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Cite this article: Holding ML *et al.* 2022 Evolutionary allometry and ecological correlates of fang length evolution in vipers. *Proc. R. Soc. B* **289**: 20221132.
<https://doi.org/10.1098/rspb.2022.1132>

Received: 10 June 2022
 Accepted: 11 August 2022

Subject Category:
 Evolution

Subject Areas:
 ecology, evolution

Keywords:
 viper, adaptation, tooth, fang, allometry

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Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.6166904>.

Evolutionary allometry and ecological correlates of fang length evolution in vipers

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Traits for prey acquisition form the phenotypic interface of predator–prey interactions. In venomous predators, morphological variation in venom delivery apparatus like fangs and stingers may be optimized for dispatching prey. Here, we determine how a single dimension of venom injection systems evolves in response to variation in the size, climatic conditions and dietary ecology of viperid snakes. We measured fang length in more than 1900 museum specimens representing 199 viper species (55% of recognized species). We find both phylogenetic signal and within-clade variation in relative fang length across vipers suggesting both general taxonomic trends and potential adaptive divergence in fang length. We recover positive evolutionary allometry and little static allometry in fang length. Proportionally longer fangs have evolved in larger species, which may facilitate venom injection in more voluminous prey. Finally, we leverage climatic and diet data to assess the global correlates of fang length. We find that models of fang length evolution are improved through the inclusion of both temperature and diet, particularly the extent to which diets are mammal-heavy diets. These findings demonstrate how adaptive variation can emerge among components of complex prey capture systems.

1. Introduction

Understanding how the functional traits of predators evolve in response to variable selective pressures links trophic ecology to the evolution and maintenance of biological diversity. Trophic traits mediate the interactions between predators and their prey, and therefore provide an opportunity to study the evolution of phenotypic diversity in relation to both abiotic and biotic pressures [1]. When alternative forms of a functional trait confer high fitness depending on ecological conditions, the power of selection to maintain diversity is amplified (e.g. bill size in Darwin's finches [2]). Predator–prey interactions are typically viewed through the lens of an escalatory (i.e. 'bigger is better') arms race. Yet, ecologically mediated specialization into multiple optima has been supported in predator–prey systems such as raptor talon shape, claw size in crabs and canine tooth length in cats [3–5]. These predator–prey interactions belong to a

class where physical damage alone incapacitates prey, but a great diversity of predator–prey interactions, spanning cnidarians to vertebrates, are mediated more by the injection of venoms into prey rather than purely by physical damage.

Fangs, stingers, spines and harpoons are used by animals to capture and kill prey. The primary function of these physical weapons is the injection of venoms that cause damage on a physiological level, attacking basal organismal processes such as hemostasis, neurotransmission and muscle function [6]. The composition and adaptive evolution of toxins has been used as a model system to investigate molecular structure–function relationships, gene family evolution and the complex pathway between genotype and phenotype [7]. There may be equally strong selection on morphological variation in the venom injection apparatus among taxa [8,9]. If prey differ in size, shape or handling requirement, alternative evolutionary optima may exist for the venom injection system, providing a route to diversification [10–12].

Viperid snakes, such as rattlesnakes, lanceheads, adders and habu have evolved modularized, solenoglyphous fangs. These teeth are hollow, positioned at the front of the mouth, and are the only teeth attached to a highly mobile maxilla bone that allows folding against the roof of the closed mouth. Extreme rotation of a viper's maxilla swings the fang forward during a strike. This folding action in vipers modularizes the fang with respect to the distance between the roof and floor of the mouth, allowing for increased fang length to evolve [13]. Despite the structural freedom to evolve longer fangs, there is considerable variation in proportional fang length relative to mandible size among species, suggesting adaptive variation.

Selection for longer or shorter fangs may come from multiple sources, and therefore, phylogenetically informed modelling across a large sample of taxa with diverse habitats and life histories is required to robustly test hypothesized correlates of fang length evolution. As gape-limited predators that swallow prey whole, maximum prey size for a viper is associated with the overall size of the snake's head [14–16]. Based on the square-cube law and isometric scaling of prey body proportions, a prey item that is twice the dimensions of another will have eight times the tissue volume, diluting both the concentration of venom and the impact of physical damage dealt by the fangs. This volumetric discrepancy across body sizes is further compounded by divergence from isometric growth—allometry—often producing more robust shapes in larger animals (e.g. disproportionately larger cross-sectional areas, thicker fat layers and tougher protective coverings [17,18]) and therefore predicting positive allometry in snake fangs. In a study of 30 species, Pough & Groves [19] showed positive allometry as well as evidence that tropical snake genera in Asia, Africa and South America show the longest relative fang lengths. However, the extent to which the observed allometric slopes were a product of static versus evolutionary allometry was unclear given wide variation in body size both within and between species. Climatic regimes are generally known to impact vertebrate and invertebrate body size distributions through factors such as heat-transfer dynamics (i.e. Bergmann's rule) or potential for evaporative water loss [20–22]. As a result, the largest prey species of several vertebrate and invertebrate groups are found at warmer temperatures, generally in the tropics.

