Snake venom potency and volume are driven by metabolism, dimensionality and prey characteristics

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Significance

Snake venom is best known for its ability to incapacitate prey, a property that makes it of both biomedical interest and public health concern. However, there is considerable variation in both the volume and potency of venom among snake species, which offers an opportunity to understand a novel aspect of predator-prey co-evolution. We investigate how this variation in snake venom is related to key characteristics of their prey, snake size, metabolic rates and complexity (dimensionality) of their habitat. Here, using comparative analysis, we show that venom has higher potency towards species closely resembling their natural diet, suggesting a co-evolution of predator to prey types, while macroecological drivers, such as habitat dimensionality and metabolic constraints shape the quantity of venom available.

Abstract

Snake venom is best known for its ability to incapacitate and kill prey. However, venom potency and volume vary greatly across species, with some species possessing venoms which are seemingly harmless, while others carry enough venom to kill vast numbers of potential prey. This highlights the need for a multi-species comparison to identify possible environmental and ecological drivers of snake venom evolution. However, studies commonly use non-native prey species as models to assess venom potency, an approach which may confound comparative analyses if the toxic effects of each snake species venom is adapted to specific prey groups. Here we compare 100 species of snake to test a range of hypotheses relating to the drivers of venom evolution. We assess potency (LD$_{50}$) by accounting for the phylogenetic distance between the often non-natural model species used to measure potency and the species naturally occurring in their diets. We also examine variation in yield among species in relation to biological and habitat characteristics. We show that snake venom potency is prey-specific, with higher potencies when venoms were tested on species phylogenetically similar to common prey species. We also show that venom yield scales positively with snake body mass supporting a link with metabolic rate, but is lower for species found in high dimensionality habitats. These results underline the importance of ecological, physiological and environmental factors in the evolution of novel predatory traits and highlight the wider potential of using venom as a system to understand the evolution of predator-prey coevolution more generally.

Introduction

Snake venom is perhaps best known for its ability to incapacitate and disrupt the physiological systems of animals. This is particularly well demonstrated by extremely lethal species, such as Russel's viper (*Daboia russelii*), which possess enough potent venom to incapacitate tens of thousands of potential prey items (Figure 1). From a human perspective this property of venom has made it not only a source of novel biomedical compounds (1) but also a major health concern, with snakebites estimated to cause up to 94,000 deaths annually (2). Although the most lethal venomous snake species often gain the most attention (3), the prey incapacitating ability of different species' venoms ranges widely, from those unable to subjugate prey larger than a few grams to those capable of subduing vast numbers of laboratory animals (4) (Figure 1). While understanding this variation is important from both a medical (2) and evolutionary viewpoint (1), little is known about what drives it. One reason for this is the lack of multi-species comparisons across taxonomically diverse groups. For example, while several studies have explored whether venoms have evolved to specifically target particular prey species these studies are typically focused at the genus level (5-8), making general inferences regarding the evolution of fundamental aspects of venom characteristics difficult (9-11). Here we conduct a comparative analysis across a taxonomically and ecologically diverse range of venomous snakes allowing us to test fundamental aspects of both the evolution of venom and predator traits in general.

Variation in predatory traits are typically associated with differences in trophic ecology. For example, selection on jaw and beak morphology in cichlid fish and birds is strongly associated with trophic factors such as prey type (12, 13). Apart from prey type, other components of trophic interactions such as search and encounter rates (14-16); the ability to spot, track and capture prey (17, 18); and the rate of ingestion of such captured prey (16, 19) are also likely to influence predatory traits. However, while morphological measures of trophic traits can be linked to such potential drivers (13) it is difficult to accurately quantify how changes in morphology map to trophic functional ability. Snake venom offers a system were foraging capabilities can be quantified directly by measuring both venom potency, such as quantified by the median lethal dose (LD_{50}), and the quantity available, allowing fundamental evolutionary drivers of venom and, in turn, predator traits in general to be tested. However, to compare such measures of venom potency across

