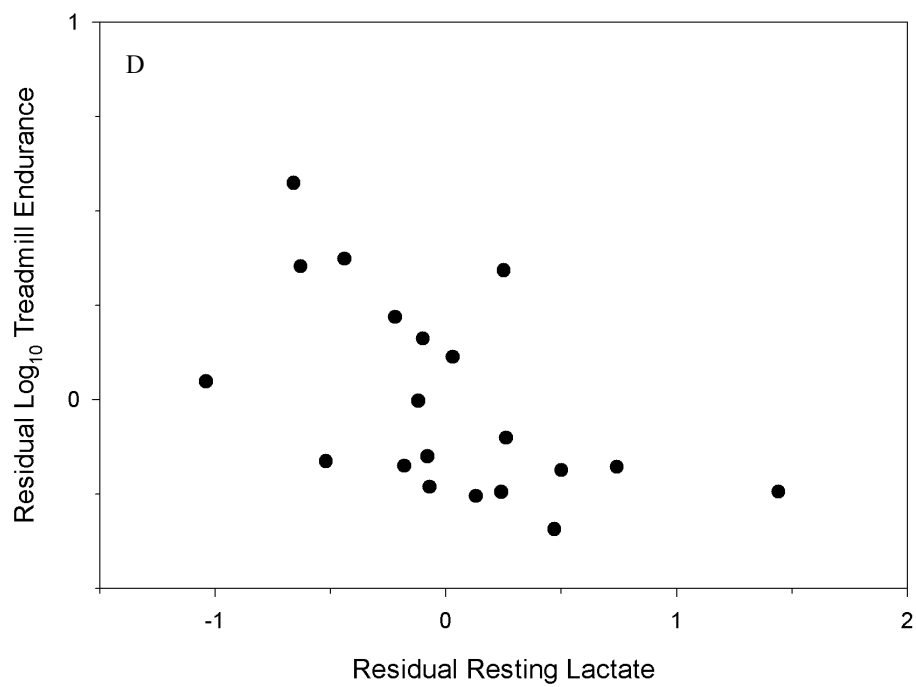
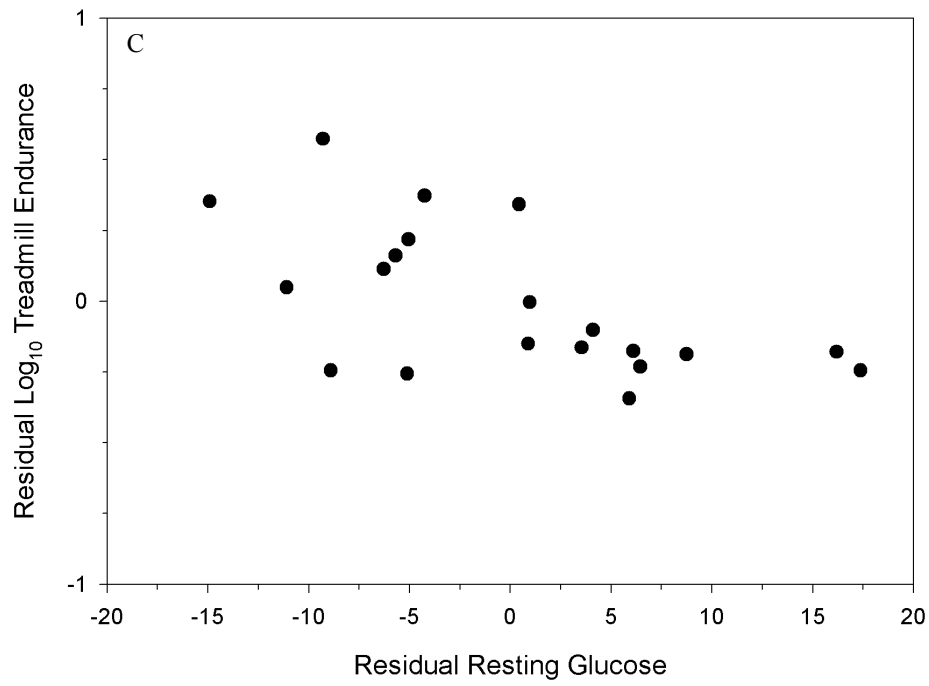


