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SYMPOSIUM INTRODUCTION

Integrating Perspectives on Animal Venom Diversity: An Introduction to the Symposium

Marymegan Daly[1,*] and H. Lisle Gibbs[†]

*Department of Evolution, Ecology, and Organismal Biology, Ohio State University, 1315 Kinnear Rd, Columbus, OH 43212, USA; [†]Ohio State University, 318 West 12th Avenue, Columbus, OH 43210, USA

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[1]E-mail: daly.66@osu.edu

Venom is a hallmark example of animal evolution: the capacity to make and use toxins has arisen via natural selection multiple times in animals as diverse as corals, snails, spiders, snakes, and mammals (Casewell et al. 2013). This diversity within the animal tree of life is mirrored by diversity at the molecular and genetic level, as the proteins that make up venoms and the genes that specify these proteins evolve rapidly to fill diverse functional roles (Sunagar et al. 2016). Because of their remarkable molecular diversity, venoms are key, albeit challenging, resource for pharmacological discovery that contribute to the development of drugs that act as anti-tumor agents, heart stimulants, and therapies for neurological diseases (Harvey 2014).

Venom biology is a multidimensional field, spanning the molecules and genes of the phenotype to the ecological consequences of its use (Calvete 2013). Those dimensions are integrated in the organisms that make and use the venom. Although there have been previous symposia and working groups devoted to venom, these have focused on either a single organismal lineage (e.g., King 2004; Kem and Turk 2009) or limited methodological approaches (e.g., Escoubas 2006; Calvete 2012). This symposium aimed to span the lineages and organizational levels at which venom is being studied and to develop links between these levels and across these lineages.

All venoms are similar in being complex cocktails of proteins and other bioactive compounds that are injected by the manufacturing animal into another animal (Casewell et al. 2013). Even when venom is not homologous in a broad evolutionary sense, the genes that are recruited into venom may belong to the same gene families. Similarities in molecular targets and the need for functional redundancy for neofunctionalizing genes may limit the pool of possible gene families from which venom genes can be recruited (Fry et al. 2009). However, as Rodríguez de la Vega points out in his symposium contribution, because toxins may be recruited convergently from within the same large gene family, it is especially important to consider genes that encode non-toxin proteins or that are nonfunctional so as not to misinterpret the level of shared evolutionary history (Rodríguez de la Vega and Giraud 2016). This caution argues against exclusive or primary reliance on databases of genes and proteins linked to venom (e.g., ConoServer: Kaas et al. 2011; ToxProt: Jungo et al. 2012) as the source of genes and proteins for comparison and underscores the importance of genomic (rather than transcriptomic) approaches to understanding the molecular origin of venom (Reyes-Velasco et al. 2015). Although these databases are an important resource for detecting and interpreting the genes that contribute to venoms, for contextualizing the genes that encode venoms, coding genes without venom function and pseudogenes are likely as important as genes with known function in venom.

The biochemical diversity of venoms poses a compelling system in which to understand the genetic and molecular origin of diversity and the ecological and evolutionary impact of this diversity (Sunagar et al. 2016). This is best understood for snakes and other lineages which have been more completely studied because they have a direct impact on human health. Nonetheless, for even these well

studied venomous lineages, the nature of genetic and proteomic diversity of venom and the mechanisms used to generate functional diversity are unclear (Hargreaves et al. 2014a; Zelanis and Tashima 2014). Mechanisms for generating diversity include exon shuffling (Siigur et al. 2001), combinatorial libraries (Olivera et al. 1995; Escoubas 2006), and accelerated rates of duplication and diversification in the gene families from which toxins are recruited (Rokyta et al. 2011; Wong and Belov 2012). However, these may not be the only means of generating molecular diversity (see Dutertre et al. 2013; Rokyta et al. 2015), and the hyper-diversity seen in snakes, spiders, and cone snails may not be characteristic of all lineages or all toxins (e.g., Sunagar and Moran 2015).

