LETTER

Snake venom potency and yield are associated with prey-evolution, predator metabolism and habitat structure

Kevin Healy,^{1,2,3*} Chris Carbone⁴
and Andrew L. Jackson¹

Abstract

Snake venom is well known for its ability to incapacitate and kill prey. Yet, potency and the amount of venom available varies greatly across species, ranging from the seemingly harmless to those capable of killing vast numbers of potential prey. This variation is poorly understood, with comparative approaches confounded by the use of atypical prey species as models to measure venom potency. Here, we account for such confounding issues by incorporating the phylogenetic similarity between a snake's diet and the species used to measure its potency. In a comparative analysis of 102 species we show that snake venom potency is generally prey-specific. We also show that venom yields are lower in species occupying three dimensional environments and increases with body size corresponding to metabolic rate, but faster than predicted from increases in prey size. These results underline the importance of physiological and environmental factors in the evolution of predator traits.

Keywords

Body size, comparative analysis, LD₅₀, macroecology, phylogenetic analysis, scaling, snake, trophic ecology, venom.

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INTRODUCTION

The ability of snake venom to incapacitate and disrupt the physiological systems of animals is one of its most defining features, with some species possessing enough venom to incapacitate tens of thousands of laboratory test animals (Fig. 1a). From a human perspective, this property of venom makes it both a source of novel biomedical compounds (Casewell *et al.* 2013) and a major health concern, with snake bites estimated to cause up to 94 000 deaths annually (Kasturiratne *et al.* 2008). Yet, not all venomous snake species possess such a lethal amount of venom towards test animals (Chippaux *et al.* 1991; Weinstein *et al.* 2011), with the ability to subjugate potential prey, ranging from the practically harmless egg-eating sea snake (*Emydocephalus annulatus*) to extremely potent species such as the many-banded krait (*Bungarus multicinctus*) (Fig. 1). While understanding this variation is important from both medical (Kasturiratne *et al.* 2008) and evolutionary (Casewell *et al.* 2013) viewpoints, much is still unknown regarding venoms ecological and evolutionary drivers.

Variation in traits associated with predation are expected to be closely linked to aspects of trophic ecology. This includes factors relating to encounter (Domenici 2001; Pawar *et al.* 2012; Kane *et al.* 2016), capture, and ingestion rates (Kiltie 2000; Carbone *et al.* 2014) along with characteristics of the prey itself. Despite the central role of venom in predation for many snake species (Casewell *et al.* 2013), the role of ecological and evolutionary drivers of venom variation is yet to be

tested in a large comparative framework. The lack of such a comparative framework has made it particularly difficult to resolve fundamental questions regarding venom evolution, such as whether the evolution of increased potency against frequently encountered prey is a general rule (Sasa 1999; Wüster *et al.* 1999; Mebs 2001).

One reason for the lack of such large-scale comparative analyses is the difficulty in conducting multi-species comparisons of venom across taxonomically diverse groups. This stems from the non-standardised choice of model species typically used to test venom potency, or using species which are not the natural diet for the snake (da Silva & Aird 2001). This can lead to the confounding case where potency measures are heavily influenced by how similar a snake's diet is to the potency test species. Here, we incorporate the evolutionary distance between a snake's diet and the model species used to measure its potency in order to allow comparisons across the taxonomic diversity of venomous snakes. Using this framework, we test a series of hypotheses relating to the drivers of both potency and venom yield in snakes (Fig. 2).

Predator–prey arms race dynamics predict the selection on venoms to be prey-specific, and, conversely, the evolution of venom tolerances in prey (Van Valen 1973). The alternative overkill hypothesis posits that once the level of lethality in a venom greatly exceeds typical feeding requirements, predator–prey dynamics play a minor role in the evolution of venom potency due to weak selection (Sasa 1999; Wüster *et al.* 1999; Mebs 2001). Evidence for both cases have been found, with prey-specificity previously demonstrated in several genera

¹Department of Zoology, School of Natural Sciences, Trinity College Dublin, Dublin 2, Ireland

²School of Biology, University of St Andrews, St Andrews KY16 9TH, UK

³School of Natural Sciences, National University of Ireland Galway, Galway, Ireland

