The biology and evolution of spider venoms

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ABSTRACT

Spiders are diverse, predatory arthropods that have inhabited Earth for around 400 million years. They are well known for their complex venom systems that are used to overpower their prey. Spider venoms contain many proteins and peptides with highly specific and potent activities suitable for biomedical or agrochemical applications, but the key role of venoms as an evolutionary innovation is often overlooked, even though this has enabled spiders to emerge as one of the most successful animal lineages. In this review, we discuss these neglected biological aspects of spider venoms. We focus on the morphology of spider venom systems, their major components, biochemical and chemical plasticity, as well as ecological and evolutionary trends. We argue that the effectiveness of spider venoms is due to their unprecedented complexity, with diverse components working synergistically to increase the overall potency. The analysis of spider venoms is difficult to standardize because they are dynamic systems, fine-tuned and modified by factors such as sex, life-history stage and biological role. Finally, we summarize the mechanisms that drive spider venom evolution and highlight the need for genome-based studies to reconstruct the evolutionary history and physiological networks of spider venom compounds with more certainty.

Key words: spider, venom, toxins, biochemistry, evolution, ecology, morphology

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I. INTRODUCTION

Spiders (order Araneae) belong to the ancient class Arachnida, which also includes the orders Scorpiones (scorpions), Solifugidae (camel spiders), Opiliones (harvestmen), Amblypygidae (whip spiders), Pseudoscorpiones (pseudoscorpions), Aracnidae and Parasitiformes (ticks and mites), Ricinulei (hooded tick spiders) and Palpigradi (palpigrades). Together with the horseshoe crabs (Xiphosura) and sea spiders (Pycnogonida), Arachnida forms the higher arthropod subphylum Chelicerata within the phylum Arthropoda (Sharma, 2018). The phylogenetic placement of the arachnids is summarized in Fig. 1. The anatomy of spiders has remained largely unaltered since their appearance during the Carboniferous (Selden & Penney, 2010; Dunlop, Penney & Jekel, 2015).

Extant spiders are divided into three infraorders: Mesothelae, Mygalomorphae, and Araneomorphae. The oldest is Mesothelae, a monotypic infraorder containing a single family (Liphistidae, the segmented trapdoor spiders) comprising ca. 140 species from Asia (World Spider Catalog, 2021). By contrast, the Mygalomorphae are distributed globally, although most of the ca. 3000 described species are found in the tropics and subtropics (World Spider Catalog, 2021). Several prominent spider families belong to this lineage, including the tarantulas (Theraphosidae), trapdoor spiders (Ctenizidae), and funnel-web spiders (Atracidae). Finally, the most diverse spider infraorder is the Araneomorphae (World Spider Catalog, 2021). This includes many fascinating and species-rich groups such as the orb-weaving spiders (Araneidae, 3066 valid species), wolf spiders (Lycosidae, 2431 valid species), and jumping spiders (Salticidae, 6346 valid species) (World Spider Catalog, 2021). Araneomorphs have adapted to many different ecological niches and have undergone a spectacular evolutionary radiation thereby evolving an array of different appearances (World Spider Catalog, 2021). Today's spiders inhabit almost all ecosystems on all continents except Antarctica (Piel, 2018; World Spider Catalog, 2021). In total, the order Araneae comprises 49581 extant cataloged species in 129 families, but up to 90000 species may remain to be discovered (Pennisi, 2017; World Spider Catalog, 2021).

Fig 1. (A) The phylum Arthropoda (arthropods) showing the relationship between the class Arachnida (arachnids) and other classes in the subphylum Chelicerata (chelicerates). The utilization of venom (v) in different taxa is highlighted. (B) Relationships of the spider infraorders and their placement within the subphylum Chelicerata.
Spiders share a conserved body plan with two functionally distinct tagmata (groupings of segments) defined as the prosoma and opisthosoma. The anterior prosoma is used for locomotion, sensing and feeding. It houses the brain, walking legs and the versatile pedipalps (Foelix, 1983; Mahmoudi et al., 2008; Calabacho-Rosa et al., 2013; Cargnelutti et al., 2018). It also carries a pair of chelicerae covering the oral cavity that have, in spiders but not most other chelicerates, evolved into the venom injection apparatus. The posterior opisthosoma is used for reproduction, digestion, excretion and respiration. It houses organs such as the book lungs, the vascular system and the digestive tract (Foelix, 1983). The ventral part of the opisthosoma houses the spinnerets, the principal components of the silk-spinning apparatus found in all spiders (Gosline, DeMont & Denny, 1986; Vollrath, 1999; Eisoldt, Smith & Scheibel, 2011).

Spiders are characterized by a predatory lifestyle in which they mostly prey on other arthropods, although some species occasionally prey on vertebrates (Nyffeler & Knörnschäd, 2013; Valde, 2020; Nyffeler & Gibbons, 2021). Several groups have evolved trophic specializations, such as feeding on other spiders (aranephagophagy, e.g. Portia spp.), ants (myrmecophagophagy, e.g. Zodarion spp.), woodlice (oniscofagophagy, e.g. Dysdera spp.), butterflies and moths (lepidotrophagophagy, e.g. Mastophora spp.) and even occasional herbivory (Forster, 1977; Yarranton, 1988; Clark, Harland & Jackson, 2000; Pekár, 2004; Žezáč, Pekár & Lubin, 2008; Meehan et al., 2009; Pekár & Toft, 2015; Nyffeler, Olsen & Symondson, 2016). However, most spiders feed primarily on insects and thus occupy an important ecological niche that maintains the equilibrium of insect populations (Foelix, 1983). The evolution of spiders has been closely linked to the evolution of their insect prey, which is supported by the observation that the evolutionary innovations of spiders often follow earlier innovations in insects. For example, the evolution of aerial foraging webs followed the evolution of insect flight, and some of the most speciose radiations of spiders have coincided with comparable radiations of insects (Vollrath & Selden, 2007; Bond et al., 2014; Misl et al., 2014; Garrison et al., 2016; Fernández et al., 2018). The close link between spiders and insects is further underlined by the negative consequences of the global insect decline, which also threatens spiders (Nyffeler & Bonte, 2020).

A key component of the significant evolutionary success of spiders in relation to their role as arthropod predators is a versatile molecular toolbox that enables chemical attacks on their prey. The two essential components are silk and venom, both of which require complex physiological systems with extraordinary molecular diversity.

