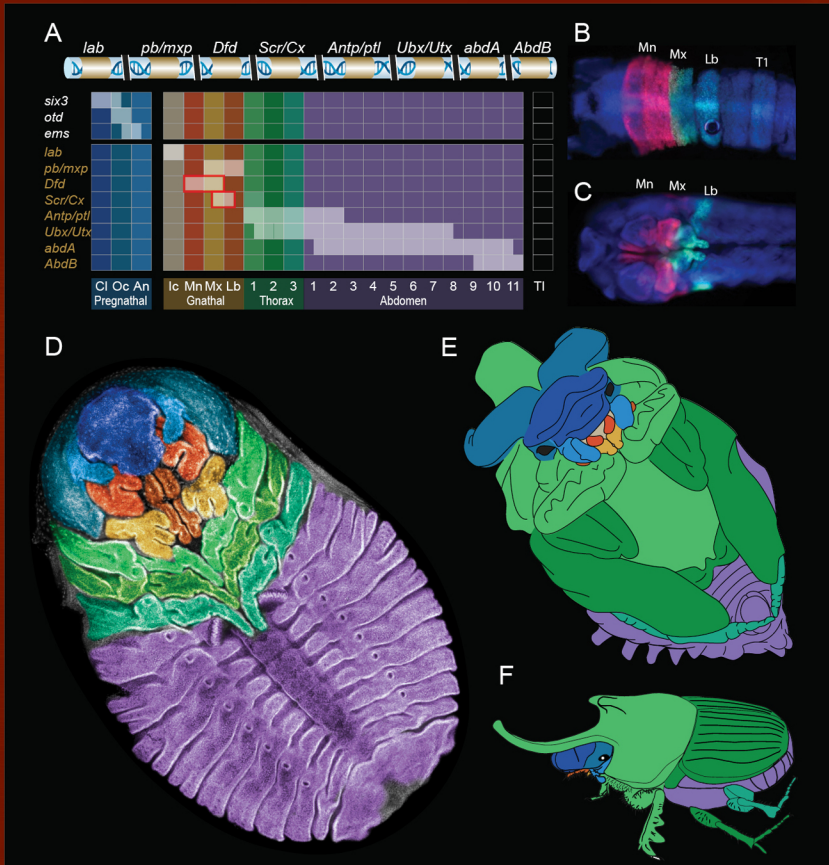


# HOX MODULES IN EVOLUTION AND DEVELOPMENT



EDITED BY  
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# 4 Duplication and Evolution of Hox Clusters in Chelicerata (Arthropoda)

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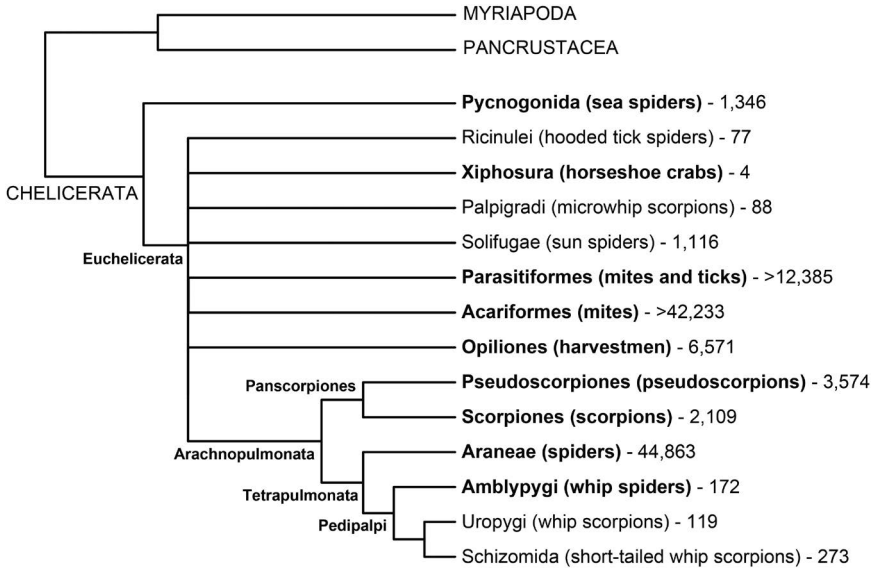
## CONTENTS

4.1 The Phylogenetic Significance of Chelicerata .....	77
4.2 Historical Studies of Chelicerate Hox Genes .....	78
4.3 The Discovery of Duplicated Chelicerate Hox Clusters.....	81
4.4 Hox Genes in Chelicerate Phylogenomics.....	84
4.5 Impact of Gene Duplications on Diversification Dynamics .....	89
4.6 Studies of Hox Function in Chelicerata.....	92
4.7 Conclusion .....	95
Acknowledgements.....	96
References.....	96

## 4.1 THE PHYLOGENETIC SIGNIFICANCE OF CHELICERATA

Within Arthropoda, the subphylum Chelicerata encompasses a broad swath of diversity, with over 120,000 described species to date. The most familiar members of Chelicerata include groups like spiders, scorpions, mites, ticks, and horseshoe crabs. Yet this lineage spans an array of rarely encountered and unusual groups, such as sea spiders (Pycnogonida), hooded-tick spiders (Ricinulei), and palpigrades (Palpigradi). The extant diversity of chelicerates is divided into 14 orders, with 2 marine groups (horseshoe crabs and sea spiders) and 12 terrestrial orders (collectively known as the arachnids). The rich fossil history of Chelicerata includes some extinct orders, including some of the largest known arthropod taxa (e.g., Eurypterida). Molecular dating based on this fossil record has supported an ancient diversification of Chelicerata that began in the Cambrian (Lozano-Fernández et al. 2020; Ballesteros et al. 2021).

In the context of evolutionary developmental biology (evo-devo), chelicerates have been targeted as exemplars in comparative analyses, largely due to their phylogenetic position (Figure 4.1). Upon the resolution of a historical debate over their



**FIGURE 4.1** Phylogeny of the chelicerate orders based on phylogenomic datasets and rare genomic changes, following Ontano et al. (2021). Numbers represent described extant species, based on Zhang (2013). Boldface text indicates orders with available genomes and/or developmental transcriptomes.

phylogenetic position within arthropods, Chelicerata are presently understood to be the sister group to the remaining Arthropoda (collectively known as Mandibulata), a result that has been robustly recovered by analyses of morphological data (Legg et al. 2013), genome-scale molecular datasets (Meusemann et al. 2010; Borner et al. 2014), and rare genomic changes (i.e., a phylogenetic data class that is typically homoplasy-free; Rota-Stabelli et al. 2011). Thus, although the majority of arthropod datasets in the evo-devo literature are drawn from insect models, polarizing developmental phenomena and establishing ancestral states toward the base of Arthropoda requires the inclusion of chelicerate data. In this regard, seminal works in arthropod evo-devo have tackled the developmental biology of spider species, focusing on reconstruction of the ancestral parasegment (Damen 2002), segment-addition mechanisms (Stollewerk et al. 2003; McGregor et al. 2008), neurogenesis (Stollewerk et al. 2001; Stollewerk 2002), and appendage development (Abzhanov and Kaufman 2000a; Prpic and Damen 2009).

## 4.2 HISTORICAL STUDIES OF CHELICERATE HOX GENES

Evo-devo datasets played an especially important role in understanding body plan evolution in chelicerates and mandibulates, specifically through the lens of Hox gene expression surveys (Damen et al. 1998; Telford and Thomas 1998; Damen and Tautz 1999; Popadic and Nagy 2001; reviewed by Hughes and Kaufman 2002a). Hox expression data were historically brought to bear upon the arthropod head problem,

which pertains to the homology of the anterior-most arthropod head segments. Whereas the mandibulate head consists of six segments and only bears sensory and feeding appendages, the anterior tagma of Chelicerata (the prosoma) typically comprises seven segments, which harbor feeding, sensory, and locomotory appendages; the prosoma is therefore more comparable to, and often synonymized with, a cephalothorax. Furthermore, it was previously thought that the brain of Mandibulata was tripartite, whereas the chelicerate brain putatively consisted only of two segmental ganglia, due to the loss of the deutocerebral (mid-brain) segment (reviewed by Popadic et al. 1998). This bipartite arrangement was considered comparable to Onychophora (velvet worms), the sister group of Arthropoda (Meusemann et al. 2010; Borner et al. 2014; Laumer et al. 2019). The alignment and homology of head segments of extant chelicerates, mandibulates, and various extinct groups was therefore unclear.

