Why So Toxic?

Venom Evolution in Animals

Pritha Kundu, Srikant Venkitachalam and T N C Vidya

In this article, we explore the selection pressures that may be shaping venom evolution and venom systems in animals. We also examine the possibility of neutral processes contributing to the persistence of apparently unnecessarily high levels of venom toxicity.

Natural Selection and Venom Evolution

Organisms across various taxa use venom in diverse roles including predation (as in most snakes), defence (e.g., the salamander, *Pleurodeles waltl*), and intraspecific competition (e.g., platypus) (see [1], *Box* 1). However, in several species, it appears as if the venom produced is much more toxic or larger in quantity than required. In this article, we try to find out if this is indeed the case.

There are, at least, two hypotheses regarding the toxicity of venom (see *Box* 2) in animals. One hypothesis is that venom is under strong natural selection (*Figure* A) and changes in venom composition arise from adaptation to the organism's changing diet or as a response to venom resistance in the prey or in the predator (in cases where venom is anti-predatory in function). Several studies have demonstrated that venom composition diversifies as a result of adaptation to specific diets in some snakes. For example, Daltry *et al* [2] found that venom composition in the Malayan pitviper varied across geographic locations based on diet differences rather than phylogenetic relationships[1] between the sampled populations. This venom–diet association seemed to be an inherited adaptation, with venom of captive individuals being very similar to that of their parents, despite being given unnatural diet in captivity. In saw-scaled vipers (genus *Echis*) also, it was seen that diversification in venom composition occurred as a response to evolutionary shifts in diets [3]. Lineages

1

2

3


[1] Pritha Kundu is a first-year integrated PhD student at the Evolutionary and Organismal Biology Unit at JNCASR, Bengaluru.

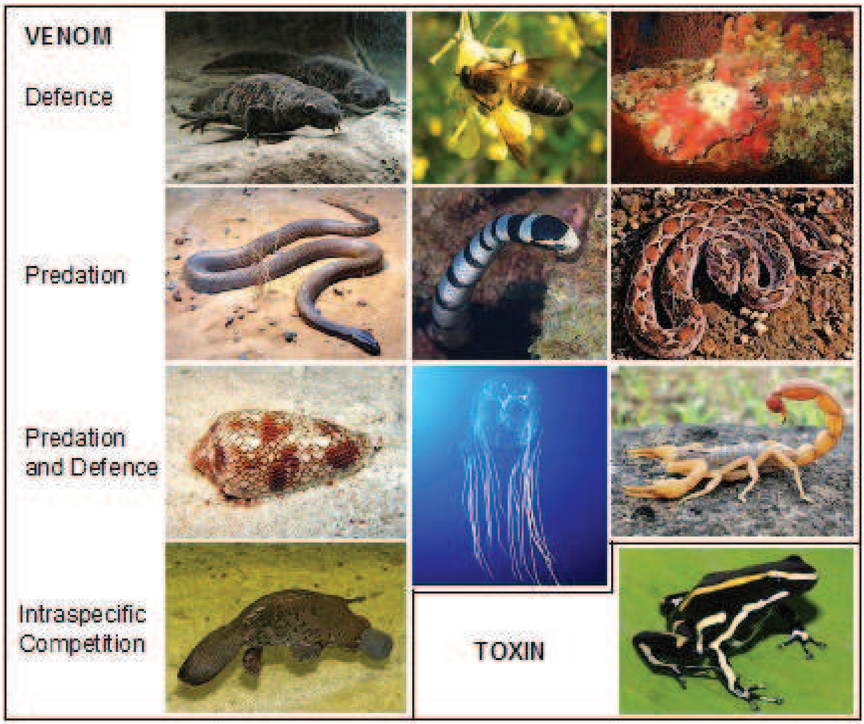
[2] Srikant Venkitachalam is a first-year integrated PhD student at the Evolutionary and Organismal Biology Unit at JNCASR, Bengaluru.