Silk production is pleisiotypic when used by the Mesothelae and Mygalomorphae for the construction of burrows, trapdoors and protective egg sacs, but apotytic functions evolved in the Araneomorphae, often involving the construction of complex foraging webs (Foelix, 1983; Vollrath & Selden, 2007; Harmer et al., 2011). Across all spiders, myriad silk types with specific functions and properties have emerged (Vollrath, 1999). All silk types are composed of repetitive protein elements that form a macromolecular protein fibre (Vollrath, 1999).

Whereas silk production is restricted to arthropods, venoms have evolved independently in more than 100 animal lineages (Fry et al., 2009; Casewell et al., 2013; Hollord et al., 2018). However, spider venoms are remarkable due to their unprecedented degree of chemical diversity. The discovery of venom glands in the Mesothelae revealed that all spiders with exception of the family Uloboridae (comprising 289 species), which have lost their venom glands secondarily, feature a functional venom system (Foelix & Erb, 2010). Spider venom can contain up to 3000 different molecules, suggesting that the prospecting of all extant spider species could yield ~10 million venom components (Saez et al., 2010; Pineda et al., 2020). Spiders therefore comprise a hyperdiverse lineage of predators with venom that is far more complex than that of most other animals (Herzig, 2019; Pineda et al., 2020). However, our knowledge of venom components is unevenly distributed, with most studies focusing on larger species or those with medical relevance. Conversely, smaller and harmless (to humans) spiders have received little to no attention thus far (Herzig, King & Undheim, 2019; Lüddecke, Vilcinskas & Lemke, 2019).

Nevertheless, increasing research effort has begun to cast light on spider venoms beyond the scope of large or dangerous taxa. The majority of previously published primary research studies and related review articles focus on translational aspects of spider venom-derived toxins, with a particular emphasis on their suitability as leads for the development of novel therapeutics and bioinsecticides. Simultaneously, some of these studies also contributed to a growing understanding of spider venom systems in a biological context and provided us with novel insights into their evolutionary history, ecology and biochemistry. Unfortunately, review articles providing a thorough synthesis of these pivotal insights into the biological aspects of spider venoms are scarce. The present review therefore aims to provide a comprehensive summary of the considerable advances made in spider venom biology over recent years.

Our review starts by discussing the venom system of spiders, describing its morphological organization and highlighting important developmental differences among lineages. This is followed by an overview of spider venom biology over recent years. Our review then goes on to describe the venom system of spiders, including its morphological organization and highlighting important developmental differences among lineages. This is followed by an overview of spider venom biology over recent years.
II. THE VENOM SYSTEM OF SPIDERS

(1) Architecture and development of the venom apparatus

The venom system of spiders is organized around the chelicerae, which differ in organization according to the lineage: while Mesothelae and Mygalomorphae possess chelicerae that are orthognathous (chelae in parallel orientation), the araneomorphs evolved chelicerae that are labidognathous (chelae are facing each other) as illustrated in Fig. 2. In spiders, the chelicerae feature two functional units that move in a jack-knife fashion. The first unit, a basal segment, is attached to the prosoma and forms a mobile base for the second unit, a fang.

Internally, the venom system of each chelicera consists of a venom gland connected to a narrow opening at the fang tip via a thin venom duct as shown in Fig. 3 (Foelix, 1983; Schmidtberg et al., 2021). Each venom gland is embedded in muscles and nerves, enabling fine control of venom release (Çavuşoğlu et al., 2005; Silva et al., 2008; Benli et al., 2013). Localization of venom glands differs between orthognathous and labidognathous types. While the venom gland of the former is localized in the basal segment of the chelicerae, it can extend into the prosoma in the latter. Venom glands in spiders are further functionally compartmentalized and their subsections produce and modify different venom components (Morgenstern et al., 2019). Despite their simple appearance and ancient origin, spider venom glands are highly complex with the ability to produce and secrete different venom mixtures. Similar specialization has been described in evolutionarily more recent organisms such as assassin bugs and cone snails, which produce defensive and predatory venoms in different glands or different parts of the same gland (Dutertre et al., 2014; Walker et al., 2018).

Little is known about the development of the venom system in spiders. In general, the development of spiders is subdivided into four main phases (Vachon, 1957; Foelix, 1983). The earliest is the pre-larva, which is followed by the larva. Both of these are embryonic stages and these animals barely resemble their adult counterparts. Thereafter, spiders enter the first complete stage of development termed the nymph stage. Nymphs already mirror adults; they are fully developed and motile, yet very small. A spider passes multiple nymph stages via ecdisis until it reaches adulthood, i.e. the final stage of the developmental process. Originally it was suggested that pre-larval and larval stages possess only undifferentiated chelicerae, venom glands and venom ducts and that these structures are only present as differentiated structures in nympha and adults (Silva et al., 2011). However, recent investigations on the Brazilian wandering spider (Phoneutria nigriventer) showed that venom glands are already present at the pre-larval stages, and that the larval venom system already comprises chelicerae, ducts and glands (Silva et al., 2011). The larval venom glands are located between the chelicerae and prosoma but subsequently migrate into the prosoma. Interestingly, toxin biosynthesis begins as early as the embryonic stage in this species (Silva et al., 2011). The very early activation of the genetic machinery that produces venom components is also highlighted by the presence of toxin-encoding messenger RNA (mRNA) in eggs of the genus Latrodectus (Buffkin, Russell & Deshmukh, 1971; Lei et al., 2015; Xu & Wang, 2016).

(2) Spider venom components and their biochemistry

Spider venoms are remarkably complex mixtures comprising thousands of different components. Many of these interact with ion channels and other receptors in the prey, thus spider venoms are primarily neurotoxic. The components fall into four main categories as discussed below: small molecules, larger proteins, cysteine-rich peptides and antimicrobial peptides (AMPs).

