



Ambush or active life: foraging mode influences haematocrit levels in snakes

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The rate at which organisms acquire resources is a critical trait and foraging mode can vary from sit-and-wait tactics to being highly mobile and active. Snakes provide a robust opportunity to examine the physiological correlates of contrasted foraging strategies. In this context, haematocrit (Hct), a proxy of blood oxygen carrying capacity, should be a reliable indicator of aerobic activity levels. We used phylogenetically informed models to examine the relationship between foraging mode and Hct in 80 snake species. After accounting for clade and habitat effects, we found a significant relationship of Hct with foraging mode; Hct is lower in snakes that ambush prey compared to active foragers across habitats. Species using both foraging tactics had marginally lower Hct than active foragers. Ambush foraging tactics are widespread in snakes, notably among low-energy specialists that usually display low feeding frequency, as well as limited activity and daily movements. Because Hct influences blood viscosity, low levels may thus be advantageous by reducing maintenance and locomotory costs. Further studies are required to better understand the implication of foraging mode on blood characteristics and other aspects of snake physiology. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, **111**, 636–645.

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INTRODUCTION

The rate of resource acquisition is a major component of individual performance and may strongly influence traits related directly to fitness, such as reproductive output (Calow, 1982; Van Noordwijk & Dejong, 1986). Resource availability is variable in space and time, and such heterogeneity favoured the emergence of contrasting life-history strategies (Roff, 1992; Charnov, Warne & Moses, 2007). Within vertebrates, variation in foraging mode provides a clear illustration, ranging from low rates of resource acquisition (i.e. sit-and-wait foragers) to highly-active foragers with high rates of resource acquisition (Huey & Pianka, 1981; Webb, Brook & Shine, 2003). For example, endothermic vertebrates (i.e. mammals and birds) demonstrate extreme specializations toward

increased activity capacities, active foraging modes, and a high rate of resource acquisition (Bennett, 1991; Koteja, 2004). Such behavioural strategies are energetically costly (i.e. increased maintenance costs) and theoretical models suggest that endothermy likely led to specialization towards higher metabolic rates and aerobic capacities (Bennett, 1991; Hayes & Garland, 1995), increased rates of food acquisition (Koteja, 2004) and intensive parental care (Farmer, 2000; Koteja, 2000).

By contrast, ectotherms can encompass a larger activity continuum. Although ectotherms are usually oriented toward energy saving strategies (Pough, 1980; Congdon, 1989), they nonetheless show substantial variation in foraging modes and rates of energy acquisition (Secor & Nagy, 1994; Webb *et al.*, 2003; Verwajen & Van Damme, 2008a). For example, some ectothermic species are capable of sustaining high activity rates. The monitor lizards in the genus *Varanus* are one such example of specializations, with

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several species (e.g. *Varanus giganteus*), being known to forage widely for prey and to have increased metabolic rates (Christian & Conley, 1994). Other ectotherms exhibit the opposite pattern; low-energy specialists are characterized by low mobility, low resource acquisition rates and, usually, sit-and-wait foraging tactics (Bedford & Christian, 1998). Activity specialization has important evolutionary implications. Low-energy strategy is often coupled with 'slow life-history traits' (Shine, 1986; Mautz & Nagy, 2000), including a low frequency of reproduction and the ability to accumulate important energy store (i.e. capital breeding; Bonnet, Bradshaw & Shine, 1998). In turn, resource capitalization is more likely to be disadvantageous for active species because of the possible impact on locomotor performances (Jonsson, 1997). Understanding both the ultimate and proximate determinants of such variation is a question that has long been of interest to evolutionary biologists (Huey & Pianka, 1981; Abrams, 1993).

Specializations associated with activity rates and foraging modes are likely to influence an organism at multiple levels of biological organization (e.g. genetic, biochemical, morphological). Important covariation exists between these levels, with selection ultimately acting most directly on overall organismal performance (e.g. whether an animal is able to capture and consume prey). For example, active foraging is usually associated with intense muscular activity and requires both increased stamina and aerobic scope (Bennett, 1991). Elevated metabolic rate and specific cellular and intracellular specializations (mitochondrial structure, density, membrane permeability) support the ATP requirements for intense activity (Else, Turner & Hulbert, 2004). Correlated morphological and physiological specializations include (but are not limited to) an increased size and efficiency of the major muscles associated with locomotion, reduced costs of locomotion, and associated changes to major organs associated with metabolic function, such as the heart and lungs (Bennett, 1991; Frappell, Schultz & Christian, 2002). Conversely, evolutionary specializations toward low activity also involve changes across multiple levels of biological organization, leading to energy saving strategies, a concomitant reduction in energy use (Pough, 1980; Peterson, 2002), and minimal maintenance costs.