⁴Institute of Zoology, Zoological Society of London, London, UK

*Correspondence: E-mail: healyke@tcd.ie

Figure 1 (a) Histogram representing the distribution of the incapacitating potential of species in our dataset of 538 potency measures for 102 species. Incapacitating potential is the animal mass (kg) which a snake's total yield of venom can impart a 50% mortality rate on. This was calculated as the mean volume of dried venom for a species divided by its lethal dose (LD₅₀) (mg kg⁻¹). The colour bar ranges from low incapacitating ability (blue) to high (red) with the corresponding colour used for the highlighted species silhouettes in both a and b. The density curves represent the distribution of incapacitating ability for each of the routes LD₅₀ was administered to the test model [red (intravenous, $n = 168$), blue (intraperitoneal, $n = 195$), orange (subcutaneous, $n = 88$) and green (intramuscular, $n = 87$)]. (b) Phylogenetic relationship between the 102 species included in the analysis. Outwards from the centre of the phylogeny the first colour band describes each species habitat followed by a band indicating the taxonomic family. The first circular bar-plot represents the mean yield for each species, with the outermost bar-plot describing the lowest LD₅₀ for a given species. Species are highlighted as silhouettes with colours matching the incapacitation ability scale from (a).

(Daltry *et al.* 1996; da Silva & Aird 2001; Mackessy *et al.* 2006; Starkov *et al.* 2007; Barlow *et al.* 2009; Richards *et al.* 2012; Vonk *et al.* 2013; Margres *et al.* 2017), while other examples have either found no such prey-specificity (Williams *et al.* 1988) or cases where the prey species have evolved tolerance towards their predator's venoms (Heatwole & Poran 1995; Biardi *et al.* 2000; Voss 2013; Pomento *et al.* 2016; Arbuckle *et al.* 2017). However, whether these cases are taxon specific or form the general rule across all venomous snakes has yet to be tested at a broad taxonomic scale. Similarly, while the amount of venom a species possess may influence its ability to capture prey (Morgenstern & King 2013), the role of snake and prey body size as well as foraging conditions have on venom yield is also poorly understood at macroecological scales.

Under the prey-specific hypothesis of venom evolution, potency is expected to be higher when measured on model species that more closely resemble the predator's diet. Given that closely related species are more likely to share physiological similarities compared to more distantly related species, the prey-specific hypothesis predicts a decrease in potency with increasing evolutionary distance between the snake's diet and the species used to measure its potency (Fig. 2). In contrast, the overkill hypothesis predicts the absence of such a relationship. Alternatively, under a focal prey resistance hypothesis, species more distantly related to the snake's diet are predicted to be more susceptible to the snake's venom due to reduced selection for venom resistance, a hypothesis with experimental support in a *Crotalus-Sciurus* system (Pomento *et al.* 2016).

As venom production incurs some metabolic cost (McCue & Mason 2006), although the level of this cost is debated (Pintor *et al.* 2010; Smith *et al.* 2014), the yield of venom a snake possesses is also likely to be under selection. Like many other trophic traits, much of this variation is likely to vary with body size according to allometric relationships of the form $Y \sim \text{Mass}^a$ (Hayes *et al.* 2002; Brown *et al.* 2004), where the value of the scaling exponent a can offer insights into the potential drivers of venom yield variation across species (Fig. 2). For example, if venom yields are constrained by rates of biological production they are predicted to increase with snake body mass according to the $\frac{3}{4}$ scaling relationship of metabolism (Brown *et al.* 2004; Isaac & Carbone 2010). In contrast, if yield is driven by predator-prey allometries a coefficient of 0.51 is predicted (Fig. 2). At the other extreme, super-linear allometries (exponents > 1) would suggest patterns associated with drivers such as sexual selection, as proposed by the weapons hypothesis (Kodric-Brown *et al.* 2006), or, in the more likely case for snakes due to limited use of venom in sexual conflict (Casewell *et al.* 2013), for use in defence that may require increased effectiveness with size, such as in the allometry of horn growth in horned lizards (Bergmann & Berk 2012).