The first expression surveys of anterior Hox genes in a spider and a mite resolved the homology of the anterior-most segments (Damen et al. 1998; Telford and Thomas 1998). It was shown that both chelicerate exemplars retained the deutocerebrum, which innervates the chelicerae (feeding appendages). These works inferred that the cheliceral segment was homologous to the antennal segment of insects (or the first antennal segment of “crustaceans”); the positional homology of the deutocerebral segment was substantiated by both (1) the absence of Hox gene expression (in both chelicerates and mandibulates, Hox expression commences in the tritocerebral segment), and (2) the innervation of the anterior-most pair of appendages by the deutocerebrum.

Similarly, Hox gene data were effectively used to address the homology of anterior segments of sea spiders. The anterior-most pair of appendages of Pycnogonida (the chelifores) were previously thought to be innervated by the protocerebrum, based upon immunohistochemical investigations of the sea spider protonymphon larva (Maxmen et al. 2005). This proposed alignment of segments would make the chelifores homologous to the “great appendages” of extinct groups like megacheirans and Radiodonta (e.g., the raptorial anterior appendages of anomalocaridids). However, surveys of Hox gene expression showed that the sea spider chelifore, like the deutocerebral appendages of other arthropods, are innervated by the second head segment ganglia and also lack Hox gene expression (Jager et al. 2006). This interpretation was subsequently validated by reexamination of sea spider larval neuroanatomy (Brenneis et al. 2008).

While Hox gene surveys proved useful for establishing an understanding of the chelicerate bauplan, these early works were limited in two ways. First, the sampling of Hox gene expression was taxonomically restricted to two orders: spiders, initially represented by the araneomorph species *Cupiennius salei* and *Steatoda triangulosa* (Damen et al. 1998; Abzhanov and Kaufman 2000a), and acariform mites, represented by the model species *Archegozetes longisetosus* (Telford and Thomas 1998). Occasional works addressing Hox expression of groups like sea spiders, scorpions, and horseshoe crabs generated data only for a subset of the Hox genes, either due to peculiarities of these groups’ ontogeny (e.g., the protonymphon larva of sea spiders has only four segments; Jager et al. 2006) or limited availability of cross-reactive antibodies for immunohistochemical assays specific to Hox genes (e.g., application

of UbdA antibody to survey *Ubx* boundaries in scorpions and horseshoe crabs; Popadic and Nagy 2001). Initially, limitations in taxonomic sampling had little impact on the Hox survey literature, as the goal was to establish chelicerate datasets as points of comparison to mandibulates, with emphasis on insects (Beeman 1987; Averof and Patel 1997; Abzhanov and Kaufman 2000b; Brown et al. 2002; Hughes and Kaufman 2002b; Angelini and Kaufman 2005; Brena et al. 2006; Janssen and Damen 2006). Nevertheless, the limited representation of chelicerates as model systems for comparative development hindered exploration of body plan disparity within diverse groups like the arachnids.

In addition to the narrow taxonomic scope of early studies, sampling of Hox gene expression was limited by the methodology available at the time. Acquisition of chelicerate Hox data relied heavily upon degenerate primer design, RACE PCR, and cloning for sequencing (reviewed by Hughes and Kaufman 2002a). Such Hox surveys risked omitting some genes in the event of failed amplifications, nor could such works guarantee that all Hox genes present in chelicerates had been discovered. As a corollary of the methodology, no information was available about the architecture of the chelicerate Hox cluster. While insect genomes were becoming available at the turn of the 21st century, the first chelicerate genome was not sequenced until 2011 (*Tetranychus urticae*; Grbic et al. 2011) and the first spider genomes until 2014 (*Stegodyphus mimosarum* and *Acanthoscurria geniculata*; Sanggaard et al. 2014). Classical model systems had already provided examples of Hox cluster disruption, such as the well-known division of the *Drosophila melanogaster* cluster into Antennapedia and Bithorax complexes, or the atomization of the cluster in *Caenorhabditis elegans* and *Oikopleura dioica* (Aboobaker and Blaxter 2003; Seo et al. 2004; reviewed by Monteiro and Ferrier 2006; Duboule 2007). Across Bilateria, the Hox cluster is presently understood to be somewhat conserved with respect to spatial and temporal collinearity, despite the loss or duplication of Hox genes, or genomic rearrangements to the ancestral architecture (Ferrier and Holland 2001, reviewed by Ferrier and Minguillón 2003). The exceptions to this rule tend to comprise rapidly developing taxa that retained spatial collinearity, but not temporal collinearity (Ferrier and Holland 2001; Ferrier and Minguillón 2003; Negre et al. 2005). Given the incidence of numerous rapidly developing arachnid groups in Chelicerata (especially parasitic groups within Parasitiformes and Acariformes), it was conceivable that Hox cluster evolution would be dynamic within Chelicerata, but such ideas could not be tested in the absence of genomic resources.

Since the publication of the first chelicerate genome (Grbic et al. 2011), the advent of short-read sequencing and whole-genome projects radically altered this landscape. Beyond providing the first insights into chelicerate Hox cluster architecture, next-generation sequencing transformed the understanding of chelicerates, with the discovery of multiple whole genome duplication events within this clade, successful resolution of some parts of the chelicerate tree of life through phylogenomic datasets, and even the unusual case (for metazoans) of reciprocal illumination between genomics and phylogeny, with Hox cluster duplications informing phylogenetic placement of some chelicerate orders.

### 4.3 THE DISCOVERY OF DUPLICATED CHELICERATE HOX CLUSTERS

Though it was initially thought that Hox genes occurred as single-copy orthologs in Chelicerata, hints of Hox gene duplications in chelicerates are scattered throughout the early Hox literature. PCR surveys of the horseshoe crab *Limulus polyphemus* revealed at least 28 homeobox fragments, corresponding to one to four paralogs per Hox class (Cartwright et al. 1993). Subsequently, Abzhanov et al. (1999) extended the PCR survey approach using universal Hox primers to spiders, discovering duplicates of *proboscipedia* and *Deformed* in *Parasteatoda tepidariorum*. They inferred that a single Hox cluster likely represented the ancestral condition of Chelicerata, with a subsequent duplication in the branch subtending modern horseshoe crabs. Despite the duplications discovered in *P. tepidariorum*, Abzhanov et al. (1999) tentatively suggested that a single Hox cluster was the most parsimonious inference for spiders, given the absence of evidence for widespread paralogy in the two spider exemplars they surveyed.

Intriguingly, a nearly contemporaneous survey of Hox expression domains in the spider *C. salei* had revealed a duplication of *Ultrabithorax* as well, with the two copies exhibiting slightly differing expression domains (Damen et al. 1998). Schwager et al. (2007) reexamined the two *Ultrabithorax* copies, in addition to detailing the expression patterns of duplicates of *Deformed* and *Sex combs reduced* in *C. salei*. Schwager et al. (2007) therefore speculated that a major duplication event, possibly a Hox cluster duplication or a whole genome duplication, may have occurred in the common ancestor of spiders. However, the limited efficiency of PCR-based surveys of Hox genes obscured direct tests of Hox cluster duplication and hindered the reconciliation of Hox duplications in spiders and horseshoe crabs.

The influx of genomic resources made possible by short-read sequencing technology greatly accelerated the potential for understanding the evolution of Hox clusters in Chelicerata. The first chelicerate genome, for the acariform mite species *T. urticae*, revealed a single Hox cluster, albeit with tandem duplications of *fushi tarazu* and *Antennapedia*, and loss of *abdominal-A* (and possibly *Hox3*; Grbic et al. 2011). The loss of *abdominal-A* in acariform mites has been linked to the loss of segmentation posterior to the second opisthosomal segment, both in *T. urticae* (Grbic et al. 2011) and *A. longisetosus* (Barnett and Thomas 2013). Nevertheless, the single Hox cluster established by the *T. urticae* genome supported the inference of an unduplicated Hox cluster in the common ancestor of hexapods and chelicerates. Meanwhile, a series of increasingly sophisticated genome assemblies for horseshoe crabs strongly supported the inference of a two-fold whole genome duplication in the common ancestor of Xiphosura (Nossa et al. 2014; Kenny et al. 2016; Gong et al. 2019; Liao et al. 2019), with more recent studies suggesting a three-fold whole genome duplication, based on the occurrence of additional isolated Hox genes on smaller scaffolds (Shingate et al. 2020a, 2020b; Nong et al. 2021). RT-PCR data from *L. polyphemus* have additionally supported the interpretation that the Hox duplicates have undergone sub-functionalization, as inferred from surveys of expression levels in individual body segments (Kenny et al. 2016).