Differences in the behaviour or morphology among specific prey types may also lead to longer or shorter fangs

in snakes with different diet compositions [10]. Recently, Cleuren *et al.* [11] used a phylogenetically broad sample of Colubroid snakes to show that two fang dimensions, elongation and sharpness, were predicted by feeding on soft prey versus scaly and chitinous prey. Here, we densely sample fang lengths of viperid snakes to reveal evolutionary trends in fang evolution within and between clades, and pair fang length with detailed quantitative rather than qualitative estimates of dietary variation to interrogate the role of taxonomic diet composition and prey features such as 'softness' in fang evolution. Our primary aims were to (i) test the role of evolutionary versus static allometry in explaining viper fang length and (ii) to test the association of climatic and dietary factors with fang length evolution in a comparative phylogenetic, model-testing framework.

2. Materials and methods

(a) Specimens and morphological measures

We sampled 1905 viperid museum specimens comprising 199 recognized species (electronic supplementary material, dataset S1 contains morphometric and collection information). For each snake, we measured the left and right fang length with a digital caliper from tip to its anterior connection to the maxilla (see electronic supplementary material, figure S1). The average of these two measurements (hereafter 'fang length') was used in subsequent analyses. We measured body size as snout-to-vent length. Finally, we photographed the dorsal aspect of the head of each snake on a 0.5 cm² grid using a digital camera. Using these photographs and the program IMAGEJ v1.52a [23], we obtained the distance from the tip of the rostrum to the start of the midpoint of the jaw rictus (see electronic supplementary material, figure S2).

A key consideration in analysing fang length evolution is to control for the size of the snake, and options include linear models with size covariates or ratios of fang length to other body size metrics. As ratios are problematic forms of control in regression models [24], regression models described below use raw fang length as the independent variable, while including head length as a covariate. We also report relative fang length as fang length divided by head length, as this proportion is an independent complex trait of each species [25], preserves the allometric component of fang length and is both functionally relevant [26] and intuitive (e.g. fang length can be discussed as a percentage of head length). We divide by head length because it physically limits the length a fang could achieve [27], and predicts gape size [28].

(b) Evolutionary allometry

We tested for evolutionary allometry by modelling the relationship between log-transformed fang length versus log-transformed mean head length across 199 species. We employed phylogenetic generalized least-squares (pgls) analyses as implemented in the nlme [29] package in R. We fitted a Pagel's λ correlation structure with the corPagel function in ape [30] using the viper phylogeny of Alencar *et al.* [31], allowing a maximum-likelihood estimate of λ during model fitting. We also used the phylRMA function in phyttools [32] to fit a phylogenetic reduced-major axis regression (RMA) model. We fitted an RMA due to its common use in allometry studies including snakes [19] but see Kilmer & Rodríguez [33] for a review of its use and drawbacks. Contributions of various types of allometry must be parsed [34]. We ruled out ontogenetic allometry by including only adult specimens in our analyses, while quantifying the potential role of static allometry by calculating allometric slopes from linear regressions of log-transformed

fang length versus log head length within each of the 54 species for which we measured more than 10 animals. If the proportionality of fang length to body size across species is primarily a product of evolutionary allometry, we would expect the interspecific allometric slope to diverge from a value of one, while most intraspecific allometric slopes will be near one (e.g. [35]).

(c) Climatic data

We used our list of measured viper species to query the Global Biodiversity Information Facility (GBIF; <https://www.gbif.org/>) for localities using `occ_search` in the `rgbif` R package, with 'has-GeospatialIssue' set to 'FALSE'. This search resulted 109 060 occurrence points. We then used the `getData` and `extract` functions from the `raster` package [36] to match all 19 bioclim variables from WorldClim (www.worldclim.org) at a 2.5 min resolution to each GBIF locality, extracting the median values of mean annual temperature (`bio1`) and mean annual precipitation (`bio12`) for each snake species (called 'temperature', and 'precipitation' henceforth). We then used the `princomp` function in base R to perform a principal components analysis on all 19 bioclim variables across the localities (electronic supplementary material, table S1) to derive an environmental PC1 (henceforth `envPC1`) that explained 91% of environmental variation present.

(d) Diet data

We gathered results from 85 previously published studies reporting gut contents of various species of vipers (electronic supplementary material, table S2). We included species for which the sum of the diet items reported was ≥ 15 (range 15–1031 prey items). Seventy-one species of vipers met our cutoff. For each species, we calculated diet composition as a per cent contribution by each of six categories. Five categories consisted of vertebrates by taxonomic class (i.e. mammals, birds, reptiles, amphibians and fish), and the sixth category summarized the contribution of arthropod invertebrates including insects, arachnids and centipedes.

