The Toxicogenomic Multiverse: Convergent Recruitment of Proteins Into Animal Venoms

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Key Words

toxin, phylogeny, evolution, convergence

Abstract

Throughout evolution, numerous proteins have been convergently recruited into the venoms of various animals, including centipedes, cephalopods, cone snails, fish, insects (several independent venom systems), platypus, scorpions, shrews, spiders, toxicoferan reptiles (lizards and snakes), and sea anemones. The protein scaffolds utilized convergently have included AVIT/colipase/prokineticin, CAP, chitinase, cystatin, defensins, hyaluronidase, Kunitz, lectin, lipocalin, natriuretic peptide, peptidase S1, phospholipase A_2, sphingomyelinase D, and SPRY. Many of these same venom protein types have also been convergently recruited for use in the hematophagous gland secretions of invertebrates (e.g., fleas, leeches, kissing bugs, mosquitoes, and ticks) and vertebrates (e.g., vampire bats). Here, we discuss a number of overarching structural, functional, and evolutionary generalities of the protein families from which these toxins have been frequently recruited and propose a revised and expanded working definition for venom. Given the large number of striking similarities between the protein compositions of conventional venoms and hematophagous secretions, we argue that the latter should also fall under the same definition.

INTRODUCTION

Venom systems are key evolutionary innovations in a broad phylogenetic range of animal lineages and are used for defense, competitor deterrence, or predation (or a combination thereof). Apart from the extensively studied, medically important clades (snakes, scorpions, and spiders), venomous animals include sea anemones, jellyfish, sea snails, cephalopods, centipedes, several insect orders, echinoderms, fish, lizards, and even some mammals (platypus and shrews). Modes of venom delivery are likewise diverse and include barbs, beaks, fangs or modified teeth, harpoons, nematocysts, pinchers, proboscises, spines, sprays, spurs, and stingers. Targets of venom action include virtually all major physiological pathways and tissue types accessible by the bloodstream.

Venom delivery systems sometimes show great variation in structure and complexity, even within a single venomous clade. Within the reptile clade Toxicofera, for example, venom was a single ancient innovation; iguanian and anguimorph lizards share a common venomous ancestor with snakes (56). In iguanian species, venom glands develop only to an incipient stage and seem to have little ecological relevance. However, anguimorph lizards (e.g., monitor lizards and Gila monsters) have a derived, encapsulated, and compartmentalized mandibular venom gland with developmentally suppressed maxillary glands (56). In contrast, snakes possess highly developed but non-compartmentalized maxillary venom glands with developmentally suppressed mandibular glands. Spread across the advanced snakes is an enormous variation of fang and venom gland morphologies (55) that arose from the ancient serpent dental uncoupling (156). Further, within the advanced snakes, divergence has resulted in four derived lineages [twice within the Lamprophiidae family (*Atractaspis* and *Homoroselaps* genera), and once each at the base of the Elapidae and Viperidae families] that possess intricate hollow-front, fanged, and high-pressure delivery systems (55).

Venoms across the Kingdom Animalia are complex mixtures that include variable combinations of proteins (ranging from multiunit globular enzymes to small peptides), salts, and organic molecules such as polyamines, amino acids, and neurotransmitters (49, 54, 72, 117, 144). Proteins found in venoms are the result of toxin recruitment events in which an ordinary protein gene, typically one involved in a key regulatory process, is duplicated, and the new gene is selectively expressed in the venom gland. In many cases, such toxin genes were amplified to obtain multigene families with extensive neofunctionalization (54, 81), followed by the deletion of some copies and degradation of others to nonfunctional copies or pseudogenes (59). The newly created toxin multigene families often preserve the molecular scaffold (including the tertiary structure) of the ancestral protein, but key functional residues outside the core scaffold are modified to acquire a myriad of newly derived activities (38, 53, 54, 59, 123).

Despite the extraordinary diversity in the structure and function of animal venom systems, several protein groups have been convergently recruited for use as venom toxins in multiple animal lineages (**Table 1**). The convergent origin of toxins across the entire metazoan spectrum suggests that there are functional and/or structural constraints on the evolution of animal venoms. Intriguingly, some of the proteins recruited as venom toxins are also recruited by hematophagous insects for utilization in their feeding secretions; with a wide range of convergent activities within the neurological and hematological systems. The aim of this review is thus twofold: (*a*) to consider the structural and functional constraints on recruitment and evolution of venom proteins and (*b*) to explore the implications of these findings for the definition of what constitutes a venomous animal.

CONVERGENTLY RECRUITED PROTEIN FAMILIES

AVIT/Colipase/Prokineticin

First isolated from black mamba venom (mamba intestinal toxin 1, MIT-1) (21, 142), AVIT peptides have subsequently been

Table 1 Convergently recruited proteins*

	AVIT	CAP	Chi	Cys	Def	Hya	Kun	Lec	Lip	Nat	PS1	PLA_2	Sm-D	SPRY
Amphibian	X													
Cephalopod		X	X			X					X	X		
Cnidarian							X					X		
Cone snail		X					X							
Fish						X		X						X
Insect (bristle)				X				X	X		X	X		
Insect (proboscis)		X		X		X	X	X	X		X	X		
Insect (stinger)		X	X			X	X				X	X		
Platypus					X					X				
Scorpion		X			X	X	X					X		
Shrew											X			
Spider	X	X				X	X(2)						X	
Reptile	X	X		X(2)	X	X	X	X	X	X	X	X(3)		X
Tick	X	X		X			X		X		X	X	X	

*Abbreviations used: CAP, CRISP (cysteine rich secretory proteins), antigen 5 (Ag5) and pathogenesis-related (PR-1) proteins; Chi, chitinase; Cys, cystatin; Def, defensin; Hya, hyaluronidase; Kun, kunitz; lec, lectin; Lip, lipocalin; Nat, natriuretic peptide; PS1, peptidase S1; PLA_2, phospholipase A_2; Sm-D, sphingomyelinase.

identified in the venom of monitor lizards as part of the Toxicofera reptile core chemical arsenal (56). Similar bioactive peptides are found in the defensive skin secretions of *Bombina* firebellied toads (Bm8, Bo8, and Bv8) (30, 31, 102). AVIT scaffold peptides found in reptile venom and *Bombina* skin secretion defensive toxins are potent agonists of mammalian prokineticin receptors, and their binding mimics the effect of an endogenous prokineticin overdose. This effect is reinforced by their highly elevated efficiency in binding mammal receptors; both Bv8 and MIT-1 bind to mammal PKR1 and PKR2 (prokineticin receptors 1 and 2) with an affinity that exceeds that of the normal mammalian peptide PK2 (prokineticin 2) by one order of magnitude and that of PK1 (prokineticin 1) by two orders of magnitude (108). Their toxicity is mainly determined by their two potent short-term physiological effects (within minutes), namely gastric smooth muscle contraction and hyperalgesia (increased pain sensitivity). In a longer time frame, Bv8 additionally shows an anorexogenic effect (108), inhibiting the feeding of the affected predator, even in food-deprived situations. Venom of both mygalomorph and araneomorph spider taxa contain peptides (MIT-like atracotoxins, ACTX) that show a similar motif as vertebrate AVIT peptides consisting of 10 conserved cysteine residues (148, 162). However, these peptides test negative in assays of PK1/PK2 activity, lack the N-terminal AVIT sequence, and are much more diverse in amino acid composition, sequence length, and scaffold shape. Consistent with the likelihood that they fulfill distinct functions, comparative three-dimensional modeling of AVIT and MIT-like ACTX peptides has shown dissimilar tertiary structures and markedly different surface chemistries. These results are perhaps not surprising because insects, which constitute the majority of spider prey, appear to lack the PK/PKR system. So far, insecticidal assays have not revealed a lethal effect, even at high doses (148). Nevertheless, the diversity of MIT-like ACTX peptides within individual species, their presence in a broad phylogenetic range of spiders (suggesting their ancient recruitment and long-term existence in spider venom), and their observed resistance to proteolytic breakdown clearly point to an adaptive role in spider venom. Recently, peptides similar to the spider venom peptides were also found in the hematophagous secretion

glands of the tick *Ixodes scapularis*, where they potentially play a role in its feeding (128).

CAP: CRISP (cysteine-rich secretory proteins), antigen 5 (Ag5), and pathogenesis-related (PR-1)

CAP [Cysteine-Rich Secretory Proteins (CRISP), Antigen 5 (Ag5), and Pathogenesis-Related (PR-1)] Proteins

Snake venom CRISP scaffold toxins with the C-terminal cysteine-rich domain (CRD) motifs inhibit a number of ion channels, including cyclic nucleotide–gated channels, ryanodine receptor channels, and Ca^{2+} and K^{+} channels (23, 24, 105, 112, 113, 158, 167, 168). Other forms of CAP scaffolds are utilized in the venoms of cephalopods, cone snails, stinging insects, scorpions, and spiders (**Figure 1**). Tex31, the first of the cone snail proteins to be isolated from the venom duct, possesses proteolytic activity, which has been attributed to the PR-1 domain (100). The cone snail proteins have a C-terminal cysteine-rich domain that is divergent from that found in the venom CRISP proteins, reflecting a different body protein being used in the toxin recruitment event. CAP domain proteins are the dominant allergy-inducing toxins in hymenopteran venoms (47).