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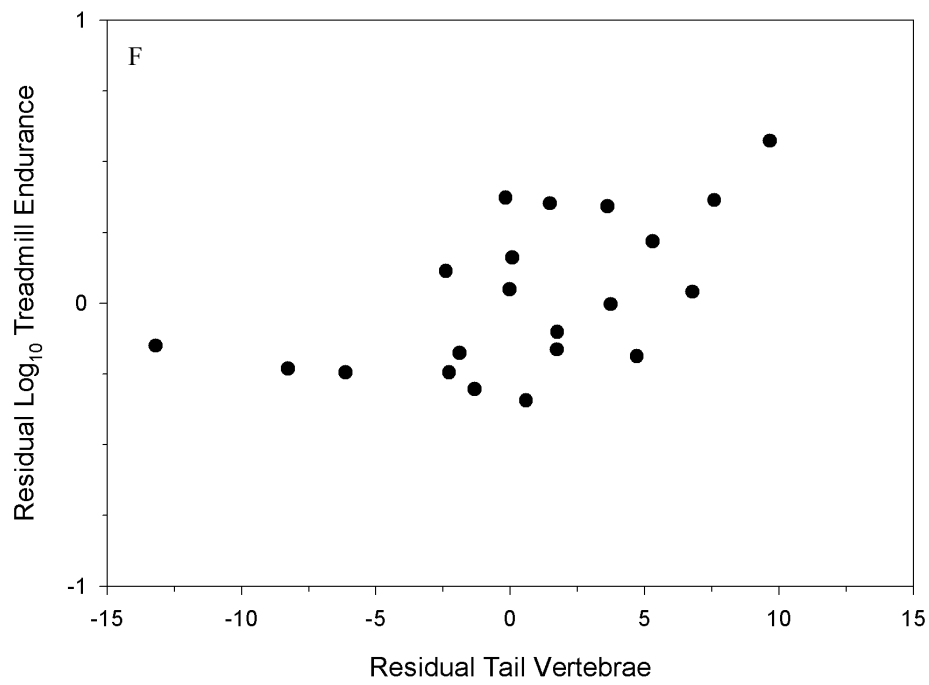