(e) Comparative analyses

We examined fang evolution and its correlates with phylogenetic comparative methods. Using Alencar *et al.*'s [31] maximum clade credibility tree, we calculated Blomberg's K [37] and Pagel's λ [38] to characterize phylogenetic signal using the `phylosig` function in the `phytools` package [32]. We then used `contMap` in `phytools` to produce a continuous trait mapping of fang length: head length ratio to the phylogenetic tree.

We evaluated support for alternative environmental models of species' mean fang length on the full set of snakes for which we could pair fang and environmental data ($N = 186$) in order to assess potential environmental correlates with the largest global sampling possible. We used phylogenetic generalized least squares (pgls), as implemented in `nlme` [29]. We fitted four models of species' mean fang length (head length alone, temperature, precipitation and `envPC1`) with species mean head length as a covariate in all models. To account for phylogenetic uncertainty in these estimates, we ran all models iteratively across the posterior distribution of 200 trees from Alencar *et al.* [31] and compare them using model weights based on Akaike's information criterion (AICc).

Having established temperature as the best abiotic environmental predictor of fang length (see Results), we next modelled fang length in the smaller dataset of 71 viper species for which we had collated diet data using pgls, which can accommodate continuous predictors including proportions [39]. Both relative fang length and diet composition, as proportion data, were logit transformed to place the proportions in a continuous rather than 0–1 bounded space [40]. Bayesian multiplicative replacement of zero values with small positive values was

done using the `cmultRepl` function from the R package 'compositions' [41] to allow both logit transformation, as well as the calculation of the first and second principal components of the compositional diet matrix (henceforth `dietPC1` and `dietPC2`) using `pcaCoDa` in the `robCompositions` package [42] in 'robust' mode (electronic supplementary material, table S3).

Each of these pgls models consisted of relative fang length as a response, and head length and temperature as covariates. Three alternative pgls models included a single diet component: proportion amphibians, proportion mammals or proportion reptiles, as these taxa loaded most strongly on the `dietPC1` and `dietPC2` (electronic supplementary material, table S3) and were not populated largely with zero values as were proportion birds and proportion invertebrates. We also tested three functional models of diet. We tested a 'fluffy' model by summing the proportions of mammals and birds as we expect higher proportions of these prey may select for longer fangs that can penetrate to the underlying tissue. Since differential fang breakage in soft versus hard prey may be important to fang length evolution [10,11], we tested a model that summed the proportion of mammals, birds and amphibians, polarizing these soft-skinned diet items against those with hard protective coatings (chitin or scales). Our third functional model summed the proportion of mammals and invertebrates (primarily centipedes) as this model demarcates dangerous prey capable of chewing on the snake that is often released quickly post-strike (e.g. [43]). Finally, we tested models that included the `dietPC1` or `dietPC2` scores summarizing variation in the entire diet matrix. These alternative diet models were compared using the AICc, alongside models that included only head length or head length + temperature as a factor to determine if our top performing diet model provided a marked improvement over a model with size and climatic data alone.

3. Results

(a) Positive evolutionary allometry in viper fangs

We found a positive allometric relationship between fang length and head length across species ($\beta_{\text{pgls}} = 1.36$, $\beta_{\text{RMA-pgls}} = 1.47$, $R^2 = 0.85$, $\lambda = 0.88$, $p < 0.0001$), indicating evolutionary allometry in the relationship between fang and head lengths, such that fangs increase in size 1.36–1.47 times the rate of heads across species (figure 1a). The result of this allometry is relatively larger fangs in larger headed snakes, on average. We found little evidence for static allometry across the snakes in our sample, as the relationship between fang length and head length was not significantly different from 1.0 for 52 out of 54 species assessed (electronic supplementary material, table S4, electronic supplementary material, figure S3), and the 95% CI for the mean of these slopes included 1.0 (figure 1b). Notably, many of the smallest species' head sizes overlapped with the largest (e.g. *Lachesis* sp. and *Bitis gabonica*) at the upper and lower bounds of their head sizes, respectively (electronic supplementary material, figure S4), while relative fang lengths clearly remained distinct. Thus, proportionality of fang length to head length in adult vipers appears to be mostly constant within species, while diverging among species.