Related proteins have been convergently recruited in the feeding secretion of animals from hematophagous taxa in three distinct insect orders: Diptera [mosquitoes (12), sandflies (75), biting midges (26), tsetse flies (86), and muscid flies (4)], Hemiptera [triatomine bugs (129)], and Siphanoptera [fleas (9)]. Within some of these taxa, multiple isoforms are present, contributing to a complex feeding-secretion protein mixture. For example, the transcriptome of *Aedes aegypti* hematophagous secretion glands includes three distinct CAP members (130), and four are found in *Anopheles gambiae* (12). The

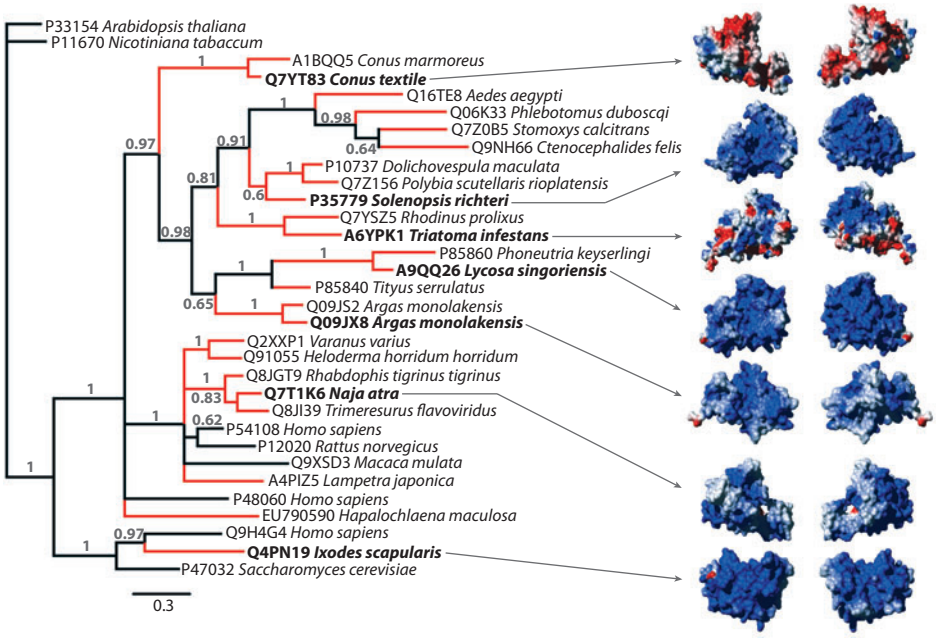

Figure 1

Bayesian phylogenetic reconstruction and molecular modeling of representative cysteine-rich secretory proteins (CRISP), antigen 5 (Ag5), and pathogenesis-related (PR-1) (CAP) proteins. Toxic mutant clades are shown in red. In the molecular modeling of representative proteins, blue surface areas indicate positive charges, red indicates negative charges, and model pairs show two sides of the protein rotated by 180°. Methods as per Reference 56.

proteins found in the hematophagus secretions of soft (*Argas*) versus hard (*Ixodes*) ticks represent separate recruitment events (**Figure 1**). The activities of CAP proteins in the insect and tick hematophagous feeding secretions remain to be elucidated. CRISP proteins have also been identified in the buccal glands of the lamprey *Lethenteron japonicum*, a hematophagous parasite of fish. Similar to the reptile toxins, the lamprey buccal secretion CRISPs block L-type voltage-gated Ca^{2+} channels (i.e., Ca_V1), acting as a vasodilator to facilitate the parasite's hematophagy (73).

All arthropod CAP proteins, whether expressed in venom or hematophagous-feeding secretions, differ from those of most other metazoan lineages in lacking a CRD domain. This feature is shared by a CAP protein secreted by the infective nematode *Ancylostoma caninum* during the larval transition to its parasitic stage (67). These worms may lack the CRD domain by common ancestry with arthropods. Both Nematoda and Arthropoda belong to the clade Ecdysozoa, so the loss of the CRD domain may have happened in an early ecdysozoan ancestor rather than in only an arthropod ancestor. According to the conventional structural definition, these Ecdysozoa sequences are not CAP proteins. However, phylogenetic analysis shows they are clearly nested within this protein family (**Figure 1**). The most common CAP/SCP topology does not contain the CRD domain; actually, the CAP-CRISP combination appears to be restricted to the metazoan lineage. Cnidarians are the only clade where a monodomain CRISP (without N-terminal CAP) has been found; therefore, the CAP-CRISP combination appears to be a metazoan innovation. Thus, the biodiversity of CAP proteins is greater than the artificially constrained definition of what constitutes such a protein type, which has focused on a scaffold derivation rather than the molecular ancestral condition.

Chitinase

Chitinase is present in the venom of the braconid wasps *Chelonus* spp. (84), which endoparasitize the lepidopteran *Trichoplusia ni*, simultaneously ovipositing eggs and injecting venom into the host's eggs. The biological role of this venom chitinase is unclear because chitin appears to be largely absent from both the oviposited wasp eggs and the egg chorion of the host lepidopteran (84). The wasp-venom chitinase has significantly diverged from nonvenom arthropod chitinases (≤45% identity at the amino acid sequence level), and thus it may have acquired new substrate specificity. Chitinase from the posterior salivary gland of the Southern Sand Octopus *Octopus kaurna* (GenBank accession number EU790591) is quite similar in form to the enzyme present in wasp venom. A highly divergent chitinase was also recently discovered in the hematophagous-feeding secretions of soft tick *Argas monolakensis* (91). Birds are the primary host for this tick, and hence the biological role of the hematophagous secretion chitinase is also unknown.

Cystatin

Cystatin-scaffold toxins are found in reptile venoms (56) and also that of the *Lonomia* caterpillar (155), with differing conservation of the characterized functional residues (45). The forms in the tick and mosquito hematophagous secretions also contain mutations in the protease-inhibiting reactive site (63, 128, 130, 173).

Defensins

Defensins are among the most widely distributed innate immunity–related antimicrobial peptides (AMPs) (164, 171). Two structural classes are recognized: the phylogenetically related α-, β-, and θ-defensins that are exclusive to vertebrates (99) and the cysteine-stabilized α/β (CSαβ) defensins that are found in plants, fungi, nematodes, and mussels as well as in the arthropod classes Insecta and Arachnida (164, 174).

The β-defensin-scaffold toxins characterized from *Crotalus* snake venoms are neurotoxins that modify voltage-gated Na^+ (Na_V)

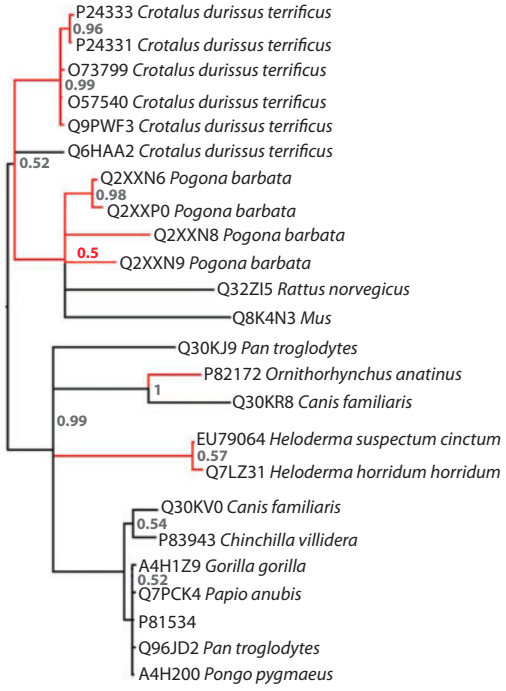

Figure 2

Bayesian phylogenetic reconstruction of representative defensin proteins. Toxic mutant clades are shown in red. Methods as per Reference 56.

channels, resulting in a potent analgesic effect and significant myotoxicity (151). Because of poor phylogenetic signal, it is unclear whether the form from *Pogona* lizard incipient venom glands represents a separate recruitment event (**Figure 2**). However, it is quite clear that a unique form in helodermatid lizard venoms, the lethal toxin 1 (LT1) multigene family, represents a separate recruitment event. This unique toxin type is constructed by four tandemly repeated β-defensin motifs, resulting in a single protein with a novel multidomain topology (Fry et al., unpublished results; Genbank accession number EU790964). This shows that high toxin diversity in venoms is attained not only by the expression of multiple paralogs, but also by domain variation in a multidomain single-product gene formed by tandem repeats within an ancestral monodomain gene. The defensin-like peptides isolated from platypus venom are related to the β-defensin class found only in vertebrates but their bioactivities remain to be elucidated (152).