snakes the route of administration (Figure 1) and the model species used to test toxicity must be standardized in some way so as not to confound comparisons (5). If venom characteristics are adapted to incapacitate prey species commonly found in their diets, we might expect the use of model species not typical of a snake species diet would result in the underestimation of its potency (1). However, the confounding effects of using measures of potency on standard laboratory model species can be minimized by controlling for the phylogenetic distance between the model species and those prey species naturally found in the snakes diet. Furthermore, this approach not only provides for the corrections to allow for comparisons across species with diverse diets but also acts as a test of whether venom evolution results in prey-specific characteristics in general.
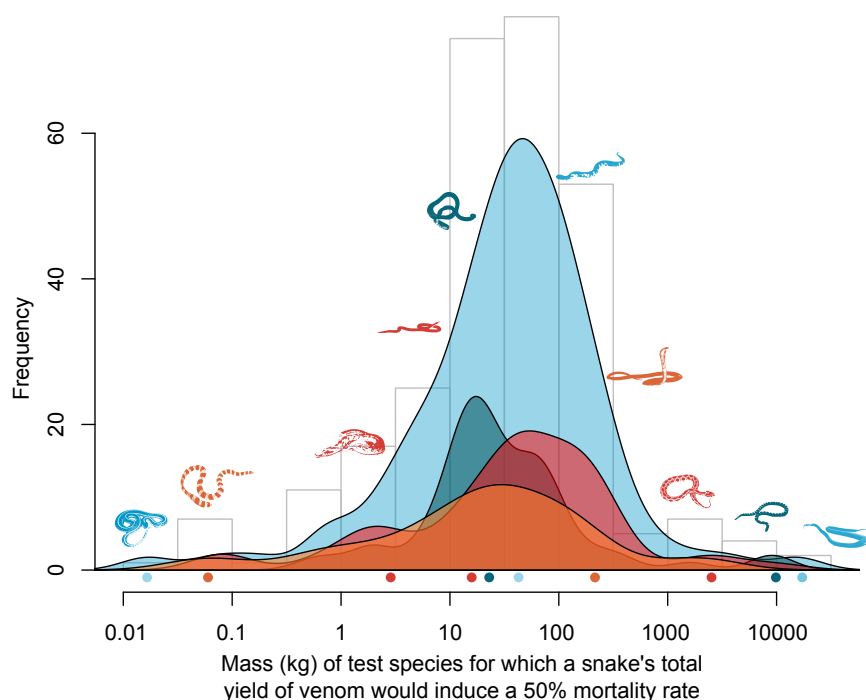

Figure 1. Histogram and density plot of the animal mass (kg) that snakes species can impart a 50% mortality rate on for 282 observations of 104 species). This was calculated as the average volume of dried venom divided by its as LD_{50} (mg/kg). Measurement routes of LD_{50} administration are shown for intraperitoneal (light blue), intramuscular IM (dark blue), intravenous (red) and subcutaneous (orange). Species form left to right are *Thamnophis elegans, Emydocephalus annulatus, Causus rhombeatus, Atractaspis bibronii, Hydrophis elegans, Agkistrodon piscivorus, Ophiophagus hannah, Daboia russelii, Bungarus multicinctus, Oxyuranus scutellatus*.

Whether snake venom potency is prey-specific as a general rule has been the subject of much debate (9-11). While prey-specificity has been demonstrated in several groups (5-8,

