

Venom evolution in snakes is driven by trophic and macroecological factors.

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This is just an example to fill in space Snake venom is well known for its ability to incapacitate prey. This property of snake venoms is the source of not only interest in its biomedical uses but also results in the major it a source of both novel biomedical compounds and as a health concern. However, despite this snake venom the evolutionary drivers behind its ability to incapacitate prey is still poorly understood. Even the role of predation as a driver of venom evolution is under question due to the range of venom potency across species, ranging from the almost nonvenomous xxx to the inland taipan to incapacitate 100,000's mice, suggesting venom is effectually under neutral selection. Whatever this drivers, venom, like all tissues, is subject to the forces of evolution, whether this is as selection to incapacitate prey, reduce the energetic costs associated with its production or through neutral drift over evolutionary time. Using a dataset of over 200 measures of Ld50 values for over 100 snake species we support the importance of predation in driving venom evolution by showing that venom is more affective in incapacitating prey species phylogenetically closer to those found in a species diet. Further to this we show that venom

metabolic rate | macroecology | venom | body mass | scaling

Introduction

Snake venom is perhaps best known for its ability to incapacitate biological systems, a property which has made it both a source of novel biomedical compounds (1) and also a major health concern due to the estimated millions of human envenomation cases each year (2). However, snake venom is also an ideal system of predatory trait evolution (1), particularly as its predatory functionality, the ability to subjugate prey, can be quantified by measured venom potency and the quantity a species possess. Despite significant developments in our understanding of the evolutionary origin (3-5) and the subsequent evolution of its complex compounds (1, 6), surprisingly little is known about the evolutionary pressures that drive this ability to incapacitate prey. For example, many species, such as the Gold-ringed cat snake (*Boiga dendrophila*), are incapable of subduing laboratory test models (7) while other species, such as Russel's viper (*Daboia russelii*), possess enough potent venom to incapacitate hundreds of thousands of potential prey items (8). This lack of a clear pattern relating to the ability to subdue prey using venom suggests drivers assumed to be important in driving predator trait evolution, such as trophic factors, are less important than expected. One potential explanation for this lack of a pattern is that weak selective pressures are caused by an "overkill" effect, were due the capacity of many species venoms to kill prey numbers far in excess of any biological requirement, such as in the case of Russel's viper, neutral evolutionary process becoming the main driver of trait variation (9, 10). Such a scenario predicts that traits relating to venom, such as potency and quantity, would be idiosyncratic in nature while also raising the question of the general importance of ecological factors on the evolution of predatory traits. Here, we use a comparative approach to test whether snake venom traits follow idiosyncratic patterns expected from neutral selection or patterns predicted from macroevolutionary and trophic theory.

As predation plays a key role in both ecosystems dynamics (11) and the evolution of the species within these ecosystems (12) much attention has been given to understanding the general patterns related to these trophic interactions and the drivers behind them. These include macroecological patterns derived from body mass and habitat complexity which predict allometric scaling relationships for traits underlining trophic interactions such as search and encounter rates (13-15); the ability to spot and track prey (16, 17); and ingestion rates (15, 18). Other general predictions for predatory traits stem from the co-evolution between both predators and their prey, were arms race dynamics may drive the evolution of predatory traits that increase the ability to capture prey and conversely the evolution of traits in prey species that increases their ability to escape (19, 20). While these two general drivers are likely to have important influences on trophic trait evolution they can be difficult to decouple as predatory traits are often difficult to quantify and compare across the large number of species required for macroecological studies. Snake venom however, offers a primarily predatory system which can be quantitatively compared across a large group of diverse species allowing the importance of both trophic and macroecological factors on its evolution to be tested.

Predatory foraging traits are expected to be adapted towards their main target prey species. For example, jaw morphology in cichlid fish is strongly selected by factors such as prey type (21). Similar selection pressures from prey type is also seen in snake venom. For example, a switch in diet from fish to eggs has resulted in the almost complete atrophy of the venom apparatus in the egg

Significance

Snake venom is best known for its ability to kill prey, a property that both makes it of biomedical interest and a public health concern. However, snake venom also offers a novel opportunity to understanding the evolution of predator traits. As a predator trait venom can be quantified by measuring its potency and quantity allowing the importance of trophic drivers, such as predators-prey arms race dynamics, and macroecological drivers, such metabolic scaling and habitat dimensionality, on its evolution can be tested. Here, using comparative analysis we show that trophic drivers result in venom evolving higher potency towards species closely resembling their natural diet and macroecological drivers shape the quantity of venom available due to habitat dimensionality and metabolic constraints.