Remarkably, most scorpion venom neurotoxins adopt the CSαβ motif, representing an explosive venom-specific gene expansion (124). Although the wide phylogenetic spread of CSαβ defensins has led some authors to propose a common and very ancient origin of these peptides (53, 175), the high sequence variation prevents reliable phylogenetic reconstructions (39). Nonetheless, bona fide CSαβ defensin peptides (i.e., those with immune-related function) have been identified in the hematophagous secretion glands of ticks and insects (e.g., 9, 14, 150, 172). Proteins that show remarkable similarity to tick defensins (more than 80% similarity) have been found in scorpion hemolymph (46), supporting a paralogous relationship between CSαβ defensins and scorpion neurotoxins (37). Indeed, gene-structure conservation and phylogenetic analyses support a paralogous relationship between scorpion defensins and three families of voltage-gated K^+ (K_V) channel blockers from scorpion venom, likely derived from two independent recruitment events (43).

In the case of mosquitoes, the hematophagous secretion defensins [e.g., defensin A1 (130)] are identical to those produced endogenously as a component of the microbial infection response. A significant volume of hematophagous secretion is reingested with either blood or sugar meals (some hematophagous flies also feed on nectars as supplementary sugar sources), and it has been assumed that these defensins (and other antimicrobial peptides/proteins including cercropins and lysozyme) inhibit bacterial growth in the insect crop or gut. Although this does not preclude a functional toxic role in the vertebrate host, it is suggestive that no hematophagous secretion-specific isoforms (such as may have been produced by selection to fit targets in the vertebrate) have been discovered yet.

Hyaluronidase

Hyaluronidase is found in the venom of toxicoferan reptiles [with isoforms sequences generated in the case of snakes (55, 65) and helodermatid lizards (Fry et al., unpublished results; Genbank accession number EU790961)], stonefish (110), and hymenopterans (61, 77) (**Figure 3**). In addition to venoms for which protein sequences have been obtained, hyaluronidase activity has been reported in octopus (147), spider (107), and scorpion venoms (15), but the corresponding enzymes have not yet been sequenced. Hyaluronidase has also been identified in the hematophagous secretion of dipteran insects (28, 129). Hyaluronidase enzymes across animal taxa show lower levels of sequence diversity than other toxins, and no new activities have been reported for either venom or body forms. Those recruited most likely act primarily as diffusion factors, enhancing tissue permeability to allow a more efficient spreading of toxins or hemostatic factors (153).

Kunitz-Type Peptides

Kunitz-scaffold toxins have been found in the venoms of snakes (55, 66), sea anemones (10, 17, 69, 101), the solitary wasp *Anopilus samariensis* (68), the scorpion *Hadrurus gertschi* (139), the polychaete worm *Sabellastarte magnifica* (UniProt accession number P84875), the snail *Conus stratius* (16), and the gorgonian coral *Melithaea caledonica* (UniProt accession number P82968). This peptide has also been recruited twice into spider venoms; the scaffold-type in the araneamorph spider *Araneus ventricosus* is phylogenetically distinct from that characterized from the mygalomorph *Ornithoctonus huwena* (**Figure 4**). There is considerable derivation of activity. For example, some snake

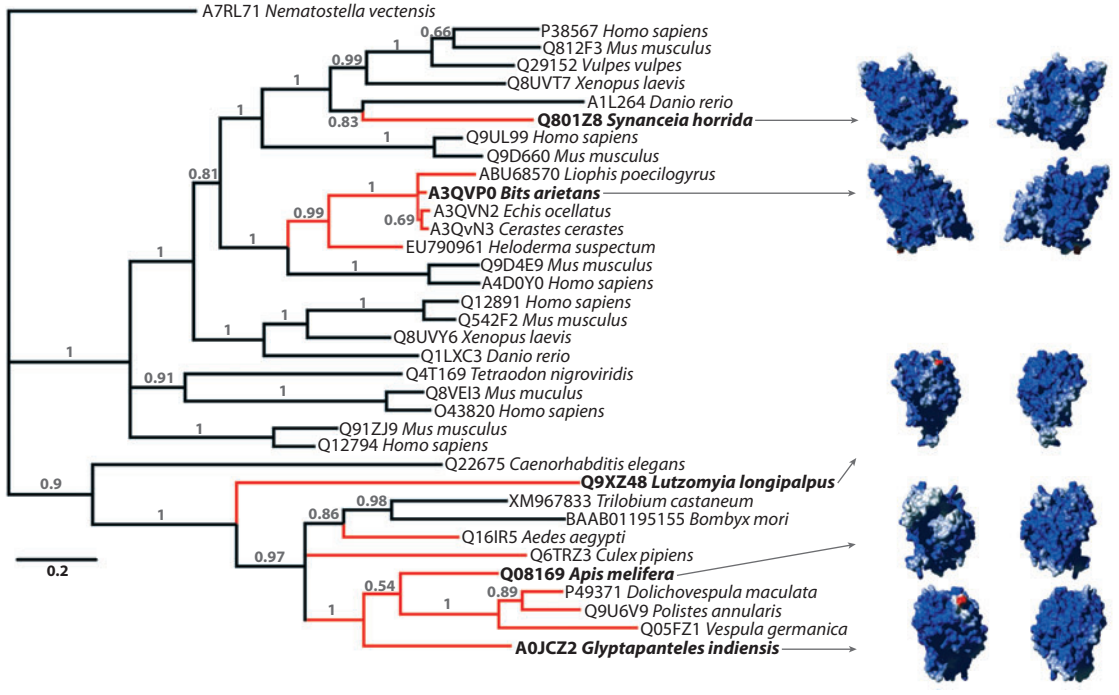

Figure 3

Bayesian phylogenetic reconstruction and molecular modeling of representative hyaluronidase enzymes. Toxic mutant clades are shown in red. In the molecular modeling of representative proteins, blue surface areas indicate positive charges, red indicates negative charges, and model pairs show two sides of the protein rotated by 180°. Methods as per Reference 56.

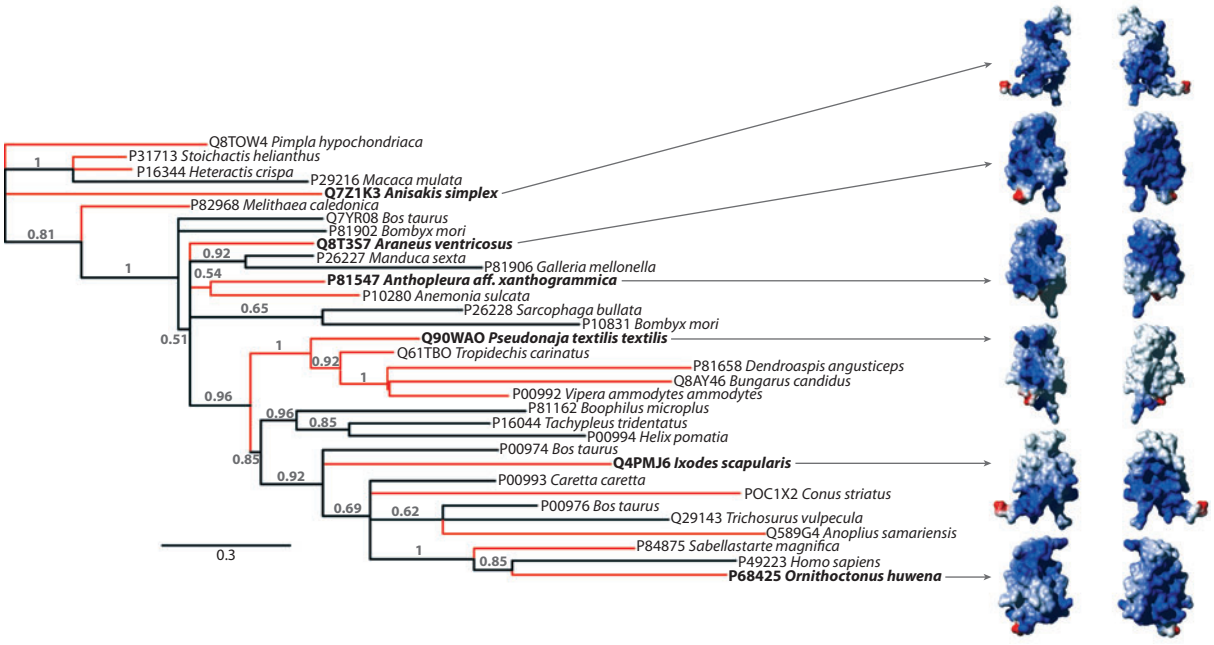

Figure 4

Bayesian phylogenetic and molecular modeling reconstruction of representative Kunitz peptides. In the molecular modeling of representative proteins, blue surface areas indicate positive charges, red indicates negative charges, and model pairs show two sides of the protein rotated by 180°. Toxic mutant clades are shown in red. Methods as per Reference 56.

venom forms inhibit the blood coagulation enzymes plasmin and thrombin (48), whereas others have neurotoxic activity targeting L-type Ca^{2+} channels (141) and K_V channels (66).

Kunitz peptides are also a major constituent of ticks and insect hematophagous secretions, inhibiting the function of blood factor Xa (26, 161). A number of other double–Kunitz domain proteins have also been identified in the hematophagy gland transcriptome of a nematoceran fly, *Culicoides sonorensis* (25). Although the functions of these proteins have not been experimentally demonstrated, three of them (named TFPI1-TFPI3) are presumed to be tissue factor pathway inhibitors on the basis of the pronounced antifactor-Xa activity of hematophagous secretion of this species and their apparent orthology (32% identity and 38% similarity at the amino acid sequence level) with the known antifactor-Xa proteins of tick feeding secretion (52).

