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Title	Snake venom potency and yield are associated with prey evolution, predator metabolism and habitat structure
Author(s)	Healy, Kevin; Carbone, Chris; Jackson, Andrew L.
Publication Date	2019-01-07
Publication Information	Healy, Kevin, Carbone, Chris, & Jackson, Andrew L. (2019). Snake venom potency and yield are associated with prey-evolution, predator metabolism and habitat structure. Ecology Letters, 22(3), 527-537. doi: 10.1111/ele.13216
Publisher	Wiley
Link to publisher's version	https://doi.org/10.1111/ele.13216
Item record	http://hdl.handle.net/10379/15180
DOI	http://dx.doi.org/10.1111/ele.13216

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Snake venom potency is prey specific with yield driven by snake metabolism and habitat dimensionality.

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Short running title
Macroecological factors drive venom evolution

Article type: Letters

Number of words in abstract: 150

Number of words in main text: 4953

Number of references: 70

Number of Figures: 4

Statement of 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.

Data accessibility statement
All data and code will be made available on publication

Keywords
Venom, Body size, Comparative analysis, Scaling, trophic ecology, Macroecology, LD_{50}, Phylogenetic analysis, Snake,

"This is the pre-peer reviewed version of the following article: Kevin Healy Chris Carbone Andrew L. Jackson (2019) 'Snake venom potency and yield are associated with prey-evolution, predator metabolism and habitat structure'. Ecology Letters, (22):527-537. ,which has been published in final form at [https://onlinelibrary.wiley.com/doi/full/10.1111/ele.13216]. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions."

Abstract

Snake venom is best 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.

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 capable of incapacitating tens of thousands of potential prey items (Figure 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 lethal amounts of venom (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 venomous species such as many-banded krait (*Bungarus multicinctus*) (Figure 1). While understanding this variation is important from

both a medical (Kasturiratne *et al.* 2008) and evolutionary viewpoint (Casewell *et al.* 2013), much is still unknown regarding its 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; Healy *et al.* 2013; Carbone *et al.* 2014); along with characteristics of the prey itself (Albertson *et al.* 1999; Cooney *et al.* 2017). Despite the central role of venom in predation for many species (Casewell *et al.* 2013), the role of these ecological and evolutionary drivers in venom variation are either still debated (Sasa 1999; Wüster *et al.* 1999; Mebs 2001) or yet to be tested in a large comparative framework. One such debated aspect is whether snake venom generally evolves increased potency against frequently encountered prey species (Sasa 1999; Wüster *et al.* 1999; Mebs 2001).

Predator-prey arms race dynamics predicts the selection of 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 venoms greatly exceed 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 (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 were the prey species have evolved tolerance towards their predators venoms (Heatwole & Poran 1995; Voss 2013; Arbuckle *et al.* 2017). However, whether these cases are taxon specific or are 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) (Figure 1), the role of snake and prey body size along with foraging conditions has on venom yield is also poorly understood at macroecological scales.

One reason for the lack of large scale comparative analyses is the difficulty in conducting multi-species comparisons of venom across taxonomically diverse groups. This stems from the non-standardized choice of model species typically used to test venom potency, such as species which are not the natural diet for the snake (da Silva & Aird 2001). This can lead to the confounding case were the measure of a venom's potency more closely relates to how similar a snake's diet is to the model species used to measure its potency. 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 of across the taxonomic diversity of venomous snakes. Using this framework, we test a series of hypotheses relating to the potency and volume of snake venoms (See Figure 2).

Under a scenario of the prey-specific evolution of venom, potency is expected to be higher when measured on model species that more closely resemble the predators diet. Hence, as closely related species are more likely to share physiological similarities in comparison to more distantly related ones, a prey specific scenario predicts a decrease in potency with increasing evolutionary distance between the snake's diet and the species used to measure its venom potency (Figure 2). In contrast, the overkill hypothesis predicts that there would be no such relationship between the similarity of a prey species to the model on which its tested (Figure 2). An alternative hypothesis is possible regarding the immunity of prey to snake venom such that species that are distantly related to those in the snake's diet may be evolutionary naive to the venoms effects and hence more susceptible. In such a scenario our framework predicts that venom potency would increase with increasing evolutionary distance between the species in the diet and the one used to measure venom potency.

