warrants further analyses which may provide useful insights into the functional regulation of these molecules.

Keywords: Tropedolaeus venom, antiplatelet phospholipases, mass profile of N-glycans
10.1016/j.toxicon.2012.04.061

61. A Phylogenetic Framework for the Study of Convergence and Divergence in Scorpion Venoms

Ricardo C. Rodríguez de la Vega 1,2, Nicolas Vidal 2
1 Laboratoire Ecologie, Systématique et Évolution, Université Paris-Sud, Orsay France
2 Département Systématique et Évolution, Muséum National d’Histoire Naturelle, Paris France
E-mail address: ricardo.rodriguez-de-la-vega@u-psud.fr (R.C. Rodríguez de la Vega).

Background: Mining on scorpion venoms using high throughput techniques has revealed a large prevalence of non canonical components in scorpion venoms, which in fact outnumb the typical toxin class. The evolutionary histories of these scorpion venom components show puzzling patterns, where both extensive divergence and recurrent recruitment are thought to have played important roles.

Methods: Non-redundant datasets were built for five protein families found in scorpion venoms from at least three different taxonomic families. Non-venom orthologs were identified by database searching and tested as proxies for rooting venom proteins phylogenetic trees. Maximum Likelihood and Bayesian trees for each protein family were reconstructed and compared to a draft species tree based on public mitochondrial sequences.

Results: High confidence rooted trees were obtained for three venom protein families. In the remaining two families, non-venom orthologs were found nested within venom clades, thus suggesting independent recruitment events.

Discussion: Mapping of venom proteins trees onto the species tree reveals a tangled pattern of continuous recruitment and lineage-specific diversification. Reconstructing the evolutionary history of venom proteins remains a difficult task due to multiple confounding factors. The use of a robust species phylogeny would certainly aid to resolve the discrepancies found in the different gene trees and help in the distinction between convergent recruitment and divergence.

Conclusions: Inasmuch genomes can be regarded as the historical records of previous selection processes, the molecules making up the venoms should reflect the history of the interspecies interactions that the producing lineages have faced. It follows that reconstruction of their evolutionary histories should 1) clarify how venoms have attained their remarkable complexity and 2) shed light over the multi-organismic processes that have shaped the evolution of venomous organisms in the context of their ecosystemic networks.

Acknowledgments:
The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement No. 246556. RCRV staying at the MNHN was funded by the Marie de Paris.

Keywords: convergence, divergence, evolution, scorpion, toxin, venom
10.1016/j.toxicon.2012.04.062

62. Tentacles of Venom: Molecular Evolution of Coleoid Venoms

Bryan G. Fry 1, Tim Ruder 1, Dessi N. Georgieva 2, David Morgenstern 3, Glenn King 3, Eivind A.B. Undheim 1,3
1 Venom Evolution Laboratory, School of Biological Sciences, The University of Queensland, St. Lucia, Australia
2 Laboratory of Structural Biology of Infection and Inflammation, Institute of Biochemistry and Molecular Biology, University of Hamburg, Germany
3 Division of Chemistry and Structural Biology, Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Australia
E-mail address: bgfry@uq.edu.au (B.G. Fry).

Background: New insights into the evolution of venom systems and the importance of the associated toxins cannot be advanced without recognition of the true biochemical, ecological, morphological and pharmacological diversity of venom systems. A major limitation of the use of venom proteins has been the very narrow taxonomical range studied. Entire groups of venomous animals remain virtually completely unstudied. One such group are the coleoids (cuttlefish, octopuses, and squid), which have only been recently revealed by us to share a common venomous ancestor.

Methods: Eleven cuttlefish, octopuses, and squid species were field collected from tropical through to polar waters, thus providing wide taxonomical and ecological coverage. Venom molecular evolution was analysed using a combined proteomics/transcriptomics approach including mass spectrometry, 2-gels, and cDNA libraries. The comparison of proteomic and transcriptomic data allowed for rapid identification of peptide/protein types present in the venoms and subsequent determination of full-length transcript sequences.

Results: The combined approach revealed not only protein/peptide types convergently recruited into the chemical arsenals of other venomous animals but also discovered novel molecular scaffolds unique to coleoid venoms. Our bioactivity studies also revealed unique temperature specific adaptations of enzymes found in the Antarctic species.

Discussion: Coleoid venoms were revealed to be as complex as other venoms that have traditionally been the recipient of the bulk of research efforts. The presence of multiple peptide/protein types convergently present other animal venoms reveals new information as to what characteristics make a peptide/protein type amenable for recruitment into chemical arsenals.

Conclusion: Coleoid venoms have significant potential not only for understanding fundamental aspects of venom evolution but also as an untapped source of novel toxins for use in drug design and discovery.

Keywords: coleoid, venom, venomomics, proteomics, transcriptomics, toxin evolution
10.1016/j.toxicon.2012.04.063

63. Discovery of the Nicotinic Receptor Toxin Anabaseine in a Polyaeferian Nemertine

William R. Kem 1, Juan Junoy 2
1 University of Florida College of Medicine, Dept Pharmacology and Therapeutics, Gainesville, FL, USA
2 Departamento de Biologia Animal, Universidad de Alcalá, Alcalá de Henares, Spain
E-mail address: wrkem@ufl.edu (W.R. Kem).