(a) Small molecules

There are several classes of abundant small-molecule components in spider venoms (Forrster et al., 2020). One important group, described as neurotransmitters in other animals, include serotonin, octopamine, 5-hydroxytryptamine, 5-methoxytryptamine, histamine, tyramine, γ-aminobutyric acid, aspartic acid and glutamic acid (McCormick & Meinwald, 1993). Another recently discovered group includes sulfated nucleosides, such as sulfated guanosine isolated from Loxosceles reclusa, and non-sulfated nucleosides, such as adenosine, guanosine, inosine and 2,4,6-trihydroxypurine in Latrodectus menacodii, and inosine in...
Parawixia bistriata (Horni, Weickmann & Hesse, 2001; Rodrigues et al., 2004). Neurotoxic alkaloids are unusual in venom systems and are more typically found in poisons, particularly those produced by amphibians (Lüddecke et al., 2018; Knepper et al., 2019). However, β-carboline alkaloids have been identified in the venom of P. bistriata (Cesar et al., 2005). The venom of Ph. nigriventer also contains the dioxopiperidine compound nigriventrine (Gomes et al., 2011). Spider venoms are also rich in citrate, inorganic ions and salts (Fenton et al., 1995; Wullschleger, Nentwig & Kuhn-Nentwig, 2005; Langenegger, Nentwig & Kuhn-Nentwig, 2019). For example, high concentrations of minerals, including Fe, Zn, Cu, Ca, Mg, Na, P and S, are found in the venom produced by Nephila spp. (Tokoro, Konc & Shioya, 1994), probably acting as co-factors that facilitate the folding and activity of toxins.

The most widely investigated small-molecule venom components are the acylpolyamines, which were discovered in the 1980s in the genera Nephila and Argiope but are now known to occur in many different species (Palma, 2012). These polyamines are insecticidal neurotoxins acting as open-channel blockers of glutamatergic and/or nicotinergic receptors (McCormick & Meinwald, 1993). Acpolyamines comprise an aromatic acyl group and polyamine chain, sometimes with an intervening linker of one or two amino acids (McCormick & Meinwald, 1993; Palma, 2012). Some feature additional amino acids on the polyamine chain, often with a terminal arginine residue. The length of the polyamine chain can range from seven atoms, as in pseudoargiopinin III, to more than 40, as in nephilatoxin-6 (McCormick & Meinwald, 1993).

**(b) Larger proteins**

The importance and abundance of large proteins in spider venom is currently a matter of debate but in some taxa they constitute key venom components (Langenegger et al., 2019). For example, the toxicity of black widow spider (Latrodecus spp.) venom in humans reflects the presence of α-latrotoxin (αLTX) that forms homotetrameric pores in the presynaptic neuronal membranes of vertebrates. This leads to the uncontrolled flux of Ca²⁺ and neurotransmitters, resulting in nociception, convulsions, and even death (Grishin, 1998; Henkel & Sankaranarayanan, 1999; Orlova et al., 2000; Ushkaryov, Rohou & Sugita, 2008). Similarly, venoms produced by the family Sicariidae contain phospholipase D (PLD), a highly cytotoxic sphingomyelin-hydrolysing enzyme illustrated in Fig. 4 (Swanson & Vetter, 2006). Finally, a cytotoxic hyaluronidase-like enzyme...
was recently shown to enhance the uptake of co-administered neurotoxins, thus acting as a spreading factor (Biner et al., 2015). With the exception of these examples, the role of larger proteins in spider venom remains ambiguous.

For example, many spider venoms contain neprilysin metalloproteases and members of the CAP (cysteine-rich secretory protein, antigen 5 and pathogenesis-related 1) family, but their molecular role remains unclear (Undheim et al., 2013; Kuhn-Nentwig et al., 2019; Langenegger et al., 2019; Zobel-Thropp et al., 2019; Lüddecke et al., 2020). Many spider venoms also contain disulfide isomerases, carboxypeptidases, and serine proteases, which may facilitate the post-translational modification and maturation of toxins (Langenegger et al., 2019). There is evidence that the chymotrypsin-like activity of one serine protease facilitates toxin maturation, but further work is required to confirm the role of other enzymes (Langenegger et al., 2018).

c) Cysteine-rich peptides

The functionally most important group of spider venom components are peptides, typically with molecular masses below 10 kDa, which are rich in disulfide bonds. Many peptides interact with ion channels and other receptors, thus representing the principal neurotoxic components of spider venoms (Langenegger et al., 2019). Cysteine-rich peptides can be assigned to different families, including the Kunitz-type serine protease inhibitors, helical arthropod neuropeptide-derived (HAND) peptides, colipase fold (MIT-1) peptides, disulfide-directed β-hairpin fold (DDH) peptides, and, most importantly, peptides with an inhibitor cystine knot (ICK) scaffold (Langenegger et al., 2019). Although many of these components have not been investigated in detail, research has focused on the ICK family, which is the most diverse, abundant, and well-characterized component of spider venom systems (Langenegger et al., 2019). The secondary structure of ICK peptides is a triple-stranded antiparallel β-sheet, and its tertiary structure is determined by at least six cysteine residues, which are oxidized to form disulfide bonds that generate the characteristic pseudoknot motif (Norton & Pallaghy, 1998; Buczek et al., 2007; Cardoso & Lewis, 2019; Langenegger et al., 2019). Most ICK peptides feature six cysteine residues and thus three disulfide bridges, but others have expanded cysteine scaffolds and/or double ICK (dICK) motifs (Escoubas et al., 2003; Bohlen et al., 2010; Pineda et al., 2014b; McCarthy et al., 2015; Chassagnon et al., 2017). ICK peptides form stable complexes with prey receptors, disrupting their normal mode of action (Klint et al., 2012; Langenegger et al., 2019). Key targets include voltage-gated sodium, potassium and calcium channels, but also acid-sensing ion channels, glutamate receptors, and transient receptor potential channels (Langenegger et al., 2019). These targets are essential for signal transduction and cellular communication in prey, so their functional disruption disturbs the physiological homeostasis of the envenomed organism. The binding of a neurotoxic ICK polypeptide is typically facilitated by the partial penetration of the cell membrane and subsequent lateral migration towards the target (Deplazes et al., 2016) followed by high-affinity and high-specificity binding (Bende et al., 2014; Langenegger et al., 2019). The physicochemical properties of ICK peptides reflect their pharmacodynamic behaviour. Given that molecular size correlates negatively with distribution time, small ICKs exert their physiological effects quickly after injection, and the exceptional stability of these molecules resists proteolytic degradation, thus maximizing their efficacy (Herzig & King, 2015). Some representative ICKs are depicted in Fig. 4.