[3] T N C Vidya teaches animal behaviour and phylogeography at JNCASR. Her primary research currently is on the social organization and behaviour of Asian elephants.

Keywords
Venom, evolution, snakes, natural selection, neutral processes.

Box 1.

Examples of animals that use toxins (yellow striped poison frog, *Dendrobates truncatus*) and venoms (all others shown), and the different functions of venom. Venom is used in defence by (L to R) the Iberian ribbed newt (*Pleurodeles waltl*), Himalayan cliff honey bee (*Apis dorsata laboriosa*), and the reef stonefish (*Synanceia verrucosa*). It is used in prey capture by (L to R) the inland taipan (*Oxyuranus microlepidotus*), banded or yellow lipped sea krait (*Laticauda colubrina*), and saw-scaled viper (*Echis carinatus*), and in both predation and defense by (L to R) the textile cone snail (*Conus textile*), box jellyfish (*Chironex* sp.), and the Indian red scorpion (*Hottentotta tamulus*). Venom is used in intraspecific competition by the platypus (*Ornithorhynchus anatinus*).



Sources of photographs: 1.Iberian ribbed newt, from http://en.wikipedia.org/wiki/File:Pleurodeles_waltl_BUD.jpg; 2. Himalayan cliff honey bee, from http://en.wikipedia.org/wiki/File:ApisLaboriosa1.jpg; 3 Reef stonefish, from http://en.wikipedia.org/wiki/File:Reef_Stonefish.jpg; 4. Inland taipan, from http://en.wikipedia.org/wiki/File:Fierce_Snake-Oxyuranus_microlepidotus.jpg; 5. Banded sea krait, from http://en.wikipedia.org/wiki. File:Laticauda_colubrina_(Zamboanguita).jpg; 6. Saw-scaled viper, from http://en.wikipedia.org/wiki/File:Saw-scaled_Viper_(Echis_carinatus)_Photographed_By_Shantanu_Kuveskar.jpg; 7. Textile cone snail, from http://en. wikipedia. org/wiki/File:Textile_cone.JPG; 8. Box jellyfish, from http://commons.wikimedia.org/wiki/File:Avispa_marina.jpg; 9. Indian red scorpion, from http://commons.wikimedia.org/wiki/File:Scorpion_Photograph_ By_Shantanu_ Kuveskar.jpg; 10. Platypus, from http://en.wikipedia.org/wiki/File:Platypus.jpg; 11. Yellow striped poison frog, from http://en.wikipedia.org/wiki/File: Dendrobates_truncatus03.jpg. All the photos are reproduced under free licenses.

of saw-scaled vipers that had shifted to feeding increasingly on arthropods (rather than on vertebrates) showed greater venom toxicity [3]. Conversely, multiple snake lineages that have undergone evolutionary shifts in their diets to include undefended prey (such as eggs) or have evolved constriction as the primary means of subjugating prey exhibit an accompanying degeneration of venom systems. This also suggests that the primary function of venom in these snakes is for prey capture rather than defence. Venom composition has also been found to vary within the lifetime of an individual. In the pitvipers *Bothrops jararaca and B. alternatus*, ontogenic variation in venom composition was found to be related to the diet in different life stages [4].

There are also several known instances of natural prey of venomous predators that develop resistance to the venom, which could result in increased venom toxicity through a coevolutionary relationship between predator and prey[2]. The prey would develop increasing levels of resistance, whereas the predator venom would

[1] Phylogenetic Relationship: This is the evolutionary relationship between two taxa. It refers to the relative time in their evolutionary history when the two taxa shared a common ancestor.

[2] Ema Fatima, Venom evolution: genetic and external factors, *Resonance*, Vol.18, No.3, 2013.

Box 2. Poison, Toxin, and Venom – Which is Which?

A *poison* is any chemical substance, of biotic or abiotic origin, which, when present in sufficient amounts, causes disturbances in the normal physiological or biochemical functioning of an organism. Therefore, a substance which is generally beneficial, can be poisonous depending on the dose (even water can be harmful in very large amounts). Moreover, a substance that is poisonous to one individual may not be poisonous to another.