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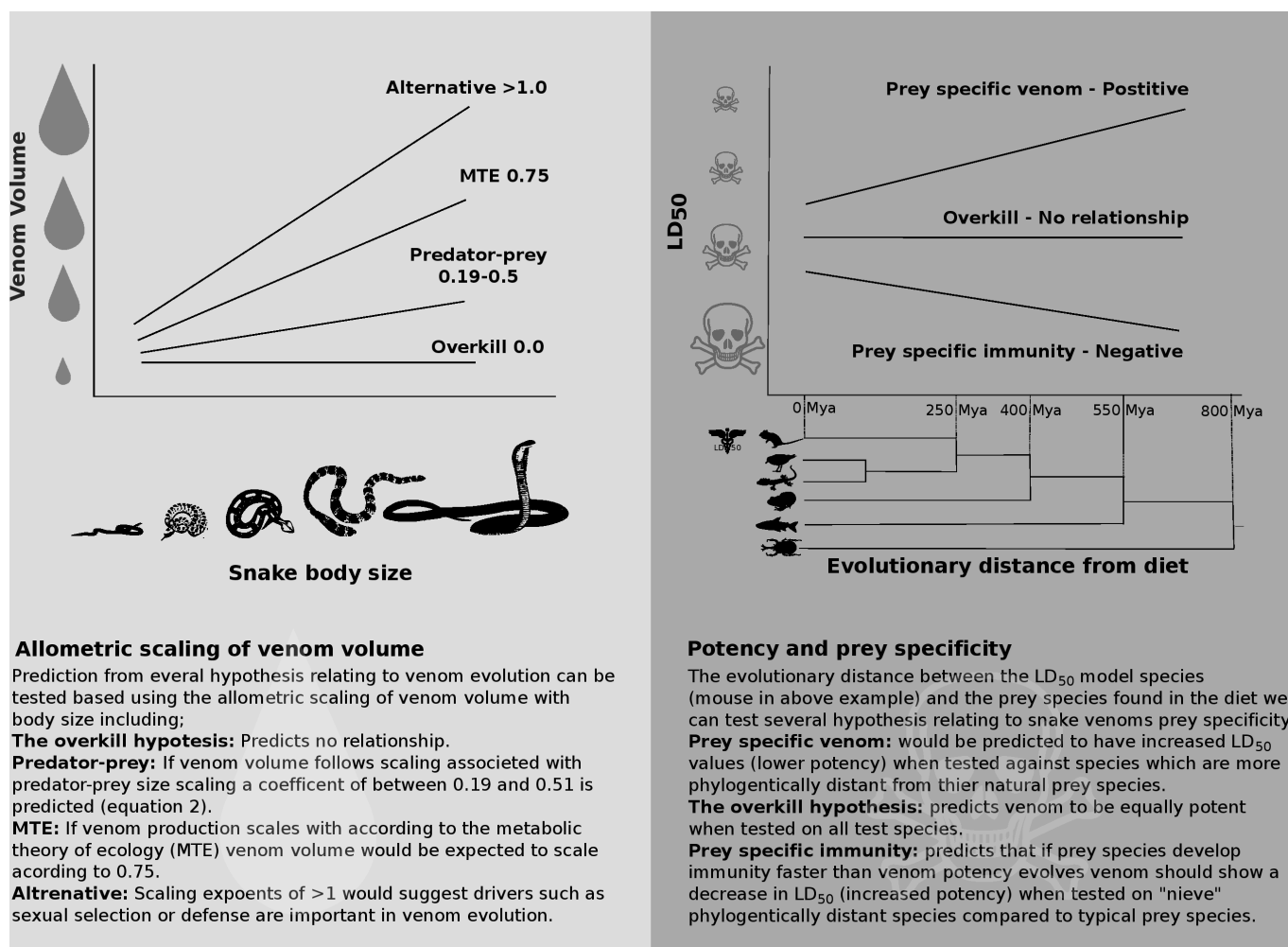


Fig. 1. Box 1. Summary of hypothesis relating to the evolution of both snake venom toxicity and volume.

eating marble sea snake *Aipysurus eydouxi* (22), demonstrating the importance of predation in venom evolution. Further such trophic selection is also seen in cases of prey-specific venoms where potency is higher when tested on species commonly found within the snakes diet, such as observed in Malayan pitvipers (23), coral snakes (24), the viper genus *Echis* (25), saw-scaled vipers (26) and insect eating *Pelias* vipers (27). However, such co-evolution is not apparent in all snake species, such as in tiger snakes where variation in venom across populations is independent of dietary differences (28). Furthermore, prey species can also respond in such co-evolutionary arms races through the development of immunity. Evidence for this is seen in venoms that show weaker potencies in potential prey items such as in the cases of Opossums and Neotropical pitvipers (29); eels and *Laticauda colubrine* (30); and between ground squirrels and rattlesnakes (31, 32). This idiosyncratic pattern of some species displaying prey-specific venom while others do not may mirror a pattern predicted from the Overkill hypotheses where neutral processes are the main driver of venom potency (10). However, many measures of venom potency are not typically tested on natural prey species leading to the question of whether these examples represent an idiosyncratic pattern expected under weak selection or whether a general pattern will emerge under appropriate analysis (ref). While measures of potency using natural prey species are becoming more common, these are taxonomically restricted making large comparative analysis difficult (24, 25). Here we account for the species that a venom's potency is tested on and measure the

phylogenetic distance between this species and the prey species within the snake's diet which, in turn, allows us to test whether venom is generally adapted towards common target prey species. However, while venom potency is an important aspect of these species ability to incapacitate prey, the volume of venom and the role of other macro-ecological drivers are also likely to be important when considering venom evolution.

The ability to subdue prey is not only dependent on the potency of a species venom but also the amount of venom available. As such, venom yield is also expected to be under positive selection from potential trophic and macroecological factors. As venom production incurs a metabolic cost (33) (although the level of this cost is debated (34)) and requires storage, the volume of venom a snake species can produce is likely to be heavily linked to one of the strongest determinants of trophic ecology; body size. In general, larger predators eat larger prey (18). If venom is under selection for its ability to kill prey it would be expected that larger snake species would need to produce larger quantities of venom to keep pace with the subsequent increased in prey size. Such an increase of venom yield to compensate for larger prey is supported by the metering of venom in response to prey size seen in several species (35, 36). However, while bigger snakes are known to have larger amounts of venom in general (37) it is not known whether venom yield scales interspecifically according to any general pattern. One prediction is that venom yield increases with snake body size with an exponent relating to their predator-prey body size scaling as described in equation 1;