Transcriptomic and genomic data for emerging arachnid model systems gradually illuminated the scale of whole genome duplications across Chelicerata. The first developmental transcriptome of a harvestman (order Opiliones) revealed ten single-copy Hox genes in *Phalangium opilio* (Sharma et al. 2012), making harvestmen and ticks (e.g., *Ixodes scapularis*) the only known chelicerate models that reflect the ancestral Hox complement of Panarthropoda (Gulia-Nuss et al. 2016). More recently, a draft genome of this harvestman species supported the inference of a single Hox cluster, albeit with four of the ten Hox genes on small scaffolds, due to fragmentation of the assembly; the remaining six Hox genes exhibited the expected pattern of collinearity, with locations of microRNAs consistent with the panarthropod groundplan (Gainett et al. 2021).

By contrast to groups like harvestmen or mites, the first developmental transcriptome of a scorpion recovered an unexpected result; embryos of the bark scorpion *Centruroides sculpturatus* were shown to retain 19 Hox genes—two copies of each Hox gene except for *Hox3* (Sharma et al. 2014b). The body plan of scorpions is notable in that they exhibit the greatest degree of heteronomous segmentation in the opisthosoma (the posterior tagma of chelicerates); this region of their body is further divided into the mesosoma (commonly called the “abdomen”) and the metasoma (the “tail”). The scorpion opisthosoma is remarkable among chelicerates in having the largest number of appendage types within Chelicerata, with embryonic limb buds giving rise to the genital operculum, the pectines, the four pairs of book lungs, and the narrowed metasomal segments. Using whole mount in situ hybridization data, Sharma et al. (2014b) showed that the four pairs of duplicated Hox genes of *C. sculpturatus* all exhibited unique spatiotemporal expression domains, with anterior boundaries corresponding to shifts in segmental or appendage identity. These data were suggestive of subfunctionalization of duplicated Hox genes in the scorpion. The expression domains of scorpion anterior Hox genes remain unknown.

With Hox duplications encountered in Xiphosura, spiders, and scorpions, a strictly traditional view of chelicerate evolution might have inferred these phenomena to reflect three separate duplications. Morphological phylogenies of chelicerates traditionally placed Xiphosura as the sister group to Arachnida; scorpions with Opiliones near the base of the arachnid tree of life; and spiders and the other tetrapulmonate orders (Amblypygi, Uropygi, and Schizomida) constituting a distantly related lineage (Shultz 2007; Garwood and Dunlop 2014). However, the proliferation of new sequencing technologies had begun to transform approaches to molecular phylogeny around this time as well, and a recurrent result in molecular phylogenomic studies of Chelicerata was the sister group relationship of scorpions and tetrapulmonates (Regier et al. 2010; Borner et al. 2014; Sharma et al. 2014a). This clade, termed Arachnoplumonata (Sharma et al. 2014a), placed spiders and scorpions much closer than previously thought, prompting the question of whether these groups underwent a shared genome duplication (Sharma et al. 2014b). While the first genomes of spiders and scorpions had been published by that time (Cao et al. 2013; Sanggaard et al. 2014), these works focused more on the genomics of venoms and silks and did not explore comparative genome architecture. The matter of shared genome duplication was duly addressed in a comparative genomic work based on the genomes of the spider *P. tepidariorum* and the scorpion *C. sculpturatus* (Schwager et al. 2017). This

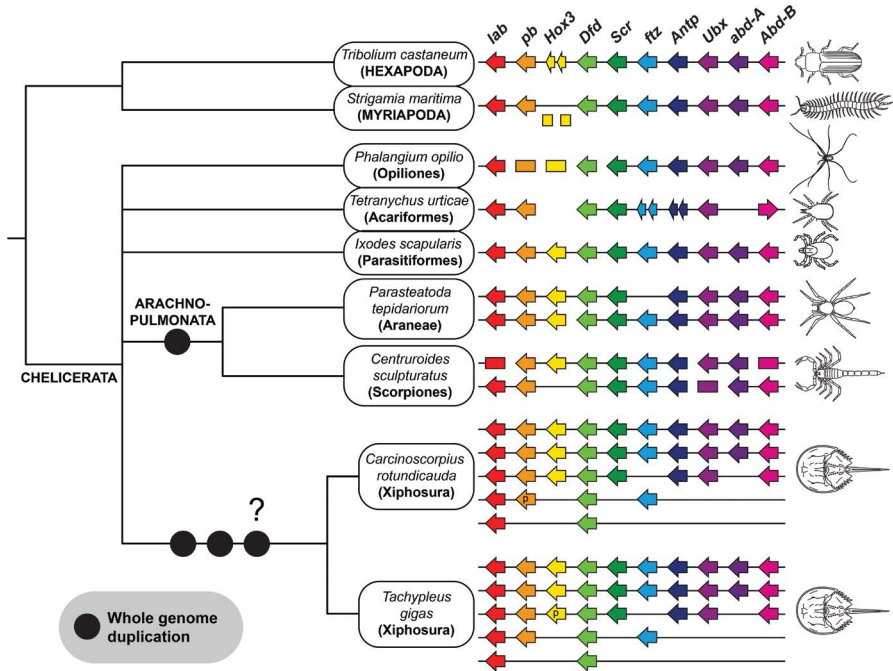
study revealed that *P. tepidariorum* retained 19 Hox genes organized on two clusters, like the 19 Hox genes of *C. sculpturatus*, albeit with some pseudoscaffolding to overcome fragmentation of the assembly (see also Pace et al. 2016; note that Schwager et al. 2017 reported 20 Hox genes in the *C. sculpturatus* genome). The expression patterns of spider Hox genes were shown to reflect similar dynamics as those of *C. sculpturatus*, and gene tree surveys across chelicerates supported the inference that spiders and scorpions shared a whole genome duplication, to the exclusion of the independent duplication events in Xiphosura (Schwager et al. 2017). Due to the lower quality of the *C. sculpturatus* genome, analyses of synteny were restricted to *P. tepidariorum*, and these showed evidence of syntenic blocks throughout the spider genome. Further support for these inferences was drawn from independent analyses of microRNA families (Leite et al. 2016) and patterns of paralogy in the homeobox gene family at large (Leite et al. 2018). More recently, chromosome-level genome assemblies of the spider species *Dysdera silvatica* and *Trichonephila antipodiana* have corroborated the inference of ancient whole genome duplication in this lineage (Sánchez-Herrero et al. 2019; Fan et al. 2021).

Recent efforts to broaden developmental genetic resources for chelicerates have further tested the inference of a shared genome duplication in the common ancestor of Arachnoplumonata. Gainett et al. (2020) generated the first developmental transcriptomes of two species of Amblypygi (whip spiders), adding a third shortly thereafter (Gainett and Sharma 2020). Ballesteros et al. (2021) similarly generated the first developmental transcriptomes of five species of Pycnogonida (sea spiders). Taken together, these works have reinforced the evolutionary scenario of an unduplicated genome in the common ancestor of Chelicerata, and whole genome duplications subtending Xiphosura and Arachnoplumonata constituting separate events. In addition, Ontano et al. (2021) generated similar resources for pseudoscorpions, with the goal of testing whether pseudoscorpions constitute derived arachnoplumonates (discussed below).