The amount of oxygen delivered to the tissues by blood is one step that potentially limits the ability of an organism to perform at a high aerobic capacity (Frappell *et al.*, 2002). Therefore, variation in blood-oxygen stores of organisms is expected to be highly correlated with metabolic scope. Haematocrit (packed blood cell volume per unit volume of blood, hereafter 'Hct') is an important correlate of blood-oxygen stores that can be affected by multiple factors, including

dehydration, pregnancy or oxygen levels (Peterson, 2002). In addition, Hct has been shown to vary with aspects of snake biology tightly associated with variation in oxygen consumption (e.g. Hct levels differ between marine/diving snakes and terrestrial snakes; Brischox *et al.*, 2011). The relative consistency of blood volume and haemoglobin-oxygen affinity between species of snake (Seymour, 1976, 1982; Heatwhole, 1999), in addition to an absence of significant amounts of myoglobin in snake muscles (Seymour, 1982), makes Hct an excellent and simple estimate of blood oxygen stores in snakes (Brischox *et al.*, 2011).

Snakes (> 3000 species; Uetz & Hošek, 2013) provide a robust model for addressing the possible correlation between foraging mode and Hct. This group spans a massive continuum of activity (foraging) levels, from extreme cases of low-energy specialists with important fasting capacities (McCue, 2007a) that usually ambush their prey, to active predators that devote a substantial part of their activity budget on foraging activities (Secor & Nagy, 1994). In the present study, we analyzed the relationship between Hct and foraging mode using a phylogenetically diverse data set derived from 80 species of snake. We use phylogenetically informed models to first determine to what extent clade membership, habitat, and foraging mode predict Hct. Second, we examine the relationship between Hct and foraging mode using phylogenetic multiple regressions. We predict that foraging mode will have a significant effect on levels of Hct in snakes. Snakes that actively pursue their prey should have significantly higher Hct levels than snakes that ambush their prey.

MATERIAL AND METHODS

HCT, HABITAT, AND FORAGING MODE

Hct and habitat data were collected from the literature and from unpublished studies (Supporting Information, Appendix S1). We used the dataset from Brischox *et al.* (2011) as a basis, which we supplemented with recently published Hct data on 10 other species (Supporting Information, Appendix S1). We also directly collected Hct values for three additional species (Supporting Information, Appendix S1). In those cases, snakes were bled by cardiocentesis within 3 min using a 1-mL heparinized syringe and a 27-gauge needle. A small amount of blood was transferred in two haematocrit tubes that were centrifuged for 3 min at 20 °C. We only considered data collected in adult snakes in a post-absorptive state; when multiple data were available for a given species, we averaged the Hct values.

We assigned to each snake species a foraging mode based on published data, field guides, and the

authors' personal experience (Supporting Information, Appendix S1). Species known to rely on sit-and-wait strategies (e.g. most viperids or boids) were classified as ambush predators, whereas species described as relying on important movement for resource acquisition were classified as active foragers (Supporting Information, Appendix S1). Four species described as using indifferently either ambush or active foraging were classified as 'mixed' foragers (Supporting Information, Appendix S1). Our final dataset consisted of 80 snake species for which we had information on Hct, habitat, and foraging mode (Supporting Information, Appendix S1).

PHYLOGENY RECONSTRUCTION

A composite 'species tree' was constructed from previously published hypotheses of relationships among snakes. Lower-level relationships (i.e. Species) were nested within a phylogenetic framework consisting of higher-level relationships (i.e. Families). Tree selection for any particular group was conducted in accordance with previously published methods (de Queiroz & Rodriguez-Robles, 2006; Gartner *et al.*, 2010) (for details on phylogeny construction, see Figure 1; see Supporting Information, Appendix S1 and S2). The final topology had 80 taxa and initially lacked branch length information.