As venom is likely a metabolically expensive resource (McCue & Mason 2006), selection is also likely to have shaped interspecific variation in the amount produced. Like many other trophic traits, much of this variation is likely to be attributed to body size (Hayes *et al.* 2002). However, while larger species are expected to produce more venom, the rate of increase of venom yield with body size can allow for insights into the driver of this variation. For example, allometric increases in the venom relating to metabolic rate would be expected to follow a scaling coefficient of 0.75 (Brown *et al.* 2004; Isaac & Carbone 2010), while scaling expected form predator-prey scaling in snakes would be expected to follow a

lower scaling coefficient of 0.51 (See Figure 2). 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 (Kodric-Brown *et al.* 2006), or defences requiring increased effectiveness with size, such as seen in the allometry of horn growth in horned lizards (Bergmann & Berk 2012).

By comparing venomous species across a wide diversity of ecologies we also test other proposed drivers of venom evolution. One such driver of venom loss is a switch to an oophagous diet of eggs. 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, as observed by its low venom yield and potency (Li *et al.* 2005) (Figure 1). Due to the reduced need to incapacitate prey items, species which display such oophagy would hence be expected to have lower potencies and venom yields. Similar to trophic ecologies associated with undefended prey items, the use of constriction seen in many venomous snakes (Shine & Schwaner 1985) may also result in similar selective releases on venom potency and yield.

While the effects of body size on trophic traits has long been realised, the importance of factors determining interactions rates has only more recently become realized. One such factor which may determine interaction rates is habitat structure (Arbuckle 2015). 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 (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.

Here we show that snake venom potency follows a general trend expected in the scenario of prey specific venom evolution. We also show that venom yield increases as expected from metabolic scaling predictions and that both venom yield and potency are driven by oophagy and the dimensionality of the environment.

Methods

Data

To test our hypotheses regarding the drivers of venom we collected 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. As a measure of venom lethality, we used median lethal dose (LD_{50}) due to its wide availability. We included intravenous (IV), subcutaneous (SC), intraperitoneal (IP) or intramuscular routes (IM) of administering the venom as other routes, such as intracerebral, were too uncommon to include within the analysis. 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 reported measurements of variability associated with each LD_{50} value and multiple measures of LD_{50} where available.

For snake body size, we used total length values from the literature and field guides as these were the most common measures available. All lengths were then converted to mass using family-level allometric scaling as described in Feldman and Meiri (2013). Dietary data of quantitative estimates of prey proportions, mainly from studies of stomach contents, were collated for each species from the literature. 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). 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 mean prey size weighted by the proportion of each prey species, genera or taxonomic group within the diet.

To test whether venom is prey specific we calculated the phylogenetic distance (Millions of years ago (Mya)) to the common ancestor of the LD_{50} 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 which could only be identified to family level or above we used phylogenetic distances calculated using TimeTree (Hedges *et al.* 2006). We then calculated a mean phylogenetic distance between the diet of each snake and each LD_{50} model used to measure its venom potency. We weighted this mean according to the proportion of each prey item in the diet so that influence of a species on the mean was dependent on how common it was in the diet. This was calculated as $D_{LD50\text{-}Diet\ (jk)} = \sum p_i d_{ik}$, were $D_{LD50\text{-}Diet\ (jk)}$ is the weighted phylogenetic distance for a focal snake species j and a LD_{50} model species k, p_i is the proportion of the diet comprised by prey item i and d_{ik} is the evolutionary distance to the common ancestor of i and k.

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.* 2012), 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 behaviour we collected data on any observation of constriction behaviour in capturing prey from the literature (Shine & Schwaner 1985).

Snake mass, prey mass, LD_{50}, and venom yield were all log_{10} transformed in order to test scaling allometry predictions. The phylogeny from Pyron and 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, LD_{50}, venom yield and body size were included in the analysis. All data is available in the supplementary information (S2).

Analysis

To test our hypotheses, we fitted Bayesian phylogenetic mixed models using the `MCMCglmm` package (Hadfield 2010) in R version 3.4.0 (Team 2016). To control for pseudoreplication due to shared ancestry between species we used the `animal` term in `MCMCglmm` (Hadfield 2010). This term uses a distance matrix of the phylogenetic distance between species to control for the potential of the 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 indicates the trait evolves according to a Brownian model of evolution, with a value close to zero indicating the trait can be treated as independent values (Hadfield & Nakagawa 2010). All models were fitted with 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). We fit separate models with LD_{50} and venom yield as response terms and a final multiple response model with both terms to account for potential co-variance between LD_{50} and venom yield.