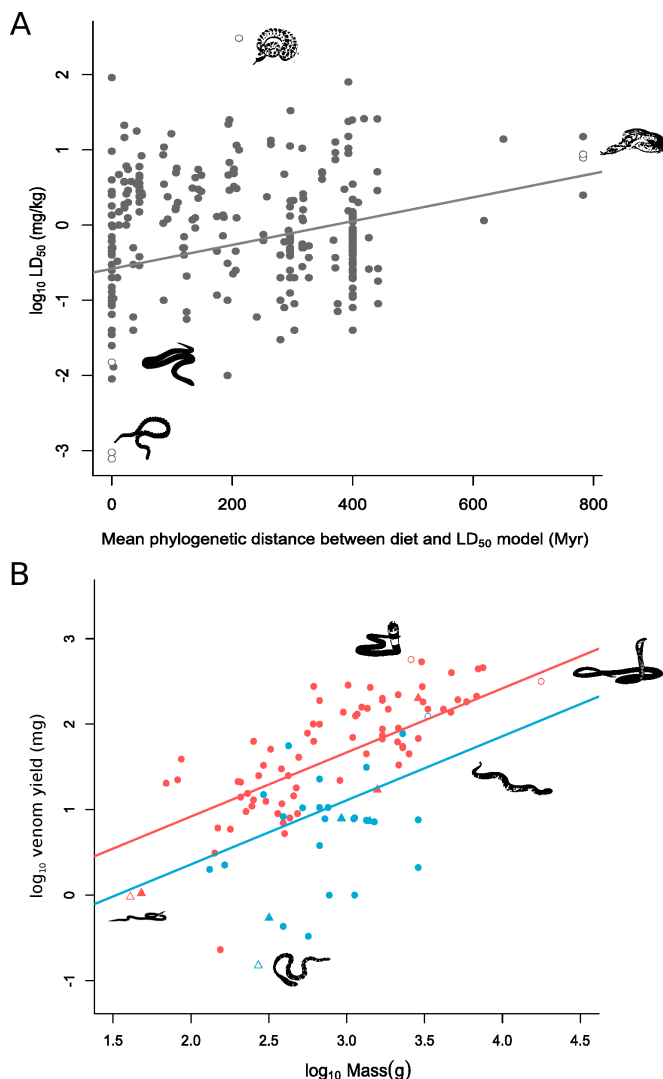


Fig. 2. (A) Mean phylogenetic distance between diet species and LD50 model (Myr) against log₁₀ LD₅₀ (intercept = -0.58, slope = 0.002). Hollow points represent silhouette species which are from left to right; *Bungarus multicinctus*; *Oxyuranus microlepidotus*; *Echis carinatus*; *Causus rhombeatus*. (B) Relationship between log₁₀ mass (g) against log₁₀ venom yield (mg). Red points and fitted line (intercept = -0.58, slope = 0.75) represent species in 2D habitats and the blue points and fitted line (intercept = -1.14, slope = 0.75) represent species in 3D habitats. Hollow points represent silhouette species which are from left to right *Atractaspis bibronii*; *Emydocephalus annulatus*; *Naja melanoleuca*; *Agkistrodon piscivorus*; *Ophiophagus hannah*. All intercepts and slopes are back transformed from the values in Table 1.

$$M_{prey} \propto M_{predator}^a$$

Were for snakes the scaling exponent a is approximately 0.88 (18). However, venom yield would not be expected to scale according to this exponent as the effects of toxicological agents also follows an allometric relationship (38) were the amount of venom required (V) to induce the same incapacitating effect on a prey of mass (M_{prey}) would be expected to follow (equation 2);

$$V \propto M_{prey}^b$$

were b is the scaling coefficient of venoms toxicological effects (38). Hence to calculate the expected allometry of venom yield with snake body mass in a case were yield increases at a rate to

match predator-prey size scaling after accounting for scaling of toxicological effects we substitute $M_{predator}^a$ from equation 1 for M_{prey} to get (equation 3);

$$V \propto M_{predator}^{ab}$$

If we take the commonly used value of 0.75 for the interspecific scaling of drug dosages (38) for b and the value of 0.68 for a from the scaling predator-prey mass relationship for snakes (18) we would expect a scaling exponent of approximately 0.51 between snake venom yield and snake mass. Other predictions include the overkill hypothesis which predicts no relationship between venom yield and prey size, or alternatively venom yield may scale according to constraints such as metabolic costs, were a scaling of 0.75 would be expected (39). At the other extreme superlinear allometries would suggests patterns associated with drivers such as sexual selection, such as proposed by the weapons hypothesis (40), or defenses requiring increased effectiveness with size, such as seen in the allometry of horn growth in horned lizards (41) (Figure 1).

Finally, an overlooked feature that may also drive the evolution of both venom quantity and toxicity is habitat structure (42). The structural complexity of a habitat, such as whether it's a 2-dimensional terrestrial surface or a complex 3-dimensional forest canopy, can influence both encounter rates (14, 18) and the escape rates of prey, with higher dimensional spaces increasing both (43, 44). Hence predators in high dimensional habitats with associated increased escape rates may compensate through larger yields of more potent venom in order to increase capture rates. For example, strike and release behaviors may be less successful in either 3-dimensional arboreal or aquatic environments requiring higher toxicities to incapacitate prey quickly. Conversely there may be less of a requirement for high potencies and large reservoirs of venom due to increases in encounter rates, and hence feeding opportunities, in high dimensional habitats which may compensate for possible increases in escape rates.

Here we test the importance of these multiple potential drivers of both venom quantity and potency in a phylogenetically corrected comparative analysis of ninety-nine species of venomous snakes. We test the importance of snake body mass; habitat dimensionality; prey type; and prey size on the variation of both venom toxicity, as measured using median lethal dose (LD₅₀), and average total venom yield. We achieve this by using a novel metric of the evolutionary distance between the model animal on LD₅₀ is measured and the typical species found in each snakes' diet in order to test the general pattern of snake venom prey-specificity which predicts higher potencies when tested on species phylogenetically close to natural target prey. This approach also allows us to control for the variance associated with the LD₅₀ model used and hence test the general influence of macroecological factors.