Using the constructed species tree, we estimated branch-lengths from node ages for the major higher-level groups in our tree using the BLADJ utility in the software package PHYLOCOM (Webb, Ackerly & Kembel, 2008). Node ages were taken from Pyron & Burbrink (2012). The root node defining the Alethinophidia was fixed using the estimated mean age (102.75 Mya), whereas the stem-group ages of the Colubroidea and of the Colubridae *sensu stricto* were set using minimum estimated ages (48.6 and 33.3 Mya, respectively).

STATISTICAL ANALYSIS

Categories for Clade, Habitat, and Activity were coded as a series of dummy variables for use in regression models (equivalent to analysis of covariance with parallel slopes). The relationship between Clade, Habitat, Activity, and Hct was examined using four models of evolution: (1) ordinary least squares (OLS) regression in which trait evolution is independent of phylogeny (equivalent to evolution on a 'star' phylogeny); (2) a Brownian motion model in which the branch lengths in our original starting tree are unchanged (equivalent to generalized least squares); (3) An Ornstein-Uhlenbeck (OU) model in which evolution of residual Hct variation is assumed to evolve towards a central optimum (stabilizing selection; Butler & King, 2004; Lavin *et al.*, 2008); and (4) a

model based upon Pagel's λ in which all internal branches are multiplied by a constant (λ).

We ran all possible iterations of our three independent variables for each of our four evolutionary models (resulting in 28 total regression models). The fit of alternate models was compared using Akaike's information criterion with a second-order correction for small sample sizes (AICc; smaller is better), Akaike weights, w_i (model probabilities; larger values are better), and evidence ratios (ER; smaller values are better). All models in the results section are compared relative to a top model with $\Delta\text{AICc} = 0$ and $\text{ER} = 1$. Finally, we examined the relationship among factors in our categorical variables using a model with all included variables (Hct = Clade + Habitat + Activity).

We used the branch-length transform parameters from OU and λ models (d and λ , respectively) to test whether phylogenetic signal (i.e. the tendency of related individuals to resemble on another) is present in the residuals of the dependent variable (Hct) in our regression. Branch length transform estimates of 1 are equivalent to a Brownian motion model (equivalent to our starting hierarchical tree), whereas values near zero indicate a 'star phylogeny', which suggests that trait evolution is independent of phylogeny. The fit of alternate evolutionary models (OU and λ) can be compared to OLS models with the same set of independent variables using a likelihood ratio test (LRT) where twice the difference in log likelihoods approximates a chi-squared statistic with degrees of freedom equal to the difference in the number of parameters between the model (1 for all analyses performed here; Pagel, 1999). A significant LRT indicates the presence of phylogenetic signal in the residuals of the dependent variable in the nonphylogenetic model (Revell, 2010). More generally, we can examine the presence of phylogenetic signal by examining the clade variable. If the clade variable is significant in a model, then phylogenetic position is also critical in predicting the dependent variables after controlling statistically for other independent variables in the regression. All analyses were conducted in either MATLAB using Regressionv2 (Lavin *et al.*, 2008) or with the 'caper' package (Orme *et al.*, 2012) in the open source programming language R (R Development Core Team, 2012).

RESULTS

Only two models, an OLS model with Clade and Habitat, and a full OLS Model (with all variables), contained substantial support ($\Delta\text{AICc} < 2$; Burnham and Anderson, 2002) (Table 1). Model probabilities (w_i) for the top two models were three- and four-fold greater than for the next best model (an OU model with Clade and Habitat). The inclusion of Activity did