(b) Phylogenetic patterns of relative fang length evolution

Our measurements of individual viperid relative fang length showed high repeatability ($R = 0.71 \pm 0.04$; electronic supplementary material, methods) indicating that species' means are informative for comparative analysis. Relative

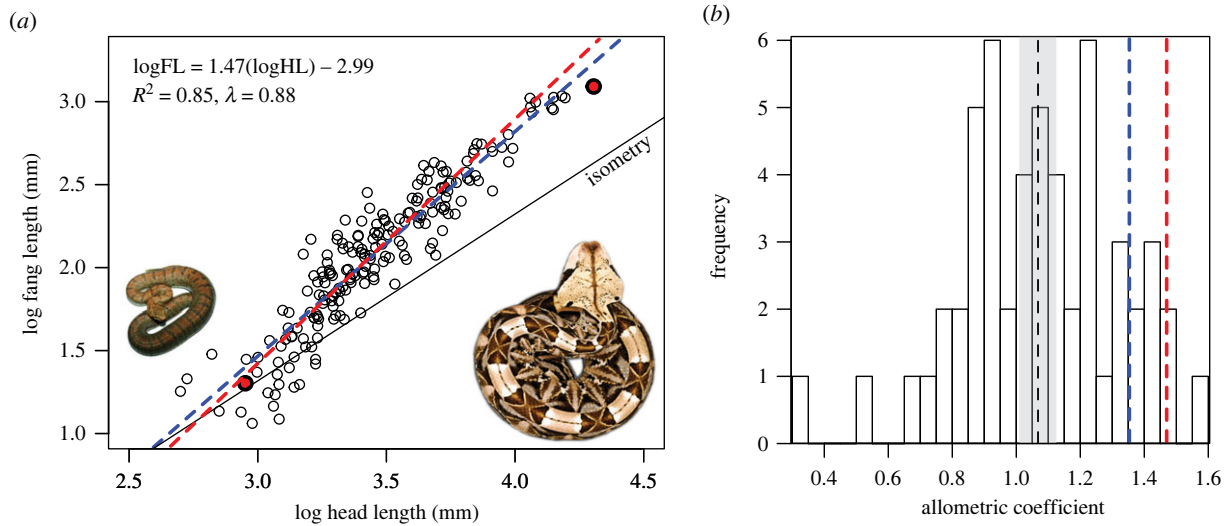


Figure 1. (a) Allometric relationship between log-transformed fang length and log-transformed head length across 199 viper species. Linear fits of both standard pgl's with a λ correlation structure (blue dashed line) and RMA pgl's (red dashed line) regression are shown. Regression equation corresponds to the RMA pgl's model fit. The black solid line shows a hypothetical isometric relationship intersecting the x -axis at the same place as the empirical relationship, to highlight the positive allometry. Large red points correspond to pictured *Crotalus transversus* (left) and *Bitis gabonica* (right) highlighting 'small' and 'large' species representatives, respectively. (b) Distribution of slope coefficients for the 54 species where greater than 10 individuals were measured. Relationships within species were not significantly different from one in 52 of 54 tests. Vertical black line corresponds to mean slope estimate of the 54 species. Shaded area indicates 95% C.I. Blue and red vertical lines correspond to the interspecific pgl's slope estimates from (a). (Online version in colour.)

fang length in vipers showed phylogenetic signal (figure 2; electronic supplementary material figure S5 accommodates visual diversity). Both Pagel's λ ($\lambda = 0.84$, $p < 0.0001$) and Blomberg's K ($K = 0.23$, $p = 0.003$) confirmed significant phylogenetic signal for relative fang length across vipers. A value of λ closer to one is reflective of the clade specific patterns of small, medium and large relative fang sizes. Relative fang length varied from only 13.6% of head length in the African amphibian specialist *Causus lichtensteini* to 37.1% of head length in the South American generalist feeder *Bothrops taeniatus*. Relatively short fangs characterize the genera *Causus*, *Vipera*, *Cerrophidion*, *Mixcoatlus*, *Gloydus* and the small montane *Crotalus*, whereas relatively long fangs characterize the genera *Lachesis* and *Protobothrops* and the subclade of *Bothrops* containing species such as *B. asper*, *B. atrox* and *B. jararacussu*. A Blomberg's K of 1 corresponds to perfect Brownian evolution along the tree, and the value of 0.23 for viperid relative fang length suggests the presence of within-clade variation in fang sizes, as is exemplified across *Bitis*, *Trimeresurus*, *Crotalus* and *Bothrops*. Within-clade variation at this level suggests potential adaptive divergence [37] in viper fang length among close relatives. To evaluate support for adaptive divergence in fang lengths, we quantified viper diets and assessed correlations between relative fang lengths, climate and taxonomic diet composition.

(c) Climatic correlates of fang length evolution

Model comparison of putative environmental predictors revealed environmental correlates of fang length evolution with small but detectable effects. The best-supported environmental model of species' mean fang length included species' mean head length ($\beta = 0.34$, $T = 37.7$, $p < 0.001$) and temperature ($\beta = 0.051$, $T = 2.7$, $p = 0.0086$, $R^2 = 92.5\%$; figure 3a,b). This model had the highest AIC model weight across the full posterior distribution of phylogenetic trees used for pgl's replicate runs. The correlation between

warmer temperatures and longer fangs is visible in figure 3b in the tendency for the darker red points to fall above the best-fit line relating fang length and head length. Unsurprisingly, the most important predictor of species' mean fang size in these models is mean head length, explaining $R^2 = 91.5\%$ of the variation in fang length in a pgl's model with head length as the sole predictor. After accounting for this size effect, our model shows that for each 1°C increase in mean annual temperature, fang length increases by 0.051 mm, meaning snakes in the warmest areas will have 1.4 ± 1.0 mm longer fangs than those in the coolest areas, on average, or around 16 per cent of the length of an average-sized fang. Therefore, environmental variables explain a rather small but detectable amount of fang length variation.