A *toxin* is any biologically active chemical substance which is produced within the body of an organism, and has adverse affects on other organisms of the same or different species. Toxins may either be produced, by active expression of genes coding for those proteins or as secondary metabolites, or acquired from the diet or environment and effectively sequestered in its body by the organism.

A *venom* is a toxin, produced by an organism in a specialized tissue, that is delivered actively, such as via bites or stings. In other words, all venoms are toxins but all toxins are not venoms. A toxin can be actively or passively harmful. A venom is always actively delivered. Most animal venoms are a mix of bioactive compounds, comprising largely of proteins and peptides. The composition and targeting of venom can be used to infer its function.

Box 2 Continued...

Box 2 Continued...

Toxicity is the extent to which a substance (poison/toxin/venom) can damage an organism. The median lethal dose, LD_{50}, is often used to indicate a substance's acute toxicity (the extent to which damage occurs within a short span of time). LD_{50} is the mass of the substance (per unit mass of the test subject) that is required to kill half the subjects of the tested population within a specified duration (see *Table* A). Therefore, there may be some subjects that die at much smaller doses and some that can survive much larger doses than the LD_{50}. More importantly, toxicity measured in a population (usually of mice or rats) may not reflect toxicity in other populations or species. Incidentally, chocolate, which many humans like, is toxic to several species including dogs and cats.

Table A.

Scientific name	Common name	LD_{50} (mg/kg)	Venom yield(mg)
	Snakes		
Bothrops alternatus	Urutu (pitviper)	15.80	60–100
Bothrops jararaca	Jararaca (pitviper)	7.00	40–70
Bungarus fasciata	Banded krait	3.60	20–114
Calloselasma rhodostoma	Malayan pit viper	23.40	40–60
Daboia russelli russelli	Russell's viper subspecies	0.75	130–250
Dendroaspis polylepis	Black mamba	0.32	50–120
Echis carinatus	Saw-scaled viper	0.15	5–48
Laticauda colubrina	Banded sea krait	0.44	_
Naja naja	Indian cobra	0.57	169–250
Ophiophagus hannah	King cobra	1.70	350–500
Oxyuranus microlepidotus	Inland taipan	0.03	44–110
	Fish		
Synanceia sp.	Stonefish	0.80	_
	Arthropods		
Hottentotta tamulus	Indian red scorpion	2.50	_
Androctonus crassicauda	Fat tailed scorpion	0.40	_
Centruroides exilicauda	Arizona bark scorpion	1.29	_
Leiurus quinquestriatus	Deathstalker scorpion	0.29	_
Parabuthus transvaalicus	Transvaal thick-tailed scorpion	4.25	_
Latrodectus mactans tredecimgluttatus	Black widow spider subspecies	0.90	_
Vespa mandarinia japonica	Japanese giant hornet	24.80	_
Pogonomyrmex maricopa	Harvester ant	0.13	0.025

constantly undergo fine-tuning to keep up its effectiveness, resulting in an 'arms race'([5], see *Box* 3, *Figure* A). The California Ground Squirrel (*Spermophilus beecheyi*) and its predator, the northern Pacific rattlesnake (*Crotalus viridus oreganus*), which have been sympatric[3] since at least the late Pleistocene, seem to be engaged in one such arms race [6]. Resistance to the venom of other rattlesnake species that are allopatric[4] to the ground squirrel is lower, suggesting evolutionary specialization for a particular venom. Another example of venom resistance is seen in the eels of genus *Gymnothorax* that venomous banded sea kraits (*Laticauda*

[3] Sympatry: Populations of related organisms that inhabit the same geographical location without inter-breeding are said to be sympatric to one another.

[4] Allopatry: Populations of related organisms inhabiting different geographical locations are said to be allopatric. Allopatry and sympatry can both give rise to speciation.