Using these corrections and a series of models accounting for phylogenetic similarity between snake species we test; (1) the overkill hypothesis: that there is no relationship between venom yield or toxicity with prey size; (2) the importance of trophic drivers on venom evolution including; that venom potency is higher (lower LD₅₀) when tested on model species phylogenetically closer to species found in the diet; and that snake species which include eggs in their diets have lower venom potencies (higher LD₅₀) or yields; (3) the importance of macroecological drivers on venom evolution including; that venom quantities are higher in species with larger prey, with a predicted scaling of approximately 0.51 or that (4) yield scales according to 0.75 as expected if metabolic cost is the main driver; (5) the importance of habitat dimensionality on venom evolution, in particular that; species in high dimensional habitats show either higher or lower potencies or venom yields depending on encounter and escape

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Table 1. Estimates and higher and lower 95% credibility intervals (CI) for LD₅₀ and mean volume. Fixed factors include mass; LD₅₀ method (subcutaneous (SC), intravenous (IV), intrapulmonary (IP) and intramuscular (IM)); habitat dimensionality (Dim- 2D and 3D); Presence of eggs in diet (Eggs in Diet) and the mean phylogenetic distance between diet species and the LD₅₀ model (Diet-LD₅₀ Dist). The random terms and the co-variance (CV) between LD₅₀ and volume are also presented. The model was run with 12,000,000 iterations with a 2,000,000 burn-in and a thinning of 5000.[AJ1]

LD ₅₀				Mean Volume		
	Estimate	Lower CI	Upper CI	Estimate	Lower CI	Upper CI
Fixed Terms						
Intercept	0.200	-0.161	0.567	0.200	-0.161	0.567
Mass	0.134	0.016	0.262	0.510	0.442	0.564
LD ₅₀ method _{SC}						
IV	-0.624	-0.842	-0.435	-0.011	-0.052	0.030
IP	-0.537	-0.746	-0.309	-0.010	-0.049	0.033
IM	-0.228	-0.455	0.049	-0.009	-0.056	0.042
Dim _{2D}						
3D	-0.202	-0.670	0.243	-0.829	-1.286	-0.396
Eggs in Diet	0.448	-0.162	0.021	-0.741	-1.325	-0.206
Diet-LD ₅₀ Dist	0.360	0.248	0.463	-0.003	-0.029	0.019
Random Terms						
Phylogeny	0.909	0.479	1.452	0.456	0.145	0.847
Phylogeny CV	-0.003	-0.290	0.301	-0.003	-0.290	0.301
Species	0.055	0.001	0.170	0.308	0.156	0.462
Species CV	0.030	-0.040	0.118	0.030	-0.040	0.118
Residuals	0.268	0.215	0.328	0.009	0.007	0.011
Residuals CV	0.003	-0.004	0.011	0.003	-0.004	0.011

[AJ1]big run!
[AJ1]big run!

rates; and finally we test (6) other potential drivers such as sexual selection and the weapons hypothesis which predict superlinear scaling of venom yields. We show that both trophic and macroecological factors are important in driving venom evolution with patterns supporting prey-specific venom in general and venom quantity scaling as predicted by metabolic cost constraints.

Results

Our final compiled dataset of venom traits and corresponding trophic and macroecological data consisted of 275 observations over 99 species which corresponds to the data used in the main analysis. We also conducted supplementary analysis where we included whether species to are known to using constricting behaviors; the inclusion of habitat type and finally an analysis including prey body size which was conducted using a reduced dataset of 177 observations across 68 species. We report the results across all models relating to the importance of each driving factor in order below.

Predator-prey coevolution

Despite the presence of only eight egg eating species in our dataset LD₅₀ was found to be significantly higher in species with eggs in their diet in both the main and constriction models, while venom yield had a negative, but non-significant, association with ovivorous behavior in all models (Table 2; Tables A2-4).

Of the species included within the analysis only 14 species had a diet completely matching that of the LD₅₀ model their venom was tested on, i.e. the LD₅₀ of a species with a diet including 100% mammals tested using a mouse model. Most species in the dataset had a diet with a least some component not matching the LD₅₀ model as reflected by a median evolutionary distance of 211.3 million years between the common ancestors of the dietary taxa and the LD₅₀ species. In all models species LD₅₀ increased with mean phylogenetic distance between the diet and the LD₅₀ model with snake species with diets phylogenetically close to the LD₅₀ model species hence having higher potencies (table 2; tableA2-

3; Figure 1B). From the main model, after back transforming the mean centered log₁₀ value, LD₅₀ was found to increase by 1.44 for every 100 million years between the species in the diet and that of the LD₅₀ model. This was after correcting for LD₅₀ injection route were intravenous and Intraperitoneal routes were found to have lower LD₅₀ values in comparison to a subcutaneous route (table 2; tableA2-3).

Macroecological drivers; body size and habitat dimensionality

The mean yield of venom, as measured using dried weight, ranged from 0.15 mg in the egg-eating sea snake (*Emydocephalus annulatus*) to 571 mg in the forest cobra (*Naja melanoleuca*). Body size, prey size and habitat dimensionality all correlated with yield. The main correlate with average yield was snake body size with a log-log scaling of between 0.74 and 0.76 across all models after back transforming (Table 2, A2-4; Figure 1). Venom yield also showed a positive increase with prey body mass, with a log₁₀-log₁₀ increase of 0.139, however only 90% of the posterior samples are above the zero threshold (Table A2). In the model corresponding to equation 2, which only included venom yield and prey mass, we found a significant exponent of 0.37 (Table A5) while in the model corresponding to equation 1 of predator prey body mass scaling we found an exponent of 0.52 (Table A6). Whether using the scaling exponent of 0.51 as calculated in equation 2 or using an exponent of 0.19 as calculated using a value of 0.37 found here between yield and prey size for *b* in equation 2 and the value of 0.52 found here between prey and predator scaling for *a* in equation 1, both values are far lower than the exponent of 0.75 between venom yield and snake body mass found across all models (Table 2, A2-4; Figure 1). Snake body size was also found to have a significantly positively correlation with LD₅₀, meaning larger snakes showed decreased potency (Table 1). However, this relationship was only significant in the main analysis (Table A2-3).

