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

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Snake venom is best known for its ability to incapacitate and kill prey. However, the trophic ability of venom is highly variable across species, with some species possessing venoms of seemingly harmless quantities and potencies, 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 of non-native prey species as models to assess venom potency, and if the toxic effects of venom are adapted to specific taxonomic groups of prey, this may confound comparative analyses. Here we test a range of hypotheses relating to the drivers of venom evolution by accounting for the phylogenetic distance between the typically non-natural model species used to measure potency and the observed dietary species in natural settings in a comparative analyses of the venom potency (LD$_{50}$) and yield of 99 species. We show that snake venom potency is prey-specific, with higher potencies when venoms were tested on species phylogenetically similar to those commonly found in their diet; and that venom yield scales positively with snake body mass as predicted by metabolic rate, but is lower for species found in habitats with higher dimensionality. These results underline the importance of ecological, physiological and environmental factors in the evolution of a novel predatory trait and highlight the wider potential of using venom as a system to understand the evolution of predator-prey coevolution in general.

Venom | Body size | Comparative analysis | Macroecology | phylogenetic analysis

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 extremely lethal venoms often gain the most attention (3), the prey incapacitating ability of different species venoms ranges widely, from those unable to subjugate animals larger than a few grams to those capable of subduing vast numbers of laboratory animals ((4) Figure 1). Understanding this variation is hence important from both a medical (2) and evolutionary viewpoint (1), yet 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 their prey these studies are typically focused at the genus level (5-8), making general inferences regarding the evolution of such fundamental aspects of venom 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, such as venom (1), are typically associated with differences in trophic ecology. For example, se-

lection 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 ingestion rates (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 and how changes in morphology map to trophic ability. Snake venom however offers a system were foraging capabilities can be quantified directly by measuring both venom potency, such as by measuring the median lethal dose (LD$_{50}$), and the quantity available. This direct measure of the predatory ability of venom hence allows for the fundamental evolutionary drivers of venom and predator traits in general to be tested. However, to compare such measures of venom across snakes the species each venoms predatory ability was tested on must be taken into account (5).

Venom is expected to have higher potencies when tested on a snakes natural prey species as venoms potencies would be selected to be effective against such species (1). In such a scenario the use of a model species not typical of a snake species diet would be predicted to result in the underestimation of potency. However, whether this potential prey-specific affect is the general rule across snakes has been the subject of much debate (9-11). For example, while prey-specificity has been demonstrated in several groups of venomous snakes (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

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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 direct predator-prey interactions result in venom evolving higher potency towards species closely resembling their natural diet while macroecological drivers, such as habitat dimensionality and metabolic constraints shape the quantity of venom available.

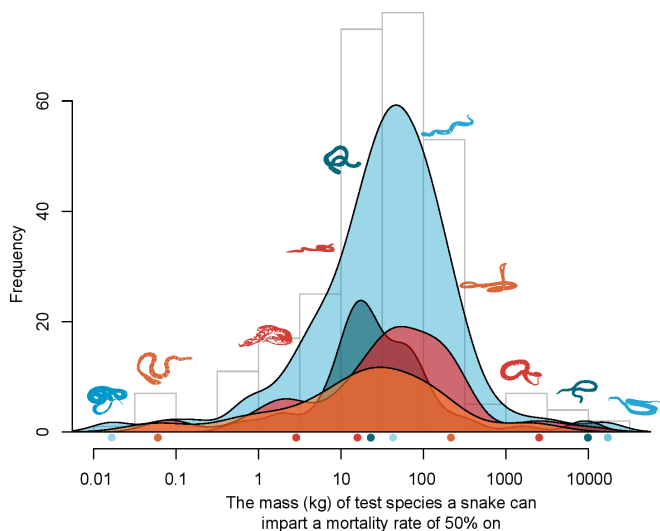**Reserved for Publication Footnotes**

Fig. 1. Histogram and density plot of the animal mass (kg) that snakes species can impart a 50% mortality rate on (282 observations, 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} are shown for IP (light blue), IM (dark blue), IV (red) and SC (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.*

race dynamics (24) predicts cases of prey-specific venoms and the evolution of prey tolerances depending on the level of selection on both predator and prey (24), while no relationship between potency and prey identity is predicted by the overkill hypothesis (9-11) (Figure 2). This prediction from the overkill hypothesis arises in a scenario were venom typically evolves levels of lethality so far in excess of trophic requirements that potential predator-prey dynamics play a minor role in further potency evolution. To both test whether any of these outcomes is the general rule with regards to venom potency and also to allow comparisons across species with diverse diets we use the evolutionary distance between the test species used to measure potency and the species typically found within each snake diet. This measure would be expected to have no relationship with venom potency if a overkill mechanism is the general driver of venom potency variation, while prey-specific venom would be expected to be higher when tested on species more closely related to their typical prey species, or lower in the case of the evolution of widespread immunity in prey species (Figure 2).