Box 3. Evolutionary Arms Race

When two species interact, adaptation in one of the species, leading to its increased fitness, may cause a selection pressure on the other species. If the other species responds evolutionarily to the selection pressure by developing some adaptive trait, this process of adapting and counter-adapting may lead to the elaboration of the traits in question. This process is called an evolutionary arms race. Such an arms race is an example of the 'Red Queen Effect', according to which organisms constantly have to adapt and evolve in order to simply survive in an environment (biotic and abiotic) that is constantly changing. The name comes from the Red Queen in Lewis Carroll's '*Through the Looking Glass*', who tells Alice "Now, *here*, you see, it takes all the running *you* can do, to keep in the same place."

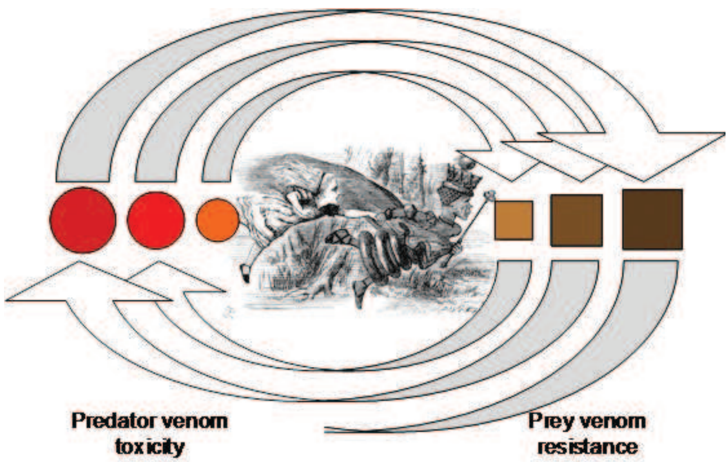


Figure A. Evolutionary arms race and the Red Queen. An example of the evolutionary arms race would be resistance (shown as squares) developed by prey, through the process of natural selection, towards venom (shown as circles) used by their predator for prey capture. This would result in selection for more toxic venom (shown as larger, deeper red circles) in the predator, which would, in turn, select for greater resistance to venom (shown as larger, darker brown squares) in prey. (Source: Centre illustration of Alice and the Red Queen from http://commons.wikimedia.org/wiki/John_Tenniel; Author: John Tenniel).

colubrina) prey upon. Eel species that were prey of the kraits were resistant to the krait venom and could withstand large doses of it, while non-prey sympatric species of eels and species of eel allopatric to sea kraits were susceptible to the venom [7].

In cases where organisms possess venom that is anti-predatory in function (in order to escape from predators), predators would be expected to develop a resistance towards venom. Studies have demonstrated that grasshopper mice (genus *Onychomys*) that eat the highly venomous Arizona bark scorpions (*Centruroides* spp.) have developed resistance to the vertebrate-specific neurotoxin that these scorpions possess [8]. It was found that the mouse species broadly sympatric with the bark scorpions showed higher resistance to scorpion venom than species allopatric with the scorpions. Moreover, even when the same species of mouse was examined from different areas, populations of the species present in areas where scorpions were present showed higher venom resistance than populations that did not coexist with the scorpions [8]. Venom resistance is also found amongst ophiophagus (snake-eating) mammals, including opossums (*Didelphidae*), mongooses (*Herpestidae*), and hedgehogs (*Erinaceidae*).

One would also expect natural selection to be acting on venom if venom synthesis were metabolically costly. A significant metabolic cost associated with venom production, leading to an increase in metabolic rates after venom extraction, has been shown in species of North American pit vipers [9]. Venom production has also been shown to be metabolically costly in scorpions. A 'metering' mechanism to regulate the amount of venom injected into the prey so that smaller amounts can be used when possible would be expected if venom production was costly. Such metering has been found in species of rattlesnakes [10], spiders [11], and a scorpion species (*Parabuthus transvaalicus*) [12]. The scorpion species, *Parabuthus transvaalicus*, was found to be able to deliver a dry (without any secretion) or wet sting, control the volume of venom delivered in the case of a wet sting (depending on the threat assessed), and also change the composition of the venom and use a metabolically inexpensive pre-venom or the

One would also expect natural selection to be acting on venom if venom synthesis were metabolically costly.

protein-rich, expensive, venom [12]. All of the above suggest that venom systems must be subject to natural selection and, therefore, species producing excessively toxic venom must be the exception rather than the norm.