20), other examples have shown either no relationship between prey and venom lethality (21), or cases were the prey species have evolved tolerance towards their predators venoms (22, 23). Of these outcomes predator-prey arms race dynamics (24) predicts prey-specific venoms or the evolution of prey tolerances depending on the level of selection on both predator and prey (24). In contrast no relationship between potency and prey identity is predicted by the overkill hypothesis (9-11) (Figure 2). The overkill hypothesis is based on the observation that venoms often display levels of lethality far in excess of the requirements needed to kill prey (Figure 1), resulting in predator-prey dynamics playing a minor role in the evolution of venom potency due to weak selection. As these hypotheses predict different outcomes when non-native species are used to test potency, we use the inclusion of the phylogenetic distance between the model species and natural prey to test between these competing hypothesis (Figure 2). In particular, the absence of a relationship between venom potency and the phylogenetic distance between prey and model species, would support the overkill mechanism, while if venom was evolved to be prey-specific, we would be expected to see higher potencies (i.e. lower LD_{50} values) in model species more closely related to their typical prey species (Figure 2).
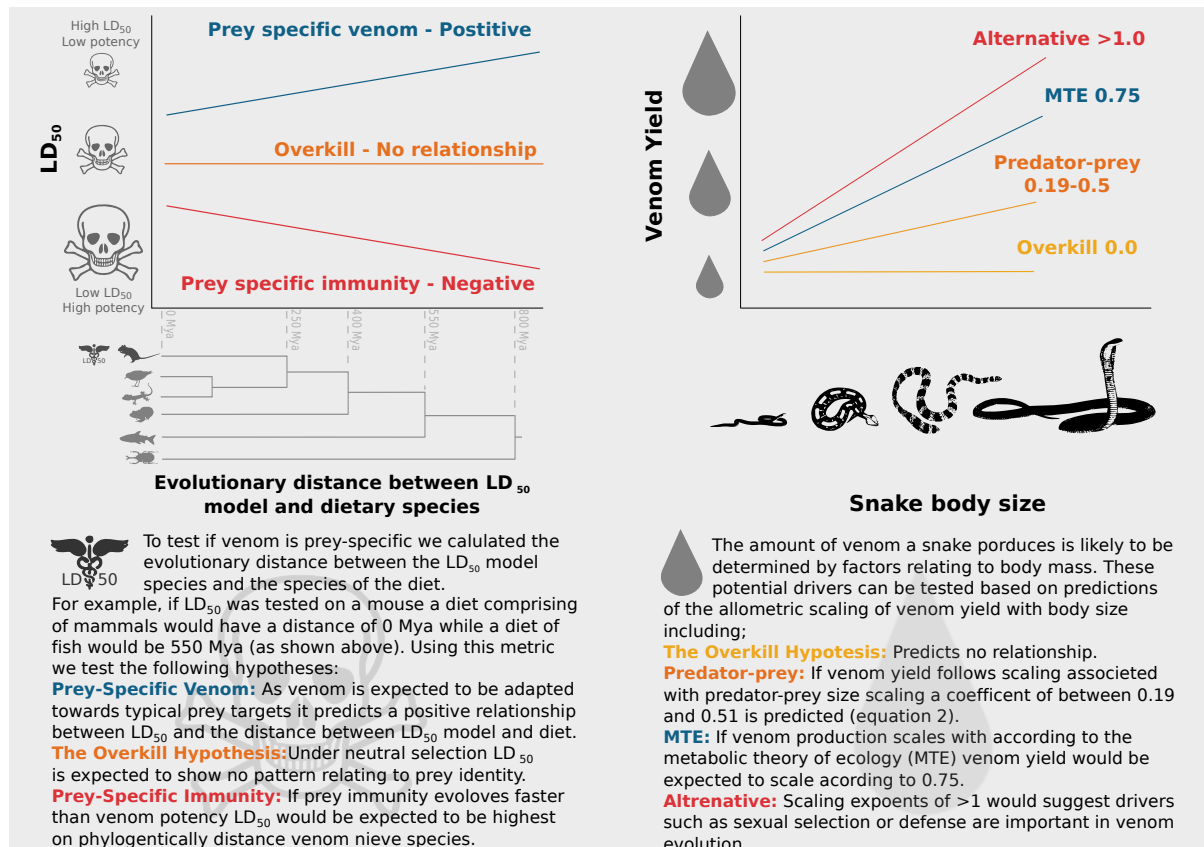

Figure 2. Summary of hypotheses relating to potential drivers of snake venom evolution.

As the ability to incapacitate prey is also determined by the amount of venom available it would also be predicted that venom yield would be under similar selective pressures. In particular, as venom production incurs an energetic cost (25) (although the level of this cost is debated (26)) and requires storage, the volume of venom a snake species can produce is likely to be linked to prey size and or metabolic rate. Both prey size and metabolic rate are strongly determined by body size (9, 19). In terms of prey size, in general larger terrestrial predators eat larger prey (19). Hence, it would be expected that larger snake species would need to produce larger quantities of venom to keep pace with subsequent increases in prey size. Metering of venom in response to prey size seen in several species (27, 28) supports the presence of such selection on venom quantity. However, while bigger snakes are known to have larger amounts of venom in general (4) it is not known whether venom yield scales interspecifically according to any general pattern.

The overkill hypothesis would predict no relationship between venom yield and prey size, or a scaling exponent with snake mass of 0. Alternatively, another prediction is that venom yield increases with snake body size with an exponent relating to their predator-prey body size scaling such as described by (equation 1);

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

Where for snakes the scaling exponent a is approximately 0.68 (19). However, venom yield would not be expected to scale according to this exponent as the effects of toxicological agents also follows an allometric relationship (29) where 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);

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

where b is the scaling coefficient of venoms' toxicological effects, commonly estimated as 0.75 (29). To calculate the expected allometry of venom yield with snake body mass in a case where yield increases at a rate to match predator-prey size scaling after accounting for the scaling of its 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 (29) for b and the value of 0.68 for a from the scaling predator-prey mass relationship for snakes (19) we would expect a scaling exponent of approximately 0.51 between snake venom yield and snake mass. Alternatively, venom yield may be predicted to scale according to constraints such as metabolic costs matched with metabolic rate, where a scaling of 0.75 would be expected (30). At the other extreme, super-linear allometries (exponents >1) would suggest patterns associated with drivers such as sexual selection, such as proposed by the weapons hypothesis (31), or defenses requiring increased effectiveness with size, such as seen in the allometry of horn growth in horned lizards (32) (Figure 2).