Lectin

Lectin-scaffold toxins in snake venoms act via a number of blood coagulation pathways (104) and are also potent anticoagulant toxins in *Lonomia* caterpillar venom (155). In contrast, the lectins in stonefish venom have myotoxic effects (89). C-type lectin proteins have also been found in the feeding secretion of blood-sucking insects, such as *Lutzomyia* sandflies (154) and *Aedes* and *Culex* mosquitoes (130, 131), and may also have an antihemostatic function (11, 130).

Lipocalin

Lonomia obliqua venom contains a number of lipocalin-scaffold variants (134, 155), and one of these, named Lopap, has a unique serine protease–like activity that activates prothrombin (127). There are currently no reports of other lipocalin-scaffold derivations with protease activity.

Several groups of hematophagous arthropods have independently recruited this protein family to serve a variety of antihemostatic functions. Arthropod lipocalins display pronounced molecular biodiversity, such as in the Reduviidae family of predatory insects, which includes the triatomids or kissing bugs (7). For example, 341 scaffold isoforms were recovered from a hematophagous secretion gland cDNA library of *Triatoma brasiliensis* (136). In *Rhodnius prolixus*, the nitrophorins, lipocalin-scaffolds with an unique heme-binding domain, function to store nitric oxide and deliver it to the skin during feeding (5, 27). These scaffold variants also sequester histamine, preventing the host response to histamine released from mast cells and platelets (133), and inhibit coagulation by interfering with factor IXa (64, 132) Another lipocalin (nitrophorin 7, without a heme group) inhibits coagulation by binding to anionic membrane phospholipids, interfering with assembly of procoagulant complexes (8). Yet other lipocalins disrupt hemostasis by sequestering ADP, thus preventing platelet activation and aggregation (50, 51), and by sequestering amines such as serotonin, thereby preventing vasoconstriction and possibly coagulation (6). Species of the bug genus *Triatoma* lack nitrophorins, but diverse lipocalins nevertheless fulfill a variety of antihemostatic roles. Characterized *Triatoma* lipocalins include pallidipin (115), triplatin (103) (which has anticollagen activity), and the antithrombin molecule triabin (114).

Bioactive lipocalin-scaffold variants also independently account for several of the pharmacological activities in the bloodmeal feeding secretion of ticks (93). Moubatin from *Ornithodoros moubata* inhibits collagen-mediated platelet aggregation (160) through a mechanism that includes binding thromboxane A2 and leukotriene B(4) (90, 96). A second lipocalin from the same tick has 46% amino acid sequence identity with moubatin, but it specifically inhibits the complement pathway by antagonizing activation of complement component C5 and antagonizing C5 activation (97, 116). Two related lipocalins, TGSP4 (from *O. savignyi*) and AM-33 (from *Argas monolakensis*), bind cysteinyl leukotrienes with high affinity (95). Cysteinyl leukotrienes, produced by neutrophils, macrophages, basophils, and mast cells in response to injury, increase endothelial permeability leading to edema; this effect would be expected to lead to occlusion of blood vessels and accumulation of a nutritionally deficient serous-rich and erythrocyte-poor fluid at the tick feeding site. Sequestration of these molecules helps ensure a nutritionally adequate meal, rich in erythrocyes (95). FS-HBP (female-specific histamine-binding protein) is a lipocalin expressed specifically by female hard ticks (*Rhipicephalus*) that binds histamine in a manner completely distinct from the *Rhodnius* nitrophorins: Rather than binding through a heme moiety, this protein has two histamine-binding pockets, one low affinity and one high affinity, in the interior of the β-barrel structure (118, 119, 135). Additionally, the lipocalins monomine and monotonin from *O. monolakensis* inhibit vasoconstriction by binding serotonin (5-hydroxytryptamine) as well as histamine (97). These molecules have a single amine-binding pocket and appear to have acquired their amine-binding properties via a separate evolutionary event distinct from the evolution of FS-HBP (97). Analysis of tick hematophagous-feeding secretion gland cDNA libraries has revealed the presence of several other lipocalins of unknown function (128). Lipocalins are also found in the feeding secretion of bloodmeal dipterans, such as the mosquito *Aedes aegypti*, and these forms presumably have anticoagulant activity (130).

Natriuretic Peptides

Natriuretic peptides have been recruited into platypus (41) and reptile (56, 57) venoms and are potent hypotensive toxins. Upstream of the natriuretic peptide-encoding region, the snakes have further evolved either BPP (bradykinin-potentiating peptides) (143) or metalloprotease-inhibiting peptides (157) that are posttranslationally liberated. The helodermatid lizards have independently evolved

PS1: peptidase S1

PLA_2: phospholipase A_2

tandemly repeated proline-rich hypotensive peptides (the helokinestatin peptides) in the same upstream region of the natriuretic peptide gene (Fry et al., unpublished results; Genbank accession number EU790965), and the additional peptides are similarly posttranslationally liberated.

Peptidase S1

The kallikrein-scaffold forms of peptidase S1 (PS1) toxins found in reptile venoms exert a broad variety of activities, ranging from liberation of kinins from circulating kininogen (ancestral toxic activity) to the cleavage of fibrinogen by a basally derived form (**Figure 5**). As in the case of the ancestral reptile venom forms, the kallikrein toxins found in *Blarina* shrews also liberate kinins (79, 80). However, the PS1 toxins found in *Lonomia* caterpillar venoms possess fibrinolytic activity (3) (**Figure 5**), thereby constituting a case of convergent derivation with the reptilian peptidases. The venom of the parasitic wasp *Cotesia rubecula* contains a PS1-scaffold-derived protein (Vn50), which, similarly to the insect PS1 homologs from which

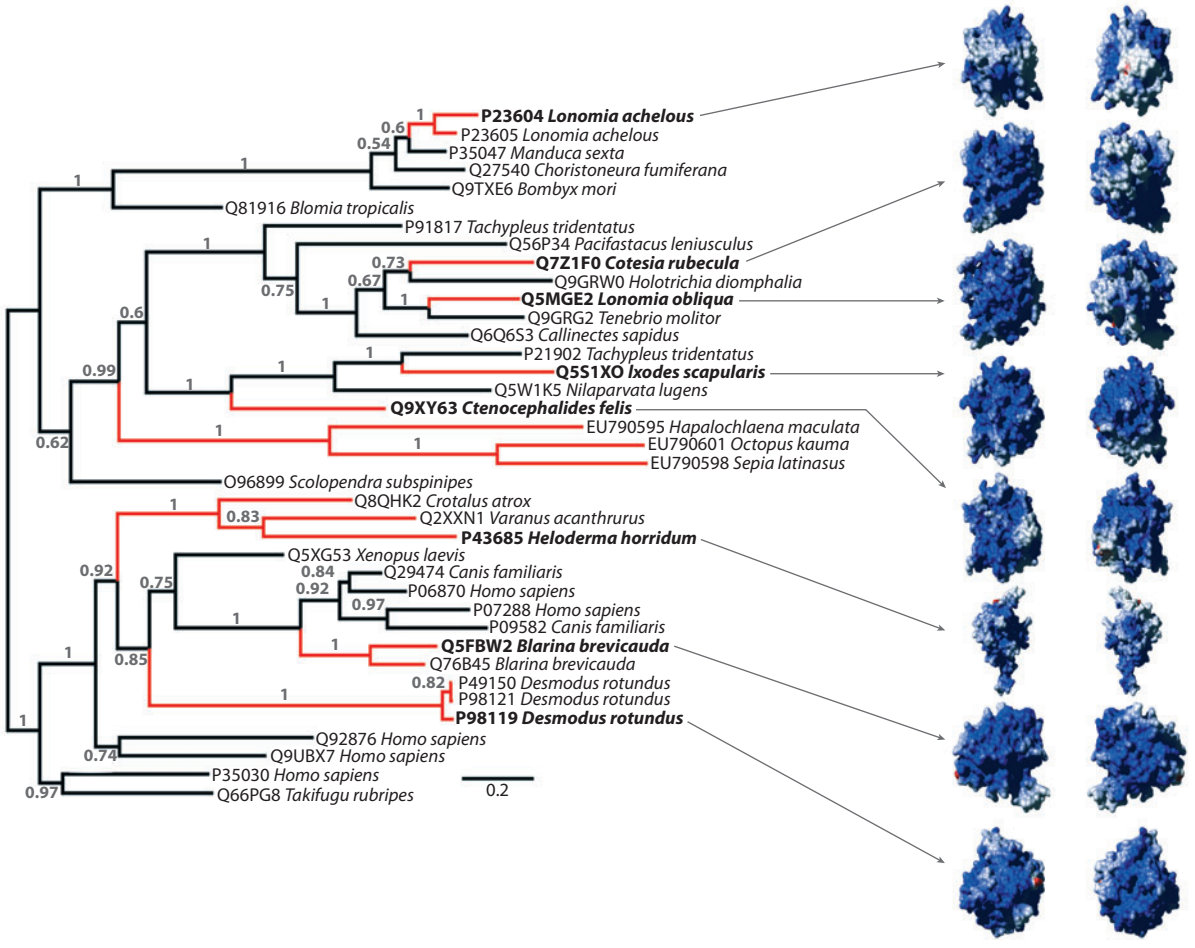

Figure 5

Bayesian phylogenetic reconstruction and molecular modeling of representative peptidase S1 enzymes. Toxic mutant clades are shown in red. In the molecular modeling of representative proteins, blue surface areas indicate positive charges, red indicates negative charges, and model pairs show two sides of the protein rotated by 180°. Methods as per Reference 56.

it evolved, lacks a functional catalytic triad (His-Asp-Ser) because the serine residue is replaced by a glycine (13). The insect PS1 homologs from which Vn50 is derived are involved in a proteolytic cascade that causes hemolymph melanization, which is a primary defense reaction upon infection or invasion by parasites. Vn50 instead inhibits hemolymph melanization, and given its strong structural similarity, it is postulated to be a competitive inhibitor of endogenous peptidase S1 homologs in the host.