LD_{50} model

For the LD_{50} model we fit the explanatory variables of habitat dimensionality (2D, 3D); the presence of eggs in the diet (absent, present); phylogenetic distance of diet species to LD_{50} model ($D_{LD50\text{-}Diet}$) and the LD_{50} route of injection for the LD_{50} model (SC, IM, IV, IP). As measures of LD_{50} can have large levels of intraspecific variation (Martinson *et al.* 2017), we include multiple measures of LD_{50} 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 LD_{50} values which also had an associated measurement error using the `mev` term in `MCMCglmm` to incorporate this variation (Hadfield & Nakagawa 2010).

Venom Yield model

For the venom yield model, we included snake body mass; habitat dimensionality (2D, 3D); and the presence of eggs in the diet (absent, present) as explanatory variables. For the subset of species which had measures of prey size for their diets we also ran a model with prey size included as an explanatory variable.

Supplementary models

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 MCMCglmm model with both factors included as response variables and snake body mass; habitat dimensionality (2D, 3D); the presence of eggs in the diet (absent, present); phylogenetic distance of diet species to LD_{50} model ($D_{LD50\text{-}Diet}$) and the LD_{50} route of injection for the LD_{50} model (SC, IM, IV, IP) as explanatory variables. To more explicitly test for a potential correlation between LD_{50} and venom yield we also fit a separate model with LD_{50} as a response variable and venom yield and the LD_{50} route of injection for the LD_{50} model as explanatory variables. Finally, we fitted a final set of sensitivity analysis including the main LD_{50} and venom yield models with constriction behaviour included as a categorical factor (absent, present); and a model with snake family included as a fixed factor.

Results

Our final dataset consisted of 538 measures of LD_{50} representing 102 species that span the full range of the evolutionary tree of venomous snakes (Figure 1, Table S1). Venom yield ranged from 0.15 mg in the egg-eating sea snake (*Emydocephalus annulatus*) to 571 mg in the forest cobra (*Naja melanoleuca*). Potency ranged from an LD_{50} of 1121 mg/kg for the Western diamondback rattlesnake (*Crotalus atrox*) when tested on the Virginia opossum (*Didelphis virginiana*), to the most venomous case of an LD_{50} of 0.00031 mg/kg in the many-banded krait (*Bungarus multicinctus*).

Prey-specific potency

We find that snake venom is prey-specific across venomous snakes with LD_{50} found to be lower, indicating higher potency, when LD_{50} was measured on animal models phylogenetically closer to the species typically found in the snake's diet (Intercept = 0.12, lower 95% CI = -0.39, higher 95% CI = 0.60, slope = 0.12, lower 95% CI = 0.04, higher 95% CI = 0.18; Figure 3, Figure 4A, Table S2). While there is large variation in LD_{50} across the span of $D_{LD50\text{-}Diet}$ in our study (Figure 4A), the phylogenetic regression identifies a strong positive relationship after accounting for the non-independence arising from phylogenetic relatedness within snakes. The increase in LD_{50} over the range of $D_{LD50\text{-}Diet}$ in our analysis is larger than the variation associated with the route venom was administered into the LD_{50} model, with intravenous and Intraperitoneal routes found to be associated with lower LD_{50} in comparison to a subcutaneous route (Figure 3, Table S2). Our analysis also found an association between potency and the nature of the prey items. Species that are known to consume eggs as part of their diet were found to be associated with higher LD_{50} values (Figure 3, Table S2). Of the random effects 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 = 0.02, higher 95% CI = 0.34, Figure 3, Table S2).

Similar results to the full model of potency was found in the sub analysis which included measurement error for 146 measures of LD_{50} for 56 species, which showed a positive relationship between LD_{50} and $D_{LD50\text{-}Diet}$ (Intercept = 1.83, lower 95% CI = 0.17, higher 95% CI = 3.85; slope = 0.13, lower 95% CI = 0.01, higher 95% CI = 0.26, Table S3); lower LD_{50} values associated with intravenous routes and higher LD_{50} values associated with oophagy (Table S3). No affect was found for the presence of constriction and the family level taxonomic group of each species when included in the supplementary models (Table S4)

Macroecological drivers; body size and habitat dimensionality

In our analysis of venom yield we find that it increases with snake body mass according to an allometry of 0.74 (Intercept = -0.65, lower 95% CI = -1.05, higher 95% CI = -0.24, slope = 0.74, lower 95% CI = 0.65, higher 95% CI = 0.81; Figure 3, Figure 4B, Table S5). This exponent

exceeds the scaling of 0.51 predicted if yield increased at a rate expected from increases in prey size (Figure 2, Eq. 3). In the model which includes prey mass we find an allometric increase of only 0.18 between venom yield and prey mass when include in the model (n = 69, Table S6). Moreover, in a separate model we find no relationship between snake mass and the mean or maximum size of their prey indicating a weak relationship between venom yield and prey size (n = 69, Table S7).