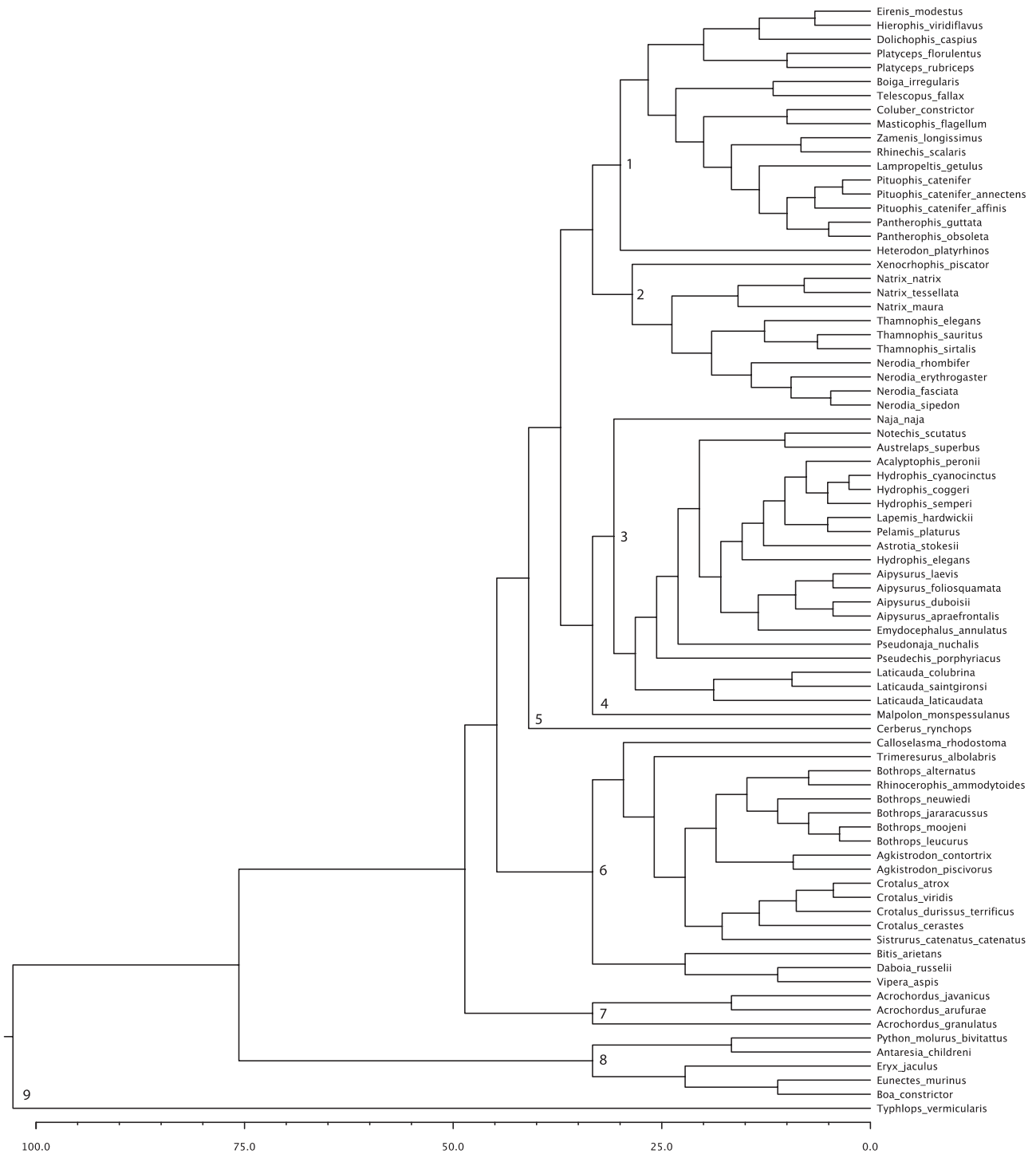


Figure 1. Phylogeny of 80 snake taxa used in the present study. Branch lengths (not shown) are in millions of years. Node numbers indicate the ‘Clades’ used in regression analysis (see Material and methods): 1 = Colubridae; 2 = Natricinae; 3 = Elapidae; 4 = Psammophiinae; 5 = Homalopsidae; 6 = Viperidae; 7 = Acrochordidae; 8 = Boidae; 9 = Typhlopidae. For complete tree file, see Supporting Information, Appendix S1.