The next most significant driver of venom yield was the dimensionality of the habitat with the 27 species in high dimensional

environments (arboreal = 9, aquatic = 18) showing lower venom yields in comparison to species in lower dimensional habitats (Table 2). A sensitivity analysis were habitat was include as terrestrial, arboreal and aquatic also showed similar significant reductions in both arboreal and aquatic habitats (Table A4).

Phylogeny, constriction behaviour and covariance between venom yield and LD₅₀

In all models there is was an intermediate to high phylogenetic signal for both LD₅₀ and venom yield with h^2 values of 0.69 for LD₅₀ and 0.49 for venom yield (Table 2, A2-4). with LD₅₀ showing a higher phylogenetic signal in all models (Table 2, A2-4). The presence of constricting behaviour was found to have no effect on either yield or LD₅₀ (Table A3). Finally, there is no significant covariance between either the residuals of phylogenetic terms of both yield and LD₅₀ across all models (Table 2, A2-4).

Discussion

Predator traits are thought to be heavily shaped by both predator-prey evolutionary dynamics and macroecological forces such as imposed by the limitations of body size and habitat structure. Here we show that traits associated with snake venom follow patterns predicted from a scenario where these factors are important evolutionary drivers of predator trait. This includes that venom potency is prey specific, with higher potencies found when venom is testes on species more phylogenetically similar to a snakes natural diet, and that venom yield scales with snake body size according to an allometry expected if energetic costs of venom production are the main driver. We also find that snake venom is influenced, as expected by the presence of ovivorous feeding behaviours and, in a less expected direction, by the dimensionality of the environment. Hence, our results show that while aspects of neutral selection, such as genetic drift, play an important role in generated the observed variance in predator traits, positive selection forces associated with trophic and macroevolutionary drivers play a key role in shaping the evolution of these traits.

Trophic factors have been known to play an important role in the maintenance of venom. This is supported by cases of its evolutionary loss with dietary changes that no longer require venom (22), the absence of an increased lifespan associated with venomous as generally seen in species with toxic defence systems (45), and also by our findings here that ovivorous feeding is associated with lower potencies and venom yield. However, the role of trophic drivers in shaping the subsequent evolution of venom has been much more hotly debated, in particularly relating to the generality of prey-specific venoms (1, 9, 10). By flipping the inconvenience usually associated with the use of non-natural test models for venom potency (24) we show that venom is generally prey-specific with higher potencies associated with species that more closely resemble a species diet. The prey-specific effect demonstrated in our analysis is also likely to underestimate the prey specific nature of venom in general due to our use of LD₅₀. While LD₅₀ measures the lethality of a venom, it is likely that venom is selected to simply subdue prey, as opposed to cause mortality, in order to reduce the chances of prey escaping or retaliating (25). Even though prey mortality achieves this, other measures of venoms ability to subdue prey, such as the speed at which a venom affects prey or a measure of its sub-lethal incapacitating effects, may give clearer reflection of venoms prey-specificity (25). However, despite such limitations, we find a clear pattern of prey-specificity suggesting that cases of non-prey specific venom (28-32) are more likely to be the exception to the general rule.

In terms of macroecological patterns, we unsurprisingly we found that larger snakes had larger quantities of venom. More surprisingly these increases did not follow the expected scaling allometry predicted from a predator-prey scaling perspective (18) with venom yield increasing faster with snake body size

than expected. Even when considering potential variation in the allometric scaling of toxicological effects (equation 2) (38), an exponent far in excess of 1 is required for b in order for the observed scaling of 0.75 between venom yield and snake mass to agree with this prediction. Furthermore, our analysis found that the predatory-prey body mass scaling of venomous snakes is much lower in comparison to snakes as a whole (18), with larger venomous snakes feeding on much smaller prey items than expected, a trend that may also explain the reduced venom potency with snake size seen in our main analysis. A more likely explanation for our results regarding venom yield scaling is that it relates to limitations relating to metabolic rate (46), which also has a similar scaling coefficient of 0.75 with respect to body mass (39, 46). In particular, it might be expected that the production of a potentially metabolically costly material such as venom (33), would follow such a metabolic scaling, particularly if the costs of venom were maintained at a constant proportion of overall energy budgets across snake species. While we focus on the average yield a snake can produce the amount of venom administered in a single bite does seem to show stronger associations with trophic factors (36). Interestingly, such a decoupling of trophic and metabolic determinants between the amount of venom in a single bite and the total reservoir may have strong implications on the predation strategies available with body size. As yield scales with body size according to a higher exponent in comparison to any trophic factor, such as prey size, larger species would be expected to have the capacity for more envenomation's before depleting their reservoir. These larger reservoirs may hence allow for strategies such as the use of multiple envenomation's on a single prey item or strike and release strategies which may require "back-up" venom for cases were prey items are not recovered.

