

## *The Evolution, Morphology and Cost-Benefit Systematics of the Venom Delivery System for Defense and Prey Capture in Viperids*

Snakes as a whole are unique among vertebrates because they are strict carnivores that swallow their prey whole (Luiselli, 2006). They have evolved highly sophisticated vertebrate musculoskeletal systems, which in turn have allowed them to develop highly specialized prey capture and feeding mechanisms (Young et al., 2002). These adaptations are exhibited in three broad categories of snakes: the constrictors, the swallows and the venomous snakes. Of the approximately 2700 recognized species of extant snakes, 450 are considered to be venomous. All venomous snakes belong to the clade Colubroidea, also known as the “advanced snakes” (Jackson, 2003). There are three taxa that are recognized within the clade Colubroidea: Viperidae, which includes venomous snakes characterized by a moveable maxilla and tubular front fangs, Elapidae, characterized by fixed tubular front fangs, and Colubridae, the largest group that includes semi and non-venomous snakes characterized by enlarged or grooved posterior fangs (Jackson, 2003). This paper will focus on the venom delivery system in the family Viperidae, specifically the evolution, mechanics and systematics of the system for the purposes of prey capture, nutrient digestion and defense.

Viperid snakes (which includes the true vipers and pitvipers) are morphologically distinct from snakes in other families. Viperids have stouter bodies, larger heads and longer jaws that allow them to swallow prey that is nearly three times larger than their own body size (Pough and Groves, 1983). It is reasonable to conclude that these morphological adaptations, along with the evolution of the venom delivery system have led this family of snakes to become one of the most specialized and derived advanced snakes, and this innovation has allowed for the venomous snakes to transition from a

mechanical to a chemical predatory strategy (Kardong and Lavin-Murcio, 1993).

However, recent studies have suggested that these adaptations have come with a cost, which will be addressed below. In addition I will review the hypotheses surrounding debate on if and how these snakes calculate and meter their venom use (to offset the aforementioned costs), and how this contributes to their fitness.

### *Evolution of the Venom Delivery System in Pitvipers*

There are only two lineages of extant reptiles that have evolved the use of venom systems, the advanced snakes and helodermatid lizards. Snakes, however, are the true masters of the venom delivery system; no other group of animals has evolved venom systems that can match that of snakes in terms of sophistication, efficiency and diversity (Jackson, 2003). The evolution of the venom system in snakes is believed to underlie the radiation of the advanced snakes (Fry et al., 2006). Using phylogenetics, Fry et al. (2006) have demonstrated that all lineages that possess a toxin-secreting gland form a clade, indicating that there was a single ancestral origin of the venom delivery system in lizards and snakes. Using cDNA libraries to characterize the toxins of several families of squamates, it was inferred that the venomous function evolved one time during the evolution of the squamates, in the clade Toxicofera (consisting of snakes, anguimorph lizards and iguanians) roughly 200 million years ago (Fry et al., 2006). This is a considerable departure from previous estimates based on the assumption that venomous function evolved independently in snakes and lizards roughly 100 million years ago.

The venom glands and fangs of viperid snakes constitute the most effective and well developed venom-delivery systems among vertebrates (Zahradnicek et al., 2008;

McCue, 2006). The venom delivery system composes roughly 15% of the total head volume of most viperids, further demonstrating the extent and evolutionary importance of this system to the fitness of these animals (McCue, 2006). The viperid venom gland is large and triangular, with the longest side of the triangle positioned by the upper lip. It is generally accepted by the scientific community that the venom gland evolved from the Duvernoy's gland, which is present in other non-venomous Colubroids (Jackson, 2003). The main venom gland is characterized by a complex tubular structure that is subdivided into several lobes by folding patterns in the outer sheath. The lumen is spacious and able to store a large amount of venom. The triangle of the gland comes to a point where the lumen becomes the primary duct. This duct passes through a mucous accessory gland into a secondary duct where it passes to the sheath of the fangs (Jackson, 2003). The venom gland in viperids is intimately involved with cranial muscles that squeeze venom out of the lumen and into the canal within the fangs by compressing the gland's muscles during biting. These cranial muscles can be divided into four functional groups, two of which are directly involved with venom deposition: the jaw adductors compress the gland and the palatal protractors serve to erect the moveable fang characteristic of viperids (Jackson, 2003).

In all venomous taxa, the fang is a modified maxillary tooth. The evolution of the maxillary tooth to provide an avenue for venom secretions is a colubroid synapomorphy (Jackson, 2003). The tubular fangs of viperids are specialized long teeth that contain a completely enclosed canal in which venom flows inside the tooth. Viperid fangs are distinguished from other taxa within the clade Colubroidia because they are actually

moveable due to the shortened rotating maxilla, and viperids have no other maxillary teeth other than their fangs (Jackson, 2003).