We also find that snake species which occupy three dimensional environments have lower venom yields in comparison to terrestrial species (B = -0.48, lower 95% CI = -0.76657, higher 95% CI = -0.17; Figure 3-4, S5-6). This difference was not found to be associated with potential differences in prey handling behaviours between these environments, with the presence of constriction in venomous snakes not found to have an effect when included within our analysis (Table S8). The phylogenetic signal associated with venom yield was moderate throughout the analysis with a h^2 of 0.46 in the main analysis (h^2 = 0.46, lower 95% CI = 0.22, higher 95% CI = 0.77, Figure 3, Tables S5,6,8). No effect was found for the taxonomic family of each snake species when included in a supplementary analysis (Tables S8).

Compensation between LD_{50} and yield

Species with less potent venoms may be expected to compensate for this through higher yields. To account for such compensatory behaviour, we ran an additional model with both LD_{50} and yield included as response variables with co-variance between the terms included within the model. We find similar results to those found in the main LD_{50} and yield models (Figure 3). Furthermore, no relationship was found between LD_{50} and venom volume in an additional analysis with LD_{50} included as a response variable and venom volume included as an explanatory factor (Table S9).

Discussion

By incorporating the evolutionary difference 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, the 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 the predatory ability of venom can be quantified and confounding effects appropriately controlled through analysis such as ours, venomous systems offer an ideal system to understand predatory-prey interactions.

One of the biggest barriers to conducting large scale comparative analysis of venom is the species used to measure its potency. 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, this data is 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 data on venom potency can be used to test fundamental hypothesis 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 & Montagutelli 2015), model species that are more closely related to the species on which a snakes venom are selected towards show higher potencies.

Such prey specific patterns in LD_{50} have previously been found when using natural prey species as potency models (da Silva & Aird 2001; Barlow *et al.* 2009). Further to the findings of prey specific nature of LD_{50} in such analysis, when the $D_{LD50\text{-}Diet}$ as calculated in this study, is highlighted for one such large study, da Silva and Aird (2001), we also find a similar pattern of increased LD_{50} with increased $D_{LD50\text{-}Diet}$ (blue triangles in Figure 4a). However, while we find a consistent prey specific pattern for LD_{50} in our analysis comparable to

previous taxon specific studies there is still substantial variation associated with LD_{50}, much of which 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 included in our analysis, such as the 0.4 to 1121 mg/kg (Githens 1935; Perez *et al.* 1979) range in the Western diamondback rattlesnake (*Crotalus atrox*). Such cases of extreme intraspecific variation are also often cases were prey have evolved particular immunity, with the high variation in LD_{50} values found in the Western diamondback rattlesnake one such example (Perez *et al.* 1979). While there are many other cases of prey resistance to snake venom (Arbuckle *et al.* 2017), we find that over the 102 venomous species tested in our analysis snake venom is general ahead in the arms race.

Another potential source of variation in LD_{50} with $D_{LD50\text{-}Diet}$ in our model is that venoms may be selected for other aspects related to incapacitating prey, such as the speed of a venom's effect (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). Hence, while different measures of the actions of venom on potential prey may improve our understanding of the selection pressures on venom, such measures are also likely to be highly variable. Such high intraspecific variation in venom potency highlights the importance of incorporating both species and measurement level variation of potencies into comparative analysis along with the need to conduct such analysis at large taxonomic scales, where the variation between species is larger than within species, such as found in our analysis. By conducting comparative analyses of venom at macroecological scales while accounting for potency and its sources of error allows for other aspects of venom to be tested, such as yield.

In terms of such macroecological patterns, unsurprisingly we found that larger snakes had larger quantities of venom. However, these increases did not follow predictions based on the observed predator-prey body size relationship in snakes (Carbone *et al.* 2014), with yield increasing far more rapidly than expected if yield was mainly driven by prey size (Figure 2). Moreover, the non-significant relationship between snake and prey size found here further

suggests the surprisingly minor role prey size may have on venom yield. Instead venom yield was found to follow the allometric scaling of 0.75 predicted from metabolic theory, assuming snakes invest a constant proportion of their metabolism to produce venom (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 previous supposed.