Table 1. Alternate regression models predicting haematocrit among 80 species of snakes

Model	Trn	d/λ^*	r^2	MSE	lnML	ΔAICc	w_i	ER
CLADE + HABITAT	OLS	NA	0.54	15.9	-214.9	0.0	0.333	1.0
CLADE + HABITAT + ACTIVITY	OLS	NA	0.57	15.3	-212.1	0.4	0.280	1.2
CLADE + HABITAT	OU	0.11	0.47	16.1	-214.8	2.8	0.084	4.0
CLADE + HABITAT	λ	0.67	0.41	17.9	-214.9	3.0	0.076	4.4
CLADE + HABITAT + ACTIVITY	OU	0.15	0.50	15.6	-212.0	3.4	0.062	5.4
CLADE + HABITAT + ACTIVITY	λ	0.71	0.45	17.1	-212.1	3.5	0.058	5.8
CLADE + HABITAT + ACTIVITY	OLS	NA	0.41	18.7	-224.7	3.6	0.056	6.0
CLADE + HABITAT + ACTIVITY	OU	0.30	0.30	18.6	-224.0	4.6	0.033	10.0
CLADE + HABITAT + ACTIVITY	λ	0.79	0.32	18.8	-224.7	6.1	0.016	21.0
CLADE + HABITAT + ACTIVITY	OU	0.31	0.17	21.3	-231.3	11.9	0.001	387.2
CLADE + HABITAT	λ	0.81	0.21	21.1	-230.4	12.5	0.001	524.8
CLADE + HABITAT	OU	0.42	0.17	21.7	-231.2	14.1	0.000	#
CLADE + HABITAT + ACTIVITY	OLS	NA	0.24	23.2	-234.7	16.5	0.000	#
CLADE + HABITAT	OLS	NA	0.26	22.9	-233.7	16.7	0.000	#
CLADE + HABITAT + ACTIVITY	λ	0.85	0.14	22.8	-234.0	17.3	0.000	#
CLADE + HABITAT + ACTIVITY	BM	1.00	0.24	23.5	-233.7	21.6	0.000	#
CLADE + HABITAT + ACTIVITY	BM	1.00	0.12	25.9	-239.2	25.4	0.000	#
CLADE + HABITAT	BM	1.00	0.15	25.6	-238.2	25.7	0.000	#
CLADE + HABITAT + ACTIVITY	OU	0.54	0.18	23.9	-229.6	29.3	0.000	#
CLADE + HABITAT + ACTIVITY	OLS	NA	0.29	24.2	-232.1	31.4	0.000	#
CLADE	OU	0.56	0.07	26.4	-234.3	33.1	0.000	#
CLADE	OLS	NA	0.21	25.9	-235.9	33.6	0.000	#
CLADE + HABITAT + ACTIVITY	λ	0.91	0.16	24.9	-232.1	34.3	0.000	#
CLADE	λ	0.91	0.05	27.3	-235.9	36.3	0.000	#
CLADE + HABITAT + ACTIVITY	BM	1.00	0.30	24.3	-230.4	36.9	0.000	#
CLADE + HABITAT	BM	1.00	0.24	25.7	-233.8	37.7	0.000	#
CLADE + HABITAT + ACTIVITY	BM	1.00	0.14	28.4	-238.3	43.8	0.000	#
CLADE	BM	1.00	0.04	30.8	-242.8	47.3	0.000	#

Models are listed in ascending order of Δ Akaike's information criterion with a second-order correction (AICc) (best model is first). Columns: Trn, transform; OLS, ordinary least squares; BM, Brownian motion (equivalent to GLS); OU, Ornstein-Uhlenbeck transform; λ , Pagel's Lambda transform; d and λ are REML estimates of OU transformation parameter and Pagel's lambda, respectively. Note that d and λ are not directly comparable themselves (see Material and methods); MSE, mean squared error (rate of evolution; Garland and Ives 2000); lnML, maximum natural log likelihood; ΔAICc , difference in model AICc score from the 'top' model; w_i , Akaike weighted probability; ER, evidence ratio. d and λ (estimated using restricted maximum likelihood) indicate the degree of phylogenetic structure present in the model. Values of 1 for both parameters indicate no branch-length transform (a Brownian motion model equal to our starting tree), whereas values of zero indicate a model in which trait evolution is independent of phylogeny (a star phylogeny). Likelihood ratio tests between our top models ($\Delta\text{AICc} < 2$) and their phylogenetic counterparts (OU and λ models with the same variables) were nonsignificant, indicating a lack of significant phylogenetic signal in the residuals of the dependent variable, Hct. The lack of significant phylogenetic signal suggests that other evolutionary processes, such as selection may be important in determining blood oxygen carrying capacity in snakes utilizing different habitats in addition to different modes of prey capture. *Estimates of d and λ are based upon restricted maximum likelihood (REML), #ER > 1000.

not substantially increase the fit of the model to the data ($r^2_{\text{Clade + Habitat}} = 0.54$ versus $r^2_{\text{Clade + Habitat + Activity}} = 0.57$).