Other potential drivers of venom evolution relate to the prey itself. For example, due to a switch in diet to one that is almost completely comprised of fish eggs, the marbled sea snake's (*Aipysurus eydouxii*) venom system has almost completely atrophied (Li *et al.* 2005) (Fig. 1). Due to the reduced need to incapacitate prey, species which display oophagy are predicted to have lower potencies and venom yields. Similar

expectations can also be made for species that use other means of incapacitating prey, such as constriction (Shine & Schwaner 1985), due to a similar selective release, or, conversely, through the need to compensate for already having lower venom potencies and yields.

While the effect of body size on trophic traits has long been realised, the importance of factors influencing the rate of interaction between predators and their prey, such as habitat structure (Arbuckle 2015), has only more recently become realised. The structural complexity of a habitat, such as whether it is a two-dimensional (2D) terrestrial surface or a complex three-dimensional (3D) forest canopy, can influence both encounter rates (Pawar *et al.* 2012; Carbone *et al.* 2014) and the escape rates of prey, with higher dimensional spaces increasing both (Heithaus *et al.* 2009; Møller 2010). While high-dimensional environments may increase the opportunity for prey escape (Møller 2010), and hence select for increased venom yields and potencies to compensate, the high encounter rates in these environments may conversely compensate for such increased potential prey escape rates.

By controlling for the model used to measure potency, we test these fundamental hypotheses of venom evolution (Fig. 2) in a phylogenetic comparative analysis of 102 species.

METHODS

Data

To test our hypotheses, we collated data on venom yield and toxicity from the literature. 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 reported measure. For venom lethality, we used median lethal dose (LD_{50}). We only used intravenous (IV), subcutaneous (SC), intraperitoneal (IP) or intramuscular routes (IM) of administering the venom. Only adult LD_{50} values were used due to ontogenetic variability in venom potency (Andrade & Abe 1999). As LD_{50} can show high intraspecific variability (Martinson *et al.* 2017) we also collated multiple measures of LD_{50} for each species and the reported measurements of variability associated with each LD_{50} value including, 95% credibility intervals, 5% Fiducial limits, standard deviation and ranges.

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 converted to mass using family-level allometric scaling as described in Feldman & Meiri (2013). We collated dietary data of quantitative estimates of prey proportions, mainly from studies of stomach contents. Only dietary analyses of adults were included in the analysis. Prey size data were included from these dietary studies when available. When prey size was not reported in the dietary studies and where prey species were identified to the species level, we used mean prey species body mass from available databases (Meiri 2010; Feldman & Meiri 2013; Myhrvold *et al.* 2015; Froese 2016). In cases where only body lengths were available for prey species, allometric scaling equations were used to convert to mass (Pough 1980; Feldman & Meiri 2013; Myhrvold *et al.* 2015). For species that

were only identified to the genus level, the genus' mean body mass was used if available. For each snake species, we calculated the weighted mean prey size W_j as $W_j = \sum_{i=1}^n (p_{ij}m_{ij})$, where the mass of each prey item (m_{ij}) was weighted by the proportion (p_{ij}) of the diet it comprised for a snake species j over the n prey items in its diet.

To test the prey-specific hypothesis we calculated the phylogenetic distance [million years ago (Mya)] to the common ancestor of the LD₅₀ model and the dietary prey items. For the phylogenetic distance between prey identified to species or genus level we used the recently published phylogenies for Mammalia (Bininda-Emonds *et al.* 2007), Aves (Jetz *et al.* 2012) and Squamata (Pyron & Burbrink 2014). For ancestral ages between major classes we used 272 Mya for the common ancestor between Lepidosauria and Archosauria (Jones *et al.* 2013), 316.35 Mya for the common ancestor of amniotes based on the fossil *Archerpeton anthracos* (Reisz & Müller 2004), 419 Mya for the common ancestor of Actinopterygians and Sarcopterygians based on the fossil *Guiyu oneiros* (Zhu *et al.* 2009), and 556.5 Mya for the fossil *Kimberella quadrata* as the common ancestor of deuterostomes and protostomes (Fedonkin *et al.* 2007). For prey items only identified to family level or above we used phylogenetic distances calculated using TimeTree (Hedges *et al.* 2006). We then calculated the mean phylogenetic distance between the diet of each snake and each LD₅₀ model used to measure its venom potency. We weighted this mean according to the proportion of each prey item in the diet. This was calculated as $D_{LD50-Diet(jk)} = \sum_{i=1}^n (p_{ij}d_{ik})$, where $D_{LD50-Diet(jk)}$ is the weighted phylogenetic distance between the diet of a focal snake species j and a LD₅₀ model species k , p_{ij} is the proportion of the j th snake species' diet comprised by prey item i and d_{ik} is the evolutionary distance (Mya) to the common ancestor of i and k .

