**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 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.

**Abstract (243 words – 250 max)**

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 (LD50) 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.

**Keywords:** Venom, Body size, Comparative analysis, Scaling, trophic ecology, Macroecology, LD50, phylogenetic analysis

**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](#_ENREF_1)) but also a major health concern, with snakebites estimated to cause up to 94,000 deaths annually ([2](#_ENREF_2)). Although extremely lethal venoms are often the focus of attention ([3](#_ENREF_3)), the ability of venom to incapacitate prey ranges widely among species, from those unable to subjugate animals larger than a few grams to species capable of subduing in excess of 10,000 laboratory animals (([4](#_ENREF_4)) Figure 1). While understanding this variation is important from both medical ([2](#_ENREF_2)) and evolutionary viewpoint ([1](#_ENREF_1)) little is known about what drives this disparity amongst species. On reason for this is the lack of multi-species comparison across large taxonomic groups. For example, while several studies have explored whether venoms have evolved higher potency towards species commonly found in the diet, these are typically focused at lower taxonomic levels ([5-8](#_ENREF_5)). Such a lack of comparisons across broad taxonomic ranges has made inferences of general patterns of the evolution of venoms functional ability difficult, so much so that the general role of prey identity on venom evolution is still under question ([9-11](#_ENREF_9)). Here we conduct a comparative analysis across a diverse range of venomous snakes allowing us to not only test patterns related to venom evolution but also test the importance of potential drivers of predator trait evolution in general.

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Figure 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 LD50 (mg/kg). Measurement routes of LD50 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*.

While venom may have defensive ([12](#_ENREF_12)) and digestive ([13](#_ENREF_13)) functions, snake venom is likely to be primarily a predatory trait ([1](#_ENREF_1)). The importance of venoms trophic function is most starkly highlighted by the atrophy of the venomous apparatus in species which have switched to undefended prey, such as in the case of the egg eating marble sea snake ([14](#_ENREF_14)). Predatory traits, such as venom, are expected to be heavily influenced by aspects relating to trophic ecology. For example, selection on jaw and beak morphology in cichlid fish and across birds is strongly associated with trophic factors such as prey type ([15](#_ENREF_15), [16](#_ENREF_16)). Apart from prey type, other factors related aspects of trophic interactions such as search and encounter rates ([17-19](#_ENREF_17)); the ability to spot, track and capture prey ([20](#_ENREF_20), [21](#_ENREF_21)); and ingestion rates ([19](#_ENREF_19), [22](#_ENREF_22)) are also likely to influence the predatory traits. However, while morphological measures of trophic traits can be linked to such potential drivers ([13](#_ENREF_13)) it is difficult to accurately quantify the importance these drivers have on shaping predatory traits. For example, while a change in diet may be associated with a change in jaw or beak morphology, how such morphological changes translate into changes in foraging abilities is less clear. Snake venom however offers a system were such foraging capabilities can be quantified directly by measuring both venom potency, such as by measuring the median lethal dose (LD50), and the quantity available. This direct measure of venoms predatory ability allows it to be compared across ecologically diverse groups such as snakes, which have habitats ranging from marine, arboreal, fossorial and terrestrial and diets ranging from fish eggs to mammals. Such an analysis would not only offer an insight to the pattern of venoms capabilities across snakes but also allow general large scale patterns of predatory traits to be tested, such as related to predator-prey dynamics and body size scaling. However, to allow such large scale analysis across snake species, how the

whether each species venom has evolved to target different prey needs to be accounted.

The lethality of venom depends on several aspects of how it was measured, such as whether it was introduced subcutaneously (SC), intravenously (IV) or using other routes ([23](#_ENREF_23)). However, the importance of the identity of the prey used to measure venom potency has been a long standing debate in the field ([9-11](#_ENREF_9)). From a trophic ecological and evolutionary perspective, it would be expected that snake venom would be selected to have higher potencies towards common target species, such as those found within its diet. Such prey-specific venoms would hence be expected to result in underestimate of the potency of venoms in species which target non-mammal prey as mice are typically used to measure within laboratory settings ([6](#_ENREF_6)). However, while such prey-specific venoms have been demonstrated in Malayan pitvipers ([7](#_ENREF_7)), coral snakes ([6](#_ENREF_6)), the viper genus *Echis* ([5](#_ENREF_5)), saw-scaled vipers ([24](#_ENREF_24)) and insect eating *Pelias* vipers ([8](#_ENREF_8)), other cases have found either no relationship between prey and venom lethality ([25](#_ENREF_25)) or a reverse case were common prey items have seemly evolved resistance towards their predators venoms, such as potentially the cases of opossums and neotropical pitvipers ([26](#_ENREF_26)) and in sea snakes and eels ([27](#_ENREF_27)). Here we use a measure of the evolutionary distance between the prey groups found within a snake species diet and the animal model used to measure its LD50 to account for these potential predator-prey dynamics. This measure of evolutionary distance between test and intended target species will not only allow for comparison of venoms across snakes but also acts as a test of various predicted outcomes relating to predator-prey dynamics (Figure 2).