Another potential macroecological factor we found shaping the available volume of venom to a species is habitat dimensionality. While we expected that species in high dimensional habitats may have higher venom yields to compensate for higher escape rates of prey (42) we found that, counter to our expectation, these species had lower yields in comparison species in low dimensional habitats (terrestrial and fossorial). This may be associated with differences in prey capturing behaviour as it might be expected that high dimensional environments require more holding behaviours during attacks in order to prevent the loss of prey, hence allowing a more accurate delivery of venom. However, the presence of constriction in venomous snakes (47), the most extreme form of prey holding behaviours, is present in both arboreal and terrestrial species and was found to have no effect when included within the analysis. Furthermore bite and release behaviours are known in arboreal species such as the black mamba (*Dendroaspis polylepis*) suggesting this behaviour is not fully restricted to low dimensional environments (48). Another potential explanation is that higher encounter rates in high dimensional environments (14) may reduce the missed opportunity of feeding cost associated with replenishing venom. Rates of replenishing venom can be substantial with estimates of replenishment rate ranging from 3-7 days (49) to 30-50 days (36, 50-52). These long periods of replenishment may hence select for larger reserves in species where encounter rates with prey are lower in order to minimise potential missed opportunity costs. While further research on the role of habitat dimensionality will allow more detailed understanding of the mechanisms driving this trend our results highlight that venom may also be selected according to factors relate to prey encounter rates.

Our analysis shows that both predator-prey dynamics and macroecological factors shape the evolution of venom in snakes. While other mechanisms, such as gene duplication events (53), are important in driving the evolution of these traits positive selection pressures are important in shaping the pattern seen in the variation of traits relating to venom. This is also expected to

be the case in other venomous groups, were patterns relating to prey specificity and energetic constraints are also likely to play key roles in their evolution (1). Examples of prey-specific venom is seen in cone snails and spiders (1), while the energetic costs of producing venom is also suggested by venom metering in taxa such as scorpions (54). The generality of prey-specific and macroecological constraints is also likely to extend across non-venomous predatory traits, such as related to tooth morphology and gap limitation scaling, or other traits such as predator pursuit speed. By using snake venom as a system of predator trait evolution we show the importance of multiple evolutionary drivers allowing not only a window into the evolution of venomous systems, but of predatory traits and trophic ecology as a whole.

Methods

Data

We collected data on venom yield and toxicity from the literature, along with our predicted drivers. We used mean dry weight (mg) extracted as a measure of venom yield as it represents the amount of active ingredients available and is the most available reported measure. As a measure of venom lethality we used median lethal dose (LD_{50}) due to its wide availability. We only included intravenous (IV), subcutaneous (SC), Intraperitoneal (IP) or intramuscular routes (IM) of injecting the venom as other routes were too uncommon to include within the analysis. We include LD_{50} values measured on all animal models as we were interested in including variation relating to the potential prey specific nature of venom.

To test whether venom is prey specific we calculated the phylogenetic distance between the model animal species used to measure LD_{50} for each snake species and the species naturally present in its diet. We calculated this as the sum of the phylogenetic distance, using mean estimates from TimeTree (55), between each prey taxa and the LD_{50} model multiplied by the proportion of each prey group reported in each snake species diet. For example, a species with a diet comprising of 20% mammals, 50% fish and 30% reptiles with a LD_{50} measured using mice would have a diet with an average phylogenetic distance of $0.2(0) + 0.5(400.1) + 0.3(296) = 288.85$ million years from the common ancestor of the LD_{50} model. Diet data was collated from the literature using studies with quantitative estimates of prey proportions, mainly from studies of stomach contents. As prey items were rarely identified to lower taxonomic levels diet was categorized into six prey categories: invertebrates, fish, amphibians, lizards, birds and mammals.

Species habitat was categorized as either terrestrial, fossorial, aquatic or arboreal based on accounts in the literature. In order to directly test the expected effect of the dimensionality of habitat environment each environment was scored, as in Pawar et al (14), with terrestrial and fossorial environments scored as two-dimensional and arboreal and aquatic scored as three-dimensional. As some venomous species also engage in constriction behavior we collected data on any observation of constriction behavior in capturing prey from the literature (47).

1. Casewell NR, Wüster W, Vonk FJ, Harrison RA, & Fry BG (2013) Complex cocktails: the evolutionary novelty of venoms. *Trends in ecology & evolution* 28(4):219-229.
2. Kasturiratne A, et al. (2008) The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. *PLoS Med* 5(11):e218.
3. Fry BG, et al. (2006) Early evolution of the venom system in lizards and snakes. *Nature* 439(7076):584-588.
4. Fry B, et al. (2015) The origin and evolution of the Toxicofera reptile venom system. *Venomous Reptiles and Their Toxins: Evolution, Pathophysiology and Biodiscovery*:1.
5. Reyes-Velasco J, et al. (2015) Expression of venom gene homologs in diverse python tissues suggests a new model for the evolution of snake venom. *Molecular biology and evolution* 32(1):173-183.
6. Dowell NL, et al. (2016) The deep origin and recent loss of venom toxin genes in rattlesnakes. *Current Biology* 26(18):2434-2445.
7. Weinstein SA & Smith LA (1993) Chromatographic profiles and properties of Duvernoy's secretions from some boigine and dispholidine colubrids. *Herpetologica*:78-94.
8. Minton SAM, Minton Jr MRSA, & Minton MR (1980) *Venomous reptiles*.
9. Mebs D (2001) Toxicity in animals. Trends in evolution? *Toxicon* 39(1):87-96.
10. Sasa M (1999) Diet and snake venom evolution: can local selection alone explain intraspecific venom variation? *TOXICON-OXFORD* 37:249-252.
11. Pace ML, Cole JJ, Carpenter SR, & Kitchell JF (1999) Trophic cascades revealed in diverse ecosystems. *Trends in ecology & evolution* 14(12):483-488.
12. Palkovacs EP & Post DM (2009) Experimental evidence that phenotypic divergence in predators drives community divergence in prey. *Ecology* 90(2):300-305.
13. Domenici P (2001) The scaling of locomotor performance in predator-prey encounters: from fish to killer whales. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 131(1):169-182.
14. Pawar S, Dell AI, & Savage VM (2012) Dimensionality of consumer search space drives trophic interaction strengths. *Nature* 486(7404):485-489.
15. Kane A, et al. (2016) Body Size as a Driver of Scavenging in Theropod Dinosaurs. *The American Naturalist* 187(6):706-716.
16. Healy K, McNally L, Ruxton GD, Cooper N, & Jackson AL (2013) Metabolic rate and body