PS1 scaffolds in cephalopod posterior salivary glands exhibit a molecular diversity comparable to the diversity observed in the PS1 scaffolds of venomous reptiles (Genbank accession number EU790592–EU790607). Their variation in intraloop (functional) residues appears to constitute an adaptive neofunctionalization pattern that seems analogous to those patterns observed in other multigene toxin families. However, knowledge of the activity of PS1 from cephalopods awaits detailed functional analyses.

The PS1 from the blood-feeding secretion of the vampire bat (*Desmodus rotundus*) is a powerful plasminogen activator (82, 138). PS1s are also widely distributed in the feeding secretions of hematophagous arthropods, including hard ticks (166), nematoceran flies [including mosquitoes (130) and biting midges (26)], and triatomine bugs (136); each of these represents a separate evolutionary recruitment event (**Figure 5**). Although the specific function of any of these proteins is not known, the presence of CUB domains (named for a class of compounds including complement subcomponents Clr/Cls, Uegf, and bone morphogenic protein-1) suggests substrate specificity (130). These serine proteases are possibly targeted to specific host proteins at the bite site, or they may be involved in activating other proteins following secretion into the host.

Phospholipase A_2

Phospholipase A_2 (PLA_2) enzymes have been convergently recruited into cephalopods, cnidarians, multiple insect orders (into the toxic arsenals associated with bristles, proboscises, and stingers), arachnids (scorpions, spiders, and ticks), and reptiles (three occasions: twice into the advanced snake venoms and once into anguimorph lizard venom). So far, Group-IA, G-IIA, G-IIB, G-III, G-IX, and G-XII PLA_2 scaffolds have been recruited into venoms (137). The molecular origin and evolutionary relationships have been investigated for G-I, G-II, and G-III PLA_2s (2, 35, 54, 58, 149). Group III PLA_2s are particularly unique in having been recruited independently into four venomous lineages. Types from sea anemone venoms do not associate phylogenetically with any of the currently defined PLA_2 types (109). Derived toxic functions in reptile venoms include antiplatelet, myotoxic, and neurotoxic activities (54, 78). The neurotoxicity of venom PLA_2s depends on their ability to bind to specific target proteins that act as receptors/acceptors in specific organs and tissues (126). After binding to the target, the toxic PLA_2 may induce its effects by mechanisms that are either dependent on or independent of its catalytic activity (78).

Sphingomyelinase D

Sphingomyelinase D (SMaseD) is a magnesium-dependent subclass of toxic enzymes until recently known only from the pathogenic bacterial genus *Corynobacterium* and from the uniquely dermonecrotic venoms in two closely related spider genera (18, 19). Smase D enzymes in both lineages contain similar C-terminal plug motifs and, in the absence of phylogenetic analyses, the disjunct presence of this motif in both lineages was put forward as evidence for a lateral gene transfer event (32). However, SmaseD homologs have recently been identified in ticks (UniProt accession number Q202J4), and BLAST searches reveal the presence of homologous enzymes in other bacteria (*Arcanobacteria* UniProt accession number Q59121) and some fungi (*Aspergillus* and *Coccidioides* UniProt accession numbers Q2UAL9, Q2UKE8, Q2U8X2, and Q1DU31)

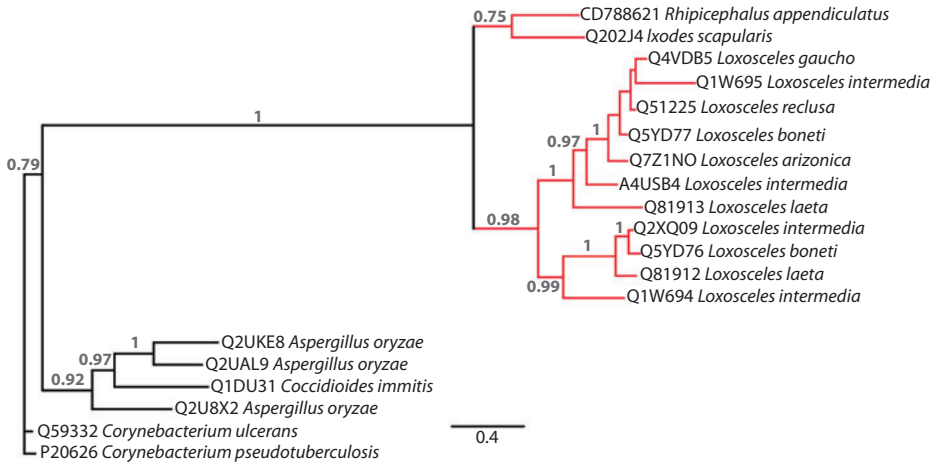

Figure 6

Bayesian phylogenetic reconstruction of representative sphingomyelinase enzymes. Toxic mutant clades are shown in red. Methods as per Reference 56.

as well (**Figure 6**). Phylogenetic analyses show that the spider and tick sequences are closely related and together form a clade that is very distinct from the bacterial and fungal enzymes, which in turn are more similar to each other. A close relationship between tick and spider enzymes does not fit with the originally postulated lateral gene transfer scenario between bacteria and spiders and would push such an event back in time to at least the last common ancestor of both arthropod lineages. If the bacterial forms were the result of lateral gene transfer, then the sequences would be nested within the spider lineages. However, strong structural dissimilarity between the arachnid SmaseD and the bacterial and fungal enzymes (evidenced by large pairwise distances and the long intervening branch in the phylogram of **Figure 6**) is consistent with a far more ancient divergence between both groups. In addition, a single lateral gene transfer event is not enough to explain the presence of homologs in fungi as well. As an alternative to the lateral gene transfer hypothesis, we postulate that the similarity between bacterial and spider SmaseD arose by convergent evolution in distinct lines of GDPD (glycerophosphodiester phosphodiesterase) enzymes. This scenario gains support from the fact that both the fungal and the tick enzymes contain C-terminal plug motifs that differ from the identical motifs in the bacterial and spider toxins. In SmaseD of both *Corynebacterium* (bacteria) and *Loxosceles* (spider), Asn residues at position 278 contribute a backbone hydrogen bond, causing subsequent residues to form a type-I β-turn, which blocks the N-terminal end of the TIM (triosephosphate isomerase) barrel (32). In the enzymes of the tick, fungi, and non-*Corynebacterium* bacteria, various other residues occupy position 278 (Asp in the tick; Ala, Lys, Ser, or Thr in fungi; and Ile in *Arcanobacterium*), potentially affecting the plugging of the barrel.

SPRY/Concavalin A–Like Lectins

SPRY toxins have been sequenced in the venoms of toxicoferan reptiles and stonefish. In reptile venoms, they are an ancestral type shared between lizard and snake venoms (56). The bioactivity of the lizard form remains to be elucidated, but the isoform characterized from *Ophiophagus hannah* snake venom produces hypolocomotion and hyperalgesia, perhaps by acting directly on the central nervous system (125). SPRY toxins in stonefish venoms produce lethal hemolysis through pore formation in cell membranes

while inducing potent endothelium-dependent hypotension and also irreversibly interfering with neuromuscular function (29, 88, 146). In addition, these toxins display edema-inducing activity, increase vascular permeability, induce endothelium-dependent vasorelaxation and platelet aggregation, and are myotoxic.

WHAT DETERMINES THE SUITABILITY OF A BODY PROTEIN FOR TOXIN RECRUITMENT?

In contrast to the tremendous diversity of venomous organisms, the number of different venom protein scaffolds appears to be rather restricted. The high proportion of convergently recruited protein families suggests that there are structural and/or functional constraints as to what makes a protein suitable for recruitment as a toxin. Although protein families that have recurrently been recruited span a broad spectrum of different structures and biochemical activities, we notice that many of them share a number of generalities.