(d) Antimicrobial peptides

Most spider venom AMPs are linear, α-helical peptides without disulfide bonds and not only display antimicrobial activity but also compromise eukaryotic cells by disrupting the integrity of their membranes, hence they are also referred to as lytic peptides (Yan & Adams, 1998; Corzo et al., 2002; Garcia et al., 2013; Langenegger et al., 2019). These short peptides may therefore serve a dual function in venom systems by defending the venom gland against microbial colonization while facilitating prey paralysis and potentially digestion (Yacoub et al., 2020). Although AMPs are present in many spider venoms, most of those identified thus far have been found in the venoms of hunting spiders (Lycosidae) and tarantulas (Theraphosidae) (Yan & Adams, 1998; Budnik et al., 2004; Santos et al., 2010; Abreu et al., 2017). The AMPs in hunting spiders appear particularly diverse, with more than 50 such peptides discovered in the venom of Lycosa sinensis (Tang et al., 2020). Some representative AMPs are shown in Fig. 4.

(3) Synergistic molecular interactions enhance the versatility of spider venom

The complexity of spider venoms is not restricted to the number and activity of components, but also extends to include synergistic interactions among these components to maximize their potency. Given the dynamic nature of the venom cocktail, these interactions are both temporally and spatially regulated, giving rise to the so-called dual prey inactivation strategy (Kuhn-Nentwig et al., 2019). This postulates that spiders inject a cocktail of neurotoxic small peptides together with an array of larger proteins that interact to facilitate two waves of activity. Some of the large proteins immediately disrupt prey physiology and metabolism, while others act to spread the neurotoxins and thus trigger a subsequent wave of paralysis (Kuhn-Nentwig et al., 2019).

Synergism in spider venom is not limited to the dual prey inactivation strategy. Indeed, the main role of some peptides is to mediate or enhance the bioactivity of others. One example is provided by the neurotoxic peptides of the wandering spider Cupiennius salei (Kubista et al., 2007). One set of C. salei toxins (CSTX type 1) acts directly on the prey, whereas CSTX type 2 peptides (CSTX-8, CSTX-12 and...
in particular CSTX-13) are minimally toxic on their own but significantly enhance the activity of co-administered type 1 peptides (Wullschleger et al., 2004, 2005; Clémençon et al., 2020). For example, the toxicity of CSTX-1 increases 65% in the presence of CSTX-13 (Wullschleger et al., 2004). Comparable effects are triggered by other C. salei venom components, including K+ ions and the cytolytic peptide cupiennin 1a (Wullschleger et al., 2005). This mechanism has been described as ‘neurotoxin merging’ and is also likely to play a role in other species that produce CSTX-related peptides in their venom (Clémençon et al., 2020; Lüddecke et al., 2020).

ICKs directly inhibit receptors in prey insects but can also act cooperatively at different time points (King & Hardy, 2013). Fast-acting toxins bind reversibly to their targets and trigger the rapid onset of neurotoxic effects, thereby achieving immediate prey immobilization (Bloomquist, 2003; King & Hardy, 2013). Subsequently, slower acting paralytic toxins bind irreversibly to the target as the effects of the fast-acting toxins decline. The paralytic chemical attack therefore involves temporal niche occupation by neurotoxins. This synergism enables rapid paralysis followed by long-term immobilization, which is useful for the storage of overpowered prey (King & Hardy, 2013).

Another important type of synergism within spider venoms is displayed by small molecule components. Acylpolyamine toxins, which modulate cationic-selective ion channels, interact with their targets via a binding site within the channel pore (Kuhn-Nentwig, Stöcklin & Nentwig, 2011). For these toxins a ligand-based activation of the respective channel is needed to make the binding site accessible. This process is facilitated by neurotransmitters (acetylcholine and glutamate) present in the venom, which open the respective channel and enable the acylpolyamine toxins to block its pore (Early & Michaelis, 1967; Schroeder et al., 2008; Kuhn-Nentwig et al., 2011; Palma, 2012). A specialized form of this mechanism is found in agedenid venom in which peptide toxins stimulate the endogenous release of glutamate and thereby enable acylpolyamine toxin binding (Adams, 2004; Kuhn-Nentwig et al., 2011).

### III. SPIDER VENOMS AS DYNAMIC SYSTEMS

Spider venoms are functional traits adapted to a specific lifestyle and reflect the ecology of the corresponding species. Many abiotic and biotic factors impose different prerequisites and constraints on functional traits during evolutionary events such as range expansion or niche partitioning. For example, dietary shifts, trophic specialization and the appearance of new predators lead to the subsequent recruitment, adaptation or loss of toxins in order to maximize defensive or predatory deployment. Such ecological influences can also result in intraspecific variability (Chippaux, Williams & White, 1991; Alape-Girón et al., 2008; Gibbs, Sanz & Calvete, 2009). Spiders are interesting models to test for intraspecific variability in venoms because they include several taxa that are currently undergoing range expansion, conquering new ecological niches or displaying sex-specific lifestyles throughout their ontogeny. The growing body of research on this topic highlights the plasticity found in spider venoms and is discussed below.

#### (1) Ontogenetic changes in venom composition

Differences in spider venom yield, toxicity and composition have been linked to life-history stages (Herzig, Ward & Dos Santos, 2004; Herzig, 2010; Santana et al., 2017; Duran, Rymer & Wilson, 2020). In the Australian tarantula Corenocnemis tropix, venom yield increases as the spider ages in concert with the growth of the venom glands (Herzig, 2010). However, the venom yield declines prior to ecdysis, probably due to the resource-intensive reconstruction of the exoskeleton and laborious moulting process, but it may also be possible that formation of the new exoskeleton physically blocks the release of venom during this period. Ecdysis and venom system maintenance therefore appear to compete for resources at this time, and metabolic resources may be withdrawn from the venom system and redirected to exoskeleton biosynthesis and growth. Similarly, quantitative and qualitative changes in venom composition have been reported during the maturation of Selencosmia crassipes (Santana et al., 2017). Several components are uniquely present at certain life-history stages, whereas the presence of larger proteins is more consistent (Santana et al., 2017). Interestingly, S. crassipes venom undergoes further compositional alterations as the spider ages, showing that compositional flexibility is maintained post-maturation (Santana et al., 2017).

#### (2) Geographic venom variation

The variability of venom profiles between allopatric populations of the same spider species has received little attention. Comparative analysis of the venom from European and North American populations of Eratigena agrestis (formerly Tegenaria agrestis) revealed no significant differences (Binford, 2001). By contrast, regional differences in venom profiles and toxicity have been reported for Loxosceles rufescens and some members of the genus Latrodectus (Planas et al., 2015; de Roodt et al., 2017). Further studies are required to determine whether geographic variations in venom composition are widespread in spiders.