This combination of high venom toxicity and large volume is puzzling.

Venom Evolution through Neutral Evolutionary Processes

A second, diametrically opposite hypothesis about the evolution of venom toxicity is that neutral evolutionary processes (such as genetic drift, see *Box 4*) may be responsible for venom evolution. The 'overkill' hypothesis suggests that since many animals seem to possess lethal toxins in quantities that are much higher than necessary to subdue their prey [13], there is probably little selection on venom toxicity. It has been observed that many venomous snakes, despite extremely lethal venom, appear to deliver to their prey, excessive amounts of venom, which may be of orders of magnitude larger than the amount of venom actually required to kill or incapacitate the prey [13]. This combination of high venom toxicity and large volume is puzzling. For example, an inland taipan (*Oxyuranus microlepidotus*) can deliver enough venom in one bite to kill over 200,000 lab mice [14]. Similarly, some scorpions that feed on insects and other invertebrates (and rarely on small vertebrates) possess venom that is also highly lethal to large vertebrates (perhaps the venom is also used for self defence). Cone snail venoms are very diverse and, although there is some specialization in feeding, venom from snails that feed on particular prey can also kill other prey: for example, snails that feed only on polychaetes can successfully immobilize fishes, and fish-hunting snails can be lethal to humans.

Therefore, in these venomous species, there appears to be no direct correlation between venom and diet. However, a tacit assumption that is often made in the overkill hypothesis is that the larger the animal, the lesser it should be affected by venom. In reality, it is possible that arthropod prey is more resistant to venoms than mammals are. Since toxicity is usually measured in laboratory mammals, it is difficult to know whether a venom is an overkill for a specific prey or not unless the effect of the venom on

Box 4. Natural Selection and Neutral Evolutionary Processes

Natural Selection: This is the process by which favoured, heritable variation is propagated across generations through the differential reproduction of individuals based on such variation. In other words, individuals that carry certain heritable traits that are best suited to the environment will have higher reproductive fitness (will leave behind more progeny) than individuals that do not carry these traits. This process is one mechanism by which populations evolve.

Neutral Evolutionary Processes: These are processes that can change the relative frequencies of heritable traits in a population across generations, not because some traits are best suited to the environment, but because of chance events. Random genetic drift can give rise to such changes. Random genetic drift refers to random changes in allele frequencies in populations because of sampling error. For instance, in a small population of an endangered tortoise, one variant of the tortoise may out-survive and reproduce another variant simply because several individuals of the other variant happened to be foraging in an area that experienced a sudden volcanic eruption. Genetic drift is more important in small populations than in large populations.

Kimura proposed the 'neutral theory of molecular evolution', according to which most evolutionary changes are a result of neutral mutations (mutations that do not have a positive or negative effect on the individual's fitness) that are acted upon by random genetic drift. If certain DNA segments are neutral, they will accumulate mutations depending on the mutation rate of the segment and will have a rate of evolutionary change that is dependent on this mutation rate and not based on any selective advantage or disadvantage. Therefore, given a certain mutation rate, populations separated by a specified amount of time will differ in their DNA segment by the mutation rate times the total length of time that the populations have been separated by. If venom was neutral, one would expect the difference in venom composition between species to reflect the times of their divergence.