Finally, an overlooked feature that may also drive the evolution of both venom quantity and toxicity is habitat structure (33). 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 (15, 19) and the escape rates of prey, with higher dimensional spaces increasing both (34, 35). 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, as measured using median lethal dose (LD_{50}), in a phylogenetically corrected comparative analysis of one hundred species of venomous snakes. Using the phylogenetic distance between species used to measure LD_{50} and dietary species, we test;

    (1) the overkill hypothesis: that there is no relationship between venom potency and the species on which it was measured or between venom yield and prey size.

    (2) the importance of trophic drivers on venom evolution including: that venom potency is higher (lower LD_{50}) 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 LD_{50}) or yields.

(3) the importance of macorecological drivers on venom evolution with scaling of venom yield with snake body mass predicted from predator-prey size scaling to be approximately 0.51; from metabolic constraints to be 0.75; and from other potential drivers such as sexual selection and the weapons hypothesis to be super-linear.

(4) the importance of habitat dimensionality on venom evolution, in particular that; species in high dimensional habitats show either higher or lower potencies.

Results

Our final compiled dataset of venom traits and corresponding trophic and macroecological data consisted of 276 observations over 100 species which corresponds to the data used in the main analysis (Table A1). We also conducted supplementary analysis where we included whether species to are known to using constricting behaviors in place of or augmenting venom delivery; the inclusion of habitat type and finally an analysis including prey body size which was conducted using a reduced dataset of 176 observations across 69 species (Table A1). 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 seven egg eating species in our dataset, LD_{50} was found to be significantly higher (lower potency) 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 (Figure 3; Tables A2-4).

Of the species included within the analysis only 17 species had a diet completely matching that of one of the LD_{50} models their venom was tested on, i.e. the LD_{50} 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 LD_{50} model as reflected by a median evolutionary distance of 211.7 million years between the common ancestors of the dietary taxa and the LD_{50} species. In all models, species' LD_{50} increased with mean phylogenetic distance between the diet and the LD_{50} model such that snake species with diets phylogenetically close to the LD_{50} model species had higher potencies (Figures 3-4a; table A2-4). From the main model, after back-transforming the mean centered log_{10} value,

LD_{50} was found to increase by 1.44 for every mean distance of 100 million years between the species in the diet and that of the LD_{50} model. The route venom was administered was also found to affect values of LD_{50} where intravenous and Intraperitoneal routes were found to have lower LD_{50} values in comparison to a subcutaneous route (Figure 3; Table A2-4).

Figure 3. Posterior distributions of LD_{50} and mean venom volume estimates (represented by dots) and higher and lower 95% credibility intervals (represented by dotted horizontal bar). Fixed factors include mass; LD_{50} 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 LD_{50} model (Diet-LD_{50} Dist). The random terms and the co-variance (CV) between LD_{50} and yield are also presented. Signifiance is determined when 95% of the data is above or below zero. The model was run with 12,000,000 iterations with a 2,000,000 burn-in and a thinning of 5000.
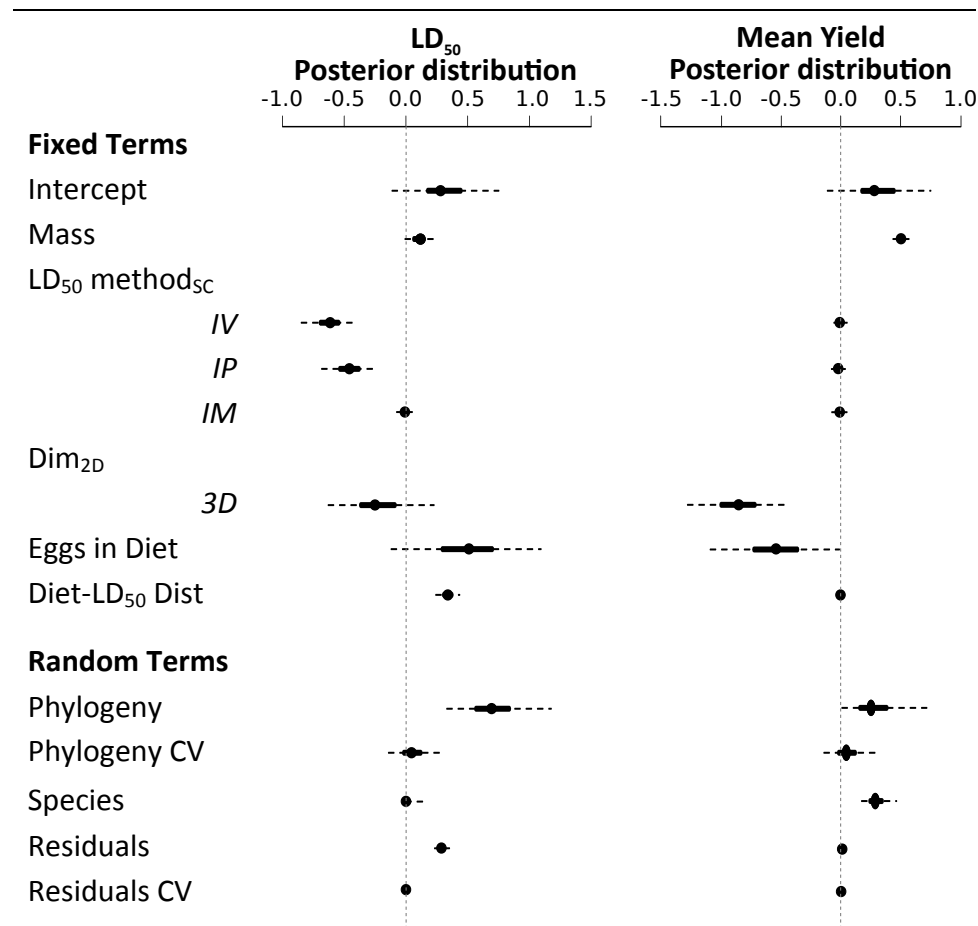
Macroecological drivers; body size and habitat dimensionality