#### (3) Venom as a sexually dimorphic trait

Most spiders are sexually dimorphic, including sexual variations in their venom profiles (De Oliveira et al., 1999; Binford, 2001; Escoubas et al., 2002; Mourao, 2007; Herzig & Hodgson, 2009). The best-studied species in this context are Australian funnel-web spiders of the genera Atrax, Iliacara and Hadronyche (family Atracidae) and the genus Misulena (Actinopodidae), which include some highly venomous members (Hauke & Herzig, 2017). Males in these
genera can cause fatal bites in humans, whereas female envenomation is much less toxic (Ishbister et al., 2005; Herzig et al., 2020). Molecular profiling has shown that females tend to produce more complex venoms than males, and that the differential toxicity towards humans is not replicated in insects (Palagi et al., 2013; Herzig et al., 2020). The greater toxicity of male venom in the families Atracidae and Actinopodidae apparently reflects the presence of specific ICKs known as δ-hexatoxins and δ-actinopodotoxins (Herzig et al., 2008, 2020; Pineda et al., 2014a) which potently inactivate tetrodotoxin-sensitive voltage-gated sodium channels in vertebrates and sodium channels in insects (Gunning et al., 2003; Nicholson, Little & Birinyi-Strachan, 2004). These ICKs are abundant in adult male venom but not in females (Herzig et al., 2008, 2020). Differences in venom composition and toxicity are not restricted to mygalomorphs, and males are not always the most toxic. For example, females of the species Ph. nigriventer (Ctenidae) and Loxosceles intermedia (Sicariidae) are seemingly more toxic than the males (De Oliveira et al., 1999; Herzig, Ward & Ferreira dos Santos, 2002). Several sex-specific components are present in Ph. nigriventer venom, one of which is found exclusively in egg-sac-carrying females (Herzig et al., 2002).

One potential explanation for the evolution of sexually dimorphic venoms is the different lifestyles of adult spiders, which in turn require different venom functions. For example, Mygalomorphae juveniles and adult females often follow a cryptic burrow-dwelling lifestyle and only leave their burrows for hunting (Santana et al., 2017). By contrast, adult males abandon their burrows and switch to an active migratory lifestyle in search of females (Santana et al., 2017). Migrating males eventually stop feeding but are more likely to encounter predators (Ishbister & Fan, 2011; Santana et al., 2017). Burrow-dwelling juveniles and females therefore benefit from predominantly prey-evoked venom, whereas males switch to a less prey-relevant armoury and instead express a venom cocktail containing more defensive toxins (Herzig et al., 2020). The potent toxicity of Ph. nigriventer and L. intermedia female venom against vertebrates indicates a similar defensive function in female araneomorphs. This is particularly important for egg-sac-carrying species that follow an active lifestyle (such as Ph. nigriventer) because the females are likely to encounter predators more frequently and have therefore evolved a powerful chemical arsenal to defend their offspring. Reproductive behaviour may also determine the venom profile. For example, male-specific venom components in the long-jawed spider Tetragnatha versicolor (Tetragnathidae) are thought to facilitate sexual communication (Binford, Gillespie & Maddison, 2016; Zobel-Thropp et al., 2018).

(4) Individual venom variability

Compared to venom profiles that differ among populations, sexes and life-history stages, the individual variation of spider venom composition has received scant attention. Venom profiles in other animals can change even within a single specimen, but little is known about such individual variation in spiders. Only recently, Hadronyche valida was shown to display a surprising degree of venom cocktail plasticity even among individuals kept under similar conditions to exclude external influences (Duran et al., 2020). Intriguingly, only 20% of the venom peptides in these individuals were common among the test specimens, whereas the remaining 80% were unique (Duran et al., 2020). Some of these identified peptides were present only at specific times, whereas others were constitutive (Duran et al., 2020). These findings suggest that H. valida produces a common ‘core venom’ that is dynamically modified with more specific molecules to maintain venom flexibility in the adults. This hypothesis needs further investigation to determine whether it also applies to other species.

IV. THE DIVERSITY OF ENVENOMATION STRATEGIES DEPLOYED BY SPIDERS

Spiders can be placed into two ecological categories. The first comprises web-building spiders, which construct foraging webs to facilitate hunting and inhabit these webs for most of their lives (Foelix, 1983). The second comprises the wandering spiders, which do not use foraging webs to capture prey (Foelix, 1983). All wandering spiders and most web-building spiders use envenomation as their major strategy to incapacitate prey, although orb-weaving spiders (family Araneidae) and some others primarily use their foraging webs and silk apparatus for this purpose (Foelix, 1983). For example, the wasp spider Argiope bruennichi traps most of its prey with silk before venom is injected (Lüddecke et al., 2020). It appears that different combinations have evolved within the family Araneidae, such as silk-first versus venom-first strategies (Foelix, 1983). In other species, silk and the venom system are literally interwoven by the incorporation of toxins into the silk strands in the foraging webs to intoxicate insect prey upon contact (Marques et al., 2004, 2005; Esteves, 2020). A more extreme variation is the silk-only hunting behaviour of the Uloboridae, which have lost their venom systems (Weng, Barrantes & Eberhard, 2006). Spitting spiders use their venom system to project a droplet of toxins and liquid silk that solidifies and subsequently paralyses the prey (Gilbert & Rayor, 1985). Bolas spiders, such as Mastophora spp., produce a silk strand with a terminal, adhesive droplet that is actively thrown towards prey (Yeargan, 1988). This is further supported by a strategy of aggressive chemical mimicry in which females release attractive volatiles that mimic the sex pheromones of their prey (Eberhard, 1977).

Many spiders use their venom not only to attack prey but also to defend against predators. Most spiders prioritize escape and only administer defensive bites if escape is not possible. Not all defensive bites lead to envenomation, similar to the ‘dry bites’ of snakes, but where venom is injected this can deter the predator long enough for the spider to escape,
or it may cause the attack to be aborted. In some cases, the predator may learn to avoid similar prey in the future. The latter can be enhanced by aposematic warning coloration or distinct defensive behaviors, such as stridulation or threat postures that advertise toxicity to the predator (Pekár, 2014). Such avoidance learning can further be supported by the pain-inducing components that are present in many spider venoms (Bohlen et al., 2010; Osteen et al., 2016). The lynx spider Peucetia viridans has evolved a remarkable defensive strategy similar to spitting cobras, in which venom is sprayed towards potential threats with great accuracy (Tinkham, 1946; Panagides et al., 2017).