First, all known toxins represent secretory proteins. The precursor peptides of all toxins contain an N-terminal signal peptide that is excised to form a functional protein. To date, there are no documented cases of any toxin precursor lacking an N-terminal signal peptide. Even if a nonsecreted body protein might have a potential toxic side effect, its gene would require the addition of a segment encoding a signal peptide either by interlocus gene conversion (nonreciprocal recombination) and retrotransposition or by exon shuffling of a signal peptide protomodule (122). Conversely, the possibility that alternative secretion mechanisms, independent of signal-peptide processing, have evolved in at least some venomous lineages cannot be eliminated. For example, the AMP buforin I, secreted in the stomach of the toad *Bufo gargarizans*, is a derivative of the nucleus-specific protein histone H2A. After proteolytic cleavage from the H2A precursor, it becomes a functional AMP (76). However, the cDNA clone of the H2A precursor transcript remarkably does not seem to contain an N-terminal signal peptide. Hence, the mechanism that governs the secretion of buforin I is currently unknown. One potential explanation is that buforin I, despite being identical to an N-terminal segment of H2A, is actually encoded by a paralogous gene that obtained a signal peptide after its duplication from the H2A gene, but this remains to be tested. Similar H2A-derived AMPs have been reported from teleost fish (20, 121), indicating that this mechanism may be widespread or evolved convergently in divergent animal lineages. If alterative modes of secretory peptide processing exist for AMPs, they may occasionally have evolved for toxins as well.

Second, protein families from which toxins have been convergently recruited are functionally versatile, but proteins within each family seem to share an underlying biochemistry that has remained remarkably uniform throughout the Kingdom Animalia (and often throughout eukaryotes). Despite evolving very different metabolic, physiological, and immunological processes and having been adapted to the ecological needs of the organisms in which they are expressed, homologs of these proteins often perform the same or very similar fundamental biochemical reactions. For example, the myriad of metabolic functions performed by peptidase S1s are all based on the same underlying biochemical reaction, which is the hydrolysis of specific peptide bonds. Similarly, the biological functions of hyaluronidase enzymes all scale down to the hydrolysis of 1–4 linkages between *N*-acetyl-β-D-glucosamine and D-glucuronate residues in hyaluronate.

The long-term evolutionary conservation of such fundamental biochemical functions and their ubiquitous importance in the Kingdom Animalia provide the necessary functional basis for a body protein in one organism to perform a similar function when introduced into another. Consequently, the bioactivity of most toxins stems from one of the following three generalized mechanisms: (*a*) structural damage caused by catalyzing the hydrolysis of a universally present substrate, (*b*) physiological

imbalance or a short-term response caused by mimicking endogenous body proteins as if they were overexpressed, or (*c*) mimicking endogenous body proteins by acting competitive inhibitors to cause an opposite physiological imbalance or disruption of a physiological response. Hyaluronidase, PLA_2, and SMAse D represent examples of toxins that act according to the (a) mechanism because they catalyze the hydrolysis of universally present substrates. The binding of Bv8 or MIT-1 toxins on mammal prokineticin receptors and the hydrolytic release of kinins from kininogen by peptidase S1 enzymes (kallikrein subtype) are examples of the (b) mechanism. Finally, the inhibition of hemolymph host defense by Vn50, which acts as a competitive inhibitor of the peptidase S1 homologs from which it evolved, represents an example of the (c) mechanism. The effectiveness of the latter two mechanisms may be amplified if the toxin mimics the action of a pleiotropic protein that governs multiple downstream metabolic pathways or if the mimicked protein plays a central role in a complex protein cascade with feedback systems (e.g., thrombin-mimicking proteins affecting the blood coagulation process). Similar multifunctionality can be attained by mimicking multiple factors that share similar structures and biochemical activities. For example, peptidase S1 toxins in reptiles and *Blarina* short-tailed shrews mimic the different activities of the related kallikrein and thrombin, thereby lysing both kininogen (vasodilation) and fibrinogen (anticoagulation). As such, the toxicity of many venom proteins that interfere with hemostasis is enhanced by the fact that they are recruited from the same protein (super)families that constitute multiple components of the involved protein cascades. Such effects for each of these three scenarios described above allow the new venom-gland protein type to provide an immediately useful toxic bioactivity. Whereas the snake three-finger neurotoxins are derivations of α-neuropeptides similar to Lynx1/SLUR (54, 71), other neurotoxin types do not necessarily represent structural or even phylogenetic relatives of the ligands they mimic. However, many neurotoxins act according to the second or third mechanism, either activating or deactivating ion channels or other receptors at neuromuscular junctions or in the central or peripheral nervous system.

Third, most toxins are recruited from body proteins involved in one or several short-term physiological processes. Because venoms are used for predation, competitor deterrence, or defense, they are effective only if they produce a rapid effect, such as fast immobilization of prey, instantaneous inhibition of blood coagulation in a host, or quick malaise or pain induction in a predator or competitor. For example, although the PK/PKR systems govern a large number of long-term physiological processes with potentially negative consequences for the host (e.g., predator anorexogenia caused by frog skin defensive AVIT peptides), the toxicity of AVIT peptides is largely determined by their short-term physiological effects (within minutes), which include powerful contraction of smooth muscle and hyperalgesia. Slow action on long-term physiological processes, lethal as they might be (e.g., accumulation of indigenous waste products or secondary systemic process after abnormal cellular depolarization), are unlikely to contribute much to the adaptive value of toxins because they are irrelevant to the basic ecological purposes of any given venom (although the anorexogenic effects have group level evolutionary benefits). This is not to say that some toxins do not have persistent effects, such as the long-term paralysis of parasitic wasps upon spider prey or, conversely, long-term paralysis by spiders of their prey. In these cases, the prey item is kept alive until the parasite eggs hatch or the spider eats its prey, respectively. Protein families that fulfill only strictly structural functions (such as keratins) or proteins that govern only time-consuming processes (such as cell growth or tissue differentiation factors) are therefore unlikely to be recruited as toxins. For a similar reason, toxin targeting is restricted to body parts that are readily accessible via the bloodstream, which also guarantees an acute effect. Most toxins additionally have an explicit extracellular substrate or site of

activity, even when they also affect intracellular processes.

Fourth, toxins seem to be recruited more frequently from body proteins with stable tertiary structures maintained by a high degree of disulfide cross-linking. The cysteine content of many animal toxins and their nontoxic relatives is remarkably high and evolutionarily conserved. Extensive disulfide cross-linking is a generalized feature of secretory proteins because it enhances molecular stability and protease resistance. In contrast, the correct folding of secreted globular enzymes is generally more sensitive to primary structure. A single mutation, such as the gain or loss of a proline or a crucial charge-state residue, may be enough to decimate the correct globular folding.

Fifth, once recruited as a functionally important component of the venom arsenal, the adaptive evolution of toxins is often reinforced by extensive gene duplication. Further duplication of the toxin gene is likely to be selectively favored because more gene copies could increase the toxin expression level in the gland and hence result in higher toxin doses and faster gland replenishment. The tandem duplication of gene copies provides the template, progressively increasing the chance for unequal crossing-over and gene conversion. This situation leads to the creation of a toxin multigene family in which differential mutations among paralogs allow them to obtain different levels of potency, functions, or complementary prey specificities (59, 81). The newly created toxin multigene families preserve the molecular scaffold of the ancestral protein but modify key surface functional residues to acquire a myriad of newly derived activities (38, 54, 59, 106). In globular enzymatic proteins, mutations are much more likely to interfere with correct posttranslational folding than is the case with extensively cysteine cross-linked proteins. Thus, a negative selection pressure operates against the duplication and diversification of globular proteins, and they are instead overexpressed but with few new gene copies. For example, no new activities have been reported for toxins based upon globular enzymes (e.g., hyaluronidase) as opposed to the extensive neofunctionalization of cysteine cross-linked enzymes (e.g., PLA_2).

One of the most intensively studied toxin structural classes, the disulfide-rich toxins of mass 3–5 kDa, which dominate the venoms of most spiders and venomous marine snails (144), appears to provide an exception to these general rules of toxin recruitment. The vast majority of these toxins contain the inhibitory cysteine knot (ICK) motif (also known as the knottin fold) that directs their three-dimensional fold (62, 120). Structurally similar peptides are also present in other arthropods and plants, where they function as either toxins targeting Ca^{2+} channels (scorpion and assassin bug toxins), antimicrobial agents (horseshoe crab tachystatin and plant thionins), or protease inhibitors (plant cyclotides). This highly compact domain (three disulfides within 20–40 amino acid residues) is quite scarce in vertebrates; the only known representative is the C-terminal domain of Agouti-related proteins, which are involved in feeding behavior and energy homeostasis (163). Based on the similarities in toxin prepropeptide transcript structure, it has been proposed that spider and cone snail ICK toxins are evolutionary related (176); however, their phylogenetic relationships, if any, are difficult to reconstruct with current methods because of their short and highly diverged sequences (36). Remote homology detection programs do not identify any spider toxin as a putative homolog if *Conus* sequences or multiple alignments are used as query, or vice versa. Some authors have proposed that the ICK is an elaboration of a simpler structural motif, the disulfide-directed β-hairpin (DDH), which is found in several vertebrate and invertebrate body proteins (159), and hence these toxins may have evolved from an ancestral DDH-containing body protein that was independently recruited into spider, scorpion, hymenopteran, and cone snail venoms. Nonetheless, convergent evolution of the three-dimensional fold—and its cysteine-pairing signature—cannot be ruled out because of the generalized scarcity of

information about nonvenom ICK-containing proteins in invertebrate genomes.