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 (Figure 3-4, Tables A2-4), an exponent far higher than the predicted scaling of 0.51 for predator-prey body size scaling (equation 3). Venom yield also showed a positive increase with prey body mass, with a $\log_{10}$-$\log_{10}$ slope of 0.139 (equating to approximately a 10% increase in venom volume with a doubling of body mass), however only 90% of the posterior samples are above the zero threshold (Table A2). Snake body size was also found to have a significantly positively correlation with LD_{50} in the main analysis, meaning larger snakes showed decreased potency (Figure 3). However, this relationship was not significant in any of the sub analysis (Table A2-4).

The next most significant driver of venom yield was the dimensionality of the habitat with the 27 species in high dimensional environments (arboreal = 7, aquatic = 20) showing lower venom yields in comparison to species in lower dimensional habitats (Figures 3-4). A sensitivity analysis where habitat was included as terrestrial, arboreal and aquatic also showed similar significant reductions in both arboreal and aquatic habitats (Table A3).
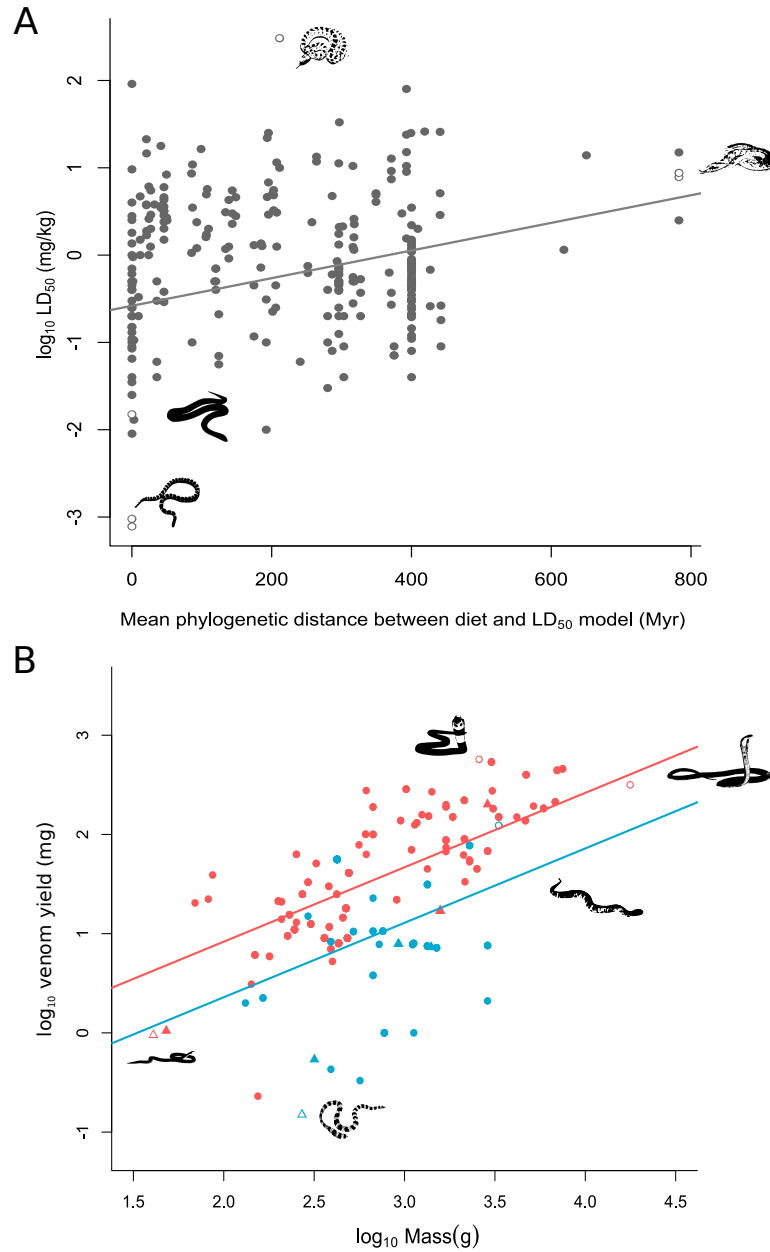
Figure 4. (A) Mean phylogenetic distance between diet species and LD50 model (Myr) against $\log_{10}$ LD$_{50}$ (intercept = -0.58, slope = 0.002). Hollow points represent silhouette species which are from left to right; *Bungarus multicinctus*; *Oxyuranus microlepidotus*; *Echis carinatus*; *Causus rhombeatus*.