Species' habitat was categorised 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.* 2012), with terrestrial and fossorial environments scored as 2D and arboreal and aquatic scored as 3D. As some venomous species also engage in constriction behaviour, we collected data on any observation of constriction behaviour from the literature (Shine & Schwaner 1985).

Snake mass, prey mass, LD₅₀ and venom yield were all log₁₀ transformed in order to test scaling allometry predictions. The phylogeny from Pyron & Burbrink (2014) was included in all analyses to account for non-independence in traits due to common descent. Only snake species which had data on the proportion of prey items in their diet, LD₅₀, venom yield and body size were included in the analysis. The Data are available in Supporting Information (Tables S2 and S3).

Analysis

To test our hypotheses, we fitted Bayesian phylogenetic mixed models (BPMM) using the MCMCglmm package (Hadfield 2010) in R version 3.4.0 (RC Team 2016). We controlled for pseudoreplication due to shared ancestry between species by using the animal term in MCMCglmm

(Hadfield 2010). This term uses a distance matrix of the phylogenetic distance between species to control for the expected similarity in trait values due to phylogenetic relatedness. We calculated the term h^2 as the relative variance attributable to the animal term (Hadfield & Nakagawa 2010). This term can be interpreted in a similar fashion to the phylogenetic lambda value, with a h^2 value close to 1 indicating a Brownian model of trait evolution, and a value close to zero indicating independence between trait values (Hadfield & Nakagawa 2010). We fitted all models using parameter expanded priors, with standard non-informative priors also tested separately to ensure that choice of prior had no effect on model results (Hadfield 2010). Choice of 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 (Brooks & Gelman 1998).

LD₅₀ models

To test the overkill, prey-specific potency and the focal prey resistance hypotheses we ran a BPMM with LD₅₀ as the response with $D_{LD50-Diet}$ as an explanatory variable (see Fig. 2). We controlled for the effect of route of injection by including it as a fixed factor (SC, IM, IV, IP). To test the oophagy hypothesis we include a fixed factor for the presence of eggs in the diet (absent, present). To test the habitat complexity hypothesis, we included habitat dimensionality as a fixed factor (2D, 3D). As measures of LD₅₀ can have large levels of intraspecific variation (Martinson *et al.* 2017), we include multiple measures of LD₅₀ for each species when available and account for this in our model, using a random effect term at the species level. We also ran a separate model for the subset of LD₅₀ values which also had an associated measurement error using the mev term in MCMCglmm to incorporate this variation (Hadfield & Nakagawa 2010). As the dissimilarity between two species may not increase linearly with phylogenetic distance, we also ran a model using the square-root transform of $D_{LD50-Diet}$ which reflects how dissimilar species are with phylogenetic distance under a Brownian model of trait evolution (Letten & Cornwell 2015).

Venom yield model

To test the allometric hypotheses regarding venom yield (Fig. 2), we ran a BPMM with mean venom yield as the response variable and snake body mass as an explanatory variable. To test the habitat complexity hypothesis, we included habitat dimensionality as a fixed factor (2D, 3D). To test the oophagy hypothesis, we include a fixed factor for the presence of eggs in the diet (absent, present). For the subset of species for which prey size could be estimated, we also ran a BPMM with mean prey size included as an explanatory variable. Finally, we tested the relationship between prey and snake body size by running two additional BPMMs with maximum and mean prey size as response variables and snake body mass as the explanatory variables.

Figure 2 Summary of the predicted drivers of venom potency and yield.

Models with response variables combining LD_{50} and yield

To test whether potential co-variance between LD_{50} and venom yield may affect the results of the main model we ran a multiple response BPMM with both factors included as response variables with snake body mass, habitat dimensionality (2D, 3D), the presence of eggs in the diet (absent, present), $D_{LD50-Diet}$ and the LD_{50} route of injection included as explanatory variables.