At the time of this writing, genomes or developmental transcriptomes are missing for only five chelicerate orders (one of which, Schizomida, is an arachnoplumionate; Figure 4.1). These datasets provide a refined understanding of the evolution of the Hox cluster across Arthropoda. In a comparative analysis of arthropod genomes, Pace et al. (2016) previously demonstrated that chelicerate and myriapod genomes generally tended to retain Hox genes in a single cluster, albeit with great variation across lineages with regard to the size of intergenic regions. With respect to chelicerates, that study compared the genomes of *I. scapularis*, the scorpion *Olivierus martensii* (formerly *Mesobuthus martensii*), and the acariform mites *T. urticae* and *Galendromus occidentalis*. The Hox cluster of *G. occidentalis* exhibited atomization (*sensu* Duboule 2007; Hoy et al. 2016), whereas *I. scapularis* retained a single Hox cluster, albeit with large intergenic regions. *T. urticae* exhibited a split cluster, with *labial* and *proboscipedia* separated from the rest of the Hox genes. The scorpion *O. martensii* was thought to possess two Hox clusters, but this reconstruction was impaired by the quality of the *O. martensii* assembly (Cao et al. 2013).

As shown in Figure 4.2, a revised view of Hox cluster organization across Chelicerata with recently published datasets supports the inference that the ancestral arthropod and the ancestral chelicerate Hox cluster consisted of ten genes. In





**FIGURE 4.2** Hox gene clusters in the genomes of Chelicerata and selected outgroups. Arrows represent direction of Hox transcriptional activity, where known. Circles represent whole genome duplications, with at least two occurring in the common ancestor of extant horseshoe crabs and another in the common ancestor of Arachnoplumonata. p: pseudo-gene. For simplicity, the genomes of *Limulus polyphemus*, *Dysdera silvatica*, *Trichonephila antipodiana*, and some acariform and parasitiform mites are not depicted, but these reflect dynamics conserved for their respective orders. References provided in text.

contrast to insects, wherein *Hox3* has undergone sequential tandem duplication followed by subfunctionalization, as well as neofunctionalization (i.e., the role of *zen* in dorsoventral patterning and *bicoid* as an anteroposterior morphogen), chelicerate genomes tend not to exhibit a history of ancient and shared tandem Hox duplicates (note that the duplications of *fushi tarazu* and *Antennapedia* in *T. urticae* appear to be lineage-specific). In systems where expression and genomic data are both available, it also has been shown that chelicerate Hox genes tend to retain temporal and spatial collinearity (Sharma et al. 2012, 2014b; Schwager et al. 2017).

#### 4.4 HOX GENES IN CHELICERATE PHYLOGENOMICS

In contrast to the botanical literature, it is not common that WGD events inform metazoan phylogeny. In part, this is because WGD events are comparatively rare in Metazoa. WGD events at the base of the vertebrates and the teleosts are well understood, but had little impact as arbiters of phylogenetic hypotheses, as the monophyly

of groups like Vertebrata and Teleostei were historically strongly supported by both morphological and molecular phylogenetic datasets.

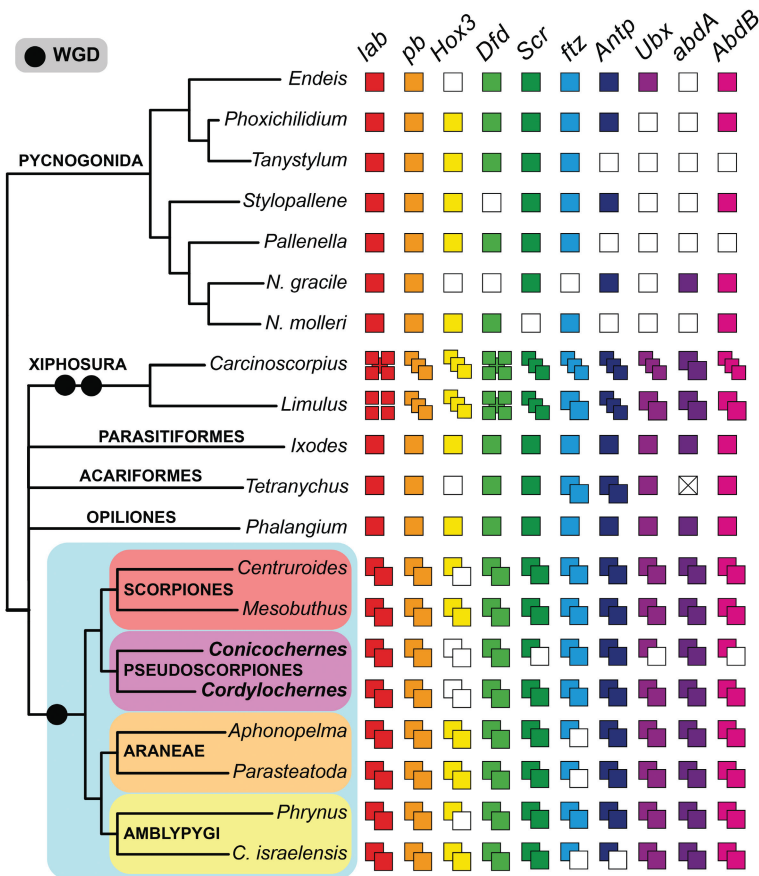
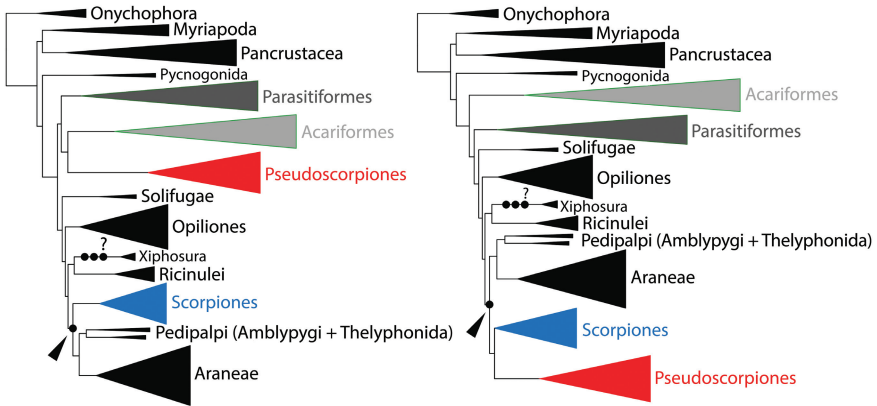
By contrast, chelicerate phylogeny remains one of the most obdurate challenges for phylogenomics. Despite the pursuit of chelicerate relationships with genome-scale datasets (Regier et al. 2010; Borner et al. 2014; Sharma et al. 2014a; Ballesteros and Sharma 2019; Ballesteros et al. 2019; Lozano-Fernández et al. 2019; Ballesteros et al. 2022), the backbone of Chelicerata is a *de facto* soft polytomy, consistent with an ancient rapid radiation (likely a Cambrian radiation; Lozano Fernández et al. 2016). Further challenges to chelicerate phylogeny come in the form of multiple fast-evolving orders that are prone to a systematic artifact called long branch attraction (LBA), a form of statistical inconsistency wherein rapidly evolving branches are artificially resolved as sister groups, with stronger support for spurious relationships despite the addition of more genes (and particularly, rapidly evolving genes). The combination of an ancient rapid radiation and LBA is especially difficult to resolve because the genes best suited to informing higher-level chelicerate relationships must evolve (1) fast enough that they capture the signature of the rapid sequence of speciation events, but also (2) slow enough that this phylogenetic signal is not eroded by saturation over much of the Phanerozoic, and (3) in a manner that evolutionary rates are homogeneous across all chelicerate orders, such that LBA is not exacerbated.