(B) Relationship between $\log_{10}$ mass (g) against $\log_{10}$ 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 Figure 3 and Table A1.

Phylogeny, constriction behaviour and covariance between venom yield and LD$_{50}$

In all models there is was an intermediate to high phylogenetic signal for both LD$_{50}$ and venom yield with phylogeny explaining 69% of the residual variation for LD$_{50}$ and 49% for venom yield, with LD$_{50}$ showing a higher phylogenetic signal in all models (Figure 2, Table A2-4). The presence of constricting behaviour was found to have no effect on either yield or LD$_{50}$ (Table A4). Finally, there is no significant covariance between either the residuals or phylogenetic terms of both yield and LD$_{50}$ across all models (Figure 2, A2-4).

Discussion

Predator traits are predicted to be strongly shaped by both predator-prey co-evolution and macroecological forces such as body size and habitat structure. Traits such as jaw or beak morphology are tightly linked to diet (13, 36), while a predators size and foraging environment also influences trophic interactions through limiting the size, encounter rate and escape rate of potential prey (15, 19, 35). Our analysis show that traits associated with venom in snakes are determined by prey characteristics, predator size and the dimensionality of the environment in which a predator forages. These results demonstrate that, contrary to the expectations of the overkill hypothesis (9-11), snake venom potency is prey-specificity in general. Also the scaling of the quantity of venom of 0.75 supports the prediction that volume is driven metabolic cost of venom but not by prey size. In addition, venom volume was affected by the dimensionality of the environment. By controlling for the variation associated with the species and administration route used to test potency, along with snake phylogeny, we show that venom systems can act as excellent systems to test the drivers of predatory trait evolution.

Predation is likely to be the primary function of snake venom (1). Perhaps the clearest evidence of venoms predatory role is seen in cases of the evolutionary loss of venom when it is no longer required to capture prey, such as seen by the almost complete atrophy of the venom apparatus in the marbled sea snake (*Aipysurus eydouxii*) due to switiching to an egg based diet (37). Similar to the marbled sea snake, we find evidence of lower potencies and yeilds in species associated with ovivorous feeding.

Beyond the role of diet in the evolutionary maintenance of venom we also find that venom is prey-specific in general, supporting a scenario were snake venom is under

selection to be more effective towards target species, and were cases of non-prey specific venom (21-23, 38, 39) represent exceptions to the general rule. These results highlight the importance of prey in shaping not just the initial evolution of venom, but also in driving its continual evolution through arms race dynamics. The general prey-specific nature of snake venom also highlights the potential utility of using diet as a criteria for species selection when bio-prospecting for novel biological compounds, as phylogenetically distant snake species with similar diets are likely to have evolved a diversity of different biological compounds which induce similar physiological effects on their shared prey species (40). Species which have previously shown no evidence of having prey-specific venoms may represent cases of the more nuanced aspects of predator-prey interactions involving venom. In particular, many species may not rely on the ability to induce mortality to capture prey but may rely on the speed of incapacitations (5). While such measures are relatively uncommon, future comparative analysis incorporating them may reveal more a nuanced role of venom in predator-prey interactions.