As the ability to incapacitate prey is also determined by the amount of venom available it would also be predicted that venom volume 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 metabolic rate. Both prey size and metabolic rate are strongly determined by body size (9, 19). In terms of prey size, in general larger predators eat larger prey (19). It would hence 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. One 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, commonly estimated as 0.75, of venoms' toxicological effects (29). Hence 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 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 (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.
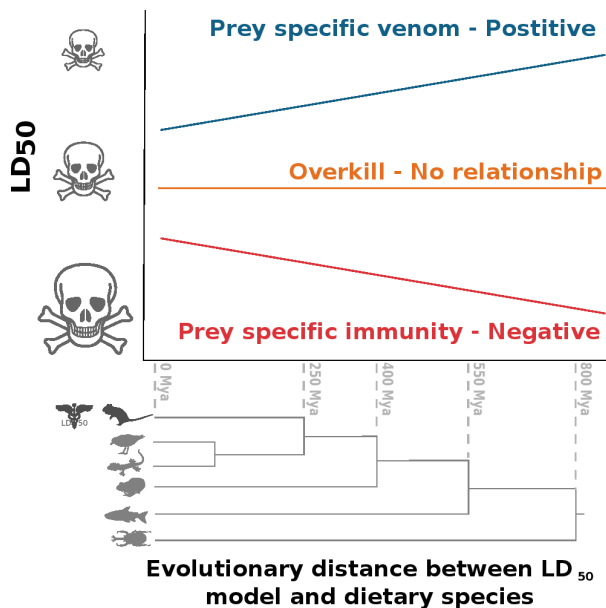
Other predictions include the overkill hypothesis which predicts no relationship between venom yield and prey size (a with a scaling exponent of 0), or alternatively venom yield may scale according to constraints such as metabolic costs, where a scaling of 0.75 would be expected (30). At the other extreme 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 1).

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 ninety-nine 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.

Prey specific venom - Postive

Overkill - No relationship

Prey specific immunity - Negative

LD₅₀ — LD_{50} (y-axis)

0 Mya · 250 Mya · 400 Mya · 550 Mya · 800 Mya

Evolutionary distance between LD₅₀ model and dietary species

To test if venom is prey-specific we caluclated the evolutionary distance between the LD_{50} model species and the species of the diet.
For example, if LD_{50} was tested on a mouse a diet comprising of mammals would have a distance of 0 Mya while a diet of fish would be 550 Mya (See methods). Using this metric we test the following hypothesis:

Prey-Specific Venom: As venom is expected to be adapted towards typical prey targets it predicts a positive relationship between LD_{50} and the distance between LD_{50} model and diet.
The Overkill Hypothesis: Under neutral selection LD_{50} is expected to show no pattern relating to prey identity.
Prey-Specific Immunity: If prey immunity evoleves faster than venom potency LD50 would be expected to be highest on phylogentically distance venom nieve species.

Alternative >1.0

MTE 0.75

Predator-prey 0.19-0.5

Overkill 0.0

Venom Volume (y-axis)

Snake body size

The amount of venom a snake porduces is likely to be determined by factors relating to body mass. These potential drivers can be tested based on predictions of the allometric scaling of venom volume with body size including;
The Overkill Hypotesis: Predicts no relationship.
Predator-prey: If venom volume follows scaling associeted with predator-prey size scaling a coefficent of between 0.19 and 0.51 is predicted (equation 2).
MTE: If venom production scales with according to the metabolic theory of ecology (MTE) venom volume would be expected to scale acording to 0.75.
Altrenative: Scaling expoents of >1 would suggest drivers such as sexual selection or defense are important in venom evolution.

Fig. 2. Summary of hypothesis relating to potential drivers of snake venom evolution.

(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 superlinear.

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

We show that both trophic and macroecological factors are important drivers of snake venom evolution.

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 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 177 observations across 68 species. We report the results across all models relating to the importance of each driving factor in order below (Table A1).

Predator-prey coevolution

Despite the presence of only eight egg eating species in our dataset, LD_{50} 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 (Figure 2; Tables A1-5).