CONVERGENCE OF ACTION

In addition to convergence of the types of peptide/proteins recruited for use in the chemical arsenals, there has also been convergence of derived activities. Kunitz-type toxins represent a striking example of convergent toxin recruitment and convergent molecular evolution to produce the same derived activity. The derived venom forms in snakes (66), sea anemones (140), and cone snails (16) block K_V channels. Snake dendrotoxins fit the functional dyad motif found in several K^+ channel blockers (33), in which the minimum pharmacophore is defined by a critical basic residue (usually a lysine) located 7 Å from a hydrophobic residue. This functional site, assisted by different constellations of secondary residues for K^+ channel binding, is quite distinct from the protease inhibition site. Hence, the same fold is being used for two distinct functions, but different side chains are involved. Sea anemones contain a range of Kunitz-type protease inhibitors (10, 17, 69, 101), some of which are also dual action polypeptides (140). For example, Kalicludines 1 and -3 (also known as AsKC1-3) not only inhibit trypsin but also block K^+ channels. However, compared with the dendrotoxins, they are weaker inhibitors of $K_V1.2$, and compared with BPTI (bovine peptidic trypsin inhibitor), they are less potent trypsin inhibitors. *Conus striatus* venom contains a Kunitz-type neurotoxin that shares 33% amino acid sequence identity with BPTI and 35% with dendrotoxin I. This toxin, named Conkunitzin-S1, blocks *Shaker* potassium channels with an IC_{50} of approximately 60 nM. The Kunitz-type toxins in mygalomorph spider venoms also have a dual activity, acting as both trypsin inhibitors and K_V channel blockers (170) For example, huwentoxin-XI, a Kunitz-type toxin from the Chinese tarantula *Ornithoctonus huwena*, competitively inhibits trypsin with K_i = 68 nM (87), and it is also a weak blocker of $K_V1.1$ channels; the epitopes responsible for protease inhibition and K_V channel block are spatially distinct and located at opposite ends of the cone-shaped molecule (170).

However, convergence of targeting also exists between derived forms of different toxin types. Notably, toxins from five distinct structural classes and phylogenetically distant organisms (CSαβ short-chain scorpion toxins, Kunitz-type snake toxins, Kuntiz-type and ICK snail toxins, and CRD-related sea anemone toxins) display the same kind of molecular determinants for K^+ channel recognition and blockage (34, 60).

Other neurological sites have also been convergently targeted at the neuromuscular junction (**Figure 7**). Virtually every site of action has been attacked on multiple occasions during evolution such as the neuronal nicotinic acetylcholine receptors convergently targeted by the κ-bungarotoxins (44) and also by conotoxins (85).

Targeting convergence can occur not only between lineages but also within a particular lineage. Two striking examples of targeting convergence within a particular lineage are the spider toxins on the one hand and the scorpion toxins on the other hand, which modulate sodium and potassium channels, respectively. The μ spider toxins are quite distinct from the δ spider toxins (such as the δ-atracotoxins, formerly known as robustoxin and versutoxin). The spider μ toxins, as in the case of the scorpion β toxins (40), cause a hyperpolarizing shift in the voltage-dependent activation of Na^+ channels, causing channel opening at lower potentials; consequently, they do not act as blockers or prolongers but more as channel activators (111). However, δ spider toxins delay Na^+ channel inactivation. Scorpion toxins that target K^+ channels act as current blockers. Most of them bind to the channel outer mouth with a homologous Lys residue plugging the ion-conduction pathway; however, some scorpion toxins do not obey this rule in spite of being bona fide blockers (38). Compelling evidence suggests that such toxins have evolved nonhomologous recognition sites that fulfill the same function (1) but through independent

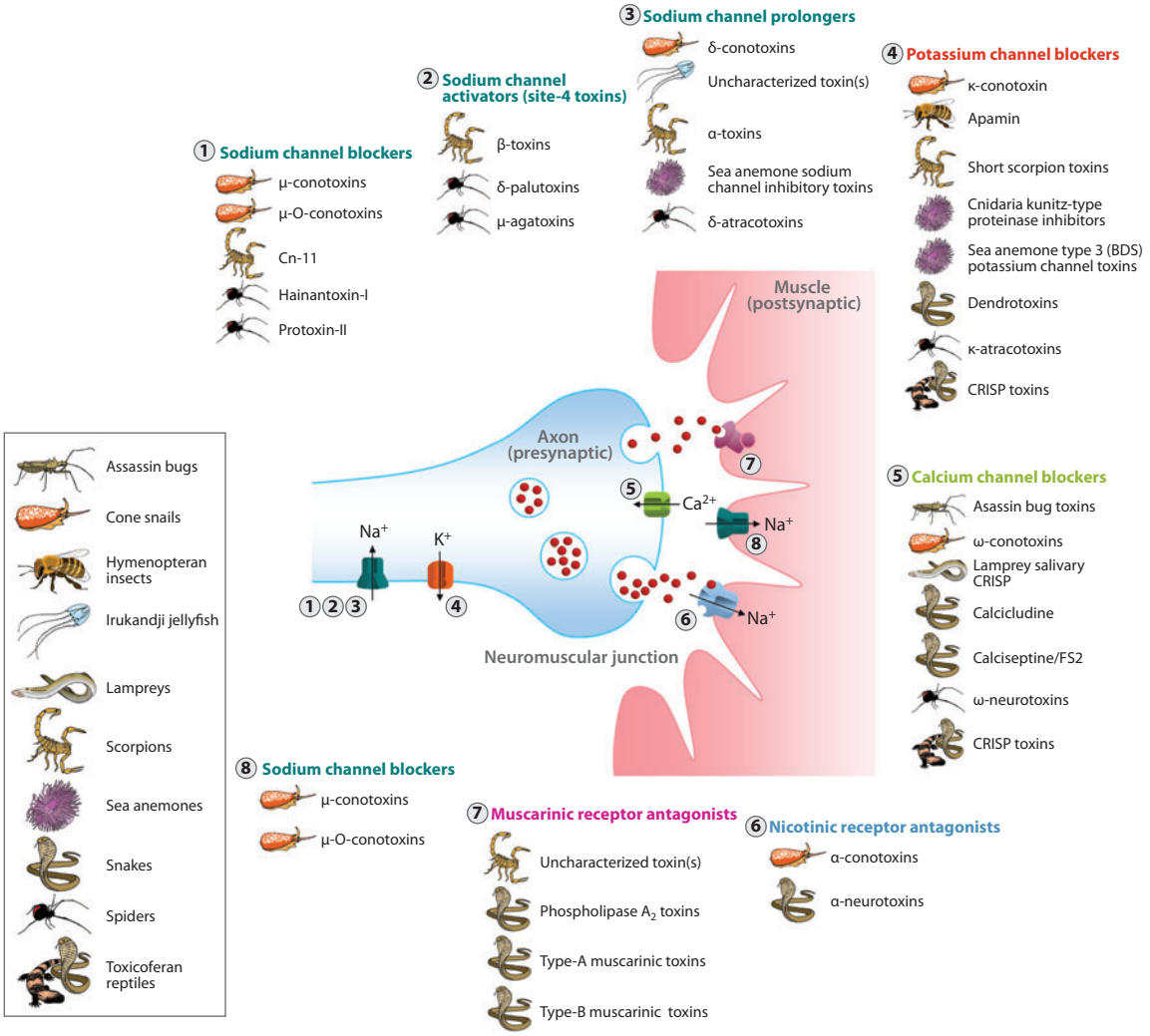

Figure 7

Sites of neurotoxic convergence.

recruitment events from ancestral body forms with immune-related function (43).

Another example of neurotoxic targeting convergence within a lineage is snake toxins targeting the muscarinic receptors. The first of these to be described included three-finger toxins from *Dendroaspis* and *Naja* species (e.g., 22), but PLA_2 toxins with muscarinic actions were discovered later (70). Further, the *Dendroaspis* muscarinic three-finger toxins are not monophyletic (59), and thus represent a fascinating case of convergence within a single clade of the same molecular scaffold being mutated two times for the same derived activity. *Dendroaspis* venoms are also extraordinary in convergently targeting L-type Ca^{2+} channels with derived forms of both Kunitz (169) and three-finger toxins (42).