(d) Dietary correlates of fang length evolution

Viper diets, as reported in the literature, varied extensively in this diverse group of snakes, including species that feed solely on mammals, amphibians or reptiles, to generalists that feed on prey from all six categories (electronic supplementary material, table S5). Piscivory was recorded in our collated diet dataset for only two species.

Diet information improved models of fang length variation among vipers. Across the full posterior distribution of trees from Alencar *et al.* [31], adding diet terms consistently improved model fit relative to the HL-only model (best in runs for 0/200 trees) and the HL + temperature model (best in 1/200 trees). There was some variability in which diet model was best across the posterior distribution of trees, which appeared to be a result of similar amounts of variance being partitioned to models where the proportion of mammals played a strong role. For example, the dangerous prey model is largely defined by the per cent mammal value, since invertebrates were comparatively rare in the dataset. The best model of fang length for the majority (54%) of trees included only the mammal portion of the diet (median Akaike weight =

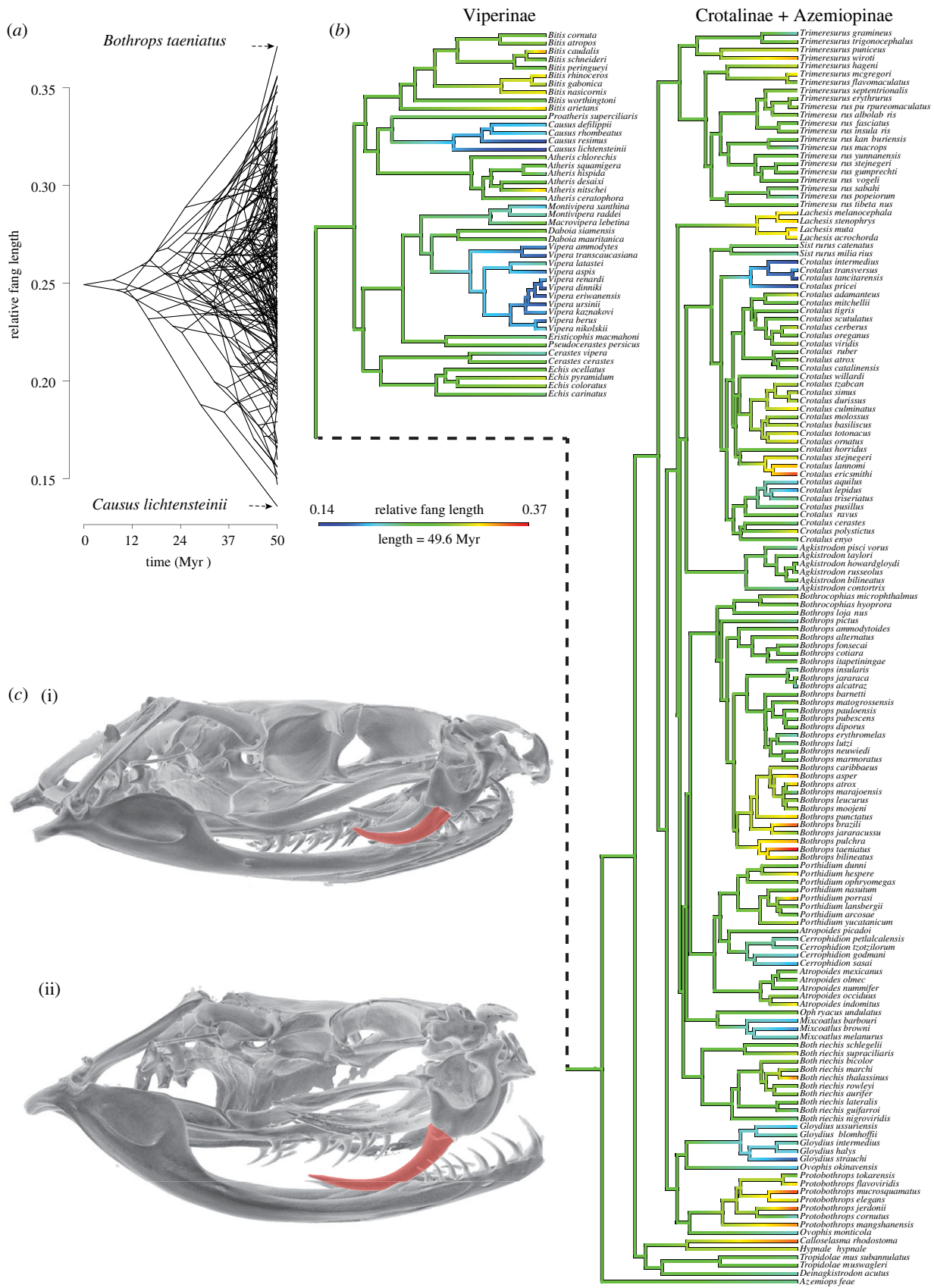


Figure 2. (a) Phenogram of relative fang length evolution across vipers. (b) Continuous trait mapping of relative fang length evolution on the best time-calibrated phylogeny identified by Alencar *et al.