For snake body size we used total length values from the literature and field guides as these were the most common measures available (See Appendix A). All lengths were then converted to mass using family-level allometric scaling (56). Prey size data was included from dietary studies when available. When prey size was not reported in the dietary studies and were prey species were identified to the species level, we used mean prey species body mass from available databases (56-58). In cases where only body lengths were available for prey species allometric scaling were used to convert to mass (57, 59). For species that were only identified to the genus level the genus mean body mass was used if possible. The estimate mean prey size for each snake species was then calculated using a weighted mean based on the proportion each prey species/genus or group within the diet.

Snake mass, prey mass, LD_{50} , venom yield and phylogenetic distance between diet and model were all \log_{10} transformed, mean centered and expressed in units of standard deviation prior to analysis. Significance was determined for the fixed effects when 95% of the data is greater or less than 0. The phylogeny from Pyron RA & Burbrink (60) was included in all analyses to account for non-independence in traits due to common descent.

Analysis

To test our hypotheses we fit Bayesian multivariate phylogenetic mixed models using the MCMCglmm package (61) in R v 3.2.4 (62). As venom yield and LD_{50} are likely to have co-evolved, both were included as response variables in a series of multivariate analysis. Phylogeny was controlled by including it using the animal term in the MCMCglmm model while variation due to multiple measures on individual species was included using a separate random term. For the main model snake body mass; LD_{50} inoculation method (SC, IM, IV, IP); habitat dimensionality (2D, 3D); the presence of eggs in the diet (absent, present); and the phylogenetic distance of diet species to LD_{50} model were included as explanatory variables to give the analysis;

(1) Yield + $LD_{50} = f(\text{Snake mass} + LD_{50} \text{ method} + \text{Presence of eggs in diet} + \text{Phylogenetic distance between diet and model species} + \text{Habitat dimensionality})$ (275 observations over 99 species).

A similar model was also fit including prey size as an explanatory model which resulted in a smaller dataset of 177 observations across 68 species. To estimate the direct scaling exponents relating to prey mass, venom yield and predator mass as referred to in equations (1 and 3) we also ran the following model;

(2) Yield = $f(\text{Prey mass})$

(3) Prey mass = $f(\text{Predator mass})$

Finally, we also fitted a final set of sensitivity analysis including the main model with constriction behavior included as a categorical factor (absent, present) and a model with habitat type included instead of habitat dimension with the levels of terrestrial; aquatic and arboreal.

All models were fitted with parameter expanded priors (Hedfield 2010) with standard non-informative priors also tested separately to ensure that choice of prior had no affect on model results. A burn-in, thinning and number of iterations was determined for each model separately to ensure effective sample sizes exceeded 1000 for all parameter estimates. We tested for convergence using the Gelman-Rubin statistic over three separate chains (63).

size are linked with perception of temporal information. *Animal behaviour* 86(4):685-696.

17. Kiltie R (2000) Scaling of visual acuity with body size in mammals and birds. *Functional Ecology* 14(2):226-234.
18. Carbone C, Codron D, Scofield C, Clauss M, & Bielby J (2014) Geometric factors influencing the diet of vertebrate predators in marine and terrestrial environments. *Ecology letters* 17(12):1553-1559.
19. Dawkins R & Krebs JR (1979) Arms races between and within species. *Proceedings of the Royal Society of London B: Biological Sciences* 205(1161):489-511.
20. Van Valen L (1973) A new evolutionary law. *Evolutionary theory* 1:1-30.
21. Albertson RC, Markert J, Danley P, & Kocher T (1999) Phylogeny of a rapidly evolving clade: the cichlid fishes of Lake Malawi, East Africa. *Proceedings of the National Academy of Sciences* 96(9):5107-5110.
22. Li M, Fry B, & Kini RM (2005) Eggs-only diet: its implications for the toxin profile changes and ecology of the marbled sea snake (*Aipysurus dybowskii*). *Journal of Molecular Evolution* 60(1):81-89.
23. Daltry JC, Wuester W, & Thorpe RS (1996) Diet and snake venom evolution. *Nature* 379(6565):537-540.
24. da Silva NJ & Aird SD (2001) Prey specificity, comparative lethality and compositional differences of coral snake venoms. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 128(3):425-456.
25. Barlow A, Pook CE, Harrison RA, & Wüster W (2009) Coevolution of diet and prey-specific venom activity supports the role of selection in snake venom evolution. *Proceedings of the Royal Society of London B: Biological Sciences* 276(1666):2443-2449.
26. Richards D, Barlow A, & Wüster W (2012) Venom lethality and diet: differential responses of natural prey and model organisms to the venom of the saw-scaled vipers (Echis). *Toxicon* 59(1):110-116.
27. Starkov VG, Osipov AV, & Utkin YN (2007) Toxicity of venoms from vipers of Pelias group to crickets *Gryllus assimilis* and its relation to snake entomophagy. *Toxicon* 49(7):995-1001.
28. Williams V, White J, Schwaner T, & Sparrow A (1988) Variation in venom proteins from isolated populations of tiger snakes (*Notechis ater niger*, *N. scutatus*) in South Australia. *Toxicon* 26(11):1067-1075.

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29. Voss RS (2013) Opossums (Mammalia: Didelphidae) in the diets of Neotropical pitvipers (Serpentes: Crotalinae): Evidence for alternative coevolutionary outcomes? *Toxicon* 66:1-6.