Of the species included within the analysis only 14 species had a diet completely matching that of the LD_{50} model 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 211.3 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 having higher potencies (Figure 2; table A3-4; Figure 1B). From the main model, after back-transforming the mean centered $\log_{10}$ value, LD_{50} was found to increase by 1.44 for every 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 2; Table A1-3).

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
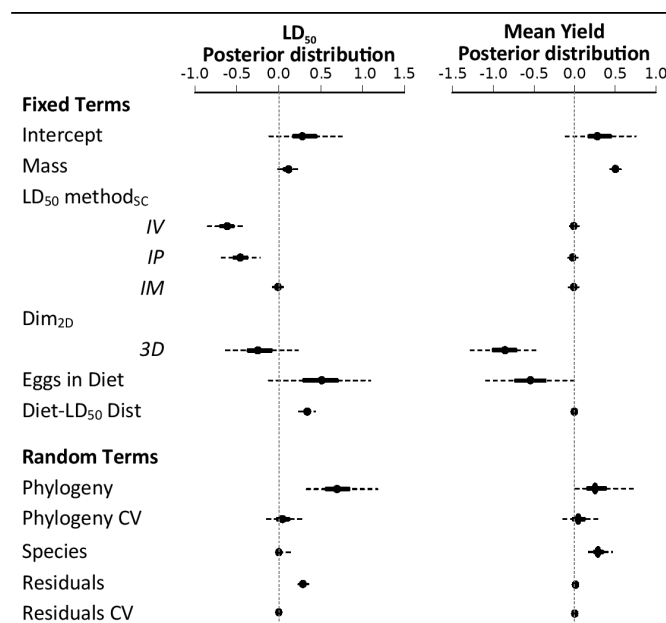
Fig. 3. Posterior distributions of LD$_{50}$ and mean venom volume estimates (represented by dots) and higher and lower 95% credibility intervals (represented by dotted horizontal bar). Fixed factors include mass; LD$_{50}$ method (subcutaneous (SC), intravenous (IV), intrapulmonary (IP) and intramuscular (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.

Fig. 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 1 and Table A1.

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, Tables A2-6), 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 A3). Snake body size was also found to have a significantly positively correlation with LD$_{50}$ in the main analysis of 99 species, meaning larger snakes showed decreased potency (Figure 3). However, this relationship was not significant in any of the sub analysis (Table A3-4).

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 (Figure 2). A sensitivity analysis where habitat was included as terrestrial, arboreal and aquatic also showed similar significant reductions in both arboreal and aquatic habitats (Table A5).

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 0.69 of the residual variation for LD$_{50}$ and 0.49 for venom yield, with LD$_{50}$ showing a higher phylogenetic signal in all models (Figure 2, A2-5). 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, A2-5).
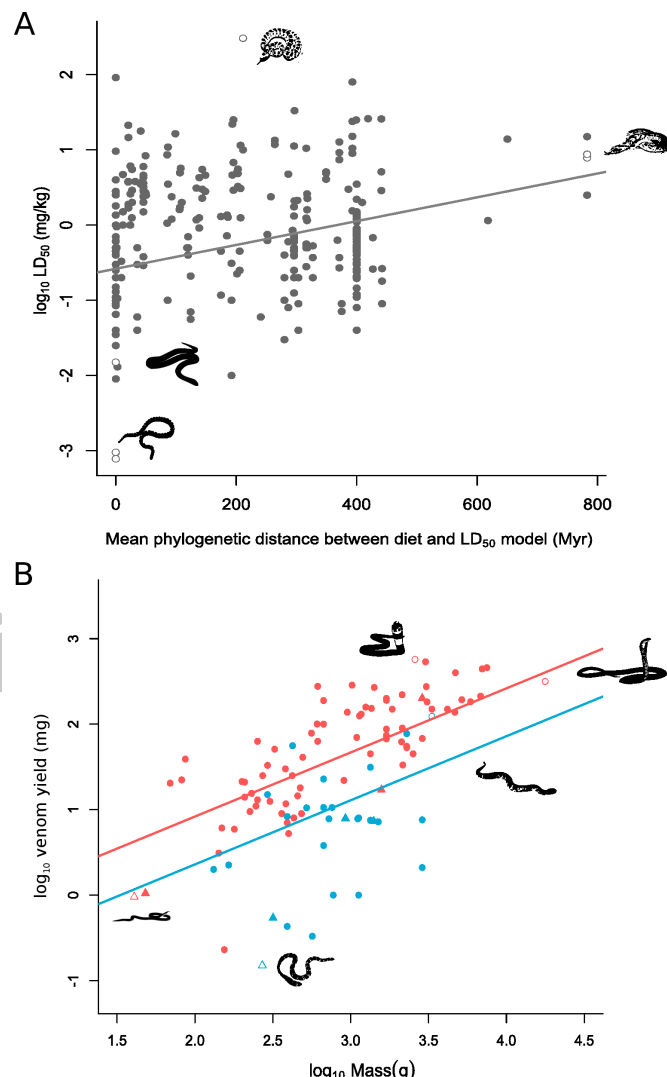
Discussion