Apart from size, habitat dimensionality was also found to influence venom yield. We expected that species in high dimensional habitats may have larger venom yields to compensate for higher escape rates of prey (Møller 2010). However, we found that species in high dimensional habitats had smaller yields in comparison to species in low dimensional habitats. This may be associated with differences in prey handling behaviours in different environments, with a potentially greater need for prey holding behaviours in high dimension environments resulting in the more accurate deliver of smaller volumes of venom. However, the presence of constriction in venomous snakes (Shine & Schwaner 1985), the most extreme form of prey holding behaviours, is present in both arboreal and terrestrial species and was also found to have no effect when included within our analysis. Furthermore bite and release behaviours are known in arboreal species such as the eastern green mamba (*Dendroaspis angusticeps*) suggesting this behaviour is not fully restricted to low dimensional environments {Branch, 1998 #69}. 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 hence select for larger venom reserves in species where prey encounter rates are low in order to minimise potential missed opportunity costs. While further research on the role of habitat dimensionality will allow more detailed understanding of the mechanisms behind this difference our results highlight that prey encounter rates may be more important than prey mass in 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 (Casewell *et al.* 2013). For example, prey-specific venom is seen in cone snails and spiders (Casewell *et al.* 2013), while the energetic costs of producing venom is also suggested by venom metering in scorpions (Nisani *et al.* 2007). Future analyses that include other venomous taxa in a comparative approach such as used here, will further test whether venom fundamentally follows 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 (Wroe *et al.* 2005), or macroecological constraints relating to pursuit speed (Domenici 2001). By using venom as a system of predator trait evolution the importance of multiple evolutionary drivers can be tested and hence offer a window not only into the evolution of venomous systems, but of predatory traits in general.

Acknowledgments

We would like to thank several people for useful discussions that have helped develop this project including Natalie Cooper, Yvonne Buckley and the members of NERD club. This work was funded by Science Foundation Ireland (K.H) and the Earth and Natural Sciences Doctoral Studies Programme with the Higher Education Authority through the Programme for Research at Third Level Institutions, Cycle 5 (PRTLI-5), and co-funded by the European Regional Development Fund, and the Marie Curie Research Grants Scheme, grant [749594] (K.H.)