Of particular interest in the scientific community is the evolution of the venom canal within the fangs of Colubroids, and through extensive research on this system, scientists have offered two different hypotheses for the evolution of the venom canal. The first states that the venom-conducting canal develops by the presence of small grooves or invaginations in the epithelial wall of the developing tooth germ that eventually make contact with and fuse to form a closed canal. The second, known as the “brick chimney” hypothesis, states that the venom conducting canal develops directly from dentine deposits as the tooth develops. This means that the fang develops from tip to base without indentations or folding on the tooth surface. Using the protein *Sonic hedgehog* as a trace, Zahradnicek et al., (2008) traced the early development of fangs of *Trimeresurus albolabris*, a pit viper, and were able to demonstrate that the canal is formed from an early folding event as predicted by the first hypothesis. Following this folding event, epithelial cells within the canal proliferate in order to widen and enlarge the canal. The cells then die via apoptosis, forming the rigid empty canal in which the venom flows.

### *Behavioral Adaptations of the Venom Delivery System*

The evolution of the venom delivery system has a twofold purpose in venomous snakes: it is used for prey capture and defense against predators. As noted above, Vipers

have morphological features that distinguish them from other snakes, and it is hypothesized that these features are specialized to vipers for enhancing their ability to capture and swallow very large prey items (in comparison to their own bodies) (Pough and Groves, 1983). The evolution of the high- pressure delivery system, in which venom is delivered very quickly by a sudden pressure surge, allows the snake to rapidly incapacitate its prey without the need for prolonged contact. For example, many venomous snakes feed by striking prey, rapidly injecting the prey with venom, and then releasing the prey, allowing it to flee until it succumbs and is immobilized by the venom. The snake will then locate the prey by following a chemical trail using strike-induced chemosensory searching, and ingest the immobilized and harmless prey. This method of prey capture limits the risk of injury to the snake by the prey that is trying to escape by eliminating the need for prolonged contact between predator and prey (Clark, 2006; Kardon and Lavin-Murcio, 1993).

In addition, the venom of snakes not only assists in prey capture, but also aids in digestion. The proteolytic venom of vipers contributes to this specialization by assisting in the digestion of the prey before it is swallowed. Specifically, the venom weakens the body wall and ruptures the internal organs of the prey, increasing the rate and efficiency of digestion (Pough and Groves, 1983). Pough and Groves (1983) demonstrated through experimentation that due to such specialized traits, vipers were able to consume their prey with one- fourth less maxillary rotations than non-vipers. This is significant because the ability of the viper to increase the size of prey consumed, while reducing the costs of handling, reduces the amount of energy the snake must expend on digestion and nutrient

degradation. It thus makes it possible for the viper to sustain itself for longer periods without the need to hunt.

As alluded to above, snakes also use their morphological venomous features in defense. For example, up to 50% of bites on humans by viperids are said to be “dry bites” in which no venom is released into the target (Kardong and Lavin-Murcio, 1993). It is hypothesized that these dry bites are the result of disturbed normal jaw mechanics in which venom would normally be secreted, and these disturbed jaw mechanics are in response to a defensive situation, rather than a predatory situation in which venom would be secreted (Kardong and Lavin-Murcio, 1993). In any event, although the venom is not injected into the target, the snake is still using its fangs and threat of envenomation to deter predators.

The evolution of the venom delivery system has allowed the venomous snakes to transition from a mechanical to chemical approach for prey capture, prey digestion and defense against predators. These adaptations have specifically resulted in the viperids becoming adept at capturing and digesting large prey items, while reducing risk and energy expenditure in the process. However, the evolution of the venom delivery system and its practical uses for venomous species is not without its costs.

### *The Costs of Venom*

Thus far, I have described the elaborate morphology and evolution of the venom delivery system, and the behavioral traits associated with this condition that have allowed these organisms to continue to be highly specialized predators and extremely well suited for their environments. However, the price of the venom system does not come cheap.

Often times the benefits of derived traits are the sole focus of interest, and the costs are overlooked. Understanding the expenses associated with producing venom is beneficial when discussing the adaptive advantages of this trait. Here, I will examine the costs of such an elaborate system, and postulate how snakes manage these expenses.

Snake venom is composed of mixtures of enzymatic proteins, complex glycoproteins, toxic oligopeptides and several other non-protein components (McCue, 2006). All of these proteins must be produced, and the up-regulation of these protein production pathways is a costly process. In addition, there are a high density of mitochondrial cells present in the secretory cells that line the venom and accessory glands, which further suggests that venom production requires significant energetic expenditure (McCue, 2006). Snakes employ a variety of strategies in order to store the venom in the lumen of the gland without harmful effects to themselves; these include maintaining extreme pH conditions in the lumen, producing chelating agents to inactivate metalloproteins, and producing and maintaining specialized enzymes that inactivate the toxins (McCue, 2006). Although the energetic expenditure of these strategies has not been scientifically determined, it is reasonable to assume based on prior knowledge of the physiology and mechanics associated with the up- regulation, feedback loops and cascade systems of protein production that this is an energetically expensive process as well.