* [31], pruned to 199 species present in our fang data. Warmer colours indicate longer fangs relative to head length. (c) Representative CT scans of two *Crotalus* species with (i) short (*C. lepidus*) and (ii) long (*C. simus*) relative fang lengths with fangs shaded red. Skull images were provided by G. Fuentes and D. Paluh. (Online version in colour.)

53%; figure 3c), whereas other means of parsing the diet data produced models that tended to perform more poorly. The second and third best-performing models were the dangerous

prey model (14% of trees) and fluffy prey model (12%). Since these outperform the mammal model only occasionally, and their proportions are highly correlated with the proportion of

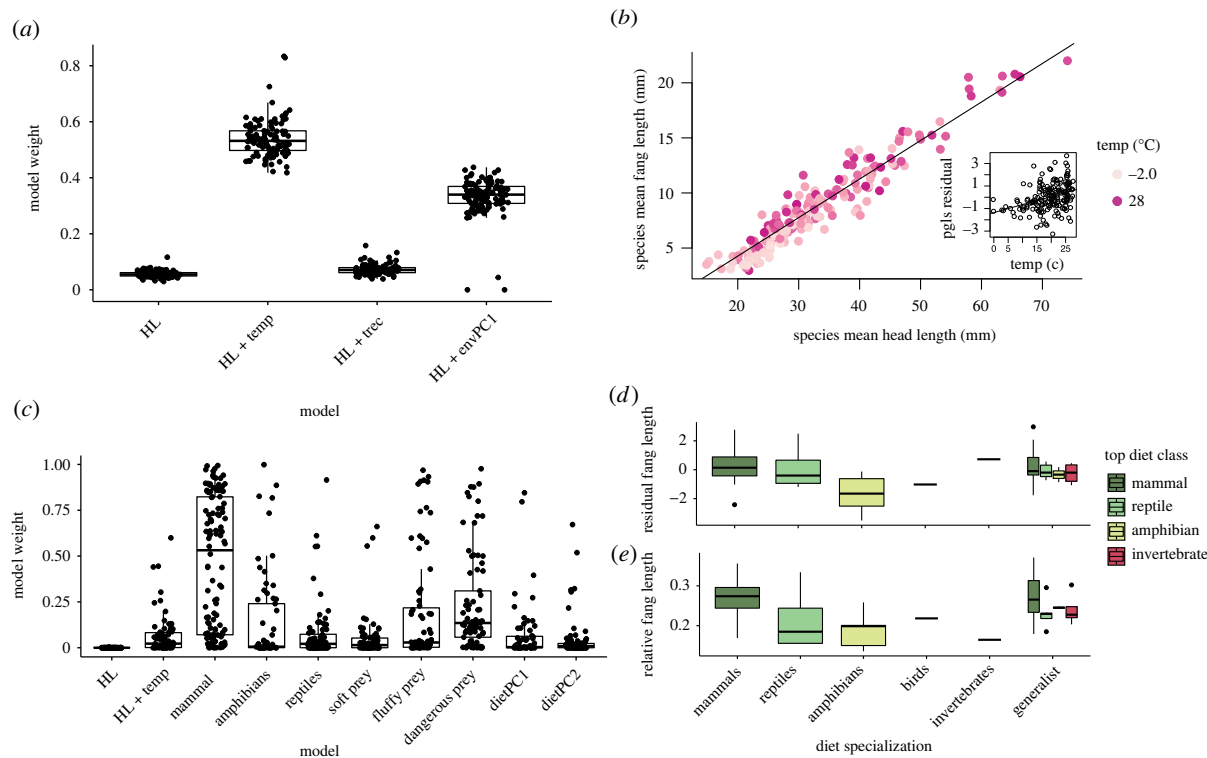


Figure 3. (a,c) Distribution of model weights from AIC model comparisons for each (a) environmental model or (c) diet model of fang length analysed using the posterior distribution of trees of Alencar *et al.* [31]. (b) Scatterplot of fang length versus head length with temperature as a colour gradient for the plotted points, and an inset showing the pgl's residuals of fang length versus head length across temperature. Finally, (d) residual and (e) relative fang length are plotted versus the most abundant prey type found in each species's diet. (Online version in colour.)

mammals (electronic supplementary material, figure S7), these results are not easily distinguishable from the role of mammals alone. Other models were rarely competitive as the top models for a given tree (figure 3c; electronic supplementary material, table S6).