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Figures

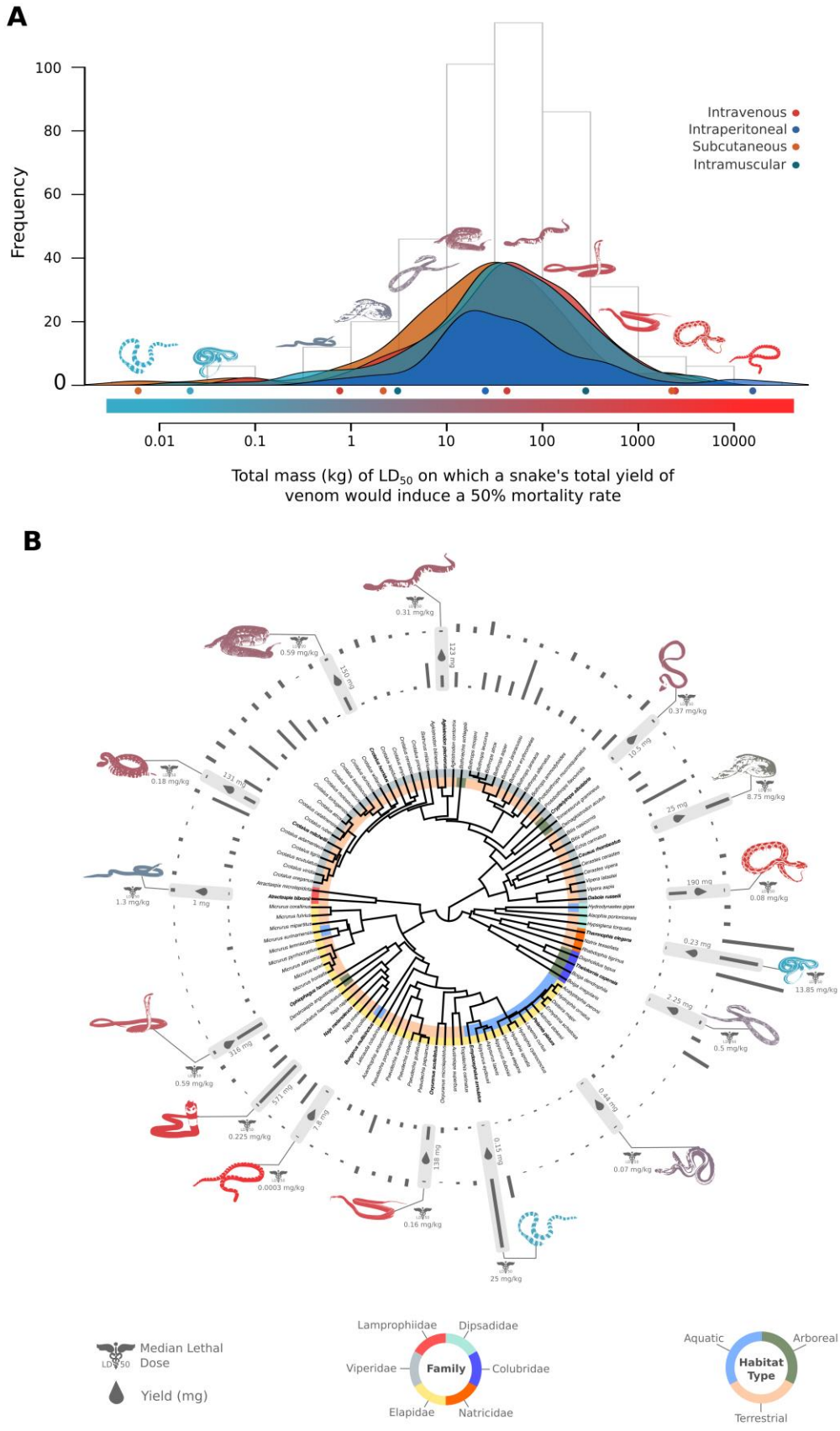

Figure 1. A. Histogram and density plot of the mass (kg) of the species potency was tested on, that a snake species can impart a 50% mortality rate (538 observations of 102 species).

The colour scale bar represents the potential incapacitating abilities across species going from low incapacitating abilities (blue) to high (red). This was calculated as the mean volume of dried venom for a species divided by its LD_{50} (mg/kg) measures. The route the LD_{50} was administered is represented by the red (IV), blue (IP), orange (SC) and green (IM) density curves and dots for highlighted species. **(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 median lethal dose (LD_{50}) for a given species. Species across range of LD_{50} and yield are highlighted as silhouettes with colours matching the colour scale incapacitation abilities in **1.A**.

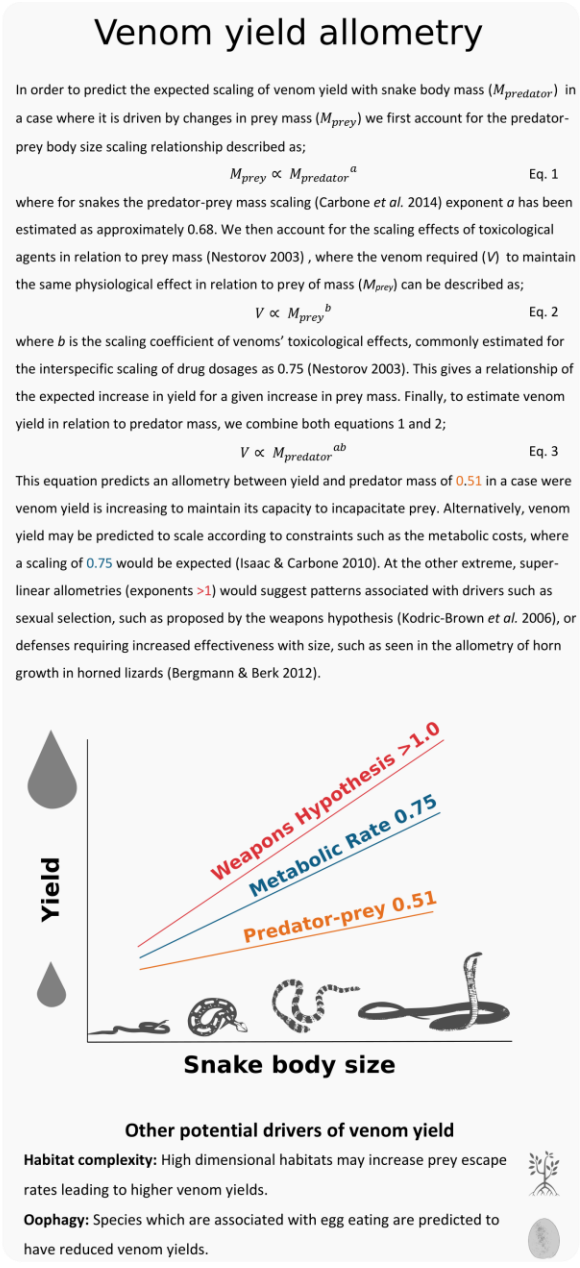

Figure 2. Summary of the predicted drivers of venom potency and yield.