30. Heatwole H & Poran NS (1995) Resistances of sympatric and allopatric eels to sea snake venoms. *Copeia*:136-147.

31. Biardi JE & Coss RG (2011) Rock squirrel (*Spermophilus variegatus*) blood sera affects proteolytic and hemolytic activities of rattlesnake venoms. *Toxicon* 57(2):323-331.

32. Poran NS, Coss RG, & Benjamini E (1987) Resistance of California ground squirrels (*Spermophilus beecheyi*) to the venom of the northern Pacific rattlesnake (*Crotalus viridis oreganus*): a study of adaptive variation. *Toxicon* 25(7):767-777.

33. McCue MD & Mason R (2006) Cost of producing venom in three North American pitviper species. *Copeia* 2006(4):818-825.

34. Pintor AF, Krockenberger AK, & Seymour JE (2010) Costs of venom production in the common death adder (*Acanthophis antarcticus*). *Toxicon* 56(6):1035-1042.

35. Hayes WK (1995) Venom metering by juvenile prairie rattlesnakes, *Crotalus v. viridis*: effects of prey size and experience. *Animal Behaviour* 50(1):33-40.

36. Hayes WK, Herbert SS, Rehling GC, & Gennaro JF (2002) Factors that influence venom expenditure in viperids and other snake species during predatory and defensive contexts. *Biology of the Vipers*:207-233.

37. Chippaux J-P, Williams V, & White J (1991) Snake venom variability: methods of study, results and interpretation. *Toxicon* 29(11):1279-1303.

38. Nestorov I (2003) Whole body pharmacokinetic models. *Clinical pharmacokinetics* 42(10):883-908.

39. Isaac NJ & Carbone C (2010) Why are metabolic scaling exponents so controversial? Quantifying variance and testing hypotheses. *Ecology Letters* 13(6):728-735.

40. Kodric-Brown A, Sibly RM, & Brown JH (2006) The allometry of ornaments and weapons. *Proceedings of the National Academy of Sciences* 103(23):8733-8738.

41. Bergmann PJ & Berk CP (2012) The evolution of positive allometry of weaponry in horned lizards (*Phrynosoma*). *Evolutionary Biology* 39(3):311-323.

42. Arbuckle K (2015) Evolutionary Context of Venom in Animals.

43. Heithaus MR, Wirsing AJ, Burkholder D, Thomson J, & Dill LM (2009) Towards a predictive framework for predator risk effects: the interaction of landscape features and prey escape tactics. *Journal of Animal Ecology* 78(3):556-562.

44. Møller A (2010) Up, up, and away: relative importance of horizontal and vertical escape from predators for survival and senescence. *Journal of evolutionary biology* 23(8):1689-1698.

45. Hossie T, Hassall C, Knee W, & Sherratt T (2013) Species with a chemical defence, but not chemical offence, live longer. *Journal of evolutionary biology* 26(7):1598-1602.

46. Brown JH, Gillooly JF, Allen AP, Savage VM, & West GB (2004) Toward a metabolic theory

of ecology. *Ecology* 85(7):1771-1789.

47. Shine R & Schwaner T (1985) Prey constriction by venomous snakes: a review, and new data on Australian species. *Copeia* 1985(4):1067-1071.

48. Branch WR (1998) *Field guide to snakes and other reptiles of southern Africa* (Struik).

49. Currier RB, *et al.* (2012) Unusual stability of messenger RNA in snake venom reveals gene expression dynamics of venom replenishment. *PloS one* 7(8):e41888.

50. Rotenberg D, Bamberger E, & Kochva E (1971) Studies on ribonucleic acid synthesis in the venom glands of *Vipera palaestinae* (Ophidia, Reptilia). *Biochemical Journal* 121(4):609-612.

51. Hayes W (2008) The snake venom-metering controversy: levels of analysis, assumptions, and evidence. *The biology of rattlesnakes*:191-220.

52. Young BA (2008) Perspectives on the regulation of venom expulsion in snakes. *The Biology of the Rattlesnakes.*, ed Hayes WK, Beaman, K.R., Caldwell, M.D., Bush, S.P. (Loma Linda University Press, California), pp 79-90.

53. Vonk FJ, *et al.* (2013) The king cobra genome reveals dynamic gene evolution and adaptation in the snake venom system. *Proceedings of the National Academy of Sciences* 110(51):20651-20656.

54. Nisani Z, Dunbar SG, & Hayes WK (2007) Cost of venom regeneration in *Parabuthus transvaalicus* (Arachnida: Buthidae). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 147(2):509-513.

55. Hedges SB, Dudley J, & Kumar S (2006) TimeTree: a public knowledge-base of divergence times among organisms. *Bioinformatics* 22(23):2971-2972.

56. Meiri S (2010) Length-weight allometries in lizards. *Journal of Zoology* 281(3):218-226.

57. Feldman A & Meiri S (2013) Length-mass allometry in snakes. *Biological Journal of the Linnean Society* 108(1):161-172.

58. Myhrvold NP, *et al.* (2015) An amniote life-history database to perform comparative analyses with birds, mammals, and reptiles. *Ecology* 96(11):3109-3109.

59. Pough FH (1980) The advantages of ectothermy for tetrapods. *American Naturalist*:92-112.

60. Pyron RA & Burbrink FT (2014) Early origin of viviparity and multiple reversions to oviparity in squamate reptiles. *Ecology Letters* 17(1):13-21.

61. Hadfield JD (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software* 33(2):1-22.

62. Team RC (2016) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

63. Brooks SP & Gelman A (1998) General methods for monitoring convergence of iterative simulations. *Journal of computational and graphical statistics* 7(4):434-455.

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