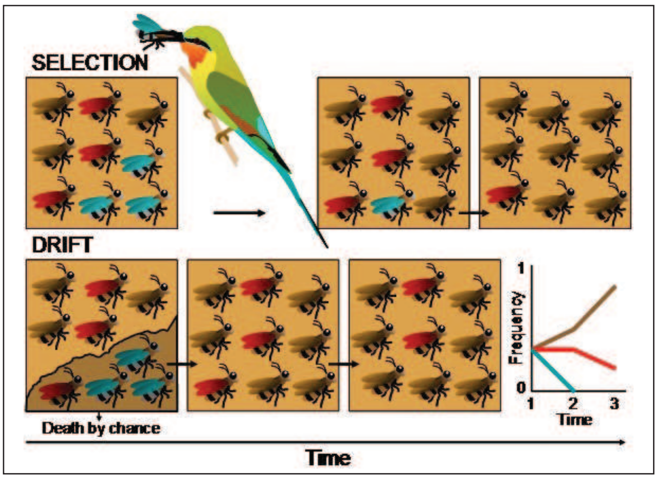


Figure A. Natural selection and random genetic drift. In the top row, the blue-winged insects, followed by the red-winged insects are preferentially eaten by a predator, resulting in the brown-winged insects increasing in frequency in the population. This is an example of directional selection. In the bottom row, part of the habitat of the insect population gets destroyed, leading to chance elimination of the blue-winged insects. Further chance variation in reproduction results in the brown-winged insects dominating the population (although there is no adaptive advantage to having brown wings). The graph shows the frequency of the three insect types across the three time points for the lower panels. The end point would be similar if such a graph was plotted for the upper panels also, but the mechanisms are very different. (Source: Artwork by T N C Vidya) natural prey is tested. It has also been found that venom toxicities may be different for preferred versus non-preferred prey.

If the overkill hypothesis were true, one would also expect venom to be metabolically inexpensive to produce.

If venom evolution was due to neutral evolutionary processes, it would result in a correlation between the amount of time for which populations/species are isolated and the divergence of their venom. Williams *et al* [15] found such a pattern, with venom proteins from tiger snakes (*Notechis* spp.) varying according to the time of isolation of populations from one another and not dependent on prey type or local ecology. However, more such studies across species are required. If the overkill hypothesis were true, one would also expect venom to be metabolically inexpensive to produce. As mentioned above, some studies have shown metering of venom and a metabolic cost associated with its production, but there are contradicting studies as well. For example, Pintor *et al* [16] inferred that the cost of venom production was small compared to the cost of shedding and digestion in the snake, *Acanthophis antarcticus*. It has also been suggested that mechanical constraints during envenomation give the appearance of venom metering.

The Way Forward

There seems to be some support for both the selection and the overkill hypotheses so far, with the evidence tilted in favour of selection. However, studies on venom evolution, in the context of ecology, are few and far between. It is possible that both mechanisms work, but because of the small number of studies so far, it is not clear which is the predominant mechanism at work. A third hypothesis could be that venom was under selection in the past but is no longer under selection, and venom composition now is loosely reflective of phylogenetic relationships between taxa.

Most studies until now have looked at the effect of venom on laboratory organisms instead of the natural fauna that the venomous organisms interact with. Thus, future studies are needed that will examine toxicity of venom to specific prey species [17]. Such studies are not easy to carry out in the laboratory, but will allow

Better insight into venom evolution would also be obtained by studying various aspects of ecology which might affect the adaptive function of venom.

for much better understanding of venom evolution than studies on the effects of venom on laboratory mice. Better insight into venom evolution would also be obtained by studying various aspects of ecology which might affect the adaptive function of venom, such as changes in diet during the organism's life, or various interactions with predators, prey, and conspecifics (individuals of the same species). Variability in venom toxicity within species and populations is also little studied so far, and more studies may help substantiate either of the two main hypotheses regarding venom evolution. There is also a great need to examine evolutionary relationships between species and their venom similarity. However, if these species are feeding on different prey, does one examine venom toxicity with respect to a single test species (such as laboratory mice) or with respect to their specific prey? This could affect the inferences of such a study. It would also be useful to evaluate the costs of venom production and storage in animals in the context of their ecology. Other aspects that might be interesting are the possible differences in evolution of venomous predators and venomous prey, mimicry of venomous organisms, and how ecological communities are structured by venom [1].