As the combination of potency, yield and the size of prey is likely to be an ecologically relevant trait, we also ran a

Venom yield allometry

Allometric scaling relationships are often used to test hypotheses regarding how traits change with body size (Brown *et al.* 2004). Such allometric relationships can be used to test hypotheses regarding the relationship between venom yield (Y) and snake body mass ($M_{predator}$). In the simplest case Y may increase with $M_{predator}$ at the same rate, which would follow the relationship:

$$Y \propto M_{predator}^1 \quad \text{Eq. 1}$$

However, the production of biological substances, such as venom, is typically constrained by energy availability as determined by metabolic rate, which in turn follows the approximate $3/4$ allometric scaling of Kleiber's law (Isaac & Carbone 2010; Brown *et al.* 2004). Hence, if Y is directly related to metabolic rate then we expect a relationship of

$$Y \propto M_{predator}^{0.75} \quad \text{Eq. 2}$$

However, Y may also be constrained by prey size (M_{prey}). To predict the expected scaling of Y with $M_{predator}$ where it is constrained by M_{prey} we first take the relationship:

$$M_{prey} \propto M_{predator}^a \quad \text{Eq. 3}$$

where for snakes the predator-prey mass scaling (Carbone *et al.* 2014) exponent a has been estimated as approximately 0.68. We then account for the scaling effects of toxicological agents in relation to prey mass (Nestorov 2003), where the Y necessary to maintain the same physiological effect for a given M_{prey} can be described as:

$$Y \propto M_{prey}^b \quad \text{Eq. 4}$$

where b is the scaling coefficient of venoms' toxicological effects, commonly estimated as 0.75 for the interspecific scaling of drug dosages (Nestorov 2003). Combining Eq. 3 and 4 gives the relationship where Y is increasing to maintain its capacity to incapacitate prey as:

$$Y \propto M_{predator}^{ab} \quad \text{Eq. 5}$$

which, substituting for a and b , predicts an allometry of 0.51. At the other extreme, super-linear allometries (exponents >1) suggest patterns associated with drivers such as defences requiring increased effectiveness with size, such as seen in the horn growth allometry of horned lizards (Bergmann & Berk 2012), or sexual selection (Kodric-Brown *et al.* 2006).

Additional hypotheses relating to venom yield

Habitat complexity hypothesis: High dimensional habitats may increase prey escape rates leading to higher venom yields.

Oophagy hypothesis: Species which are associated with egg eating are predicted to have reduced venom yields.

BPMM with a response variable of the incapacitating ability of each snake species towards its prey. We calculated incapacitating ability I for each LD_{50} measure i of a focal snake species j as $I_{ij} = \frac{Y_j}{LD_{50ij}}$, where Y is the snake species' mean venom yield. We then divide this by the mean weighted prey mass of its diet to estimate the number of prey items it can impart a 50% mortality rate on. We fitted the same explanatory factors as the multiple response BPMM.

Figure 3 Posterior distributions from the lethal dose (LD₅₀) and mean venom yield models, with modes 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), intraperitoneal (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 ($D_{LD50-Diet}$). The random terms are also presented. Significance is determined when 95% of the posterior estimate is above or below zero.

Supplementary models

To test the constriction hypothesis, we ran the main LD₅₀ and yield BPMMs with constriction behaviour included as an explanatory variable (absent, present). To test for potential family level taxonomic effects, we ran BPMMs with family level included as an explanatory variable in each of the main models. To test for the sensitivity relating to the environment species were categorised in, we re-ran the main yield model with the semi-aquatic species, *Agkistrodon piscivorus*, *B. multicinctus* and *Hydrodynastes gigas*, recategorised to terrestrial environments. Finally, as *Didelphis virginiana* is known to be a predator of *Crotalus atrox* and has a level of resistance to its venom, we re-ran the main LD₅₀ model excluding this species.