Keywords: nicotine, nemertine, polystyliferan, anabaseine
10.1016/j.toxicon.2012.04.064
Background: Nemertines, a phylum of predominantly marine worms, prey on other animals and defend themselves with toxins. The anoplan nemertines lack a proboscis stylet for puncturing their prey and secrete neurotoxic and cytotoxic peptides. The hoplonemertines are enoplon (armed) worms that have a proboscis armed with one more stylets. Hoplonemertines are systematically divided into the relatively diverse order Monostylifera that is relatively common in certain benthic zones and the less readily collected order Polystylifera that are bathypelagic as well as benthic in distribution. Monostylifera nemertines are known to produce alkaloidal toxins (including anabaseine) that affect nicotinic acetylcholine receptors and pyridine chemoreceptors (Bacq, 1936; Kem, 1971; Kem and Soti, 2001; Kem et al., 2006). Anabaseine has been a lead compound in the design of alpha7 nicotinic receptor antagonists to treat disorders of cognition such as Alzheimer’s disease and schizophrenia (Kem et al., 2004; Freedman et al., 2008).

Methods: We recently obtained two live specimens of the sublittoral benthic polystyliferan Paradrepanophoros crassus (PC) from the northwest coast of Spain. Bacq (1936) found this species to contain a substance(s) active on nicotinic autonomic ganglia. We used Ehrlich’s reagent (Kem et al., 1971) for anabaseine detection and HPLC and mass spectrometric methods for isolation and identification of anabaseine and related alkaloids. Ethanolic preservation was used to extract the toxins.

Results: We have demonstrated that PC contains high concentrations of anabaseine in its body proper and proboscis but lacks 2,3’-bipyridyl, nemertelline and other alkaloids found in the benthic monostyliferan Amphiporus angulatus.

Conclusions: Our data indicate that the biosynthetic machinery for producing anabaseine probably was acquired by a common ancestral hoplonemertine before the evolutionary divergence of these two hoplonemertine orders.

Keywords: anabaseine, nicotinic, nemertine
10.1016/j.toxicon.2012.04.064

64. Centipede Venoms: Old and Unusual

Eivind A.B. Undheim1,2, Alun Jones1, John W. Holland1, Rodrigo A.V. Morales3, Brit Winnen1, Bryan G. Fry2, Glenn F. King1

1 Division of Chemistry and Structural Biology, Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Australia
2 Venom Evolution Laboratory, School of Biological Sciences, The University of Queensland, St. Lucia, Australia
3 Monash Institute of Pharmaceutical Sciences, Parkville VIC, Australia
E-mail address: e.undheim@imb.uq.edu.au (E.A.B. Undheim).

Background: At 420 million years old centipedes represent one of the oldest extant arthropod venom systems. However, despite the ancientness of these venoms, almost nothing is known about their components or molecular evolution.

Methods: Five centipede species were selected to provide both wide taxonomic coverage and a phylogenetic timeline to date evolutionary events. Emphasising the Scolopendridae due to their large size, availability, and clinical importance, the following species were selected to represent over 400 million years of evolution and to allow for comparisons at the order, subfamily, genus, and species level: Theraphopoda sp., Ethmostigmus rubripes, Cormocephalus westwoodii, Scolopendra alternans, and Scolopendra morsitans. Milked venoms were analysed using a combined proteomics/transcriptomics approach including mass spectrometry, 2-gels, and cDNA libraries. The comparison of proteomic and transcriptomic data allowed for rapid sequence determination and, crucially, identification of posttranslational modifications.

Results: We provide the first comprehensive insight into the chilopod “venome”, revealing novel scaffolds unique to centipede venoms as well as scaffold types convergently recruited into other venoms. We also present multifunctional transcripts and transcripts with tandemly repeated sequences, which are unusual to invertebrate venoms.

Discussion: Centipede venoms appear to differ substantially from the venoms of other arthropods in the abundance of high molecular weight components. The ancient evolutionary history of centipedes is also apparent from the differences at the highest taxonomic level, which diverged about 400 mya.

Conclusion: The presence of a wide range of novel proteins and peptides in centipede venoms highlights these animals as a rich source of novel bioactive molecules. Understanding the evolutionary processes behind these ancient venom systems may not only aid in directing bioprospecting efforts, but it will also broaden our understanding of which traits make proteins and peptides amenable to neofunctionalisation.

Keywords: centipede, venom, venomics, proteomics, transcriptomics, toxin evolution
10.1016/j.toxicon.2012.04.065

65. The Phylogenetic Scale of Venom Variation in Haplogyne Spiders

Greta J. Binford, Miles Dale, Andrew Wood, Jared Delahaye, Ian Voorhees, Jennifer Mullins, Pamela A. Zobel-Thropp
Lewis & Clark College, Dept. Biology, Portland, OR, USA
E-mail address: binford@lclark.edu (G.J. Binford).

Background: Haplogynes are a higher-level clade of araneomorph spiders that includes many taxa of interest with respect to their venom composition. Some notable haplogynes include pholcids (“daddy long legs” or cellar spiders), plectreurids, and families in the scytodoid superfamily such as spitting spiders (Scytodidae) and the sicariid family that includes brown recluse (Loxosceles) and six-eyed sand spiders (Sicarius).

Methods: With a goal of analyzing the phylogenetic scale of venom variation in spiders in general, we are comparing venom gland transcriptomes and proteomes from representatives of this group selected based on their phylogenetic position. Our data include some comparisons among relatively closely related taxa (common ancestor within the last 30 million years) and among other more