McCue (2006) devised a series of experiments to investigate the cost of snake venom production by tracing and quantifying the increased metabolic expenditure in snakes that had their venom extracted during the initial 72 hours of venom replenishment. Wild captured pitvipers representing the species *Crotalus atrox*, *Crotalus horridus* and *Agkistrodon contortrix* were acclimated to the laboratory conditions for three months

before their resting metabolic rates were measured as the control. Venom was extracted by anesthetizing the snakes using Isofluroane, then manually extracting the venom by applying pressure to the venom glands. Venom was extracted from a total of 33 snakes. Using a paired *t*-test, McCue (2006) found that the hourly rates of oxygen consumption in individual snakes following venom extraction were significantly higher compared with the resting metabolic rates measured for the same individual snakes. Snakes that were replenishing their venom stores had an approximately eleven percent increase in mean hourly metabolic rates over the 72-hour period compared to trials when no venom was extracted. The process of venom replenishment requires up to two weeks, and therefore the data associated with the initial 72 hours of replenishment represents a large underestimate of the total costs incurred for full replenishment. Not only do these data overwhelmingly support the hypothesis that venom production is an extremely energetically costly process for the snake, but it explains why it is apparent that snakes conservatively meter their venom.

During the last 30 years, scientists have examined the venom delivery system in snakes by quantifying the amount of venom injected into the target using enzyme-linked immunosorbent assays of targets (Young et al., 2002). Through these analyses, scientists have concluded that snakes conservatively meter their venom. This conclusion is based on a series of factors including the size of the target species and notably, the behavioral context of the strike (whether the strike is defensive or aggressive).

There are two major hypotheses that have been proposed to explain this phenomenon in snakes. The first is the venom-metering hypothesis, which proposes that snakes have the cognitive ability to control how much venom is ejected from their fangs



into the target, and make such decisions based on the prey size and or behavioral context of the strike (Young and Zahn, 2001). The second hypothesis, known as the pressure-balance hypothesis, attributes the differences in venom deposition to a physiological variation in strike movements and the surface features of the target, with an emphasis on the role of peripheral resistance (Hayes et al., 2008; Young et al., 2002; Young et al., 2003). Essentially, the significant difference between the venom-metering and the pressure balance hypotheses is that the latter can explain differential venom injection without the notion of decision making on the part of the snake. Although these two hypotheses are often pitted against one another as alternative explanations, they are not mutually exclusive. Critics of the venom-metering hypothesis argue that there is an absence of evidence to support that differential venom gland contraction is a conscious act on the part of the snake.

Young et al. (2002, 2003) performed experiments with viperids and continue to assert that the venom-metering phenomenon is supported by the pressure balance hypothesis, stating, “the observations and experimental results of the current study are all consistent with the pressure-balance model” (Young et al., 2003). However, Hayes et al. (2008) continue to argue that venom metering can occur through multiple mechanisms based on the behavioral context of the bite; these mechanisms include venom gland contraction, controlling how long the fangs remain in contact with the target, and the duration flow/number of pulses of venom delivered. In defensive contexts, the duration of fang penetration and venom flow is much shorter than in prolonged bites, which led to the conclusion that the differences in venom delivery between brief and longer bites cannot be explained solely from differential pressures on the fang sheath, as the pressure

balance hypothesis suggests (Hayes et al., 2008). Furthermore, Hayes et al. (2008) explain that the cognitive control of just one of the aforementioned key features of biting would be a sufficient mechanism for regulating venom delivery.

These two hypotheses represent different views of analysis (cognitive versus physiological) and are therefore not mutually exclusive. Although Young et al. (2002; 2003) heavily support the pressure balance theory, Hayes et al. (2008) suggest that no one of these hypotheses is more important than the other, but rather are different ways of explaining venom regulation. In fact, Hayes et al. (2008) maintain the view that both cognitive and physiological functions are responsible for the venom-metering phenomenon. As of date, no one hypothesis is widely accepted by the scientific community as to how snakes meter their venom. Researchers have gathered evidence that supports both hypotheses, and at this time it is not possible to make a definitive determination as to which theory best represents how snakes meter venom. This will surely be a continued area of research in the following years. However, there is an abundance of evidence to suggest that snakes meter their venom as a result of the energetic cost of production, and this theory is widely accepted in the scientific community.

The evolution of the highly specialized venom delivery system in viperids is a remarkable accomplishment that has allowed this group of animals to become highly specialized and successful predators, perfectly suited for their environments. In addition to developing these advanced systems of prey capture, viperids have also developed ways in which to manage their resources and reduce energy expenditure towards producing venom. This resource managing further illustrates the adaptability of this organism and

illustrates why this animal is one of the most successful and derived organisms in existence.