Perhaps the simplest yet most debated prediction relating to the role of predator-prey dynamics in the evolution of venom potency is that of the overkill hypothesis ([9](#_ENREF_9), [10](#_ENREF_10)). This hypothesis posits that venom typically evolves levels of lethality so far in excess of trophic requirements that any potential predator-prey dynamics play a minor role in further potency evolution (Figure 2). However, while this hypothesis may hold for exceptionally potency venoms, many species show intermediate level of lethality (Figure 1). Moreover, the alternative hypothesis that venom potency is selected towards common prey species, has gained support by the several cases of snake species displaying such prey-specific venom since this initial debate ([5](#_ENREF_5), [6](#_ENREF_6), [8](#_ENREF_8)). Whether these cases of prey-specaificty are the general rule rather than the expection is not known. However, the gernality of either prey-specificity or overkill effects can be tested based on whether venoms measured using species evolutionary distant from those found in the diet are lower than those tested on species closely related. In particular as closely related prey species are more lilkey to have similar physiologies prey-specific venoms would be expected to show higher potencies on test models simlar to their prey while if the overkill hyptheis sis the genral rule, no such effect would be expected (Figure 2).

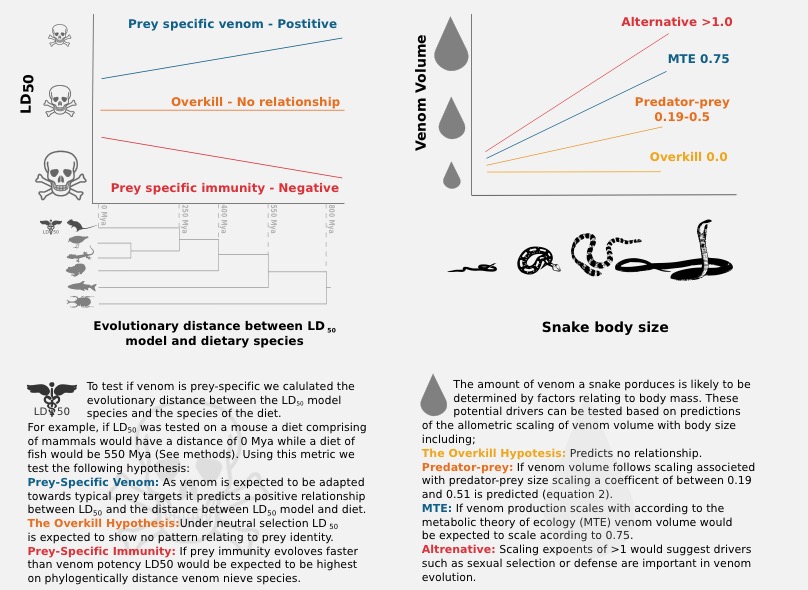


Figure 1. Summary of hypothesis relating to potential drivers of snake venom evolution.

As the ability to incapacitate prey is also determined by the amount of venom available it would also be predicted that venom volume would be under similar selective pressures. In particular, as venom production incurs an energetic cost ([28](#_ENREF_28)) (although the level of this cost is debated ([29](#_ENREF_29))) 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](#_ENREF_9), [22](#_ENREF_22)). In terms of prey size, in general larger predators eat larger prey ([22](#_ENREF_22)). 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 ([30](#_ENREF_30), [31](#_ENREF_31)) supports the presence of such selection on venom quantity. However, while bigger snakes are known to have larger amounts of venom in general ([4](#_ENREF_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);

Where for snakes the scaling exponent *a* is approximately 0.88 ([22](#_ENREF_22)). However, venom yield would not be expected to scale according to this exponent as the effects of toxicological agents also follows an allometric relationship ([32](#_ENREF_32)) where the amount of venom required (*V*) to induce the same incapacitating effect on a prey of mass (*Mprey*) would be expected to follow (equation 2);

where *b* is the scaling coefficient, commonly estimated as 0.75, of venoms’ toxicological effects ([32](#_ENREF_32)). 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 from equation 1 for to get (equation 3);

If we take the commonly used value of 0.75 for the interspecific scaling of drug dosages ([32](#_ENREF_32)) for *b* and the value of 0.68 for *a* from the scaling predator-prey mass relationship for snakes ([22](#_ENREF_22)) 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 ([33](#_ENREF_33)). 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 ([34](#_ENREF_34)), or defenses requiring increased effectiveness with size, such as seen in the allometry of horn growth in horned lizards ([35](#_ENREF_35)) (Figure 1).