We next centred and standardized the HL, temperature and logit-transformed mammal proportion of the diet so that we could compare effect sizes among variables across the full posterior distribution of trees from Alencar *et al.* [31]. The standardized regression coefficient is largest for head size (median = 3.84), followed by temperature (0.74), and then proportion mammals in the diet (0.50; electronic supplementary material, figure S8). Back transformation of the logit-transformed coefficient revealed that, controlling for head size and temperature, we expect fangs in snakes that do not eat mammals to be smaller by between 1.3 and 1.8 mm compared to snakes that feed solely on mammals, which would amount to approximately 18% of the length of an average fang. As a *post hoc* assessment, we classified each viper species by the most abundant prey category in its diet, considering any snake where a single taxonomic group made up more than 70% of the diet a specialist, and classifying others as generalists. Dietary trends in fang length evolution showed that vipers feeding predominantly on mammals have longer fangs (when controlling for other factors) than species feeding on other taxa, particularly amphibian-eating taxa. These trends are repeated both in the mammal specialist group and again within the generalists when categorized by the most abundant taxa in their diets (figure 3d,e; electronic supplementary material, figure S9). Taken together, our assessment of correlations between snake ecology and fang length suggests that living at

warmer temperatures and feeding predominantly on mammals is associated with longer fangs.

4. Discussion

The fangs of vipers are highly specialized dental structures, injecting venom from a duct that passes through the maxilla and empties into the top of the hollow fang. Despite the function of puncture and injection of venom seemingly being simple, fangs vary extensively in shape and length [10,11,27,44]. Our work quantitatively assessed several potential correlates of fang length evolution to provide insights into the adaptive significance of this key functional trait linking predator to prey. We found clear evidence for positive evolutionary allometry, as well as abiotic and biotic correlates of fang length that suggest adaptive variation. Cundall [27] suggested the use of natural history information to explore the functional roles of fang length variation in vipers. Our study represents this kind of integration of densely sampled morphological, phylogenetic and ecological information, revealing trends that highlight fang morphology as subject to divergent natural selection pressures as snake species vary in both size and ecology.

(a) Evolutionary allometry of viperid fangs

Pough & Groves [19] were the first to show evidence for positive evolutionary allometry in the relationship between fang and head length using a dataset of 30 viperid species, but their analyses included a small proportion of described species and did not account for phylogenetic non-independence due to shared ancestry. Our increased species

sampling of 199 species and phylogenetically informed assessment of the allometric slope coefficient leads to a reduced estimate of the allometric coefficient (1.47 in our study versus 1.77 for [19]), but one where fangs still grow nearly 1.5 times faster than heads over the range of head sizes in our study. Furthermore, we use intraspecific regressions to estimate the slope coefficient across the range of adult body sizes to show that much of the pattern in our data is specifically attributable to evolutionary allometry, with fixed patterns of relative fang length variation between species, and not an extension of conserved trajectories of static allometry [44,45]. The result of the allometric trajectory across species is exaggerated fang size disparity between the smallest and largest headed viperid species. As they are explicitly involved in trophic interactions, viper fangs provide an example of a trophic trait showing positive evolutionary allometry resulting from natural selection.

Larger headed snakes will also be those that eat the largest prey, as prey size and snake head size are correlated [14,15]. While prey are not relatively more massive for large snakes [16], a prey animal of twice the linear dimensions of a smaller individual of the same shape will be eight times more voluminous. Larger prey therefore contain more internal tissue upon which venom must act and may simply require a deeper bite to allow rapid spread of venom in prey.

(b) Climatic and dietary correlates of fang length evolution

Pough & Groves's [19] observation that tropical snakes tended to have larger fangs led us to consider potential abiotic environmental predictors of fang length. Though their observations held, in that vipers living in warmer environments tended to have the largest fangs relative to body size, this effect size is small explaining differences in fang length of about 16% for an average-sized fang. Still, this observation represents a rare documentation of climate predicting functional trait variation in a predator guild.

The abiotic environment appears to impact body size in all major taxa preyed upon by vipers, although the specific relationships are taxon specific. For example, centipedes tend to reach their maximum sizes in warmer areas [46]. Frogs and toads are largest in the warm tropics, while overall trends in body size are best predicted by clines in the potential for desiccation [21]. Small rodents tend to be largest where primary productivity is highest, which includes tropical areas, and generally are larger at warmer temperatures [22]. The taxonomic specificity of the relationship between climate and species' body size might form the basis of the small effect of our measured variables on fang size. One caveat of our approach is that the concentration of only certain genera in the tropics could mean the small correlations observed here are due to uncontrolled phylogenetic effects. Dense geographical sampling of wide-ranging tropical taxa such as *Bothrops* sp. may help resolve this in future work.