The estimated d and λ values from OU and λ versions of the top two models were small (Table 1) and not significantly different from zero (based upon likelihood ratio tests; not shown), indicating that the phylogeny describing residual covariance was almost a star with very little hierarchical structure

(note that d and λ values themselves are not directly comparable).

Partial regression coefficients for factors of Clade, Habitat, and Activity are shown in Table 2. The overall regression model was always highly significant, regardless of whether OLS, OU or λ models were used (Table 2). Among the clades used in the present study, only acrochordids showed increased Hct after controlling for the effects of Habitat and

Table 2. Full ordinary least squares (OLS) regression models and regression models after adjusting branch-lengths (Fig. 1; see also Supporting Information, Appendix S2) using either an Ornstein-Uhlenbeck (OU) process or Pagel's lambda (λ) (see Material and methods)

Variables	OLS				OU ($d = 0.149$)				λ ($\lambda = 0.71$)				d.f.
	β	SE	F	P	β	SE	F	P	B	SE	F	P	
y-intercept	28.24	0.96	866.30	< 0.01	27.96	1.25	497.55	< 0.01	28.22	4.04	48.81	< 0.01	1,65
Clade													
Acrochordidae	16.71	5.08	10.81	< 0.01	17.58	5.38	10.66	< 0.01	18.16	6.15	8.73	< 0.01	
Boidae	2.38	3.19	0.56	0.46	2.65	3.25	0.67	0.42	2.26	4.22	0.29	0.59	
Elapidae	-2.51	2.20	1.30	0.26	-2.34	2.42	0.93	0.34	-2.58	3.19	0.65	0.42	
Homalopsidae	-1.94	4.60	0.18	0.67	-0.83	4.90	0.03	0.86	-0.62	5.48	0.01	0.92	
Natricinae	3.99	2.75	2.10	0.15	3.95	3.06	1.66	0.20	4.39	3.56	1.52	0.22	
Psammophiinae	-3.24	4.03	0.65	0.42	-2.96	4.24	0.49	0.49	-2.70	4.71	0.33	0.57	
Typhlopidae	2.76	4.03	0.47	0.50	3.04	4.24	0.51	0.48	2.78	7.06	0.15	0.70	
Viperidae	0.82	2.54	0.10	0.75	1.18	2.68	0.19	0.66	1.82	3.78	0.23	0.63	
Habitat													
Aquatic	-23.65	5.84	16.41	< 0.01	-24.25	6.20	15.31	< 0.01	-24.71	6.55	14.23	< 0.01	
Marine	5.90	2.21	7.12	< 0.01	5.07	2.45	4.28	0.04	5.08	2.46	4.25	0.04	
Semi-Aquatic	-2.82	2.30	1.51	0.22	-2.94	2.41	1.49	0.23	-3.16	2.36	1.80	0.18	
Activity													
Ambush	-5.34	2.51	4.53	0.04	-5.36	2.51	4.56	0.04	-5.77	2.51	5.28	0.03	
Mixed	-0.75	2.55	0.09	0.77	-0.51	2.69	0.04	0.84	-0.99	2.93	0.11	0.74	
Model													
			33.19	< 0.01			24.93	< 0.01			20.51	< 0.01	3,75

Significant partial regression coefficients in all three models are shown in bold. Regression coefficients reflect changes in the dependent variable with respect to a reference group; here, we use 'Colubrinae', 'Terrestrial', and 'Active' as our reference categories for Clade, Habitat, and Activity, respectively, because we considered that a generalist, active foraging and terrestrial colubrid best represented the 'generalized snake condition'. All tests are two-tailed. Acrochordids are a unique group of fully aquatic snakes with unusual biology and life histories; thus, regression coefficients for aquatic animals should be interpreted with caution (Brischoux *et al.*, 2011).

Activity. Among habitats, marine animals showed significantly increased Hct, whereas aquatic animals showed significantly decreased levels (Brischoux *et al.*, 2011).