RESULTS

Our dataset consisted of 538 measures of LD₅₀ representing 102 snake species that span six families (Fig. 1, Table S1). Venom yield ranged from 0.15 mg in the egg-eating sea snake (*E. annulatus*) to 571 mg in the forest cobra (*Naja melanoleuca*). LD₅₀ ranged from 1121 mg kg⁻¹ for the Western diamondback rattlesnake (*C. atrox*) when tested on the Virginia opossum (*D. virginiana*), to 0.00031 mg kg⁻¹ in the many-banded krait (*B. multicinctus*) when measured on the White-rumped munia (*Lonchura striata*). Mammals comprised the majority of LD₅₀ model species in the dataset (80% of all measures), followed by fish (6.7%), Reptiles (6.3%), Aves (5.7%), amphibians (0.9%) and arthropods (0.4%) (Table S1).

LD₅₀ models

We found that the prey-specific hypothesis was supported with lower LD₅₀ values, indicating higher potency, found when LD₅₀ was measured on animal models phylogenetically

closer to the species typically found in the snake's diet ($D_{LD50-Diet}$ slope = 0.12, lower 95% CI = 0.04, higher 95% CI = 0.19, $n = 538$ measures for 102 species; Figs 3 and 4a, Table S2). The phylogenetic regression identified a positive relationship between LD₅₀ and $D_{LD50-Diet}$, with the increase of 5.5 in LD₅₀ over the range of $D_{LD50-Diet}$ larger than the 3.9 difference in LD₅₀ associated with the route venom was administered (Figs 3 and 4a). As expected IV ($n = 168$) and IP ($n = 195$) routes were associated with lower LD₅₀ values when compared to a SC ($n = 88$) route (Fig. 3, Table S2). While limited by sample size, our analysis also found support for the oophagy hypothesis with species that consume eggs ($n = 7$ species) found to be associated with higher LD₅₀ values (Fig. 3, Table S2). Variation associated with phylogeny was found to account for more variation than within-species variation, with a moderate phylogenetic signal between that of a full Brownian evolution and full independence of the trait ($h^2 = 0.43$, lower 95% CI = 0.18, higher 95% CI = 0.69, Fig. 3, Table S2).

Similar results to the full model of potency were found in the sub-analysis which included measurement error for 146 measures of LD₅₀ for 56 species (Table S3). This included support for the prey-specific potency hypothesis, the oophagy hypothesis along with similar effects relating to the route venom was administered (Table S3). In the analysis using the square root transform of $D_{LD50-Diet}$ we found results qualitatively similar to those in the main LD₅₀ analysis (Table S4).

Venom yield models

We found support for the metabolic rate hypothesis of venom yield scaling with an allometry of 0.74 between yield and snake body mass (slope = 0.74, lower 95% CI = 0.66, higher 95% CI = 0.82, $n = 538$ measures for 102 species; Figs 3 and 4b, Table S5). This exponent exceeded the scaling of 0.51

Figure 4 (a) Mean phylogenetic distance between diet species and lethal dose (LD₅₀) model ($D_{LD50-Diet}$) against $\log_{10}$ LD₅₀. The positive relationship supports the prey-specific hypothesis [intercept (intravenous) = -0.4 , slope = 0.12]. Hollow points represent silhouetted species which are, from left to right; *Oxyuranus scutellatus*; *Crotalus horridus*; *Bungarus multicinctus*; *Emydocephalus annulatus*; *Daboia russelii*; *Thamnophis elegans*; *Causus rhombeatus*. (b) Relationship between $\log_{10}$ mass (g) against $\log_{10}$ venom yield (mg). The higher intercept for species two-dimensional habitats (red points and fitted line intercept = -0.65 , slope = 0.74) compared to species in three-dimensional habitats (blue points and fitted line: intercept = -1.13 , slope = 0.74) supports the differential habitat hypothesis. The oophagy hypothesis was supported by the low yields observed in oophagous species (triangles). Hollow points represent silhouetted species which are, from left to right; *Atractaspis bibronii*; *Bothrops ammodytoides*; *Thamnophis elegans*; *Causus rhombeatus*; *E. annulatus*; *Daboia russelii*; *Hydrophis elegans*; *Naja melanoleuca*; *Agkistrodon piscivorus*; *Ophiophagus hannah*. All intercepts and slopes are derived from the values in Fig. 3, with model fit incorporating random effects and other marginal effects as outlined in the main model (see Methods). The *Micrurus* genus is highlighted by blue circles in (a) as an example of the importance of accounting for such marginal effects and as a comparison to a previous study on LD₅₀ in the group by da Silva & Aird (2001).