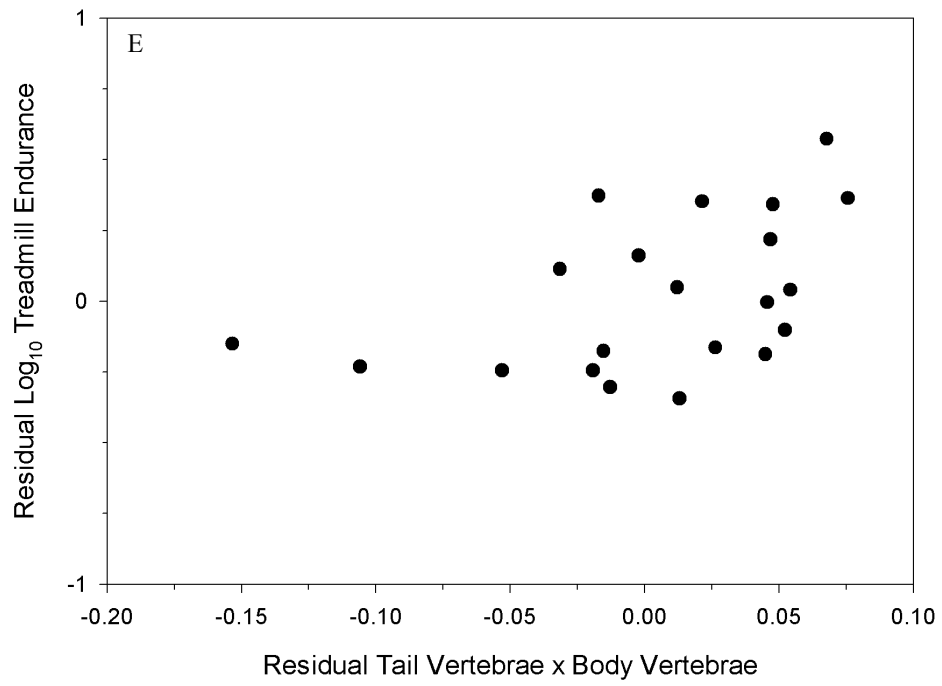
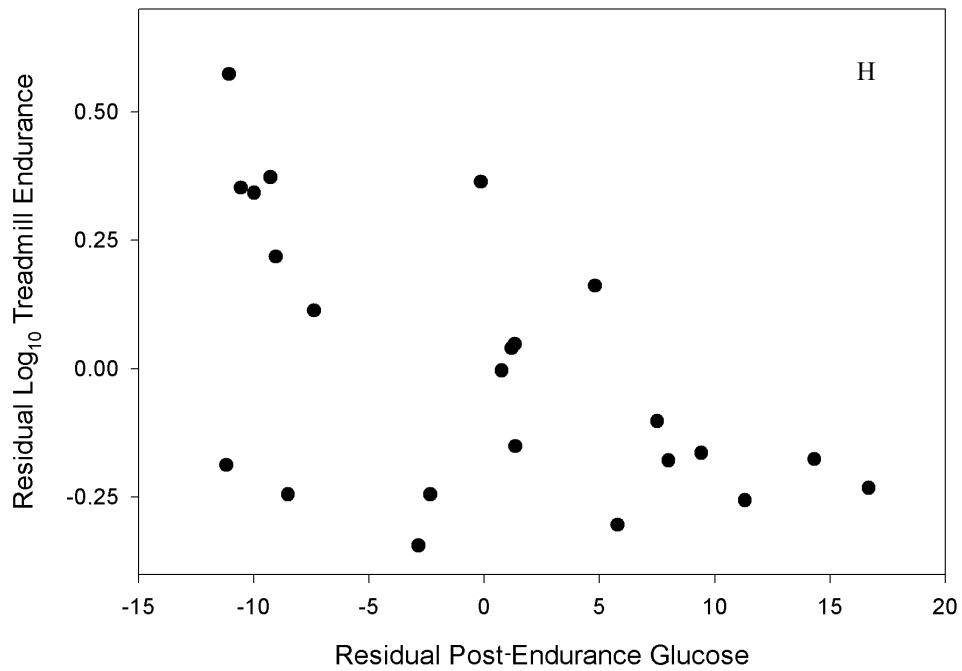
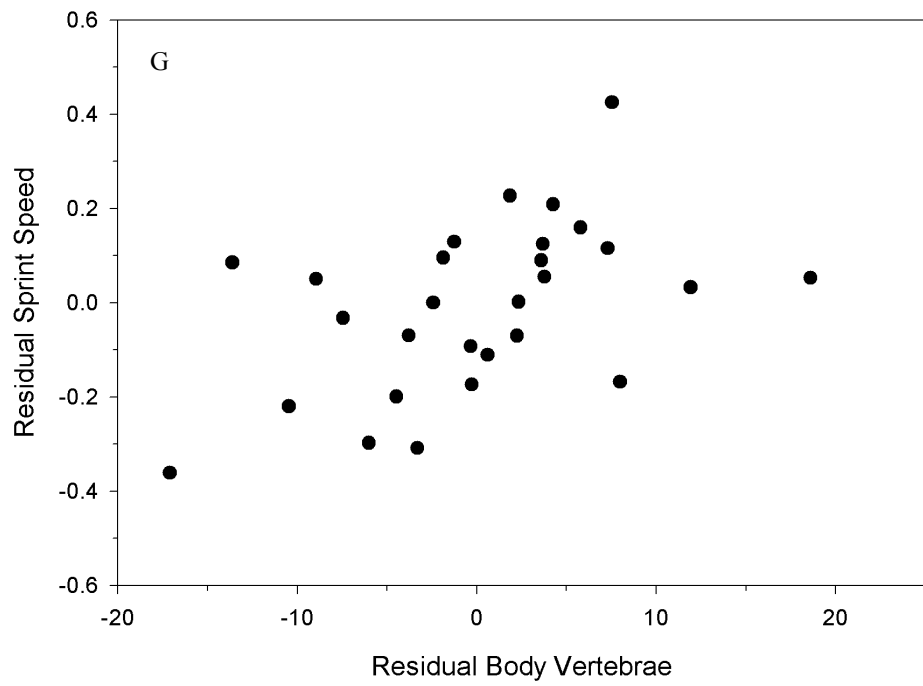


