Dispatch

Venom Evolution: Gene Loss Shapes Phenotypic Adaptation

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Snake venoms are variable protein mixtures with a multitude of bioactivities. New work shows, surprisingly, that it is the loss of toxin-encoding genes that strongly influences venom function in rattlesnakes, highlighting how gene loss can underpin adaptive phenotypic change.

Venoms are important evolutionary innovations found scattered across the animal kingdom. From taxa as ancient as cnidarians, all the way through to our more recent piscine, amphibian, reptilian and mammalian vertebrate relatives, we find species that inject venom toxins into other animals for defensive, predatory or reproductive purposes [1]. Venoms are typically mixtures of toxic protein and peptide constituents and are thought to have evolved via a process of frequent gene duplication coupled to accelerated sequence evolution [1]. This expansion of toxic constituents is thought to have underpinned the evolution of new, often synergistic, protein functions. Surprisingly, in this issue of *Current Biology*, Dowell *et al.* [2] report that this adaptive process can also act in reverse, whereby frequent gene loss is responsible for shifting venom bioactivity away from an ancestral phenotype to a derived adaptive state.

Snake venom evolved at least 60 million years ago [3] — perhaps even as far back as 170 million years ago [4] — and it is the most well-studied of all animal

venom systems. This is in part due to the high incidence of snakebite envenomings and deaths that occur each year in tropical regions of the world. Currently, it is estimated that as many as 94,000 people die annually, with many more suffering long-term morbidity as a result of the toxic effects of venom [5]. Medically important snakes use elegant hollow fangs located at the front of the upper jaw to inject venom expelled from the venom gland into target animals. The venom is primarily used for prey capture and it is often complex in terms of composition and, crucially, is highly variable between species. In fact, venom variation has been described at every taxonomic level in snakes, including inter- and intra-specifically, and it has even been reported to change over the lifetime of a single individual [6], making it an ideal model for studying genotype–phenotype interactions. Indeed, variation relates not only to the composition of the toxin-encoding genes themselves, but also their translated toxic proteins and the biochemical function of venom. It has therefore been postulated that, in addition to genotypic variation, post-genomic processes may influence venom composition in some [7] (but not all [8]) cases, but are likely to be particularly influential in those species exhibiting evidence of ontogenetic change [9].

Despite the relatively large number of protein components found in snake venom (~50–200), such toxins are actually encoded by relatively few gene families. However, these families, which include metalloproteinases, phospholipases, serine proteases and three-finger toxins, are multi-locus in nature. They appear to have originated from certain genes that were co-expressed in the ancestral venom gland and other body tissues, followed by their increased expression in the venom gland, and in some cases, the loss of their low-level expression in other tissue types [10–12]. Subsequently, many toxins have diversified by a process of frequent gene duplication, postulated to have occurred via the ‘birth and death’ model of gene evolution [13],

and resulting in a suite of related venom toxins that are heavily expressed in the venom gland [14]. Often, these paralogous genes show evidence of having evolved under the influence of positive selection, with amino-acid changes frequently observed in regions of the molecule that are found on the surface of the protein structure [14,15]. This, in turn, seemingly facilitates the evolution of new protein functions, and related venom toxins often exhibit distinct, sometimes synergistic, bioactivities on crucial physiological pathways [1]. Well-studied examples of this include clotting-factor-activating and fibrinogenolytic metalloproteinases and neurotoxic and myotoxic phospholipase A_2 (PLA_2) toxins, which have evolved from constitutive housekeeping 'ADAM' and PLA_2 genes, respectively [3]. However, the current scarcity of genomic information from venomous snakes means that the details of these processes are poorly understood; for example, it remains unclear which mechanisms underpin gene duplication events and how frequent and important gene losses might be to the venom phenotype.

Dowell *et al.* [2] have addressed some of these fundamental questions by studying toxin-encoding genes in North American rattlesnakes (Viperidae: *Crotalus* spp.). Rattlesnakes are an ideal model for studying venom variation. Whilst venom from a number of species, including the Eastern (*Crotalus adamanteus*) and Western (*C. atrox*) diamondbacks, function in a typically 'viperid' manner by disrupting haemostasis, the venoms of many other rattlesnakes (such as the Mojave rattlesnake, *C. scutulatus*) are unusual because they have neurotoxic effects. In these cases, venom-induced neurotoxicity is caused by a heterodimeric venom toxin that consists of one acidic and one basic PLA_2 protein stuck together. By sequencing the genomic regions known to encode PLA_2 toxins from multiple rattlesnake species it was inferred that ancestral rattlesnakes had in fact seven PLA_2 genes, including the two

involved in the formation of the neurotoxin, and were therefore likely neurotoxic (Figure 1). Crucially, since that time, the Eastern and Western diamondbacks and the Mojave rattlesnakes have each differentially lost a number of entire PLA_2 genes, resulting in subsets of different PLA_2s expressed in the venom gland. While the Mojave rattlesnake has retained the acidic and basic genes required to produce the neurotoxin, the Eastern and Western diamondbacks have lost both of these genes, resulting in the complete loss of neurotoxic venom activity (Figure 1).