predicted if yield increased at a rate expected from changes in prey size (Fig. 2, eqn. 5). In the model which included prey mass, we also found an allometric increase of only 0.18 between venom yield and prey mass ($n = 369$ measures for 65 species, Table S6). Moreover, we found no significant statistical relationship between snake mass and the mean or maximum size of their prey indicating a weak relationship between venom yield and prey size when controlling for phylogeny ($n = 65$ species, Table S7). We also found that snake species which occupy 3D environments have lower venom yields by approximately half an order of magnitude in comparison to terrestrial species ($B = -0.56$, lower 95% CI = -0.83 , higher 95% CI = -0.25 , $n = 538$ measures for 102 species; Figs 3 and 4b, Table S5). The phylogenetic signal associated with venom yield was moderate throughout the analysis with a h^2 of 0.49 in the main analysis (Fig. 3, Table S5).

Models with response variables combining LD₅₀ and yield

In the analysis with both LD₅₀ and yield included as response variables with co-variance between the terms included we found qualitatively similar results to those in the main LD₅₀ and yield models (Table S8). We also found no relationship between LD₅₀ and venom yield in an additional analysis with LD₅₀ included as a response variable and venom volume included as an explanatory factor (Table S9).

In the incapacitating ability analysis, we found further support for the prey-specific hypothesis with incapacitating ability found to have a negative relationship with $D_{LD50 - Diet}$ (396 measures, 71 species, Table S10). We also found a positive relationship between incapacitating ability and body size, support for the oophagy hypotheses (7 oophagous species, Table S10), and to a lesser extent as the higher 95% CI of the posterior overlapped zero, for the habitat complexity hypothesis (15 species in high dimensional habitats, Table S10).

Supplementary analysis

The results found in both the LD₅₀ and yield models were qualitatively replicated in each of the sensitivity analysis. We found no support for the constriction hypothesis (Tables S11 and S12) along with no qualitative change to the results in the models that included taxonomic family as a fixed effect (Tables S11 and S12), the environmental designation sensitivity analysis (Table S13), or in the analysis which excluded *D. virginiana* (Table S14).

DISCUSSION

By incorporating the evolutionary distance between what a snake eats and the species on which its potency was measured, we show that venom is generally prey-specific and driven by snake size, oophagous behaviour, and dimensionality of the environment. 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 (McGee *et al.* 2015; Cooney *et al.* 2017), while a predator's size and foraging environment also influences

trophic interactions through limiting the size, encounter rate, and escape rate of potential prey (Møller 2010; Pawar *et al.* 2012; Carbone *et al.* 2014). Here, we show that, in contrast to predictions relating to the overkill hypothesis, snake venom is also driven by such ecological pressures. These results not only help us understand the drivers of variation of venom in snakes but are also likely to apply to other venomous animals. Moreover, as a venom's ability to incapacitate a given prey item can be quantified, and confounding factors appropriately controlled for, it offers an ideal system to understand predator-prey interactions.

Historically, venom potency has been measured using laboratory species, in particular rodents as they allow for comparisons to human physiology due to our shared mammalian ancestry (Uhl & Warner 2015). While there has been a recent shift towards the use of natural prey models, which can account for the species specific effects of venoms found here, these data are still unavailable for the majority of venomous snakes (da Silva & Aird 2001; Barlow *et al.* 2009). We demonstrate that, by accounting for how closely related a model species is to natural prey species, historical potency data can be used to test fundamental hypotheses regarding snake venom and predator-prey interactions at the macroecological scale. Similar to the use of medical model species that are more closely related to humans in order to mimic expected organismal responses (Barré-Sinoussi & Montgutelli 2015), model species that are more closely related to the species on which a snake's venom are selected towards show higher potencies.