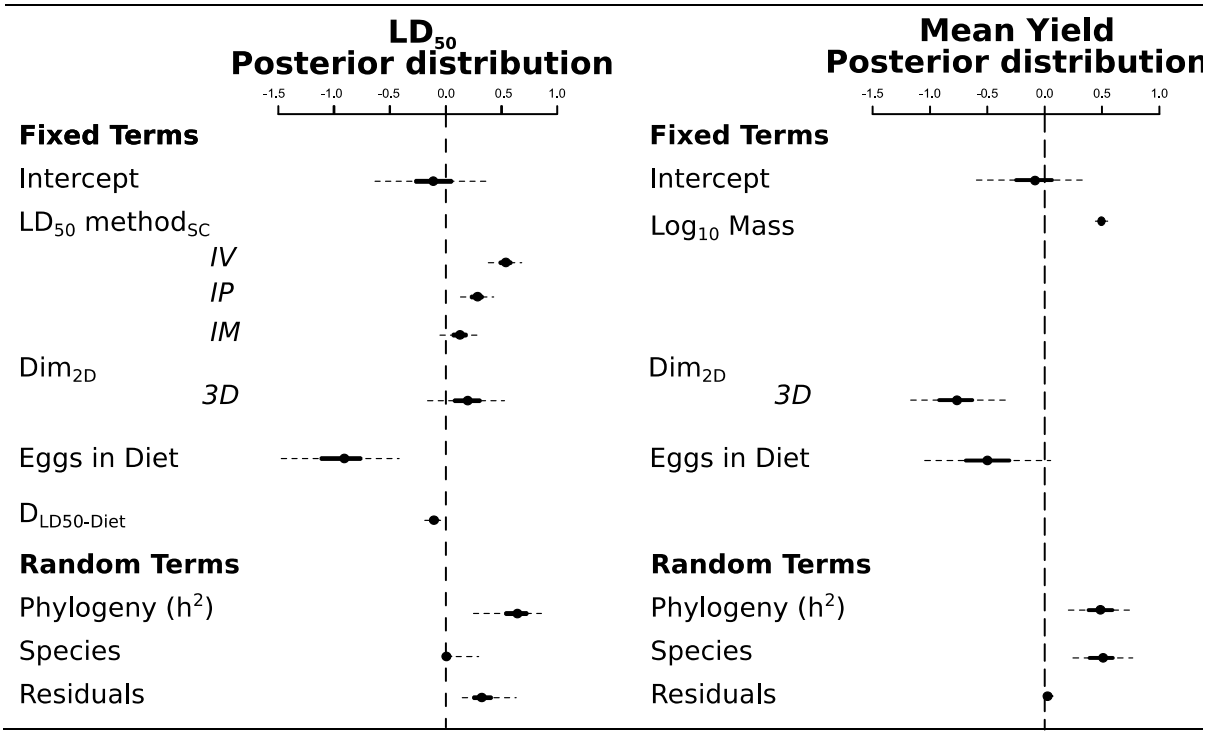

Figure 3. Posterior distributions form the LD_{50} and mean venom yield models, with modes represented by dots and higher and lower 95% credibility intervals represented by dotted horizontal bar. Fixed factors include mass; LD_{50} method (subcutaneous (SC), intravenous (IV), intraperitoneal (IP) and 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 LD_{50} model ($D_{LD50\text{-}Diet}$). The random terms are also presented. Significance is determined when 95% of the posterior estimate is above or below zero.