Suggested Reading

[1] N R Casewell, W Wüster, F J Vonk, R A Harrison and B G Fry, Complex cocktails: the evolutionary novelty of venoms, *Trends in Ecology and Evolution*, Vol.28, No.4, pp.219–229, 2012.

[2] J C Daltry, W Wüster and R S Thorpe, Diet and snake venom evolution, *Nature*, Vol.379, pp.537–540.

[3] A Barlow, C E Pook, R A Harrison and W Wüster, Coevolution of diet and prey-specific venom activity supports the role of selection in snake venom evolution, *Proceedings of the Royal Society Biological Sciences*, Vol.276, No.1666, pp.2443–2449, 2009.

[4] D V Andrade and A S Abe, Relationship of venom ontogeny and diet in *Bothrops*, *Herpetologica* Vol.55, No.2, pp.200–204, 1999.

[5] E D Brodie III and E D Brodie Jr., Predator-prey arms races, *Bioscience*, Vol.49, No.7, pp.557–568, 1999.

[6] J E Biardi, R G Coss and D G Smith, California Ground Squirrel (*Spermophilus beecheyi*) blood sera inhibits crotalid venom proteolytic activity, *Toxicon*, Vol.38, No.5, pp.713–721, 2000.

[7] H Heatwole and N S Poran, Resistances of sympatric and allopatric eels to sea snake venoms, *Copeia,* Vol.1995, No.1, pp.136–147, 1995.

[8] A H Rowe and M P Rowe, Physiological resistance of grasshopper mice (*Onychomys* spp.) to Arizona bark scorpion (*Centruroides exilicauda*) venom, *Toxicon*, Vol.52, No.5, pp.597–605, 2008.

[9] M D McCue, Cost of producing venom in three North American pitviper species, *Copeia*, Vol.4, pp.818–825, 2006.

[10] W Hayes, Venom metering by juvenile prairie rattlesnakes, *Crotalus* v. *viridis*: effects of prey size and experience, *Animal Behaviour*, Vol.50, No.1, pp.33–40, 1995.

[11] L Kuhn-Nentwig, J Schaller and W Nentwig, Biochemistry, toxicology and ecology of the venom of the spider *Cupiennius salei* (Ctenidae), *Toxicon*, Vol.43, No.5, pp.543–53, 2004.

[12] Z Nisani and W Hayes, Defensive stinging by *Parabuthus transvaalicus* scorpions: risk assessment and venom metering, *Animal Behaviour*, Vol.81, No.3, pp.627–633, 2011.

[13] D Mebs, Toxicity in animals. Trends in evolution?, *Toxicon*, Vol.39, No.1, pp.87–96, 2001.

]14] A Broad, S Sutherland and A Coulter, The lethality in mice of dangerous Australian and other snake venom, *Toxicon*, Vol.17, No.6, pp.661–664, 1979.

[15] V Williams, J White, T Schwaner and A Sparrow, Variation in venom proteins from isolated populations of tiger snakes (*Notechis ater niger*, *N. scutatus*) in South Australia, *Toxicon*, Vol.26, No.11, pp.1067–1075, 1988.

[16] A F V Pintor, A K Krockenberger and J E Seymour, Costs of venom production in the common death adder (*Acanthophis antarcticus*), *Toxicon*, Vol.56, No.6, pp.1035–1042, 2010.

[17] D P Richards, A Barlow and W Wüster, Venom lethality and diet: differential responses of natural prey and model organisms to the venom of the saw-scaled vipers (*Echis*), *Toxicon*, Vol.59, No.1, pp.110–116, 2012.

Address for Correspondence
T N C Vidya
Evolutionary and Organismal Biology Unit
JNCASR, Jakkur
Bengaluru 560 064, India.
Email: tncvidya@jncasr.ac.in