Finally, an overlooked feature that may also drive the evolution of both venom quantity and toxicity is habitat structure ([36](#_ENREF_36)). 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 ([18](#_ENREF_18), [22](#_ENREF_22)) and the escape rates of prey, with higher dimensional spaces increasing both ([37](#_ENREF_37), [38](#_ENREF_38)). 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 (LD50), in a phylogenetically corrected comparative analysis of ninety-nine species of venomous snakes. Using the phylogenetic distance between species used to measure LD50 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 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 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 in driving venom evolution with patterns supporting prey-specific venom in general and venom quantity both scaling as predicted by metabolic cost constraints and lower in higher dimensional habitats.

**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 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, 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 (Figure 2; Tables A1-5).

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 such that snake species with diets phylogenetically close to the LD50 model species having higher potencies (Figure 2; table A3-4; 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 estimate is made after correcting for LD50 injection route where intravenous and Intraperitoneal routes were found to have lower LD50 values in comparison to a subcutaneous route (Figure 2; Table A1-3).

Figure 2. Posterior distributions of LD50 and mean venom volume estimates (represented by dots) and higher and lower 95% credibility intervals (represented by dotted horizontal bar). 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 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.

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**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, 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 log10-log10 slope of 0.139 (equating to an approximate 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 LD50, meaning larger snakes showed decreased potency (Figure 3). However, this relationship was only significant in the main 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).

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Figure 3. (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 Figure 1 and Table A1.

**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 h2 values of 0.69 for LD50 and 0.49 for venom yield (Figure 2, A2-5). with LD50 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 LD50(Table A4). Finally, there is no significant covariance between either the residuals of phylogenetic terms of both yield and LD50 across all models (Figure 2, A2-5).

**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. We found that venom potency is prey specific, with higher potencies found when venom is tested 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 (exponent of XX). We also found 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 ([14](#_ENREF_14)), the absence of an increased lifespan associated with venomosity as generally seen in species with toxic defence systems ([12](#_ENREF_12)), 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](#_ENREF_1), [9](#_ENREF_9), [10](#_ENREF_10)). By flipping the inconvenience usually associated with the use of non-natural test models for venom potency ([6](#_ENREF_6)) 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 ([5](#_ENREF_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 a measure of its sub-lethal incapacitating effects, may give clearer reflection of venoms prey-specificity ([5](#_ENREF_5)). However, despite such limitations, we find a clear pattern of prey-specificity suggesting that cases of non-prey specific venom ([25-27](#_ENREF_25), [39](#_ENREF_39), [40](#_ENREF_40)) are more likely to be the exception 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 ([22](#_ENREF_22)) 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) ([32](#_ENREF_32)), a value far in excess of 1 is required for *b*, the scaling of toxicological effects, in order for the observed scaling of 0.75 between venom volume and snake mass to agree with this 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 in comparison to snakes as a whole ([22](#_ENREF_22)), with larger venomous snakes feeding on much smaller prey items than expected. This lower scaling of prey size may 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 ([41](#_ENREF_41)), which also has a similar scaling coefficient of 0.75 with respect to body mass ([33](#_ENREF_33), [41](#_ENREF_41)). In particular, it might be expected that the production of a potentially metabolically costly material such as venom ([28](#_ENREF_28)), 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 administrated in a single bite does seem to show stronger associations with trophic factors ([31](#_ENREF_31)). 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 ([36](#_ENREF_36)) 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 ([42](#_ENREF_42)), 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 ([43](#_ENREF_43)). Another potential explanation is that higher encounter rates in high dimensional environments ([18](#_ENREF_18)) 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 ([44](#_ENREF_44)) to 30-50 days ([31](#_ENREF_31), [45-47](#_ENREF_45)). 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 ([48](#_ENREF_48)), 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, where patterns relating to prey specificity and energetic constraints are also likely to play key roles in their evolution ([1](#_ENREF_1)). Examples of prey-specific venom is seen in cone snails and spiders ([1](#_ENREF_1)), while the energetic costs of producing venom is also suggested by venom metering in taxa such as scorpions ([49](#_ENREF_49)). The generality of prey-specify and macroecological constraints is also likely to extent 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 (LD50) 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 LD50 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 LD50 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 ([50](#_ENREF_50)), between each prey taxa and the LD50 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 LD50 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 LD50 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 ([18](#_ENREF_18)), 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](#_ENREF_42)).

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 ([51](#_ENREF_51)). 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 ([51-53](#_ENREF_51)). In cases were only body lengths were available for prey species allometric scaling were used to convert to mass ([52](#_ENREF_52), [54](#_ENREF_54)). 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, LD50, venom yield and phylogenetic distance between diet and model were all log10 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 ([55](#_ENREF_55)) 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 ([56](#_ENREF_56)) in R v 3.2.4 ([57](#_ENREF_57)). As venom yield and LD50 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; LD50 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 LD50 model were included as explanatory variables to give the analysis;

1. Yield + LD50 = *f*(Snake mass + LD50 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;

1. Yield = *f*(Prey mass)
2. 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 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 ([58](#_ENREF_58)).

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