Haematocrit varied between levels of activity after controlling statistically for effects of Clade and Habitat use. As predicted active foraging snakes had significantly increased Hct relative to snakes that ambush their prey. Snakes using a mixed strategy were not significantly different from active foragers (although the sign of the regression coefficient was always negative indicating marginally less Hct than active foraging snakes). The overall regression model was always highly significant.

DISCUSSION

Activity specialization is under multiple selective pressures and involves integrated changes in morphology, aerobic scope, and behaviour (Bennett, 1991; Goolish, 1991; Porges *et al.*, 2003; Johnson *et al.*, 2008; Clemente, Withers & Thompson, 2009). Haematocrit, as a proxy of the amount of oxygen carried in the blood, is a relevant parameter that should reflect foraging mode. For example, high Hct observed in endotherms compared to ectotherms is interpreted in the frame of superior activity capacity (Pough, 1980) and increased energy requirements. In snakes, previous studies have demonstrated relationships between habitat use and Hct (Brischoux *et al.*, 2011). Despite wide variation in foraging tactics in snakes, however, no study has yet attempted to examine the influence of foraging mode on correlates of activity metabolism in a broad, comparative context.

We found that only two OLS models showed substantial support in our model comparison (Models 1 and 2) (Table 1), suggesting weak phylogenetic signal in haematocrit and a stronger, adaptive role for habitat. Clade effects, although significant in both top OLS models, were driven primarily by the acrochordids, a group that is known to be very atypical in terms of their ecology, morphology, and physiology compared to other caenophidians (Houston & Shine, 1994). Besides acrochordids, the other eight clades showed similar Hct values after controlling for other variables in the model. Habitat had a strong influence on blood oxygen carrying capacities (Brischoux *et al.*, 2011). Marine snakes show significantly higher Hct, and this trait has been interpreted as an adaptation to the physiological constraints of diving (Brischoux *et al.*, 2011).

After accounting for habitat effects, we found that active foragers showed, on average, 14%, 23%, and 16% higher Hct than ambush foragers, respectively, for terrestrial, semi-aquatic, and marine snakes (Fig. 2), therefore supporting our hypothesis. Mixed

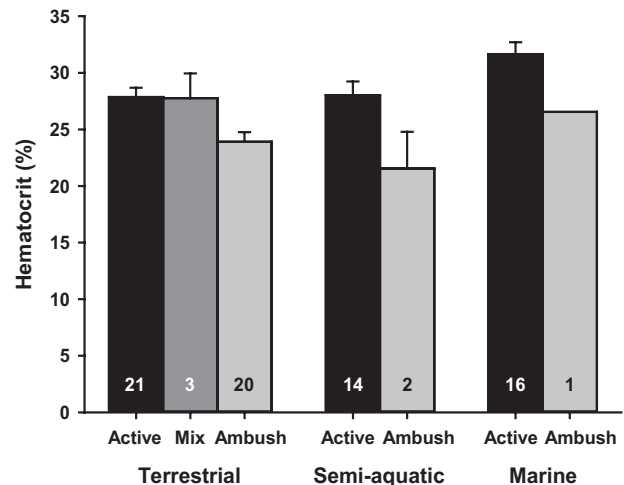


Figure 2. Haematocrit (mean \pm SE) in relation to foraging mode within three main habitat types. Numbers in bars indicate the sample size (number of species).

strategy was only documented for terrestrial snakes and occupied an intermediate position. Foraging mode has been associated with other marked physiological and morphological specializations in squamates (Garland & Else, 1987; Porges *et al.*, 2003; Verwajen & Van Damme, 2008b) to which variation in Hct can be added. Previous studies have demonstrated that active foragers have increased aerobic scopes and can sustain prolonged activity compared to more sedentary or reclusive species (Bennett & Licht, 1972; Pough, 1980; Andrews & Pough, 1985; Mautz & Nagy, 2000). For example, in varanid lizards, active foraging is associated with high maximum metabolic rates and prolonged endurance (Clemente *et al.*, 2009). Similar observations exist for snakes (Secor & Nagy, 1994). Active foragers are characterized by increased daily movements and sustained activity levels for resource acquisition (Clemente *et al.*, 2009). In more active animals, increased Hct is directly correlated to increased transport of oxygen to the tissues, increased ATP production, and sustained aerobic activity.

