Below we detail how we will address each of concerns raised by the reviewers and the editor. Editor and Reviewer comments are in orange with our responses given in blue.

Overall Rating: Reject with no chance for re-submission   
  
Editor Comments: In this manuscript, the authors test hypotheses intended to explain the variation in the potency and yield of snake venoms across 100 snake species, using data from the literature and phylogenetic comparative methods. The results support the idea that variation in venom toxicity and yield are shaped by use of different prey species, snake size, and habitat dimensionality. The manuscript was reviewed by three outside referees. Although all three found the work interesting (as did I), two of the three reviewers recommended rejection. The negative reviews cite various issues, including problems with the overall predictions, the phylogenetic correction of LD-50 scores, biases in taxon sampling, and limitations of LD-50 data in general. In theory, some of these issues could be addressed in a revision, but the majority of reviewers did not generally think the manuscript was of sufficient quality for PNAS (making the eventual acceptance of a revised manuscript seem very unlikely). Therefore, I must recommend rejection. I wish the authors all success in revising their manuscript for submission to another journal, and I hope that these reviews will be helpful in that endeavor.

We thank the editor and reviewers for their time and interest in our work. We believe that each of the concerns raised by the reviewers leading to the rejection decision can be addressed on re-submission. In particular, the quality of this manuscript will be substantially increased through implementing new statistical analysis and addition text relating to each of the reviewers concerns. We outline how we will address each of the reviewers concerns in order below.

Reviewer #1:   
  
Suitable Quality?:No   
  
Sufficient General Interest?:Yes   
  
Conclusions Justified?:Yes   
  
Clearly Written?:Yes   
  
Procedures Described?:Yes   
  
Supplemental Material Warranted?:Yes   
  
Comments : The question of what are the evolutionary drivers of snake venom toxicity is a question of broad interest to both evolutionary biologists who study predatory adaptations and for biomedical scientists seeking to understand causes of venom toxicity to humans. The authors present a novel use of comparative analyses to get at "big picture" causes of variation in venom toxicity through capitalizing on the large amount of data on venom toxicity to non-model "prey" species such as lab mice. The results are fascinating but I am ultimately concerned about details related to the predictions, source of the data and methods of analyses. In some ways the results of the ms are a "big picture" story whereas my concerns are "small picture" in scale and I cannot pinpoint how what I am concerned about can explain away the patterns that the authors find. This is frustrating because as I said above the results are fascinating and could be true. But my concerns limit my enthusiasm for publishing this work in a very high-profile journal like PNAS.

We thank the review for their comments and enthusiasm towards our work.

Specific issues:   
  
Predictions are weak - As described in Fig. 1 both positive and negative relationships support a prey specificity prediction depending on whether in general the evolution of resistance is significant or not. Sorting out whether this is case requires knowledge of the frequency with which resistance evolves in natural prey yet this information is not available at present. The all-encompassing nature of this present prediction makes rejecting the prey specificity hypothesis difficult and so weakens the significance of the results.

Unfortunately, we believe this to be a case of misunderstanding with regards to our predictions as we make three clear predictions with regards to the relationship between LD50 and the phylogenetic distance between the LD50 model species and the snakes prey (See Figure 1). Both positive and negative relationships do not both predict prey specific venom with only a negative relationship supporting the hypothesis that venom is prey specific. A positive scaling on the other hand would reject the prey-specific venom hypothesis instead supporting that venom is not responding to evolutionary changes in prey immunity in response to selection from venom. This scenario would support a naive prey hypothesis and requires no prey-specific evolution of venom. Hence all but a negative scaling supports the prediction of a prey specific venom. We will make these points clearer in the text.

The flip side is that support for the overkill hypothesis could also be explained as a result of species-wide variation in the presence or absence of resistance. For example if half the species surveyed had resistant prey and half did not the net result of species wide-comparisons like those reported here would be support for the overkill hypothesis when in fact prey specificity existed.