Such prey-specific patterns in LD₅₀ have previously been found when using natural prey species as potency models (da Silva & Aird 2001; Barlow *et al.* 2009). Moreover, we find that when the focal group of da Silva & Aird (2001) is highlighted in our analysis (blue circles in Fig. 4a), they also display an increase in LD₅₀ with $D_{LD50 - Diet}$ similar to the pattern we find. However, while we find a consistent prey-specific pattern for LD₅₀ in our analysis that is comparable to previous taxon specific studies, there is still a substantial amount of unexplained variation associated with LD₅₀ in our model. Much of this variation is likely to stem from context specific predator-prey interactions within species, such as demonstrated by phenotype matching (Holding *et al.* 2016). Such cases are likely to be the source of the large intraspecific variation of potency seen in some species in our analysis, such as the range of potencies in the Western diamondback rattlesnake (*C. atrox*) due to being tested on both a typical prey species and on one of its predators, the Virginia opossum, which has evolved resistance to its venom (Voss 2013). Another potential source of variation in LD₅₀ with $D_{LD50 - Diet}$ is that venoms may be under natural selection for other aspects related to incapacitating prey, such as the speed of their effects (Barlow *et al.* 2009). However, even this measure shows large variation across studies. For example, in *Echis* species a prey-specific effect was found when using time to incapacitate as a metric in one study (Richards *et al.* 2012) but not in another (Barlow *et al.* 2009). The use of linear or Brownian models of evolution to calculate $D_{LD50 - Diet}$ values in our analysis is unlikely to capture these and other genetic, biotic and abiotic sources of variation in the composition and functionality of venoms (Holding *et al.* 2018). While our

study shows that using large comparative approaches can identify general patterns in venom evolution, the inclusion of different functional aspects of venom along with more complex models of its evolution are likely to further clarify the level of context specificity in venoms.

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 mass relationships in snakes (Carbone *et al.* 2014), with yield increasing far more rapidly than expected if yield was mainly driven by prey size (Fig. 2). Moreover, the non-significant relationship between snake and prey size found here further suggests the surprisingly minor role prey size may have on the scaling of venom yield in snakes. Instead, yield was found to follow the $\frac{3}{4}$ allometric scaling predicted from metabolic theory, assuming snakes invest a constant proportion of their metabolism to produce venom (Brown *et al.* 2004). This scaling signifies that the metabolic costs of venom (McCue & Mason 2006) may have a more significant role in the evolution of venom than previously supposed. Other drivers which may contribute to this scaling include the allometry of traits such as head size, however head size has only been found to have minor effects when tested (Mirtschin *et al.* 2002).

Apart from size, habitat dimensionality was also found to influence venom yield. While we expected that species in high dimensional habitats may have larger venom yields to compensate for higher escape rates of prey (Møller 2010), we found that they had smaller yields in comparison to species in low dimensional habitats. This may be associated with a potentially greater need for prey holding behaviours in high dimension environments, such as in arboreal habitats (Deufel & Cundall 2006), which in turn allows for the more accurate delivery of smaller volumes of venom. However, bite and release behaviours are known in arboreal species such as the eastern green mamba (*Dendroaspis angusticeps*), suggesting this behaviour is not fully restricted to low dimensional environments (Branch 1998). An alternative explanation of these results is that higher encounter rates in high-dimensional environments (Pawar *et al.* 2012) may represent a case of foraging optimisation (Stephens & Krebs 1986). If expected foraging opportunities are high, the cost of losing a prey item by using less venom may not exceed the energetic costs associated with venom production. Furthermore, large reservoirs of venom may also be costly as venom replenishment times can be substantial, with estimates ranging from 7 days (Currier *et al.* 2012) to 30–50 days (Hayes *et al.* 2002; Hayes 2008). Long periods of replenishment may 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 is required our results highlight that prey encounter rates may be an important factor driving venom yield 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. For example, prey-specific venom is seen in cone snails and spiders (Casewell *et al.* 2013), and the energetic costs of producing venom is also suggested by venom

metering in scorpions (Nisani *et al.* 2007). By extending the comparative approach used here to other venomous groups, the universality of these patterns in venom evolution can be tested. Furthermore, elements of prey-specificity and macroecological constraints are likely to apply to non-venomous predatory traits. By using venom as a model system of predator trait evolution, the importance of multiple evolutionary drivers can be tested, and offer a window into both the evolution of venomous systems, and predatory traits generally.

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AUTHORSHIP

KH collated the dataset and conducted the analysis. All authors contributed to the analysis, design, discussion and interpretation of the results, and writing of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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