VI. THE VENOM OPTIMIZATION HYPOTHESIS AND THE ECONOMIC UTILIZATION OF VENOM

Venoms are powerful evolutionary adaptations for predation and/or defense, but they are also physiologically expensive. The energy invested into venom systems (venom apparatus, toxin expression and secretion) reduces the resources available for other physiological processes (Nisani & Hayes, 2011; Nelsen, Kelln & Hayes, 2014). Accordingly, the wandering spider C. salei uses its venom as economically as possible, deploying small amounts against weaker prey and larger quantities against more resistant prey, a strategy known as the venom optimization hypothesis (Wigger, Kuhn-Nentwig & Nentwig, 2002). The conservation of venom resources has been investigated in other taxa (Kuhn-Nentwig & Nentwig, 2002). The conservation of venom resources has been investigated in other taxa (McCue, 2006; Nisani, Dunbar & Hayes, 2007; Enzor, Wilborn & Bennett, 2011; Morgenstern & King, 2013; Smith, Ortega & Beaupre, 2014). Although not all studies support the hypothesis (Smith et al., 2014), the concept of frugal venom deployment is widely accepted (Morgenstern & King, 2013). In spiders, the hypothesis is supported by a range of physiological adaptations. For example, some neotropical members of the Theraphosidae have convergently evolved urticating setae (Foley et al., 2019), which are projected towards a potential threat and cause irritation upon contact (Kaderka et al., 2019). Such adaptations make defensive bites mostly unnecessary and thus reduce venom expenditure. This hypothesis is supported by the lower median venom yields that are obtained from new-world tarantula subfamilies compared to their old-world relatives (Hauke & Herzig, 2021).

Spiders with trophic specializations usually produce unique venom components that are highly effective against their preferred prey while constraining their activity against other prey (Pekár et al., 2018b). Accordingly, the venom profiles and venom system morphology of specialist and generalist spiders follow different trends, with specialists evolving simpler venoms and smaller venom glands (Pekár et al., 2018a). These findings can be interpreted in light of the venom optimization hypothesis as follows: when a spider specializes, venom components that affect non-preferred prey will become dispensable and will be purged from the venom to save resources. Only the prey-specific toxins will remain, and these will evolve to optimize their activity against the preferred prey. As a consequence of this streamlining process, venom complexity and the venom system as a whole are likely to become simpler over time in prey-specialised spiders.

VI. ON THE ORIGINS OF AN ARSENAL: WHERE DO SPIDER VENOMS COME FROM?

Venom toxins are proteins and peptides that have evolved from ancestors with normal physiological functions via recruitment processes such as gene duplication and subsequent neofunctionalization and/or subfunctionalization, leading to the weaponization of these proteins as toxins (Fry et al., 2009). Other processes involved in toxin gene evolution include single gene co-option, horizontal gene transfer, and de novo evolution (Casewell et al., 2011; Casewell, 2017; Drukewitz et al., 2019). The evolutionary mechanisms that have been revealed to act on spider venom systems are discussed in more detail below.

(1) The evolution of venom delivery systems in spiders

A tight evolutionary connection exists between the morphology of a venom delivery system and the included components (Schendel et al., 2019). Thus, a thorough understanding of the evolutionary processes that gave rise to venoms will afford a similar understanding regarding their linked injection systems.

For many animal lineages including spiders (Starr, 1985; Kukuk et al., 1989; Fry et al., 2006; Vonk et al., 2008; Zancolli & Casewell, 2020), little is known about the origin of their venom delivery apparatus. It is not yet clear from which ancestral organ the venom system is derived and different possibilities exist. One possible evolutionary origin is the salivary glands, similar to those of ticks (Kirkland, 1971; Cooms & Roshdy, 1973). This hypothesis is supported by the localization of spider venom glands within the chelicerae, the role of salivary glands in the evolution of venom systems in other animals, and the common ancestry of ticks and spiders (Low et al., 2013; Sharma, 2018; Casewell et al., 2019; Langenegger et al., 2019). Another hypothesis is that the spider venom system evolved from the silk-producing glands present in early chelicerates. In sea spiders, the larvae of some species possess secretory silk glands in their chelae (Bogomolova, 2007), which may have been repurposed as venom glands in spiders. Such a close connection between the silk and venom systems is implied on multiple levels. For example, both arise from the ectoderm during development and toxin genes are located in close proximity to silk genes on spider chromosomes (Foelix, 1983; Sheffer et al., 2021). Further, silk-related mRNAs are expressed in spider venom glands and spitting spiders produce silk
proteins in their specialized venom glands enabling them to spit toxic silk droplets towards their prey (see Section IV) (Gilbert & Rayor, 1985; Babb et al., 2017). In addition, an evolutionary link between the venom and silk systems in spiders may explain the presence of toxins incorporated into the foraging webs of some species (see Section IV) (Marques et al., 2004, 2005; Estoves, 2020). Testing these, and other hypotheses, on the evolution of spider venom systems remains an important area for future studies.

While the origin of venom delivery systems remains unresolved, it is apparent that the subsequent modification of the injection apparatus facilitated at least one major evolutionary transition in spiders. An arguably key event in the history of spiders was the rapid diversification that occurred after the rise of modern araneomorphs (Dimitrov & Hormiga, 2021). More than 90% of all extant species are araneomorphs (World Spider Catalog, 2021) and thus they are largely responsible for the great success of the spider lineage. Major differences between modern araneomorphs and their primitive relatives in the Mesothelae and Mygalomorphae infraorders are the arrangement of the chelicerae as well as the size and placement of the venom apparatus. In the primitive spiders, small venom glands are located within the basal part of the chelicerae. Especially in the most primitive infraorder Mesothelae they are very small and presumably have only modest functionality (Foelix & Erb, 2010). By contrast, in Araneomorphae the venom glands are enlarged and extend into the prosoma. It is assumed that this anatomical migration of the venom storage and production system, together with its enlargement, enabled the reduction in size and body mass that was required for successful utilization of foraging webs. This process therefore was pivotal for the adoption of a web-based lifestyle (Langenegger et al., 2019). It is notable that the migration of the venom glands from the basal part of the chelicerae into the prosoma is recapitulated during the development of araneomorphs as exemplified in Ph. nigri- venter (Silva et al., 2011). Here, the venom glands are located adjacent to the chelicerae at the larval stage but subsequently move towards the prosomal cavity as the embryo matures (Silva et al., 2011).