As a result, the composition of genes in a phylogenetic dataset, the substitution model, and the algorithmic approach all strongly affect the inference of chelicerate relationships (Sharma et al. 2014a). Chelicerate phylogeny is contentious, with historical and recent molecular matrices disputing the status of arachnid monophyly, due to the nested placement of Xiphosura within the arachnids (Wheeler and Hayashi 1998; Giribet et al. 2002; Ballesteros and Sharma 2019). It was previously shown that genes supporting arachnid monophyly are in the minority across phylogenetic datasets and these genes do not exhibit statistically better properties (e.g., saturation rate; taxon occupancy; compositional heterogeneity) than genes recovering horseshoe crabs as derived arachnids (Sharma et al. 2014a; Ballesteros and Sharma 2019). By contrast, Lozano-Fernández et al. (2019) discovered a matrix supporting arachnid monophyly when analyzed under a particular approach, but this result was sensitive to model, taxon, and matrix choice. One of the shortcomings of these previous works was that they lacked representation of all extant chelicerate orders, namely, miniaturized groups like palpigrales and schizomids. These taxa were sequentially added to phylogenomic matrices in later works (e.g., Ballesteros et al. 2019; Howard et al. 2020). However, the recovery of arachnid monophyly remained contentious, with some works unable to recover this relationship altogether (e.g., Ballesteros et al. 2019; Noah et al. 2020; Ontano et al. 2021; Ballesteros et al. 2022; Ban et al. 2022) and others able to recover it only with certain models and upon excluding some arachnid orders (e.g., Howard et al. 2020). While the monophyly of Arachnida continues to be widely accepted in the literature, the most comprehensive phylogenetic datasets of Chelicerata to date have been unable to recover this relationship and have observed little evidence that arachnid non-monophyly is attributable to an artifact (Ballesteros et al. 2022). Apart from the monophyly of Chelicerata, Euchelicerata (the non-Pycnogonida chelicerates), Tetrapulmonata (spiders and four other arachnid orders that plesiomorphically bear

four book lungs), and relationships within the tetrapulmonates, few higher-level nodes in the chelicerate tree of life have been satisfactorily resolved.

Given these disputes and the inherent recalcitrance of the basal phylogeny of chelicerates in the phylogenomic era, it may be surprising that any further progress has been made in chelicerate phylogeny. Nevertheless, Hox (and other developmental patterning) genes have played a central role in resolving two additional nodes within Chelicerata. The first phylogenomic analyses of chelicerates recovered the sister group relationship of Scorpiones + Tetrapulmonata with strong support (Regier et al. 2010; Sharma et al. 2014a). This clade, Arachnoplumonata (*sensu* Sharma et al. 2014a), was united by the presence of book lungs (eight book lungs occur in scorpions). This relationship was initially considered counterintuitive, given the historical interpretation that scorpions were the sister group of the remaining arachnids (Weygoldt and Paulus 1979; but see Shultz 1990, 2007). The discovery of duplicated Hox genes in scorpions as well as spiders, together with Hox expression data, strongly suggested a shared rare genomic change uniting these taxa (Schwager et al. 2007; Sharma et al. 2014b), to the exclusion of groups like mites and harvestmen (Telford and Thomas 1998; Sharma et al. 2012). This paralogy was shown to be systemic, affecting the rest of the homeobox family, as well as miRNA families (Leite et al. 2016, 2018). The extent of this shared rare genomic change was revealed through the genomes of the model spider *P. tepidariorum* and scorpion *C. sculpturatus* (Schwager et al. 2017). Additional expression data and gene tree analyses from non-Hox homeobox genes (e.g., *extradenticle*, *homothorax*) from spider, scorpion, and harvestman exemplars supported the inference of a systemic duplication, followed by ancient subdivision of spatiotemporal domains (Pechmann and Prpic 2009; Turetzek et al. 2017; Nolan et al. 2020). Taken together, the systemic evidence of the shared genome duplication served as a complex phylogenetic meta-character, potentially consisting of hundreds or thousands of synapomorphies; postulating parallel or convergent acquisition of these genomic and developmental traits in tetrapulmonates and scorpions became an untenable position.

Drawing upon this approach, Ontano et al. (2021) reexamined the position of Pseudoscorpiones, one of three fast-evolving arachnid orders that are highly prone to LBA. Across phylogenomic datasets, pseudoscorpions typically clustered with Acariformes or Parasitiformes, another pair of long-branch orders (Figure 4.3). However, certain subsets of phylogenomic matrices, especially those filtered for slowly evolving genes, would recover pseudoscorpions within Arachnoplumonata, as the sister group to scorpions (Figure 4.3). Reasoning that a placement within Arachnoplumonata would be evidenced by the signature of whole genome duplication, Ontano et al. (2021) generated the first developmental transcriptome and draft genome for Pseudoscorpiones and examined these for the retention of ancient paralogs. To these surveys, they added the first developmental transcriptomes for Pycnogonida (Ballesteros et al. 2021a), Amblypygi (Gainett and Sharma 2020; Gainett et al. 2020), and a mygalomorph spider (Setton et al. 2019). Consistent with the placement of pseudoscorpions within Arachnoplumonata, Ontano et al. (2021) discovered in the developmental transcriptome of the species *Conicochernes crassus* (1) two paralogs of every Hox gene except for *Hox3* (Figure 4.3), (2) retention of 40% of homeobox gene duplicates that were shared with at least one other



**FIGURE 4.3** Hox gene duplications as rare genomic characters in chelicerate phylogeny. Top: Two competing hypotheses of pseudoscorpion placement, either clustering with other long-branch orders (top left) or with scorpions (top right), as a function of internal taxonomic sampling. Arrowhead indicates the location of the arachnoplumate WGD. Bottom: Incidence of Hox genes across Chelicerata. Note the duplicated Hox genes of pseudoscorpions, consistent with the placement of this group within arachnoplumates. Multiple icons on branches subtending Xiphosura represent a likely three-fold whole genome duplication event in the common ancestor of the four extant species. (Modified from Ontano et al. 2021).

arachnoplumonate order, and (3) retention of duplicates of all leg-patterning genes with known arachnoplumonate-specific division of spatiotemporal expression patterns (Turetzek et al. 2017; Nolan et al. 2020). These inferences of shared duplication were substantiated by the topologies of gene trees, particularly of leg-patterning genes. Despite the degree of fragmentation of the draft genome of *Cordylochernes scorpioides*, Ontano et al. (2021) also discovered the retention of duplicated miRNA families previously shown to be duplicated in spiders and scorpions (Leite et al. 2016), further supporting the inference that pseudoscorpions constitute derived arachnoplumonates. Intriguingly, phylogenomic analyses conducted by Ontano et al. (2021) showed that the clade Pseudoscorpiones + Scorpiones could be consistently recovered by traditional molecular matrices, but only if pseudoscorpions were densely sampled (effectively, breaking the long branch subtending this order).

The work of Ontano et al. (2021) was able to leverage Hox gene duplications (a *de facto* readout of whole genome duplication) as an arbiter of phylogenetic placement, resolving a relationship that traditional phylogenomic methods could not redress conclusively. While such uses of partial or whole genome duplication are well known in botanical and mycological phylogenomics (e.g., Salichos and Rokas 2013; Leebens-Mack et al. 2019), they are rarer in the metazoan literature, due to the comparative scarcity of WGD events in Metazoa. Notably, an unusual change in the sequence of Hox6 (*fushi tarazu*) was similarly used to argue for the placement of chaetognaths, another enigmatic taxon, within Gnathifera (Fröblius and Funch 2017), with subsequent validation from phylogenomic analyses (Marlétaz et al. 2019).

The utility of Hox gene duplications as phylogenetic characters in Chelicerata may not yet be fully expended. It is generally presumed that the shared whole genome duplication initially identified in spiders and scorpions is restricted to Arachnoplumonata (i.e., the common ancestor of these two orders). This is substantiated by the absence of systemic paralogy (e.g., duplicated Hox clusters) in Acariformes (Grbic et al. 2011), Parasitiformes (Pagel Van Zee et al. 2007; Hoy et al. 2016; Gulia-Nuss et al. 2016), and most recently, the first Opiliones genome (Gainett et al. 2021). In fact, it is entirely possible that the putative “arachnoplumonate duplication” occurred more deeply in the tree. Currently, developmental transcriptomes and genomes are missing for the arachnid orders Solifugae (sun spiders), Ricinulei (hooded tick spiders), and Palpigradi (microw hip scorpions)—all three of which exhibit unstable phylogenomic placements (Ballesteros and Sharma 2019; Ballesteros et al. 2019; Ballesteros et al. 2022). The hypothetical discovery of shared genome duplication between arachnoplumonates and any of these poorly studied trio of orders would similarly affix them as part of a strongly supported clade in the chelicerate tree of life. Given that some phylogenomic analyses consistently recover Ricinulei as the sister group of horseshoe crabs (which exhibit their own independent whole genome duplication events), developmental transcriptomes and genomes for this order are of especially high priority for higher-level chelicerate phylogeny. Specifically, the hypothetical discovery of rare genomic changes uniting Ricinulei (or other arachnid orders) with Xiphosura would effectively sound the death knell of the Arachnida concept.

