Evolution: Gene Co-option Underpins Venom Protein Evolution

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Venoms contain variable mixtures of bioactive proteins. New work shows that parasitoid wasp venom toxins evolve by the co-option of genes rather than the canonical process of gene duplication. These findings suggest co-option may be an unappreciated process underpinning protein neofunctionalization.

Venoms are key evolutionary adaptations that are injected into other animals for predatory, defensive and/or reproductive purposes [1]. The venoms themselves often consist of highly variable mixtures of protein and peptide components (toxins) that act on a multitude of receptors to perturb physiological systems in the envenomed victim [1,2]. Our understandings of the origin and evolution of venom proteins are predominately restricted to relatively few animal lineages that use their venom for predatory purposes (e.g. snakes, spiders, cone snails) [3–5]. These studies suggest that the process of gene duplication has been central to generating the suite of toxins observed in venom today. Surprisingly, in this issue of *Current Biology*,Martinson *et al.* [6] report that the evolution of venom toxins in parasitoid wasps is largely reliant on the co-option of single copy genes, and therefore has predominantly occurred independent of gene duplication events. These findings suggest that co-option may be an overlooked mechanism underpinning the evolution of new gene functions.

The importance of venom as an adaptive trait is highlighted by the many independent occasions with which this character has evolved over evolutionary time, with venomous lineages punctuated throughout both invertebrate (e.g. spiders, scorpions, cephalopods, bees, wasps) and vertebrate (e.g. fish, snakes, lizards, mammals) branches of the tree of life [1]. Despite this diversity, the vast majority of venom evolution studies have focused on lineages that are medically-important to humans; as certain species of snake, scorpion, spider and cone snail are capable of causing lethality. While these studies suggest that venom toxins have evolved by a multitude of processes, including gene duplication, recombination, alternative splicing, exon shuffling and co-option [1,7], it is gene duplication that has been most commonly invoked as the predominant mechanism underpinning venom protein evolution.

 Venom toxins appear to have originated from genes co-expressed in ancestral venom glands and other physiological tissues, followed by their selection for increased expression in the venom gland and, potentially, the loss of their low-level expression in other tissue types [8–12]. Subsequently, many toxins have further diversified by the process of gene duplication, and are therefore said to follow the ‘birth and death’ model of gene evolution [13]. In addition, many of these paralogous genes show evidence of accelerated sequence evolution or ‘hypermutation’, which accumulate in a highly episodic fashion [14], indicative of adaptive change [3,4,14]. However, recent research suggests that gene duplications may actually be of immediate importance for the selection of increased expression levels rather than generating sequence diversity [12]. Nonetheless, subsequent sequence change is often accelerated and directed towards modifications of the protein surface, and this process seemingly facilitates the evolution of new protein functions by enabling interactions with new physiological targets [1,14]. Millions of years of evolutionary time punctuated by these various processes have therefore resulted in many venoms containing toxins with common evolutionary origins that exhibit different functionalities, though collectively they may act synergistically on the same pathways to markedly perturb physiological processes [1,2,15]. However, the scarcity of genomic information from venomous animals, coupled with a biased foci towards medically important lineages, mean that the mechanisms underpinning venom evolution in general remain poorly understood, particularly in those animals that do not use venom for securing a meal.

 In this issue of *Current Biology*, Martinson *et al.* [6] investigated the evolution of venom toxin encoding genes in parasitoid wasps and found that, in contrast to the mechanisms described above, the co-option of single copy genes underpins the molecular composition of their venom. Parasitoid wasps (Fig. 1) employ their venom in an atypical manner, by using it to create a suitable environment for the development of their larvae, in contrast to predatory or defensive purposes. Consequently, only female parasitoids are venomous, and the injection of their venom has been demonstrated to alter the metabolism, immunity and gene expression of their insect hosts, resulting in behavioural changes that keep these animals alive for prolonged periods of time, facilitating the development of their larvae [16,17]. Parasitoid wasps were used as the model for this study because closely related wasps (even congeners) are known to parasitise different insect hosts, and therefore there are anticipated pressures on the venom to adapt in terms of both composition and function in response to these different targets.

To investigate venom evolutionary processes, Martinson *et al.* [6] compared the transcriptomic and proteomic composition of the venom gland and venom from four closely related parasitoid wasp species (deepest divergence occurred ~4.9 mya). One of their most interesting findings is the extensive amount of toxin turnover observed between these related species. A total of 406 putative venom toxins were found across all four species, which formed 134 homologous groups. However, only 33 of these groups were actually conserved across all four species, demonstrating that extensive venom variation has evolved over relatively short periods of evolutionary time. Further emphasising this finding is evidence that over 40% of the toxins found in the venom of the two most closely related species studied, *Nasonia vitripennis* and *N. giraulti* (which only diverged ~1.6 mya), were different.

 Such high rates of gene turnover therefore provide a model system for examining how these toxins have been ‘recruited’ for roles in venom. By analysing genomic data, Martinson *et al.* [6] show that a large proportion of ‘new’ venom toxins are actually single copy genes and have therefore not evolved via the canonical pathway of gene duplication coupled to sub- and/or neo-functionalization, but have instead been co-opted for a role in venom. In fact, only four of the 53 recently gained venom genes identified in their study exhibited unequivocal evidence of gene duplication. Further bucking the traditional venom evolution trend is the absence of any signals of accelerated sequence evolution acting on these genes; a characteristic often found in the duplicated venom toxins identified in predatory venoms [1,3,14]. Furthermore, using mating experiments, Martinson *et al.* [6] were able to demonstrate that the rapid turnover of these co-opted venom toxins is primarily the result of cis-regulatory changes in the expression of these genes in the venom gland.

 Martinson *et al.* [6] propose that the differences in the processes underpinning the evolution of toxin genes in parasitoid wasps and previously studied venomous animals may be the result of differences in venom function. It is hard to dispute this assertion, given the nuanced and atypical effect that the venom of parasitoid wasps has on their insect hosts. However, in a wider context, these findings raise important questions relating to the processes that other venomous animals may have used to generate their venom toxins - are parasitoid wasps outliers due to the unusual ecological role of their venom, or is co-option a key recruitment mechanism in non-predatory venoms in general? For example, the co-option of venom toxins has previously been described in animals that do use venom for predation [4], but was found to be a minor mechanism underpinning the evolution of ancillary rather than key toxins (which evolved via gene duplications). Contrastingly, studies on the platypus, a venomous mammal that also uses its venom in a sex-specific manner for reproductive purposes (male competition), also revealed that gene duplications have only played a minor role in the evolution of their venom toxins [18]. Consequently, the future study of animal lineages that use their venom for defensive purposes seems particularly important in order to more broadly correlate mechanisms of toxin evolution with the ecological function of venom systems.

 In summary, the findings of Martinson *et al.* [6] seem likely to stimulate a new research pathway relating to how venom toxins evolve. This is important because, on the one hand, venom toxins represent some of the most rapidly diversifying proteins in the animal kingdom [3], making them an ideal model for studying molecular mechanisms of adaptation. Whereas on the other hand, there is growing evidence that many toxin genes are actually co-expressed in multiple tissue types (albeit at significantly higher levels in the venom gland), providing a framework for exploring how the expression of genes encoding toxic molecules are differentially regulated for internal (physiological) and external (venom) purposes [4,6,8–11]. Finally, these results are also important for the much broader question of how proteins evolve new functions. While gene duplication and alternative splicing are typically invoked as major mechanisms underpinning protein neofunctionalization [19,20], this study suggests that the process of co-option should be re-evaluated as a potentially important method by which genes can acquire novel functions.

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