Finally, sites of the coagulation cascade are also convergently targeted (**Figure 8**). Kunitz-domain proteins in snake venoms, for example, are similar to tick and biting midge hematophagous secretion proteins in mimicking the activity of endogenous TFPI (tissue

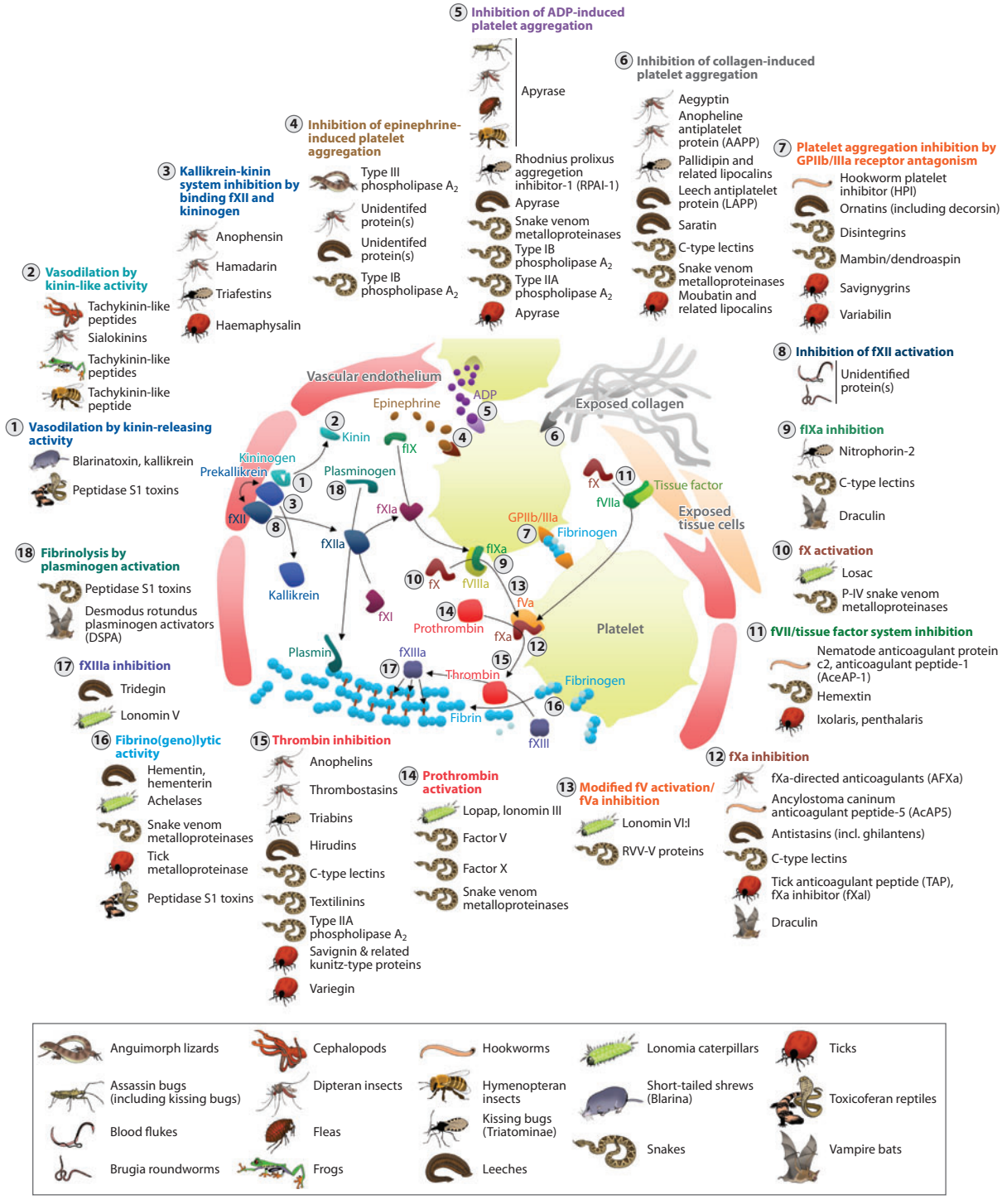

Figure 8

Sites of hematoxic convergence.

factor pathway inhibitor) by efficiently shutting down coagulation at the point of initiation. Using various protein scaffolds as templates, antiplatelet action mediated by the RGD tripeptide motif has also been a convergent target by snakes (74), leeches (83), and arthropods, including ticks (92) and horseflies (165). As with neurotoxicity, targeting convergence can occur within a single venomous clade, such as the RGD-containing distintegrins in viper venoms (74) and the RGD-containing three-finger peptides in mamba venoms (98).

WHAT DEFINES AN ANIMAL AS VENOMOUS OR A PROTEIN AS A TOXIN?

The convergent recruitment of different protein types for use as toxins raises the fundamental question of how to define an animal as venomous. Variables such as relative lethality to a prey item or to a potential predator are arbitrary and obscure evolutionary relationships. We reject the suggestion that rapid death should be the determinant of whether a toxic secretion should be classified as a venom. Apart from the difficulty in defining rapid death, such an overly restrictive and arbitrary definition obscures the evolutionary homology among the toxins, thus contributing little to our understanding of venom origin and evolution. The biological reality is complex, especially if we consider that the same secretion may have vastly different effects on different recipient animals. For example, the secretion of the Australian Paralysis Tick *Ixodes holocyclus* induces lethal paralytic neurotoxicity in humans as well as in a variety of non-native livestock and domestic animals, and thus clearly fits the conventional definition of venom. However, paralysis is not induced when these ticks feed on their natural hosts, including bandicoots, wallabies, and other marsupials (145). Tick hematophagous secretions contain a plethora of molecules that inhibit host hemostasis, immune response, and pain (so as to escape detection). A coevolutionary chemical arms race with native host animals has likely resulted in an especially potent secretion in *I. holocyclus* to overcome resistant native prey. Therefore, non-native hosts are likely to be far more sensitive because they are immunologically naive. In addition, the susceptibility of individual hosts can be affected by prior history of exposure to ticks and seroconversion, so susceptible hosts can become resistant to paralysis (145). The tick, therefore, is confronted with a highly variable landscape of blood-meal hosts, ranging from highly susceptible to highly resistant. Because fitness is maximized when the tick can obtain a sufficient meal for reproduction regardless of the resistance of the host, selection favors the evolution of a potent pharmacopoeia, sufficient to allow feeding on resistant hosts but collaterally toxic to susceptible hosts.

Any venomous animal faces the same issues: Whether the venom is used for feeding or defense, it must work on the most resistant targets so effects will be more extreme on more susceptible animals. With this in mind, we regard venom as a secretion, produced in a specialized gland in one animal, and delivered to a target animal through the infliction of a wound (regardless of how tiny it) a venom must further contain molecules that disrupt normal physiological or biochemical processes so as to facilitate feeding or defense by the producing animal. By extension, toxins should be regarded as particular examples of intergenome active elements by means of their action on the extraorganismal space (Gene Ontology term number 0043245). This definition, based on biological function as opposed to an anthropocentric view of toxicity, recognizes that there is a vast range of effects of envenomation, from the hardly noticeable subversion of hemostatic defenses produced by a mosquito to the lethal effects of venomous snakes. Accordingly, we regard the feeding secretion of hematophagous specialists (e.g., arthropods or leeches) as a specialized subtype of venom.

Our broadened concept of venom is validated by the finding that many of the same classes of proteins are represented in the hematophagous secretions of blood-feeding arthropods and in more classical venoms of

reptiles and other organisms. Specifically, 11 of the 14 protein families discussed above are represented in at least one group of blood feeders, and nine of these families are found in two or more groups. In addition, protein families recruited in both conventional venom and hematophagous secretions of blood-feeding taxa often show the exact same biological activities. For example, a recently identified CRISP protein found in the buccal gland secretion of the sea lamprey (a blood parasite of fish) is not only homologous to CRISP toxins in toxicoferan reptiles, but also shows the same voltage-gated Ca^{2+} channel blocking activity and capacity to inhibit smooth muscle contraction (73). Despite the fact that this muscle contraction probably fulfills different adaptive roles in the two taxa (vasodilation to facilitate blood feeding in lamprey versus low blood pressure and prey immobilization in reptiles), they both meet the same overarching biological function: predation. Similar parallel recruitments in conventional venom systems and hematophagous sialomes are linked to the three major processes of hemostatic responses to vascular injury: vasoconstriction, platelet aggregation, and blood coagulation (**Figure 8**). Striking examples include (*a*) vasodilatory components (tachykinins, kallikreins, and natriuretic peptides in various venomous animals versus sialokinins in mosquitoes), (*b*) inhibitors of ADP-induced platelet aggregation (apyrases in the venom of stinging insects and assassin bugs versus the hematophagous secretions of blood-feeding ticks, dipterans, fleas, and bugs), (*c*) platelet IIb–IIIa glycoprotein antagonists (snakes versus blood-feeding nematodes, ticks, and leeches), (*d*) thrombin inhibitors (viperid snakes versus leeches, ticks, dipterans, and triatomine bugs), (*e*) fibrino(geno)lyic proteases (toxicoferan reptiles and *Lonomia* caterpillars versus leeches), and (*f*) plasminogen activators (snakes versus vampire bats). Failure to recognize these numerous parallelisms between conventional venoms and secretions adapted to blood-feeding would be based on the arbitrary discrimination of their adaptive roles and would disregard their overruling similarities in terms of protein composition, bioactivity, and predatory function.

Acceptance of the broader definition proposed here expands our sample of venomous animals and increases the number of known independent occasions in which venom has evolved. This expansion of our sample size of venoms and venomous proteins will improve our understanding of factors underlying the evolution of venoms and their associated proteins.

SUMMARY POINTS

1. Convergently recruited proteins share several conserved features: a secretory protein ancestor, functionally versatile protein ancestors with a fundamentally conserved basal activity, extensive disulfide cross-links, stable molecular scaffolds, and once recruited, adaptive evolution generates a suite of novel isoforms with neofunctionalization.
2. In addition to convergence of protein types utilized, convergence of toxic targeting has occurred extensively.
3. The convergence between venomous and hematophagous animals allows for an expanded definition of venom, thus recognizing the vast biodiversity of toxicity levels and types.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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