Diversity in the structures used to inject venom add a second level of complexity to understanding the venom phenotype. These delivery mechanisms may be highly conserved across a lineage at a broad level (e.g., the rear fangs of snakes: Vonk et al. 2008; the nematocysts of Cnidaria: Fautin 2009). In gastropods, the venom apparatus varies (Castelin et al. 2012), but this variation corresponds to variation in the function and diversity of venom (Gorson et al. 2015). In their contribution, Smith et al. (2016) exploit this link between anatomy and function to gain perspective on the diversity of venomous fishes, finding evidence for venom delivery structures across lineages, including those whose members are rarely collected alive or whose physiology and ecology are unknown. Phylogenetic perspective can serve as a means of bioprospecting for new venomous lineages, leveraging centuries of anatomical study to identify venomous species and lineages and the historical contexts in which they have arisen.

The diversity and evolution of venom have both phylogenetic and functional components, reflecting the lineage of the organism producing the venom and the specific interactions between the venomous animal and its target. The ubiquity of venom across Metazoa reflects several independent originations, although the number and nature of these is controversial (e.g., Fry et al. 2003; Hargreaves et al. 2014b). Broad similarities may obscure important differences at finer scales: within many lineages, venom is widespread but has multiple origins. In the contributions to this volume, this is demonstrated by Rodríguez de la Vega and Giraud (2016) for genes and by Smith et al. (2016) for morphology. However, Sanz-Soler et al. (2016) find deep homology at the genetic level, identifying genes implicated in venom that are shared across anapsid, diapsid, and archosaurian reptiles.

Data gathered through genomic and transcriptomic studies can be harnessed to address questions about the evolution and diversity of venom only if the function of genes and products are known. This requires knowledge of venom-producing organisms, their anatomy, and their biology, including how venom mediates their interactions with prey, predators, and conspecifics. Functional information is key for diverse and significant directions in venom studies ranging from annotating genomes (Aloy et al. 2001) to inferences about evolutionary and ecological mechanisms for maintaining variation (e.g., Valentin and Lambeau 2000; Rodríguez de la Vega and Possani 2005) yet is often poorly-known relative to the molecular processes that generate variation in the venom phenotype. In their contribution, Miller et al. (2016) describe such functional context for scorpions and their mammalian prey, but in doing so, highlight the complicated path between function and phenotype: the ecological differences do not map to phenotype and function in the way predicted by theory. A different mismatch between function and phenotype comes from the contribution of Ames and Macrander (2016) who find evidence for a venom gland that serves a dual function, contributing to the secretion of digestive enzymes within the gut and venoms exported to the tentacles.

An integrative approach offers important conceptual and practical advantages for future studies of venom biology. Historically, research on venom has emphasized its impact on human health: first as a challenge and later as a palliative (e.g., Fox and Serrano 2007; King 2011). In particular, taxa of critical phylogenetic position in clades show ecological innovations may be compelling targets for ''bio-prospecting'' (e.g., Smith and Wheeler 2006; Verdes et al. 2016). In this symposium, Gorson and Holford (2016) provide a perspective on the ways in which venom differs among gastropods of the family Terebridae. Specifically, the close relationship between these snails and the better-characterized cone snails (Holford et al. 2009) allows these comparisons to be extended in phylogenetic and ecological space. Phylogenetic and ecological perspective can enhance prospecting for pharmacologically interesting proteins, and may be critical in identifying novel venom genes through comparative analysis (Fry 2005; Moran et al. 2008; Whittington et al. 2010).

Beyond these applications, as highlighted by the presentations and discussion in this symposium, venom is a compelling model system in which to investigate fundamental and integrative questions in evolutionary biology. The genetic, proteomic, and functional data available for venom make it highly

tractable as a system in which to address fundamental questions about molecular evolution of rapidly-evolving gene families, the molecular basis of adaptive variation, and the role that key adaptive innovations play in phylogenetic diversification. Inferences about metabolic costs and benefits of deploying venom in snakes and spiders has informed several hypotheses about the adaptive value of using venom (e.g., Wigger et al. 2002); the relative metabolic cost of venom in other animals is unclear but highly relevant to any inference about behavioral and biological strategies for optimizing dose or use (Morgenstern and King 2013). In the final contribution, Holding et al. (2016) highlight this potential in their review on what is known about the molecular and evolutionary basis of venom resistance in mammals which serve both as prey and predators of venomous snakes. They develop a case for using venom resistance as a model for understanding the molecular basis of complex coevolutionary adaptations in predators and prey by linking the phenotypes across hierarchical levels and explicitly invoking how coevolutionary theory can be used to generate precise and testable hypotheses for the interplay between variation in venom and resistance in its target.

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