These results are unexpected. While gene losses are explicitly inferred by the 'birth and death' model frequently invoked to drive snake venom toxin evolution [7,13,16], this study is the first to show that differential gene loss is capable of moulding the biochemical phenotype of venom. The results are in contrast to the canonical assumption that gene duplication underpins toxin and functional variation and, consequently, provide a paradigm shift for the field. In a wider context, this study also provides one of only a few well-described examples of how gene loss can be associated with adaptive changes to specific phenotypes [17,18].

Dowell *et al.* [2] go one step further by predicting the mechanism responsible for causing genotypic variation in toxin genes. They propose that non-allelic homologous recombination was likely responsible for causing the genetic rearrangements that led to both the ancestral duplication and more recent loss of rattlesnake PLA_2 genes. This hypothesis is supported by evidence that transposable elements, including both class I retrotransposons and class II transposons, occur in hotspots in the genetic regions interspersing the PLA_2 genes: they therefore seemingly provide the substrate for gene duplication and deletion via non-allelic homologous recombination during meiosis.

The question that remains unanswered is why have some rattlesnake species lost the ancestral neurotoxic venom activity that has been retained and is utilised in so many of their counterparts? Dowell *et al.* [2] suggest that this loss of neurotoxicity is likely adaptive, and that dietary variation and/or predator/prey interactions might be responsible for driving the observed genotypic variation. This is not an unreasonable assumption given prior reports of correlations between diet and venom composition [1,19] and evidence of prey (and some predator) species developing strong resistance to viperid venoms [20]. Future comparative research incorporating both natural history information on prey composition and experimental evidence of venom toxicity to different prey items would likely reveal the adaptive basis for such divergent venom phenotypes.

In summary, venoms are complex cocktails, and their composition and therefore bioactivity is underpinned by seemingly complex and variable interactions between genes, their expression, their translation and their post-translational modification. Evidence that the loss of genes also has a strong influence on shaping venom phenotypes further reinforces the value of using animal venom systems to understand adaptation in the natural world.

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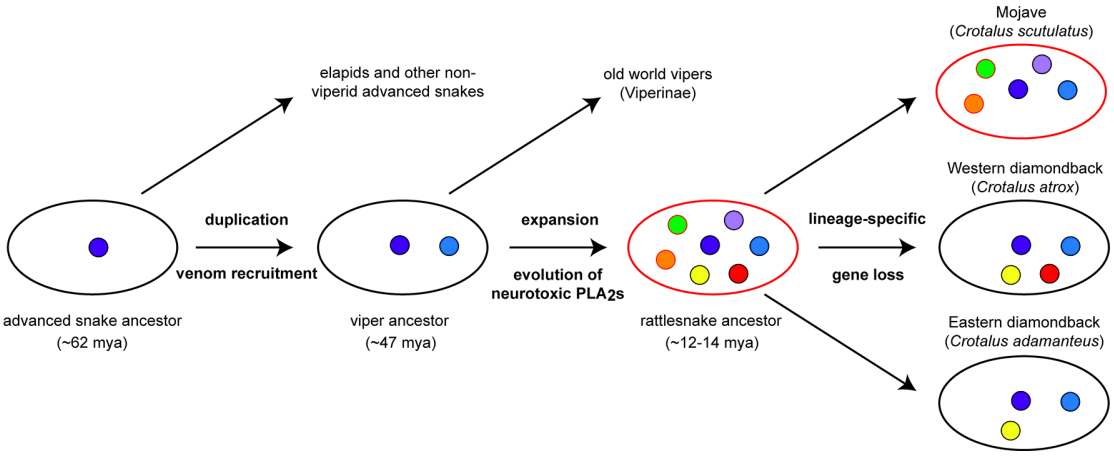

Figure 1. Simplified schematic of the evolution of venom PLA_2 genes in rattlesnakes. Coloured circles represent the different PLA_2 genes described by Dowell *et al.* [2], including the acidic and basic subunits of the neurotoxic PLA_2 complex (green and orange). Red outlines indicate neurotoxic venom activity.

In Brief:

Snake venoms are variable protein mixtures that cause a multitude of bioactivities. Surprisingly, it is the loss of toxin-encoding genes that strongly influences venom function in rattlesnakes. These findings highlight how gene loss can underpin adaptive phenotypic change.