In terms of macroecological patterns, unsurprisingly we found that larger snakes had larger quantities of venom. However, these increases did not follow predictions based on the observed predator-prey body size relationship in snakes (19), with yield increasing far more rapidly than expected. Instead venom yield was found to follow the allometric scaling of 0.75 predicted from metabolic theory, assuming snakes invest a constant proportion of their metabolism to produce venom (30, 41). This scaling signifies that the metabolic costs of venom may have a more significant role in the evolution of venom than previous supposed. For example as yield increases with body size according to a higher exponent than prey size, larger species may be expected to have the capacity to envenomate more prey items before depleting their reservoir in comparison to smaller species which may be constrained to something closer to a one shot strategy.

Apart from size, habitat dimensionality was also found to influence venom yield. We expected that species in high dimensional habitats may have higher venom yields to compensate for higher escape rates of prey (33), however, we found that these species had lower yields in comparison to species in low dimensional habitats (terrestrial and fossorial). This difference may be associated with differences in prey handling behaviours in different environments, with the potentially higher requirement of prey holding behaviours in high dimension environments resulting in more accurate deliver their smaller volumes of venom.

However, the presence of constriction in venomous snakes (42), the most extreme form of prey holding behaviours, is present in both arboreal and terrestrial species and was also found to have no effect when included within our 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 (43). Another potential explanation of these results is that higher encounter rates in high dimensional environments (15) may reduce the missed opportunity of feeding costs associated with replenishing venom. Rates of replenishing venom can be substantial with estimates ranging from 3-7 days (44) to 30-50 days (28, 45). These long periods of replenishment may hence select for larger venom reserves in species where prey encounter rates are low 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 behind this difference our results highlight that prey encounter rates may be an important factor in venom evolution.

While our analysis demonstrates the importance of trophic and macroecological drivers in snake venom evolution these drivers are also expected to influence the evolution of venom in other taxa (1). For example, 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 scorpions (46). Future analyses that include other venomous taxa in a comparative approach such as used here, will further test whether venom fundamentally follows such similar patterns. Certain elements of prey-specify and macroecological constraints are also likely to generally apply across other non-venomous predatory traits. For example, possible predator-prey arms dynamics relating to bite force and prey size (47), or macroecological constraints relating to pursuit speed (14). By using venom as a system of predator trait evolution the importance of multiple evolutionary drivers can be robustly tested and hence offer a window not only 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 administering 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 (48), 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 (15), 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 (42).

For snake body size we used total length values from the literature and field guides

as these were the most common measures available. All lengths were then converted to mass using family-level allometric scaling (49). 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 (49-51). In cases were only body lengths were available for prey species allometric scaling were used to convert to mass (50, 52). For species that were only identified to the genus level the genus mean body mass was used if available. 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. All data is available in the supplementary information (S2).

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 (53) was included in all analyses to account for non-independence in traits due to common descent.

Analysis

To test our hypotheses we fitted Bayesian multivariate phylogenetic mixed models using the MCMCglmm package (54) in R v 3.2.4 (55). 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. The relative variance attributable to the phylogenetic random effect component (H^2) was calculated as the ratio of variance explained by phylogeny to the sum of phylogenetic variance, species variance and residual variance. 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(Snake mass + LD_{50} method + Presence of eggs in diet + Phylogenetic distance between diet and model species + Habitat dimensionality) (276 observations over 100 species).

A similar model was also fitted including prey size as an explanatory model, which resulted in a smaller dataset of 176 observations across 69 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(Prey mass)

(3) Prey mass = f(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 effect 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 (56).

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Supplementary Information (S1)

Table A1. Breakdown of species in dataset. Numbers in brackets refer to number of observations

Category	Number of species	
	Full analysis	Prey size analysis
LD$_{50}$ method		
Subcutaneous (SC)	47	31
Intravenous (IV)	68	47
Intraperitoneal (IP)	58	35
Intramuscular (IM)	24	15
LD50 model		
Amphibian	2	2
Arthropod	1	0
Bird	5	2
Fish	9	3
Lizard	9	5
Mouse	100	69
Environment		
Terrestrial	74	56
Arboreal	7	6
Aquatic	20	7
Habitat Dimensionality		
Low (1)	73	56
High (3)	27	13
Eggs present in Diet	7	7
Constriction observed	5	2
Total	100 (276)	69 (176)

Table A2. Estimates and higher and lower 95% credibility intervals (CI) in model including prey sizes as a fixed factor. Fixed factors include mass; LD_{50} 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 variance (CV) between LD_{50} 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.