Comparing taxonomic and functional models of the relationship between fang length and diet composition supported the contribution of mammals alone as the best diet model of species' fang length evolution. The effect of feeding on mammals equates to modest increases of approximately 18% fang length for an average length fang. Notably, this variation exists in addition to the allometric component of fang length variation, which is inextricably associated with

diet since larger snakes tend to eat more mammals. The clearest distinction of fang length by diet occurs when comparing mammal-eating to amphibian-eating viperids. Amphibians represent the most extreme differentiation from mammals in terms of prey morphology, as amphibian skin is unprotected by layers of either fur, feathers, scales or chitinous armour. Taken together, shorter fangs in amphibian-eating snakes suggests evolution from longer to shorter fangs when specializing more on amphibians, potentially due to combinatorial selection from thinner integuments and a bite-and-hold prey handling behaviour [47].

Mammals also present a prey morphology characterized by a low average surface area to volume ratio, which may be expected to favour longer fangs regardless of taxonomic affinity. Lizards and centipedes are very common in many viper species' diets and are more elongate prey compared to mammals. When feeding on elongate prey, relatively short fangs will penetrate to the deepest layers of prey tissues for intra-muscular or intra-coelomic injection of venom, whereas relatively longer fangs may be needed for the deepest possible injection into large rodents, rabbits or the largest-bodied lizards and anurans. Short fangs may also be less likely to break when meeting tough arthropod exoskeletons or reptile scales [10,11], or during extended struggles with bird, reptile, amphibian or arthropod prey that are often bitten and held until death [47,48]. Finally, longer fangs may assist in making contact with more evasive mammalian prey (e.g. [49]), as occurs with the evolution a jaw mobility in some fishes [50,51]. The evolution of fang length based on taxonomic diet composition may therefore stem from a complex suite of these putative selective forces, of which none are mutually exclusive [11].

Previous work has jointly emphasized the ability of snakes to dynamically reposition fangs during a strike [27,52] and the stochastic elements of the strike that often lead to very frequent off-target fang placement in prey tissue [27,53,54], calling into question a selectionist view of fang length evolution. Yet, if fangs evolved neutrally, we would not expect fang morphology to covary with diet, as we show here. On average, deeper injection of venom is more lethal [55], so relatively wide prey might indeed exert selection on fangs, where a difference of 1 mm in length makes injection of venom in deeper tissues layers more likely. It is also important to note that fang curvature has demonstrated functional significance in strike kinematics [44,56], and this feature of fang shape might also interact strongly with prey morphology and envenomation strategy, representing a crucial examination for future work. In particular, curvature may delimit short-fanged reptile specialists from short-fanged amphibian specialists, given that stout, curved fangs can reduce breakage [10]. Generally, functional studies of penetrance and breakage of differently shaped fangs will be crucial to a deeper understanding of the nature of selection on fang shape and the function significance of variation when biting different prey taxa.

Broadly, our results suggest that snake fang length may present an axis for trophic diversification of snakes in a phenotype that forms the physical interface of predator-prey interactions. Our correlative results lay out a continuum of relative fang lengths that have evolved repeatedly alongside shifts in life history (e.g. smaller fangs and amphibian eating in *Causus*, *Ovophis* and *Gloydus*). Predator and prey traits have been best studied in the context of coevolution, where trait matching hypotheses for continuous traits provide the

highest opportunity for the generation and maintenance of trait diversity, as compared to 'bigger is better' arms-race dynamics, which often reduce phenotypic diversity [57]. While we lack the detailed information on prey shape that is required to liken snake fang length to the diversity in hummingbird bill length as it relates to flower length, our results support complex natural selection on fang length associated with overall head size and dietary ecology.

Data accessibility. All data have been made available either in the manuscript or as electronic supplemental material [58].

Authors' contributions. M.L.H.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, visualization, writing—original draft and writing—review and editing; V.C.T.: data curation, investigation, resources and writing—review and editing; O.Z.: conceptualization, data curation, investigation, resources and writing—review and editing; J.L.S.: conceptualization, data curation, investigation, methodology, resources and writing—review and editing; R.M.R.: data curation, formal analysis, investigation, methodology, resources and writing—review and editing; A.J.M.: data curation, formal analysis, methodology, visualization and writing—review and editing; M.P.H.: data curation, methodology, resources and writing—review and editing; C.L.P.: data

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All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. The authors declare they have no competing interests.

Funding. This work was funded by National Science Foundation Post-doctoral Fellowship in Biology: Use of Biological Collections grant no. 1711141 to M.L.H.

Acknowledgements. We are grateful for extensive comments from Erich Hofmann, and to the curatorial staff at the Museum of Vertebrate Zoology at Berkeley, Illinois Natural History Survey, California Academy of Science, Los Angeles County Museum, Arizona State University, Florida Museum of Natural History, UT Arlington Amphibian and Reptile Diversity Research Center, V. N. Karazin Kharkiv National University Museum of Nature and the Instituto Butantan for facilitating the use of their collections.

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