Conversely, ambush foragers usually show reduced activity and movements, have lower rates of energy acquisition, and lower resting metabolic rates. Sit-and-wait foragers typical have 'slow' life-history traits (Webb *et al.*, 2003) and energy saving tactics that minimize maintenance costs (Pough, 1980). For example, they down-regulate maintenance requirements during fasting periods (Secor & Nagy, 1994; Secor, Stein & Diamond, 1994; McCue, 2007b). Activity relies on brief episodes of locomotion between extended periods of immobility (either for foraging or sheltering). Sit-and-wait foragers cannot sustain prolonged bouts of muscular activity and are sensitive to

exhaustion as a result of lactate accumulation (Pough, 1980). Because they naturally do not maintain high levels of activity, elevated blood oxygen capacity is not likely to be relevant for ambush foragers and may even be constraining. Blood viscosity increase nonlinearly with haematocrit levels (Snyder, 1971; Viscor *et al.*, 2003) and lowered resistance to blood flow may therefore be one adaptive aspect of energy saving (Pough, 1980; Stark & Schuster, 2012). Lower Hct may provide significant advantages by reducing vascular resistance. Finally, booids and viperids represent low-energy specialists (Bedford & Christian, 1998; Zaidan, 2003; McCue, 2007b) and these two clades show the lowest Hct on average (Brischoux *et al.*, 2011: fig. 2).

It is worth noting that Hct is a labile physiological parameter that responds to multiple factors including age, body size, sex or reproductive status (Pough, 1980; Seymour, 1982; Garland & Else, 1987). Hct also responds to fluctuations in the environment, nutritional state, and seasons (Snyder, 1971; Peterson, 2002). For example, dehydration induces significant hemoconcentration and increases Hct levels (Peterson, 2002). Similarly, ambient temperature can induce important adjustment in blood Hct and viscosity (Viscor *et al.*, 2003) in both endotherms and ectotherms. Haematocrit levels can also change rapidly as demonstrated in a racer (*Coluber constrictor*) that is a typical active forager (Stinner & Ely, 1993). This species dynamically not only responds to handling stress by increasing blood pressure and heart rate, but also haematocrit levels. Haematocrit change can also reflect physiological processes and nutritional health (Peterson, 2002). Infrequent feeders such as pythons experience high costs of digestion and nutrient uptake. Secor *et al.* (2001) demonstrated a significant postprandial haematocrit decline (25%) in relation to up-regulation of the digestive tract after feeding in *Python molurus*, whereas Overgaard *et al.*, (1999) were unable to document such a response in the same species. The consistency of haemoglobin-oxygen affinity between species has yet to be investigated for the large number of the species that we studied, and cautious interpretation of Hct levels is therefore required because of the potential influence of the confounding factors listed above. Nevertheless, the influence of such factors is unlikely to remove the signal of increased Hct that we discovered in the present study and our findings support our initial prediction that foraging mode is likely influenced by this important physiological parameter.

A possible limitation of the present study was the classification of activity into three clear-cut categories. Indeed, more subtle variations can exist (Cooper, 2005). For example, typical 'racers' in colubrids and

elapids usually show preferences for high body temperatures when active in the field, high locomotor performance, and rely on speed to chase their prey (Whitaker & Shine, 2002; Lelièvre *et al.*, 2010). Other active foragers, such as colubrid 'rat snakes' have lower preferred field active body temperatures, are less mobile, and use cryptic strategies that minimize exposure (Carfagno & Weatherhead, 2008; Lelièvre *et al.*, 2011). Marked eco-physiological differences exist between these two 'substrategies', including contrasted energy expenditure and Hct levels (Lelièvre, 2010). Important variation may also occur within species between populations (Schuett *et al.*, 2013), and there may be significant genetic contribution to variation in Hct values reflecting local adaptation. Further studies are required to better understand the nature and the direction of the selective pressure acting on Hct and the importance of foraging mode, as well as exposure tactics (i.e. conspicuous or elusive life styles). Proximate physiological conflicts between oxygen transport, blood viscosity, and temperature deserve specific attention (Snyder, 1971; Viscor *et al.*, 2003).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Dataset and foraging mode classification.

Appendix S2. Details on phylogeny construction.