	LD_{50}			Mean Volume		
	Estimate	Lower CI	Upper CI	Estimate	Lower CI	Upper CI
Fixed Terms						
Intercept	0.325	-0.162	0.777	0.325	-0.162	0.777
Mass	0.07	-0.091	0.204	**0.525**	**0.429**	**0.612**
LD_{50} method$_{SC}$						
IV	**-0.617**	**-0.864**	**-0.375**	-0.016	-0.101	0.0713
IP	**-0.603**	**-0.862**	**0.350**	-0.033	-0.122	0.069
IM	0.231	-0.509	0.087	-0.026	-0.131	0.090
Dim$_{2D}$						
3D	-0.149	-0.651	0.389	**-0.992**	**-1.493**	**-0.495**
Eggs in Diet	0.402	-0.227	1.018	**-0.582**	**-1.179**	**-0.0492**
Diet-LD_{50} Dist	**0.364**	**0.247**	**0.492**	-0.005	-0.053	0.038
Prey mass	0.078	-0.122	0.253	0.139	-0.016	0.305
Random Terms						
Phylogeny	0.844	0.381	1.335	0.414	0.001	0.996
Phylogeny CV	0.084	-0.166	0.332	0.084	-0.166	0.332
Species	0.029	0.001	0.108	0.221	0.002	0.430
Residuals	0.237	0.177	0.300	0.027	0.020	0.034
Residuals CV	0.002	-0.013	0.018	0.002	-0.013	0.018

Table A3. Estimates and higher and lower 95% credibility intervals (CI) in model including habitat with the levels of terrestrial, aquatic and arboreal. Fixed factors include mass; LD_{50} 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 LD_{50} model (Diet-LD_{50} Dist). The random terms and the co-variance (CV) between LD_{50} 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.

	LD_{50}			Mean Volume		
	Estimate	Lower CI	Upper CI	Estimate	Lower CI	Upper CI
Fixed Terms						
Intercept	0.312	-0.099	0.704	0.312	-0.099	0.704
Mass	0.112	-0.019	0.222	**0.508**	**0.439**	**0.576**
LD_{50} method$_{SC}$						
IV	**-0.634**	**-0.845**	**-0.405**	-0.002	-0.052	0.059
IP	**-0.456**	**-0.666**	**-0.230**	-0.021	-0.080	0.038
IM	-0.149	-0.397	0.105	-0.013	-0.083	0.046
Dim $_{terrestrial}$						
Aquatic	-0.393	-0.938	0.192	**-1.112**	**-1.587**	**-0.638**
Arboreal	0.133	-0.606	0.821	**-0.947**	**-1.489**	**-0.369**
Eggs in Diet	0.489	-0.079	1.066	**-0.529**	**-1.048**	**-0.032**
Diet-LD_{50} Dist	**0.340**	**0.244**	**0.439**	-0.005	-0.030	0.019
Random Terms						
Phylogeny	0.739	0.357	1.187	0.255	0.025	0.544
Phylogeny CV	0.062	-0.145	0.283	0.062	-0.145	0.283
Species	0.035	0.001	0.121	0.315	0.179	0.453
Residuals	0.290	0.236	0.352	0.016	0.013	0.019
Residuals CV	0.002	-0.008	0.013	0.002	-0.008	0.013

Table A4. Estimates and higher and lower 95% credibility intervals (CI) in model including constriction as a fixed factor. Fixed factors include mass; LD_{50} 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 LD_{50} model (Diet-LD_{50} Dist). The random terms and the co-variance (CV) between LD_{50} 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.

	LD_{50}			Mean Volume		
	Estimate	Lower CI	Upper CI	Estimate	Lower CI	Upper CI
Fixed Terms						
Intercept	0.330	-0.162	0.736	0.330	-0.162	0.736
Mass	0.115	-0.006	0.247	**0.501**	**0.446**	**0.560**
LD_{50} method$_{SC}$						
IV	**-0.6341**	**-0.856**	**-0.421**	-0.009	-0.047	0.032
IP	**-0.456**	**-0.682**	**-0.230**	-0.008	-0.051	0.037
IM	-0.148	-0.055	0.045	-0.006	-0.055	0.045
Dim$_{2D}$						
3D	-0.178	-0.627	0.272	**-0.859**	**-1.314**	**-0.385**
Eggs in Diet	0.686	0.096	1.336	-0.508	-1.086	0.032
Diet-LD_{50} Dist	**0.331**	**0.243**	**0.424**	-0.002	-0.022	0.016
Constriction$_{no}$						
present	-0.092	-0.754	0.506	-0.321	-1.004	0.253
Random Terms						
Phylogeny	0.802	0.385	1.230	0.327	0.008	0.700
Phylogeny CV	0.074	-0.160	0.319	0.074	-0.160	0.319
Species	0.034	0.001	0.116	0.342	0.001	0.519
Residuals	0.290	0.233	0.350	0.009	0.001	0.011
Residuals CV	0.003	-0.004	0.011	0.003	-0.004	0.011