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Review

The venom optimization hypothesis revisited

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ABSTRACT

Animal venoms are complex chemical mixtures that typically contain hundreds of proteins and non-proteinaceous compounds, resulting in a potent weapon for prey immobilization and predator deterrence. However, because venoms are protein-rich, they come with a high metabolic price tag. The metabolic cost of venom is sufficiently high to result in secondary loss of venom whenever its use becomes non-essential to survival of the animal. The high metabolic cost of venom leads to the prediction that venomous animals may have evolved strategies for minimizing venom expenditure. Indeed, various behaviors have been identified that appear consistent with frugality of venom use. This has led to formulation of the "venom optimization hypothesis" (Wigger et al. (2002) Toxicon 40, 749–752), also known as "venom metering", which postulates that venom is metabolically expensive and therefore used frugally through behavioral control. Here, we review the available data concerning economy of venom use by animals with either ancient or more recently evolved venom systems. We conclude that the convergent nature of the evidence in multiple taxa strongly suggests the existence of evolutionary pressures favoring frugal use of venom. However, there remains an unresolved dichotomy between this economy of venom use and the lavish biochemical complexity of venom, which includes a high degree of functional redundancy. We discuss the evidence for biochemical optimization of venom as a means of resolving this conundrum.

1. Introduction

Venoms are extremely complex mixture of proteins and non-proteinaceous compounds. Recent studies revealed around a thousand peptides in the mass range 1–10 kDa in the venom of the Australian funnel-web spider *Hadronyche versuta* and more than 600 in the venom of the related spider *Atrax robustus* (Escoubas et al., 2006). Similarly, 380–630 peptides were identified in the venoms of five different scorpions in the family Buthidae (Nascimento et al., 2006). These diverse peptides target a myriad of receptors and ion channels, making them ideal for pharmaceutical (King, 2011) and agrochemical (King and Hardy, 2013) research. However, it also raises a biological conundrum: multiple toxins often bind to the same molecular target, resulting in a high degree of functional redundancy. For example, analysis of the Tox-Prot database (Jungo et al., 2012) reveals 14 toxins from the scorpion *Leiurus quinquestriatus hebreus* that all act on site 4 of the insect voltage-gated sodium (Na_V) channel (for reviews of Na_V channel pharmacology, see Catterall et al., 2007; King et al., 2008).

Many studies have been conducted to understand the evolutionary processes that drove the generation of this venom complexity (for reviews see Sollod et al., 2005; Fry et al., 2009), especially considering the rarity of such extreme functional redundancy in most other systems (Nowak et al., 1997; Pal et al., 2006). Some studies have attributed the complexity of venoms to a molecular "arms-race" in which the processes of toxin mutation and counter-mutation of the toxin target result in strong directional selection (Kordis and Gubensek, 2000; Juarez et al., 2008). However, there is currently a paucity of studies supporting

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this view. For example, although some studies have shown positive selection of toxins from Asian scorpions that act on Na_V channel receptor sites 3 (Weinberger et al., 2010) and 4 (Tian et al., 2008), no studies have revealed positive selection of the same toxins in scorpions from other geographical locations such as the Americas, despite the availability of transcriptomic data. Similarly, although the ArachnoServer database (Wood et al., 2009; Herzig et al., 2011) contains 800 curated spider-toxin sequences, there is currently only one study showing positive selection of spider-venom proteins (Binford et al., 2009).

A recent study points to a considerable heterogeneity in the evolutionary selection pressure applied to toxins that belong to the same toxin family, act on the same molecular target, and come from the same scorpion species (Weinberger et al., 2010). This heterogeneity is directly linked to the role of these toxins in natural envenomations. Previous studies have not incorporated biological/physical factors into their evolutionary analyses, but rather have treated the different toxins disconnected from the ecological context of venom use, which is highly variable. For example, in the emperor scorpion *Pandinus imperator*, venom is used exclusively by juveniles (Casper, 1985), with adults discontinuing predatory use of venom. In most other scorpions, the stinger is used at least once in most predatory encounters, regardless of age. This difference in venom use may result in different evolutionary trajectories for the venom toxins. On a larger scale, knowledge of venom use, target specificity, and other ecological factors are missing from molecular evolutionary studies of scorpion toxins.