Predator traits are thought to be heavily shaped by both predator-prey arms race dynamics and macroecological forces such as imposed by the limitations of body size and habitat structure. Here we show that snake venom follows patterns consistent of a predatory trait which has evolved under such selection pressures, with venom potency found to be prey-specific in general and the energetic costs of venom and habitat dimensionality found to be drivers of venom yield. Overall, these results show that by controlling for the species used to measure potency in comparative analysis across large taxonomic groups, hypothesis regarding the evolutionary drivers of venom can be robustly tested.

The importance of trophic factors in the evolutionary maintenance of venom is well demonstrated by cases of dietary switches

to immobile or unprotected prey, such as seen by the almost complete atrophy of the venom apparatus in the marbled sea snake (*Aipysurus eydouxii*) due switching to an egg based diet (36). This maintenance mechanism is also supported by our findings that ovivorous feeding is associated with lower potencies and venom yield. However, beyond the role of maintaining the possession of venom the role of trophic drivers in shaping venoms subsequent evolution 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 (6) we show that venom is generally prey-specific. Moreover, our analysis is likely to underestimate the generality of the prey-specific nature of venom due to our use of LD_{50}. While LD_{50} measures the lethality of a venom it is likely that venom is selected to simply subdue prey, in order to reduce the chances of prey escaping or retaliating (5). 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 measures of sub-lethal incapacitating effects, may further our understanding of how predator-prey dynamics shape venom potency (5). However, despite such limitations, we find a clear pattern of prey-specificity suggesting that cases of non-prey specific venom (21-23, 37, 38) are more likely to be exceptions to the general rule.

In terms of macroecological patterns, unsurprisingly we found that larger snakes had larger quantities of venom. More surprisingly these increases did not follow the expected scaling allometry of 0.51 from a predator-prey scaling perspective (19) with venom yield increasing faster with snake body size than expected. Even when considering potential variation in the allometric scaling of toxicological effects (equation 3) (29), a value far in excess of 1 is required for the scaling of toxicological effects (*b*), in order for our results to match the predator-prey scaling prediction: a situation that seems unlikely. Furthermore, our analysis revealed that the predatory-prey body mass scaling of venomous snakes, (*a* in equation 3) is much lower than expected, based on comparisons to previous body mass scaling conducted on all snakes (19), meaning that larger venomous snakes feed on much smaller prey items than predicted for their size. This lower scaling of prey size may explain the reduced venom potency with snake size seen in our main analysis.

While venom yield did not follow predictions arising from predator-prey body mass scaling it did match an allometry of 0.75 expected from metabolic theory (30, 39). This suggests that the scaling of metabolic rate with body size and the related scaling of the production of a energetically costly material such as venom (25), is a more direct driver of venom yield than trophic drivers such as prey size. However, while the total amount of venom available to a snake may be such metabolic scaling, there is evidence that the amount administered in a single bite is more closely related to prey size (28). Such a decoupling of trophic and metabolic between the amount of venom in a single bite and the total reservoir may have interesting implications on the predation strategies available with body size. As yield scales with body size according to a higher exponent than prey size, larger species would 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.

Another 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 (33) 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 (40), 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 (41). Another potential explanation is that higher encounter rates in high dimensional environments (15) may reduce the missed opportunity of feeding cost associated with replenishing venom. Rates of replenishing venom can be substantial with estimates ranging from 3-7 days (42) to 30-50 days (28, 43). These long periods of replenishment may hence select for larger 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 (44). Future analysis 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 (45), 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 (IM) routes 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 (46), 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 (40).

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 (47). 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 (47-49). In cases were only body lengths were available for prey species allometric scaling were used to convert to mass (48, 50). 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 (51) 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 (52) in R v 3.2.4 (53). 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) (275 observations over 99 species).

A similar model was also fitted 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(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 (54).

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