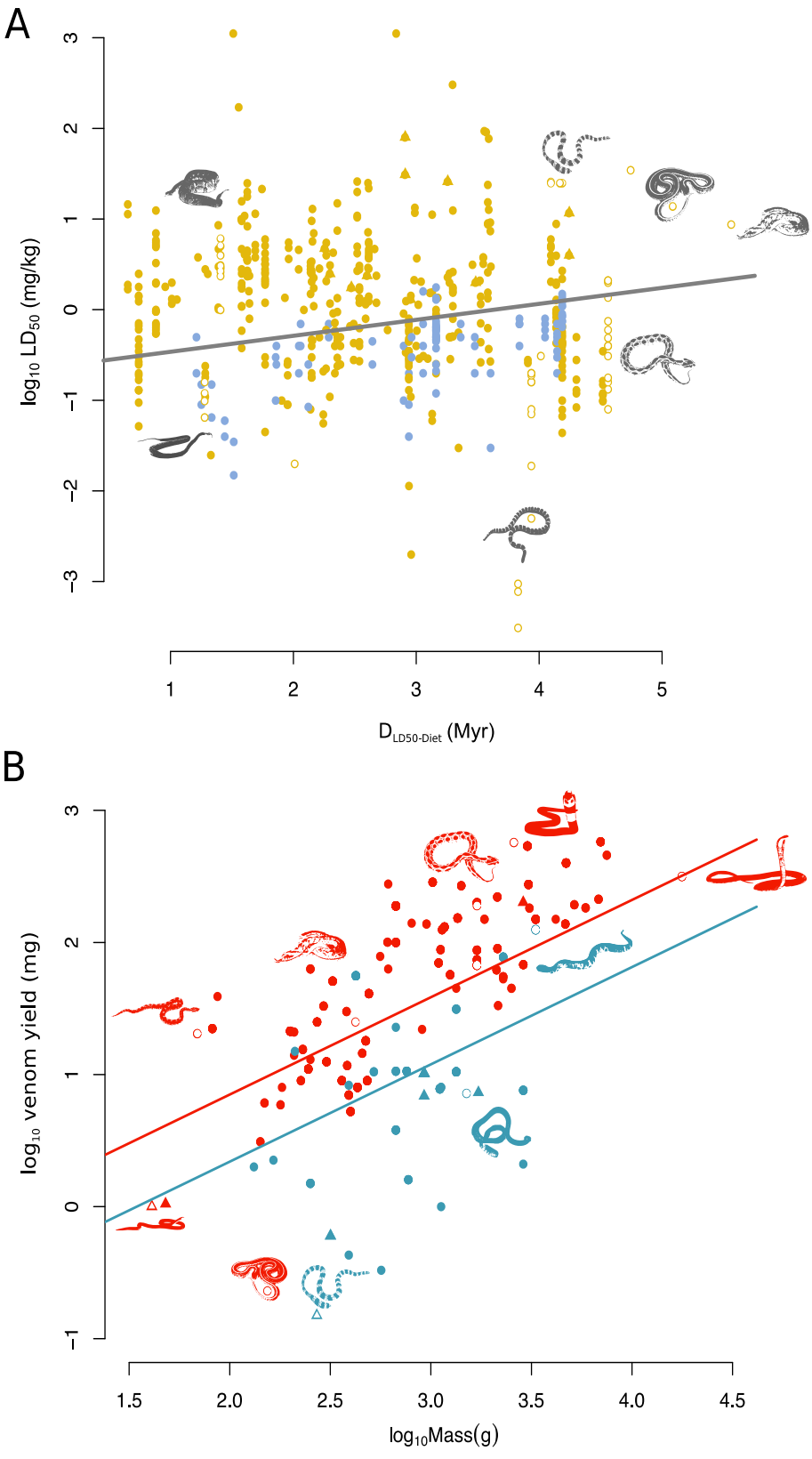

Figure 4. (A) Mean phylogenetic distance between diet species and LD_{50} model ($D_{LD50\text{-}Diet}$) against $\log_{10}$ LD_{50} (intercept (IV) = -0.4, slope = 0.12). Hollow points represent silhouette species which are from left to right; *Oxyuranus scutellatus*; *Crotalus horridus; Bungarus*

multicinctus; *Emydocephalus annulatus*; *Daboia russelii*; *Thamnophis elegans*; *Causus rhombeatus*. (B) Relationship between $\log_{10}$ mass (g) against $\log_{10}$ venom yield (mg). Red points and fitted line (intercept = -0.65, slope = 0.74) represent species in 2D habitats and the blue points and fitted line (intercept = -1.13, slope = 0.74) represent species in 3D habitats. Triangles represent observed cases of ovophagy. Hollow points represent silhouette species which are from left to right *Atractaspis bibronii*; *Bothrops ammodytoides*; *Thamnophis elegans; Causus rhombeatus*; *Emydocephalus annulatus*; *Daboia russelii; Hydrophis elegans*; *Naja_melanoleuca*; *Agkistrodon piscivorus*; *Ophiophagus hannah*. All intercepts and slopes are from the values in Figure 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 triangles in (A) as an example of the importance of accounting for such marginal effects and as a comparison to a previous study on LD_{50} in the group by da Silva and Aird (2001).