#### 4.5 IMPACT OF GENE DUPLICATIONS ON DIVERSIFICATION DYNAMICS

The evolutionary significance of whole genome duplications is that a swath of new genes and modules (e.g., Hox genes; MADS-Box genes) is thought to provide new potential for diversification—either in terms of speciation or complexity of body plans and gene regulatory networks (Ohno 1970; Wagner 1994; Taylor and Raes 2004; Magadum et al. 2013). The majority of genes is typically lost after a whole genome duplication, with the probability of gene loss estimated around 80% in vertebrate genomes (Dehal and Boore 2005; Pasquier et al. 2017). However, a subset of retained duplicated genes can undergo subfunctionalization or neofunctionalization, in addition to simply being retained as two “similar” copies (Lynch and Conery 2000). As a result, gene family expansions and genome duplications are associated with evolvability (Holland et al. 1994; Conant and Wagner 2003; Wagner et al. 2003; Irish and Litt 2005) and increased rates of evolution via the unlocking of developmental innovations (Irish 2003; Fried et al. 2004; Wagner et al. 2005; Pasquier et al. 2017).

Despite the intuitive logical bridge between genome duplication and evolvability, a clear link between gene duplication and diversification (either of species-level diversity or of morphology) has not been well established. Much of the debate concerning this correlation has focused on the vertebrate and angiosperm literature (Meyer and Schartl 1999; Zhou et al. 2001; Donoghue and Purnell 2005; Crow and Wagner 2006). Teleost fishes exhibit the highest proportion of species-level diversity within the vertebrates, concomitant with a cumulative three-fold whole genome duplication (Crow et al. 2005). However, as a group, vertebrates are greatly outnumbered by lineages with single Hox clusters, such as nematodes and insects. Outgroups to vertebrates, such as echinoderms or mollusks, exhibit much greater disparity of body plans and no dearth of morphological innovations (Crow and Wagner 2006). Moreover, at a more granular level, the impact of gene family expansion on diversification rate is not always intuitive, even in the case of gene families thought to bear directly upon the ecological success of organisms. As an example, it was previously thought that recent expansion of neurotoxins in *Conus* snails, together with diversifying selection, had facilitated the rapid diversification of this group (Duda and Palumbi 1999). However, a recent investigation of *Conus* venom complexity showed no clear statistical association between toxin gene diversity and speciation rate (Phuong et al. 2019). By contrast, in plants, multiple episodes of polyploidization have been identified across the angiosperm tree of life (Soltis and Soltis 1999; Soltis et al. 2009), and it has long been thought that there is a positive association between species richness and incidence of polyploidy (Otto and Whitton 2000; Vamossi and Dickinson 2006; Walden et al. 2020; but see Wood et al. 2009; Mayrose et al. 2011). Crow and Wagner (2006) proposed that heightened net diversification rates in the wake of an angiosperm whole genome duplication may reflect different mechanisms that reduce risks of lineage extinction, such as functional redundancy of gene copies, robustness to deleterious mutations, and increased potential for adaptation.

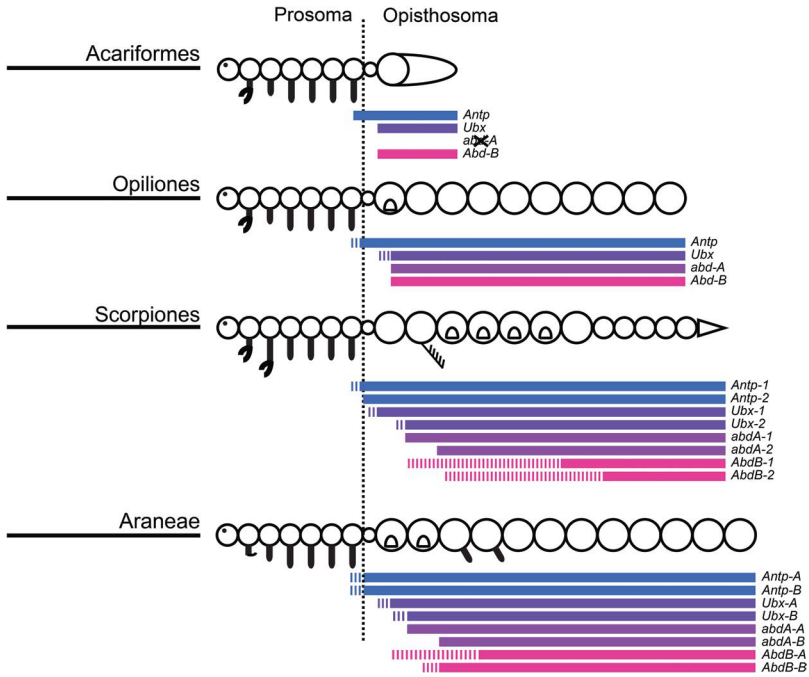
The identification of multiple whole genome duplication events in Chelicerata offers badly needed data points within Metazoa for understanding the impact of gene duplication on net diversification rate. Described species richness in Chelicerata is

strongly asymmetrical, with the largest orders (Araneae; Acariformes) dwarfing the smallest (Xiphosura; four extant species) by four orders of magnitude (Figure 4.1). Comparison of these lineages' extant species richness immediately disfavors the interpretation that whole genome duplication is causally linked to speciation rate or morphological disparity; horseshoe crabs, despite a likely threefold whole genome duplication, are the epitome of low net diversification rate, high lineage turnover, and external morphological stasis. The relatively recent estimated age of at least one of the Xiphosura duplications (Cretaceous; Obst et al. 2012), as compared to the arachnoplumonate duplication event (pre-Silurian; Schwager et al. 2017), does not mitigate this observation; the horseshoe crab genome duplications are older than most of the angiosperm whole genome duplication events, giving this relictual arthropod lineage sufficient time for morphological innovation after rapid accrual of new genes. The fossil record also discourages extending to Xiphosura the mechanisms for lowered extinction rate proposed by Crow and Wagner (2006); fossils of Xiphosura show that this lineage included a large number of species and genera since their appearance in the Ordovician, but this record does not support a post-Mesozoic increase in net diversification rate.

Within the terrestrial chelicerates, the correlation between species richness and a history of whole genome duplication (i.e., membership in the clade Arachnoplumonata) buckles further. Spiders (ca. 50,000 spp.) are slightly more diverse than acariform mites (ca. 42,000 spp.) with respect to the number of described species (Zhang 2013), but it is likely that the true diversity of Acariformes is much greater. Within arachnoplumonates, three smaller orders (Amblypygi, Uropygi, and Schizomida) each include fewer than 300 species, whereas pseudoscorpions (ca. 4,000 spp.) and scorpions (ca. 2,400 spp.) are comparatively larger groups. Outside of arachnoplumonates, diverse orders include the aforementioned Acariformes as well as Parasitiformes (ca. 15,000 spp.) and Opiliones (ca. 7,000 spp.). But the apulmonate arachnids also include taxa like Palpigradi and Ricinulei, small orders with less than 100 described species. These patterns suggest no clear correlation between whole genome duplications and extant diversity.

The link between whole genome duplication and body plan disparity (and/or complexity) is also disfavored by macroevolutionary patterns within Chelicerata. Spiders exhibit marked body plan disparity and an array of evolutionary innovations that are anatomical, biochemical, and behavioral (e.g., venoms; silks; web morphology). But the body plan disparity of mites is no less complex, with specific reference to their mouthparts, cuticular ornamentation, and appendage modifications (Evans 1992). Inversely, arachnoplumonate groups like Amblypygi and Uropygi exhibit conserved patterns of morphological evolution (without major differences in body plan organization across families), whereas Opiliones exemplify comparatively greater body plan disparity, with constituent lineages exhibiting remarkable modifications of specific appendage pairs, patterns of dorsal sclerotization, and a plethora of sexually dimorphic traits.