(2) The role of recruitment and neofunctionalization

A growing body of evidence suggests that many spider toxins evolved from neuropeptides, which represent promising evolutionary blueprints for toxins because they control vital processes. Accordingly, the dysregulation of neuropeptide activity disrupts neuronal chemistry and thus triggers neurotoxicity. Recruitment into the venom system would involve the accumulation of mutations that alter their surface chemistry and thus weaponize them by converting them from signalling molecules into unregulated agonists or inhibitors. The most prominent examples are HAND peptides that have been recruited into the venom systems of spiders and centipedes by the weaponization of ion transport peptide/crustacean hyperglycemic hormone (ITP/CHH) neuropeptides (Undheim et al., 2015). The same family of neuropeptides has been recruited to the Theridiidae venom system, where they evolved into the latrodecin (McCowan & Garb, 2014). The analysis of wasp spider (A. bruennichi) venom has also shown that peptides with similarity to neuropeptides from Camponotus ants (ITG-like peptides), diuretic-hormones and insulin-like growth factor-binding proteins have been recruited into the venom apparatus (Lüddecke et al., 2020).

Neofunctionalization is not restricted to the recruitment and modification of non-toxic components. Existing toxins can also undergo neofunctionalization to acquire new activities and functions. One example is the δ-hexatoxins of funnel-web spiders, which evolved from an insecticidal ICK used for hunting into a defence toxin that is used to deter vertebrate predators when the spiders are seeking mates (Herzig et al., 2020). This may explain the potentially fatal effect of δ-hexatoxins in humans (Ibister et al., 2005). However, given the absence of primates in the evolutionary history of funnel-web spiders, the potent toxicity of these toxins in humans may be an evolutionary coincidence based on the similarity of target predator and human receptors (Herzig et al., 2020). A similar coincidence may also explain the toxicity of some Theraphosidae venoms (Hauke & Herzig, 2021).

(3) Gene duplications in the evolution of ICKs

ICKs are the most abundant cysteine-stabilized peptides in nature; they are found in the venoms of many spiders and other animals, and have also evolved diverse functions in plants, fungi, bacteria and viruses (Undheim, Mobli & King, 2016). A fundamental obstacle hindering the evolutionary analysis of ICKs is the pseudoknot motif and its disulfide bonds, which are largely responsible for defining the tertiary structure of these peptides. Amino acid substitutions can therefore accumulate with little impact on structure, leading to profound diversity (Olivera et al., 1995; Sollod et al., 2003; Kozinsky-Atias & Zilberberg, 2012; Sunagar et al., 2013; Sunagar & Moran, 2015; Undheim et al., 2016). This challenge has been addressed recently by the application of structural venomics’ (a combination of venom transcriptomics, proteomics and structural biology) in Hadrolyche infensa to shed light on ICK evolution (Pineda et al., 2020). This approach showed that most ICK peptides are descendants of a single weaponized ICK lineage that underwent duplication and structural diversification, giving rise to a variety of peptides with elaborate ICK folds following their recruitment as venom components (Pineda et al., 2020). The ancestral ICK toxin was proposed either to contain a fourth disulfide bond that stabilized its β-sheet (lost in some descendants) or the typical three disulfide bonds with the fourth evolving independently at least twice (Pineda et al., 2020). Domain duplication would then explain the dICK peptides (Pineda et al., 2020). Structural venomics thus provides evidence that gene duplication is an important process in the evolution of spider venom and other venomous lineages. Similarly, gene duplication was proposed as an explanation for the evolution of αLTX in Latrodectus spp.
However, it is important to note that the confident reconstruction of gene evolution processes requires genomic data. Results based exclusively on venomic data sets need to be interpreted with caution as assembly artifacts and overinterpretation can easily blur the evolutionary signature and lead to false assumptions. At least one ancient whole-genome duplication event is also thought to have occurred in the lineage leading to spiders and scorpions, providing the foundation for extensive neo-functionalization (Schwager et al., 2017).

(4) Venom gene evolution by horizontal gene transfer

Horizontal gene transfer has contributed to the evolution of some venom components, including PLD in the family Sicariidae (Cordes & Binford, 2018). PLD was traced to a single proteobacterial ancestor, from which it appears to have radiated widely, at least partially facilitated by horizontal gene transfer (Cordes & Binford, 2018). Horizontal gene transfer has also been proposed to explain the origin of α-LTX, based largely on the complete genome sequence of Parawixia tepidariorum (Gendreau et al., 2017; Schwager et al., 2017).

(5) Selection pressures acting on spider venoms

It is a long-standing paradigm that venoms evolve rapidly under strong positive selection (Lynch, 2007; Gibbs & Rossiter, 2008; Juárez et al., 2008; Sunagar et al., 2013). Indeed, the evolution of many venoms may conform to the ‘Red Queen hypothesis’ given that prey species are constantly co-evolving with their predators (Van Valen, 1974). This hypothesis predicts that a set of antagonistic evolutionary traits in different species exert reciprocal positive selection pressures on each other and therefore drive the evolution of their counterparts. The traits that evolve as ‘Red Queens’ are therefore trapped in evolutionary arms races. In the light of animal venoms, it is commonly assumed that predator venoms and the venom resistance of prey species are examples of such an evolutionary arms race. The subsequent escalation of toxicity and toxin resistance are therefore principal forces leading to the diverse and powerful pharmacopeia observed in extant venoms (Van Valen, 1974; Fry et al., 2015; Holford et al., 2018). However, this concept of reciprocal evolution is entirely derived from studies based on evolutionarily recent classes of animals, particularly reptiles (Sunagar & Moran, 2015). By considering the selection pressures on venom genes throughout the animal kingdom, recent studies have shown that selective pressures differ between recent and more ancient clades (Sunagar & Moran, 2015). Whereas venom genes in young clades tend to evolve under strong positive selection, older lineages such as spiders show signatures of purifying selection. This led to the proposal of a two-speed model of venom gene evolution, which predicts that the diversifying nature of positive selection mostly acts during the early stages of ecological specialization within a given species (Sunagar & Moran, 2015). This period of diversification is followed by an extended stage of purifying selection that imposes heavy constraints on the venom system. Although the selection pressures on spider toxins have not been investigated broadly throughout the spider tree of life, the few studies performed to date largely support this two-speed evolutionary model (Pineda et al., 2014a; Sachkova et al., 2014; Sunagar & Moran, 2015; Herzig et al., 2020; Lüddecke et al., 2020).