Such species variation in resistance would indeed make it more difficult to reject the overkill hypothesis as the lack of a relationship could either represent a true overkill effect or a case were half the species had resistant prey and rest had prey-specific venom. We will add in our manuscript that this problem would indeed make it hard to support the Overkill hypothesis should we have found it. However, we do find a negative relationship in all our models supporting prey-specific venom. Hence this potential extra difficulty in rejecting a scenario of no-relationship strengthens our findings that overall prey specific venom is the general rule for snakes and not the exception.   
  
LD50 scores are highly variable at evolutionary scales that are much less the broad phylogenetic scale dealt with here - In our experience, comparisons among congeneric prey (including mammals) can show variation 4 to 5-fold differences in LD-50 scores using same injection method and venom source and so standardizing everything to lab mice as representative mammals could be quite misleading (see Mackessy, S.P., Sixberry, N.M., Heyborne, W.H., and T. Fritts. 2006. Venom of the Brown Treesnake, Boiga irregularis: Ontogenetic shifts and taxa-specific toxicity. Toxicon 47: 537-548 for comments about the inappropriateness of inbred lab stains for assessing function toxicity of venoms). This makes sense because the strong selection pressures imposed by the high fitness consequences of predator-prey interactions mean that a phenotypic trait that summarizes the results of this interaction (like LD50) is likely to be highly labile making it potentially inappropriate for the large-scale comparisons reported here. Consistent with idea is that venom genes are among the most rapidly evolving genes studied in vertebrates again consistent with the observation that toxicity is a phenotype that is evolutionarily labile.

We agree that venom is incredibly variable and evolves rapidly. Indeed venoms variability and extreme range was one of the primary reasons we conducted this analysis, as we outline in the introduction. However, while we only mentioned it briefly in the results section, we find that LD50 shows a high phylogenetic signal in all our models. Moreover LD50 was found to have a high phylogenetic signal (lambda ~ 0.7), comparable to that typically found for traits such as body size, suggesting that LD50 shows constraints in values changes as expected under an Brownian motion evolutionary model. We now realize that this finding suggesting that LD50 is far more conserved than we would have expected is particularly interesting to the field. In the case of a re-submission we will expand far more on this finding in the text and convert our phylogenetic variances into lambda values which would allow for easier comparison on how evolutionarily conserved LD50 is in comparison to other traits.

Phylogenetic correction of LD-50 scores is certainly wrong in some cases. For example, taking a paper that is cited (Gibbs and Mackessy 2009) mouse and lizard LD-50 values across closely-related Sistrurus rattlesnakes are comparable whereas those for frogs are very different (almost 100x greater). At minimum applying the correction from mouse LD 50s would vastly overestimate the toxicity to lizards I agree this is a single case but there could be others. It just seems highly unlikely to mean that something as labile as LD50 could be conserved enough to be accurate corrected by phylogenetic distances of millions of years.

As stated above LD50 is relatively highly conserved across the entire phylogeny of snake species within our analysis, hence LD50 can be conserved over within groups of venomous snakes over large periods of evolutionary time. However, the reviewer does highlight a separate point with regards to certain prey physiologies potentially showing particular resistance patterns independent of evolutionary distance. To address this we have already run preliminary models with an additional factor in the analysis of the predominant prey species class (Reptile, Mammal etc) for each species. We find the same results as described in our main analysis. A full description of this additional analysis will be include in any re-submission.

Finally, this study is (of necessity) heavily biased toward certain groups of venomous snakes. Coral snakes (Micrurus) and rattlesnakes (Crotalus and Sistrurus) represent something like 60% of the species surveyed at least based on my count within injection types) and so largely reflects patterns in those groups rather than venomous snakes as a whole. A possible bias due to this aspect of the study is that the coral snake paper is a famous go-to citation for evidence for prey specificity of venom due to a lack of other convincing studies. Thus the pattern could be more a reflection of a pattern in coral snakes rather than venomous snakes as a whole. Perhaps this could be assessed by analyzing subsets of the data - perhaps Crotalus, Micurus and others.

We have conducted preliminary analysis which include a facto which includes the class of each snake as an additional factor. We again find the same results as the main model. We also point out that one of the main reasons for applying a phylogenetic correction is to correct for pseudo-replications due to how closely related species. Hence our correction would also account for such a potential in taxonomic bias. We are however happy to also include separate sub analysis of the main groups as suggested by the reviewer in a re-submission.