What impact, then, did Hox gene duplication have on the evolution of Arachnoplumonata? In the case of posterior patterning of scorpions, Sharma et al. (2014b) contended that the duplication of Hox genes and their subsequent subfunctionalization was essential to establishing the heteronomous segmentation and novel appendage identities of the scorpion opisthosoma. This hypothesis, while consistent with the expression patterns of scorpion posterior Hox genes, cannot be tested further at



**FIGURE 4.4** Hox gene duplications correlate with complexity (heteronomous segmentation) of the chelicerate opisthosoma (the posterior tagma). References provided in text.

present due to the lack of functional tools in *C. sculpturatus*. Intriguingly, a comparable set of expression dynamics, as shown in [Figure 4.4](#), occurs during embryogenesis in *P. tepidariorum*, with each of the 19 Hox genes exhibiting unique spatiotemporal domain boundaries (Schwager et al. 2017). As a result, a unique combination of Hox transcripts occurs in the first five opisthosomal segments, which consist of the pedicel (the reduced stalk-like segment connecting the prosoma to the opisthosoma), the first and second pairs of respiratory organs, and the first and second pairs of spinnerets. One possibility is that the differentiation of the spinnerets, which vary widely in structure, number, and arrangement across the spider tree of life, was a key step to unlocking the evolutionary potential of this highly successful group of chelicerates. Specifically, duplicates of the Hox genes *Ultrabithorax* and *abdominal-A*, whose anterior boundaries span the second through the fifth opisthosomal segments, must be investigated functionally in the context of spinneret-bearing segment fate specification. Such an investigation is imperative for linking the evolutionary origins of spiders with the deployment of duplicated Hox genes. As a corollary, the expression patterns of these genes must also be investigated in tetrapulmonates like Uropygi and Amblypygi, which bear respiratory organs on the same segments as spiders, but lack paired ventral organs on the remaining posterior segments. The establishment of the first developmental genetic resources for a whip spider species proffers a ready avenue for such a comparative investigation (Gainett and Sharma 2020). Overall, a revitalized and concerted effort to understand the functions of every Hox gene of model species like *P. tepidariorum* and *P. opilio* is sorely needed. Such



an effort is imperative for testing hypotheses about the role of sub-/neofunctionalized Hox gene duplicates as drivers of developmental and body plan innovations in Arachnoplumonta.

#### 4.6 STUDIES OF HOX FUNCTION IN CHELICERATA

Within arthropods, broad understanding of Hox cluster architecture, gene function, and regulation is largely informed by datasets from insects (e.g., Lewis 1978; McGinnis et al. 1984; Merrill et al. 1987, 1989; Brown et al. 2002; Angelini et al. 2005; Shippy et al. 2008) and the crustacean model *Parhyale hawaiensis* (Liubicich et al. 2009; Pavlopoulos et al. 2009; Martin et al. 2016). By contrast, functional data are comparatively limited in chelicerates and altogether nonexistent for Myriapoda. A seminal work in the chelicerate Hox literature first addressed the role of *Antennapedia* (*Antp*) in patterning opisthosomal identity in the spider *P. tepidariorum*. Khadjeh et al. (2012) showed that knockdown of *Antp* (via maternal RNA interference [RNAi]) resulted in an ectopic appendage pair on the first opisthosomal segment (the pedicel), whereas double-knockdown of *Antp* and *Ultrabithorax* (*Ubx*) resulted in ectopic appendages on both the first and second opisthosomal segments (with the latter bearing a small, rudimentary appendage bud). These ectopic appendages expressed both *Deformed* (*Dfd*) and *Sex combs reduced* (*Scr*), which are associated with the walking legs in wild type spider embryos; these results suggested that *Antp* and *Ubx* suppressed the expression of prosomal Hox genes in the opisthosoma. Khadjeh et al. (2012) concluded that the function of spider *Antp* reflected convergence with respect to the function of insect *Ubx* (i.e., repressing appendages on posterior segments). While a triple knockdown of *Antp*, *Ubx*, and *abdA* was trialed, this experiment did not yield a phenotype discernible from the *Antp+Ubx* double knockdown.

At the time, the scale of Hox gene duplications was not known in *P. tepidariorum*; a previous work had reported the duplication of some Hox genes, but not *Antp* (Abzhanov et al. 1999). The Hox genes analyzed by Khadjeh et al. (2012) corresponded to the “-A” copies designated by Schwager et al. (2017). It is presently unknown how knockdown of *Antp-A* affected its paralog (*Antp-B*), as well as *Dfd-B* and *Scr-B*. Subsequently, Pechmann et al. (2015) showed that knockdown of *labial-1* (*lab-A*, *sensu* Schwager et al. 2017) resulted in the loss of the pedipalps and first walking leg, and the diminution of those appendages’ segments. At the time of that study, the existence and expression pattern of the *lab-2* paralog was known, but RNAi against *lab-2* did not result in a phenotype. Moreover, knockdown of *lab-1* did not abrogate the expression of *lab-2* or *Dfd* in the walking leg segments. In that same work, Pechmann et al. (2015) showed that knockdown of *Dfd* (*Dfd-A*, *sensu* Schwager et al. 2017) resulted in the homeotic transformation of the first walking leg into pedipalpal identity, with corresponding ectopic expression of *lab-1* in the transformed first walking leg. Pechmann et al. (2015) interpreted these data to mean that *lab-1* was necessary for tissue development in the pedipalpal and first walking leg segments, as well as for establishing pedipalpal fate. Curiously, no homeotic pedipalp-to-chelicera transformations were recovered in the phenotypic spectrum for *lab-1*. Pechmann et al. (2015) suggested that these data closely paralleled the dynamics of *lab* in the fruit fly *D. melanogaster*, wherein *lab* mutants exhibit defects in head

involution during embryogenesis, but not homeosis (Merrill et al. 1989); homeotic transformations are only observed in the posterior adult head of the fruit fly for a subset of hypomorphic *lab* alleles. By contrast, in the milkweed bug *Oncopeltus fasciatus*, neither maternal nor embryonic RNAi against *lab* resulted in a phenotype. Pechmann et al. (2015) postulated that a homeotic function for *lab* in the chelicerate head may occur later in embryogenesis, but this observation could not be tested due to embryonic lethality incurred by *lab-1* knockdown in developing spiders.

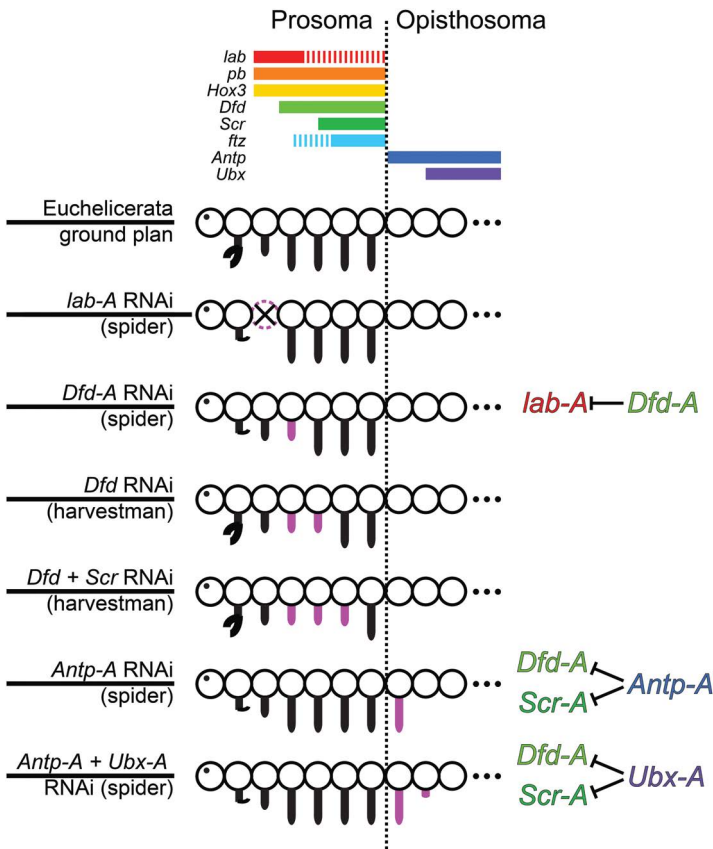
To date, these two works remain the only published studies of Hox function in spiders. Knockdowns of *P. tepidariorum* *Dfd-B*, *pb*, and *Scr* have been trialed in unpublished experiments, but resulted in no phenotypes (M. Pechmann, *personal communication*). I previously attempted to knockdown the *P. tepidariorum* paralogs of *Abdominal-B* (both individually and targeting the two copies simultaneously), but these experiments similarly yielded no phenotypes, either in my hands or in a colleague's (E.E. Schwager, *personal communication*). Thus, of the 19 Hox genes of *P. tepidariorum*, functional data are only available for four.