In this review we aim to summarize current knowledge on the biology of venom use by examining three orders of venomous animals whose venoms systems have evolved independently—spiders, scorpions, and snakes. We first present data on the metabolic cost of venom use and what is known about the way venomous animals manage this cost before attempting to connect these data to what is known about the evolution of venom proteins.

2. The metabolic cost of venom use

The ecological advantages conferred by the possession of a venom system are evident from the extraordinarily diverse range of animals that have evolved venoms for the purposes of predation, defense, or competitor deterrence (King, 2011). Venom can be broadly defined "as a secretion, produced in a specialized gland in one animal and delivered to a target animal through the infliction of a wound, which contains molecules that disrupt normal physiological or biochemical processes so as to facilitate feeding or defence by the producing animal" (Fry et al., 2009). Based on this definition, the extant suite of venomous animals includes annelids, cnidarians, echinoderms, molluscs, vertebrates, and arthropods. These animals have evolved a wide variety of venom delivery systems, including barbs, beaks, fangs, harpoons, pincers, proboscises, spines, spurs, and stingers (Fry et al., 2009).

The use of venom as a chemical weapon allows the venom-producing animal to refrain from competing with potential prey or predators on a physical level (except at the moment of envenomation), thereby transferring the "battleground" into the envenomated animal. However, venom is not a magic weapon, and there are multiple examples of secondary loss of venom. For example, a dietary shift in the marbled sea snake (*Aipysurus eydouxii*) to eating eggs rather than the egg layers has resulted in the loss of active venom (Li et al., 2005). Venom has also been secondarily lost in uloborid spiders which instead kill their prey by wrapping them tightly in hackled silk (King, 2004). These secondary losses suggest that venom use comes with a considerable biochemical price.

Few studies have assessed the metabolic cost of venom regeneration, which encompasses two factors—the regeneration of the actual venom components themselves as well as the secretory and processing machinery associated with toxin production. The actual amount of venom produced appears to be relatively small compared to the body weight of the envenomator. The wet mass of venom appears to never exceed 0.5% of body weight in snakes (Morrison et al., 1983; Pe and Cho, 1986; Mirtschin et al., 2006), scorpions (Yahel-Niv and Zlotkin, 1979; Nisani et al., 2007), and spiders (Malli et al., 1998). Nevertheless, the metabolic cost of venom production is not trivial. McCue and Mason found that the metabolic rate (MR) of snakes was increased by 11% on average during the three days over which venom regeneration was measured (McCue and Mason, 2006). This is a significant metabolic cost when one takes into account the fact that venom regeneration takes considerably longer than three days, with RNA synthesis alone taking >4 days (Rotenberg et al., 1971). Full regeneration appears to take >28 days in snakes (Rotenberg et al., 1971; Oron and Bdolah, 1973), and as much as 85 days in theraphosid (mygalomorph) spiders (Perret, 1977), although experiments done with the araneomorph spider *Cupiennius salei* revealed a recovery of venom toxicity within 16 days (Boevé et al., 1995). Measurements of amino acid incorporation in viperid venoms indicate that venom regeneration in these snakes takes more than 22 days and up to 41 days (Oron and Bdolah, 1973). An increased MR over the long time period required for venom regeneration suggests that maintenance of a venom system represents a considerable metabolic load.

Pintor et al. (2010) argue somewhat differently. They found that the MR was elevated 21% above normal during the first three days of venom regeneration in the death adder *Acanthophis antarcticus*, but then the MR returned to resting levels, resulting in a modest overall metabolic cost for venom regeneration. They calculated the cost of venom regeneration at 26% of the cost of the digestion of a small prey (5% of snake weight) and 6% of the cost of shedding, suggesting that the cost of venom regeneration was insignificant in context of the overall metabolism of the snake. However, the authors milked only 1.54 mg of venom (dry weight) prior to studying venom regeneration. Two different sources (Freeman and Kellaway, 1934; Mirtschin et al., 2006) indicate that the average glandular content of these snakes is 42–45 mg of venom (dry weight), consistent with the observation that *A. antarcticus* injects a mean of 42 ± 16 mg of venom during a single predatory bite of an adult mouse (Morrison et al., 1983). This indicates that only 3–4% of the total venom was depleted in the Pintor study.