I realize this is a negative review. I emphasize that I think the study is interesting and certainly feel the work would be of broad interest providing that the shortcomings (at least as I see them) are carefully laid out.

We thank the review for their useful comments which we think will substantially increase the impact and quality of our manuscript.

Reviewer #2:   
  
Suitable Quality?:Yes   
  
Sufficient General Interest?:Yes   
  
Conclusions Justified?:Yes   
  
Clearly Written?:Yes   
  
Procedures Described?:Yes   
  
Supplemental Material Warranted?:Yes   
  
Comments : This is a highly interesting study, with important findings for the field. The topic of venom adaptation to prey has been contentious and heavily debated over the years, with the weight of evidence from small scale comparative studies broadly supporting the hypothesis that venoms composition is likely to be focused towards prey. This study provides a valuable large-scale comparison and provides evidence supporting this hypothesis. Consequently, I believe this paper will be highly influential. The findings relating to both mass/metabolism are less novel (i.e. anticipated), but the habitat dimensionality results are surprising and will undoubtedly stimulate new research.

We thank the reviewer for their positive comments on our manuscript.

I have one major concern (and a number of minor points) with the analyses presented here and it relates to how the potency model and phylogenetic distance data is utilised. The authors split out models in to classes (e.g. mammals, reptiles, etc) and assigned phylogenetic distance based on dietary composition, again to these classes. As an aside, this needs to be more explicit in the manuscript and the methods. Anyhow, the majority of the potency data comes from rodent (mice) work, yet snakes eat a variety of mammals, but in all cases, irrespective of prey, the phylogenetic distance would equal 0. This is a major limitation, albeit completely understandable given that the majority of dietary studies will not classify prey items down to species levels. However, for example, for the Australian snakes their mammalian prey items actually diverged from eutherians (i.e. the mouse model) over 175 million years ago, yet the phylogenetic distance assigned in the analyses would remain as 0. There is therefore great potential for this data to skew the results, depending on the timing of diversification of prey items within those classes from the model used for testing venom potency. I am not sure how the authors can deal with this, but I think some text addressing this limitation is absolutely required in the manuscript.

We agree with the reviewer that this is a limitation to our analysis. We thank the reviewer for highlighting this issue and we will go one step further than addressing it in the text by also conducting addition analysis using more detailed measures of the phylogenetic distances between the LD50 model and the prey type. We will utilize the higher quality data used in the prey size sub analysis which will allow use to measure the phylogenic distance to the level of genus. We believe that such an analysis is more likely to improve the signal we observe in our original analysis as the use of distance to taxonomic class is likely to mask a prey-specific signal in snake species specializing on mammals.

Minor comments:

We thank the reviewer for meticulously highlighting each minor error which we will correct in any resubmission.

Introduction: Reference 3 is inappropriate for this text. Colubrid snakes are not the most lethal and don't gain much attention at all.

This reference was intended to point to another paper which makes a similar argument that the most lethal snakes get more attention despite the interesting nature of many less lethal species. We will make the intention of this reference far cleaerer.

Figure 2: suggest removing the skull and bones and drop water marks from behind the text to make it easier to read.

We will happily change the figure to increase its readability.

Figure 3: In the legend it states that significance is determined when 95% of the data is above or below zero. I suggest annotating the figure in some way to further highlight these results to the reader.

We will indicate “significant” effects more clearly by using bolded lines and symbols

Discussion: "For example as yield increases with body size according to a higher exponent than prey size, larger species may be expected to have the capacity to envenomate more prey items before depleting their reservoir in comparison to smaller species which may be constrained to something closer to a one shot strategy." Is there any evidence to support this statement? It is assumed, perhaps well-established, that snakes are capable of biting and delivering venom multiple times, and I find it hard to believe that small snakes cannot do this. Unless there is direct evidence of this, I suggest modifying "one shot strategy" to something less extreme.

We will happily modify the text from “one shot strategy” to a more toned down version such as a referring to the condition as “a reduced capacity for multiple envenomation events”   
  
Discussion: "bite and release behaviours are known in arboreal species such as the black mamba (Dendroaspis polylepis)". Black mambas are not arboreal and nor were they incorporated into your study. I am assuming you mean D. angusticeps here.