An evolutionary trajectory largely shaped by purifying selection was demonstrated for α-LTX (Garb & Hayashi, 2013) as well as ICKs from the lynx spider Oxyopes takobius (Sachkova et al., 2014). The evolutionary analysis of toxins from Australian funnel-web spiders also supports the role of negative selection in the evolution of spider venom molecules such as δ-hexatoxins and their homologs in the families Atracidae, Actinopodidae, Barychelidae and Macrothelidae, as well as κ-hexatoxins in the Atracidae, whereas ω-hexatoxins from the same spiders appear to evolve under periodic positive selection (Pineda et al., 2014a; Herzig et al., 2020).

(6) The significance of genomic data to unravelling spider venom evolution

Although spiders are well known as venomous predators, their ecological importance, evolutionary success, and the molecular diversity of their venoms are often neglected. Most previous investigations have focused on the translational potential of spider venom peptides, particularly those with biomedical or agrochemical applications (Saez et al., 2010; King & Hardy, 2013; Saez & Herzig, 2019). More recent studies have also considered the biological importance of spider venoms in terms of ecology, ethology and evolution. These investigations have shown that spider venoms are dynamic systems in which the diverse chemical components work synergistically at different levels and according to the purpose of the venom (Wigger et al., 2002; Morgenstern & King, 2013; Kuhn-Nentwig et al., 2019; Langenegger et al., 2019; Clémençon et al., 2020; Duran et al., 2020). The components of spider venom thus vary among populations, sexes, developmental stages and even individuals (Binford, 2001; Herzig et al., 2008, 2020; Santana et al., 2017; Duran et al., 2020). These studies are encouraging, but their results must be interpreted with caution because only a small fraction of overall spider diversity has been investigated thus far. Furthermore, methods are not yet standardized to ensure consistent sample sizes and physiological states for venom glands, and this may be difficult to achieve when studying free-living specimens in their natural habitat. Therefore, it is unclear whether current concepts of spider venom plasticity can be generalized to all members of the order Araneae.

The investigation of spider venom evolution has progressed significantly in recent years and much more is now known about the underlying processes. However, comprehensive analysis requires high-quality whole-genome data from a broader range of venomous species because comparative studies addressing toxin gene evolution are still

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restricted to selected taxa (Drukewitz & von Reumont, 2019). Most insights into venom gene evolution are currently derived from proteotranscriptomic data, which must be interpreted with caution. Although several spider genome sequences are now available, the data only sparsely cover spider diversity (Sanggaard et al., 2014; Babb et al., 2017; Gendreau et al., 2017; Schwager et al., 2017; Garb, Sharma & Ayoub, 2018; Sánchez-Herrero et al., 2019). Additional genome sequences and comparative whole-genome studies will shed more light on the evolution of spider venom genes. For example, the detailed analysis of exon regions, scaffolds and microsynteny could determine whether the diversity of ICK genes and their microsyntenic flanking regions will shed more light on the evolution of spider venom genes. If so, this would be reflected in the phylogenetic relationships among the ICK genes and their microsyntenic flanking regions. Alternatively, the short ICK motif may arise frequently by chance (de novo gene evolution), in which case phylogenetic analysis would not recover any related clades and the flanking regions would be dissimilar. To make matters more complex, it is possible that ICKs undergo multimodal evolution, with de novo genes undergoing subsequent duplication and divergence. The broad genomic comparison of venom-related genes in tandem with proteotranscriptomics may reveal signatures related to particular evolutionary processes, such as de novo evolution, horizontal gene transfer and gene duplication/gene loss, allowing us to verify the proposed importance of positive and negative selection on spider venom.

VII. CONCLUSIONS

(1) Spiders are among the most successful evolutionary lineages of venomous animals with significant ecological roles. Their success story as predatory arthropods is unambiguously built upon their diverse venoms. Nevertheless, most research on spider venoms concentrates on taxa with medical significance. Only recently has a larger diversity of spiders begun to be studied for their venoms. This increased taxonomic diversity provides further insights into spider venom biology and evolutionary ecology. It appears that spider venoms are dynamic systems, which can be altered by intrinsic and environmental cues. The combination and alteration of venom components that enables synergistic attacks on prey, predators or competitors is known from other taxa, but has scarcely been explored in spiders.

(2) Spiders evolved an array of ethological specializations to facilitate envenomation and to conserve resources during venom deployment, including venom spitting, urticating setae, aposematism and stridulation. These behavioural innovations effectively supplement their venom system and are used in a variety of defensive and predatory scenarios. The presence of such specializations highlights that spiders are capable of actively deploying their venom in a context-dependent manner to maximize the efficiency of their chemical arsenal. Moreover, the acquisition of some supplemental traits, such as urtication or extensive silk-spinning, may impose reciprocal selection on the venom system. These may have dramatic impacts on the venom system and could even cause its reduction or complete loss in some species.

(3) The application of a variety of state-of-the-art methodologies has cast light on the selective forces behind the evolution of spider venom systems and highlighted the dynamic and multimodal evolutionary trajectory of toxin genes. Seemingly, their tremendous diversity is reflected in the multitude of evolutionary mechanisms at play in spiders. It became particularly clear that spider venoms are strongly influenced by ecological niche and life-history aspects. Whether the natural mode of action and evolutionary function of spider venom can be interpreted requires a sufficient knowledge of the natural history of the respective species.

(4) The evolutionary ecology of spider venoms and their components remains an understudied field with many unanswered questions. For instance, the venom compositions of the overwhelming majority of spider lineages are still unknown and thus our understanding of spider venoms on a broad taxonomic scale remains limited. It is obvious that refinement of the venom apparatus was pivotal for the emergence and great success of extant spiders, yet the evolutionary origin of the spider venom system remains speculative. Herein, we outline some of the most pressing questions for future research and provide several for testing.

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