Figure 4.8 Continued.



Concluding Remarks

My dissertation has focused on the locomotor biology of snakes, considering first the relationship between habitat, behavior, and morphology among species and second, on variation in morphology and its effect on variation in performance among individuals. The biology of snakes, in general, is poorly understood relative to many other tetrapod groups (Greene 1997), but investigations of locomotion are likely to yield important clues regarding form-function relationships in snakes. Studies of locomotion are an ideal platform from which to explore ecomorphology because locomotion is so critical to an organism's survival (Dickinson 2000). As depicted in Figure 0.1 of this dissertation, natural and sexual selection often act most directly on the behavior of an organism. Behavior is, in turn, limited by the performance capacity of the organism; performance itself is determined by the synergistic effect of many lower-level traits (Arnold 1983; Garland and Losos 1994). All of these traits—behavior, performance, and morphology—are likely to be influenced by variation in habitat, which may strongly influence the selective regime of an organism.

In chapter one, I test the adaptive hypothesis that a snake's heart position will vary with habitat. Snakes may be particularly vulnerable to the affects of gravity because they are essentially long, fluid-filled tubes (Lillywhite 1993). Thus, arboreal snakes are thought to have more anteriorly placed hearts to reduce the work done by the heart to pump blood to the brain. Several studies have demonstrated significant functional differences in cardiovascular physiology among snakes from different habitats, but none has used modern phylogenetic statistical techniques to assess whether this variation may

reflect, at least in part, phylogenetic history and relatedness. Within our sample, we found arboreal animals to not have hearts significantly closer to the head. Further, we found that incorporating phylogenetic relationships in our statistical models drastically improved their fit to our data. Chapter two responds to criticism of this research.

In chapter three, we use a similar approach to chapter one to address several form-function hypotheses relating to the axial musculature of snakes. The major epaxial muscles are responsible for generating propulsive forces during lateral undulation in snakes (Jayne 1982). However, these muscles are also important in certain behaviors, including constriction and, in conjunction with the vertebrae, determine flexibility. We assessed whether variation in muscle lengths was associated with variation in habitat, and whether it varied between constrictors and non-constrictors. We found strong evidence that habitat influences the evolution of muscle morphology in our sample of snakes, but also that phylogenetic inheritance was important. Further, we found evidence that constrictors have shorter muscle lengths, putatively to increase flexibility.

In the fourth and final chapter, we examine the underlying basis of locomotor variation in the corn snake, *Pantherophis guttata*. Understanding variation among individuals has been a cornerstone of evolutionary biology since Darwin first formulated his theory of natural selection (Darwin 1959). In snakes, the mechanics of locomotion are generally well understood (Gans 1962). We still lack, however, a fundamental understanding of the source of locomotor variation within species of snakes.

We examined the relationship of metabolic rate and locomotor performance to several morphological, physiological, and biochemical measures. We found extensive

variation in most traits. Although we did not find support for relationships among our measures of metabolism and performance, we did find several unique correlations between lower-level traits and metabolism or performance.

The major conclusions of this dissertation are two-fold. First, adaptive hypotheses regarding form-function relationships and habitat can only be answered in a comparative context that incorporates phylogenetic history. Second, corn snakes have extensive individual variation in locomotor performance, and that variation is demonstrably related to variation in several lower-level traits. And, these lower-level predictors are not necessarily the same as those found in other studies of snakes (e.g., Arnold and Bennett 1988; Garland 1988).

Locomotion will continue to be of great interest to biologists. First, it is ecologically important to organisms (Dickinson et al. 2000). Further, it is an integrative and complex trait, and a complete understanding of the locomotor biology of an organism requires an approach that examines many levels of biological organization simultaneously (Garland and Kelly 2006). Finally, myriad questions remain regarding how locomotion varies in relation to habitat, function, behavior, and other aspects of ecology and evolution (Feder et al. 2010).

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