Part of the difficulty may be related to the penetrance of RNAi via maternal injection of double-stranded RNA in *P. tepidariorum*. It is generally known that numerous candidate genes will frequently not yield discernible loss-of-function phenotypes in this species, with estimates of knockdown success ranging from 5% to 20% across experimental screens (Y. Akiyama-Oda and H. Oda, *personal communication*; M. Pechmann, *personal communication*; E.E. Schwager, *personal communication*). Genes that are strongly maternally expressed are thought to be more resistant to knockdown, as are genes that exhibit consistently high expression levels late in embryogenesis (Pechmann et al. 2011; N.M. Prpic-Schäper, *personal communication*), whereas one research team that has pioneered the understanding of chelicerate appendage development has experienced greater success with RNAi (~80% of genes yielding discernible phenotypes; N.M. Prpic-Schäper, *personal communication*). Embryonic injection is feasible in *P. tepidariorum*, but is technically challenging, with typical experiments exhibiting lower rates of penetrance than successful maternal injections (e.g., Pechmann et al. 2011). For genes that retain their duplicates, it is also possible that knockdown of one paralog is rescued by the compensatory function of the other (or that the two copies exert combinatorial effects), though few empirical cases of this phenomenon have been published in chelicerates (Benton et al. 2016).

The recently established harvestman model system *P. opilio* offers a chelicerate exemplar with an unduplicated genome and the ancestral complement of ten Hox genes. Notably, whereas the walking legs of *P. tepidariorum* are morphologically very similar at the completion of embryogenesis, individual leg pairs of *P. opilio* are distinguished from one another by their relative lengths and the unique number of tarsomeres (subdivisions of the last leg segment) of each leg pair. Gainett et al. (2021) recently leveraged these features of harvestman biology to address the functions of *Dfd* and *Scr* via embryonic RNAi. They found that knockdown of *Dfd* resulted in homeotic transformations of the first and second walking legs to pedipalpal identity. This contrasts with the function of spider *Dfd-A*, which only affects the first walking leg (Pechmann et al. 2011). The discrepancy may reflect subfunctionalization of the two spider *Dfd* copies, although a test of this hypothesis via double knockdown of *Dfd-A* and *Dfd-B* has not yet been performed to my knowledge.

Gainett et al. (2021) also found that knockdown of harvestman *Scr* had no discernible impact on prosomal patterning. To test for the possibility of functional redundancy with *Dfd*, they performed a double knockdown of both *Dfd* and *Scr*. This experiment resulted in homeotic transformation of the first three walking leg pairs into pedipalpal identity, suggesting that both *Dfd* and *Scr* are necessary for the specification of third walking leg identity in chelicerates. These dynamics closely parallel the functional redundancy of other pairs of adjacent Hox genes in arthropods (e.g., *Ubx* and *abdA* in suppressing appendage identity in the insect abdomen; *Antp-A* and *Ubx-A* in suppressing appendage identity in the spider opisthosoma; Angelini and Kaufman 2005; Khadjeh et al. 2012).

Outside of these few data points (Figure 4.5), studies on the function of chelicerate Hox genes remain unexplored. Capitalizing upon the advent of CRISPR-Cas9-mediated mutagenesis in mites (Dermauw et al. 2020) and ticks (Sharma et al. 2022),



**FIGURE 4.5** Summary of Hox RNAi experiments in Chelicerata and known regulatory interactions (right of corresponding experiment). While expression domains of prosomal Hox genes are shown for reference, note that no functional data exist for chelicerate homologs of *pb*, *Hox3*, or *ftz*. Purple icons indicate structures affected by homeosis. References provided in text.

Sharma et al. (2022) recently trialed knockouts of *pb*, *Antp*, and the leg patterning gene *dachshund* in *I. scapularis*. Oddly, while on-target mutagenesis of these target genes was demonstrated through genotyping by sequencing, *dac* and *Antp* mutants of *I. scapularis* exhibited no morphological phenotypes. For the *pb* experiment, Sharma et al. (2022) reported that a small number of mutant ticks exhibited an enlarged hypostome (cheliceral complex). This phenotype is unexpected and counter-intuitive because arthropod Hox genes are not expressed in the deutocerebral segment (the chelicera-bearing segment) in early development (Damen et al. 1998; Telford and Thomas 1998; Jager et al. 2006; Sharma et al. 2012). Neither the absence of phenotypes in the *Antp* and *dac* experiments, nor the effect of *pb* knockout on the chelicerae, are easily explained in this study. One possibility is that the categorization of the phenotypic spectrum may have been impacted by the decision to score these experiments only at hatching; it is possible that Sharma et al. (2022) did not observe a considerable portion of knockout phenotypes that led to embryonic lethality. As examples, severe RNAi phenotypes of *lab-1* in the spider, or of the Hox co-factor *homothorax*, do not survive to hatching at all (Pechmann et al. 2015; Sharma et al. 2015).

Regardless, future studies must endeavor to fill in the gaps in the knowledge of chelicerate Hox function, as a prerequisite to understanding gene regulation, especially in groups with duplicated Hox clusters. The condition of single-copy Hox genes in the harvestman, together with the high rate of penetrance of embryonic RNAi in *P. opilio* (80% of trialed genes, across experiments), makes this system a promising platform for illuminating the Hox logic of the chelicerate body plan, as well as examining the regulatory architecture of chelicerate Hox genes that retain the ancestral unduplicated condition.

## 4.7 CONCLUSION

The field of chelicerate evo-devo and phylogenomics, and the attendant understanding of Hox cluster evolution in this lineage, is quintessentially a celebration of the power of genomics and modern sequencing approaches. The present proliferation of ultra-long read sequencing technologies heralds the beginning of taxonomically comprehensive comparative genomic approaches to understanding Hox cluster evolution in this curious and ancient group of animals (e.g., Sánchez-Herrero et al. 2019; Fan et al. 2021). Such approaches break the impasse imposed by small-bodied, rare, and/or cryptic chelicerate groups like Ricinulei, Palpigradi, and a broad swath of acariform and parasitiform mite families, for which obtaining cDNA or developmental transcriptomes from embryos is improbable or practically unfeasible. Beyond informing the evolution of Hox clusters and gene families, a broader genomic representation for Chelicerata holds the potential for providing new sources of phylogenetic characters. Rare genomic changes such as WGD events, shared tandem duplications and gene family expansions, and acquisition of microRNAs may adjudicate between competing hypotheses at the base of the euchelicerate radiation (i.e., the relationships of Xiphosura and the apulmonate arachnid orders), which constitutes one of the most recalcitrant soft polytomies in the metazoan tree of life.

Yet, advances in genomics must be paralleled by concomitant developments in functional genetics and expansion of toolkits in chelicerate emerging model

organisms. The recent advent of Cas9-mediated gene editing in a mite and a tick model are promising developments for chelicerate evo-devo. Adapting such approaches may circumvent limits to phenotypic penetrance and knockdown efficiency posed by dsRNA-mediated RNA interference in the spider *P. tepidariorum*. A high-value target for future research efforts remains an understanding of regulatory interactions between Hox genes of the same cluster *versus* regulation across clusters in groups like spiders and scorpions.

The discovery of the chelicerate WGD events, together with the establishment of new emerging model organisms and the proliferation of genomic and functional resources in the decade since the first chelicerate genome (Grbic et al. 2011), bespeak an ideal study system for deciphering the genetic and evolutionary consequences of gene duplications in a variety of developmental contexts. It is all the more propitious that the age of the arachnoplumonate duplication is approximately equal to those at the base of the vertebrate tree; future comparisons of chelicerate and vertebrate genomic and functional datasets may lead to further insights and emergent patterns of the consequences of metazoan WGD events. Taken together, the next decade of revitalized research on the comparative development, genomics, and functional genetics of Chelicerata will likely be one of transformative insights that stem from a once poorly-studied group of arthropods.

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