Thank you for highlighting this. We of course meant D. angusticeps and will amended the text to read so.

The text of the manuscript needs work. There are a lot of typos and poorly phrased sentences. I've outlined those I spotted below:

We will take particular care in improving the standard of writing in the manuscript.

Introduction: Russell's viper is misspelled.   
  
Introduction: "snake venom offers a system were foraging" should be "where"   
  
Introduction: "the importance of macorecological" should be "macroecological"   
  
Results: "where intravenous and Intraperitoneal" - intraperitoneal does not need to be capitalised.   
  
Results: "to have a significantly positively correlation" - significantly positive correlation"?   
  
Discussion: "snake venom potency is prey-specificity in general" - "prey-specific"?   
  
Discussion: "we find evidence of lower potencies and yeilds" - "yields"   
  
Discussion: ""and were cases of non-prey specific" - "where"   
  
Discussion: "resulting in more accurate deliver their smaller volumes of venom" - sentence doesn't make sense.   
  
References: In general these need tidying up - there are a number where issue and page numbers are missing, journal names are wrong (TOXICON-OXFORD) and where author names are misspelled (Wuester).   
  
Figure 1 legend: the is an open parentheses missing. "Species form left" should be "from".   
  
Figure 2 legend: "scaling expoents" should be "exponents"

Reviewer #3:   
  
Suitable Quality?:No   
  
Sufficient General Interest?:No   
  
Conclusions Justified?:No   
  
Clearly Written?:No   
  
Procedures Described?:Yes   
  
Supplemental Material Warranted?:Yes   
  
Comments : An interesting manuscript which attempts to develop a model using exisiting LD50 data to test the hypothesis that snake venom has evolved to target particular prey species (NB. this is generally the accepted view). Parameters collected from the literature include venom yield, snake length, prey type/size, and habitat.

There are a number of assumptions made in the collection of data, when exact data were unavailable, which complicate the interpretation/extrapolation of data.

We would be happy to address any particular limitations or complications identified with regards to the data collection.

In addition, the validity of the LD50 test per se is open to question. The LD50 measures death of an animal (normally mouse) over a defined period of time (e.g. 24-48 hours) but does not take into account how rapid the death is (e.g. 5 minutes or 24 hours) or what toxins are responsible for the death (e.g. neurotoxins, pro-coagulants). It is likely that some toxins are prey specific while others are not. These limitations are not adequately addressed in the manuscript.

We agree with reviewer 3 comment that LD50 is limited in some respects as a measure of venom toxicity from an ecological viewpoint. In fact we make this very point in the discussion of our manuscript as we state

“In particular, many species may not rely on the ability to induce mortality to capture prey but may rely on the speed of incapacitation (5). While such measures are relatively uncommon, future comparative analysis incorporating them may reveal a more nuanced role of venom in predator-prey interactions.”

In the case of resubmission, we will expand on this point highlighting not only the need to conduct a similar study to ours when other measures become more available but also on the limitations of not just LD50 but also of these other measures of toxicity. For example, in Barlow et al 2009 the researchers use both LD50 and the speed with which venoms incapacitated and killed as measures of venom toxicity. While the researchers find evidence of prey specific venom using Ld50 they find no such evidence using time to incapacitation. This highlights that time to kill may not always be the best measure to capture prey-specific venom.

We also agree that the role of particular toxins in the prey-specific nature of venom would be an interesting avenue of research. However, including this into a comparative study is not a trivial task as the complex biochemical make-up of snake venom and its variation within and across species is still poorly understood. A particular example of this is highlighted by the recent discovery in the well know species, , which displays a venom primary composed of neurotoxins in one population while a population only 50km away displays a primarily hemotoxic venom. Such variation and complex combinations of these venoms would be difficult to include in an analysis. However, we will expand on this interesting line of study within the discussion.

There are a number of spelling errors in the manuscript including 7 errors in the text accompanying Figure 2.

We thank reviewer 3 for pointing this out and we will resolve to correct and improve the text throughout the manuscript.