Chelicerata

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INTRODUCTION

Chelicerate Phylogenetics

Chelicerata is a subphylum of arthropods that includes terrestrial as well as marine animals. Both the fossil record and molecular data place the origin of the chelicerates over 500 million years ago in the Cambrian (e.g., see Dunlop 2010; Rota-Stabelli et al. 2013). It has been shown that the chelicerates are a monophyletic group, and although they have previously been grouped together with the myriapods as Myriochelata, it is generally accepted that chelicerates represent the sister group of Mandibulata (pancrustaceans and myriapods) (Friedrich and Tautz 1995; Cook et al. 2001; Giribet et al. 2001; Hwang et al. 2001; Pisani et al. 2004; Dunn et al. 2008; Meusemann et al. 2010; Regier et al. 2010; Rota-Stabelli et al. 2011).

The chelicerates constitute two sister groups, the euchelicerates (Weygoldt and Paulus 1979) and the pycnogonids (sea spiders) (Fig. 5.1), which are united morphologically by the anterior-most pair of chelate appendages: the cheliceres of the former and the chelifores of the latter (reviewed by Dunlop and Arango 2005; Edgecombe 2010). This conclusion is supported by both neuroanatomy and Hox gene expression (Jager et al. 2006; Manuel et al. 2006; Brenneis et al. 2008).

There are more than 100,000 described species of chelicerates (Dunlop 2010) that can be subdivided into 14 recognised orders (Fig. 5.1;

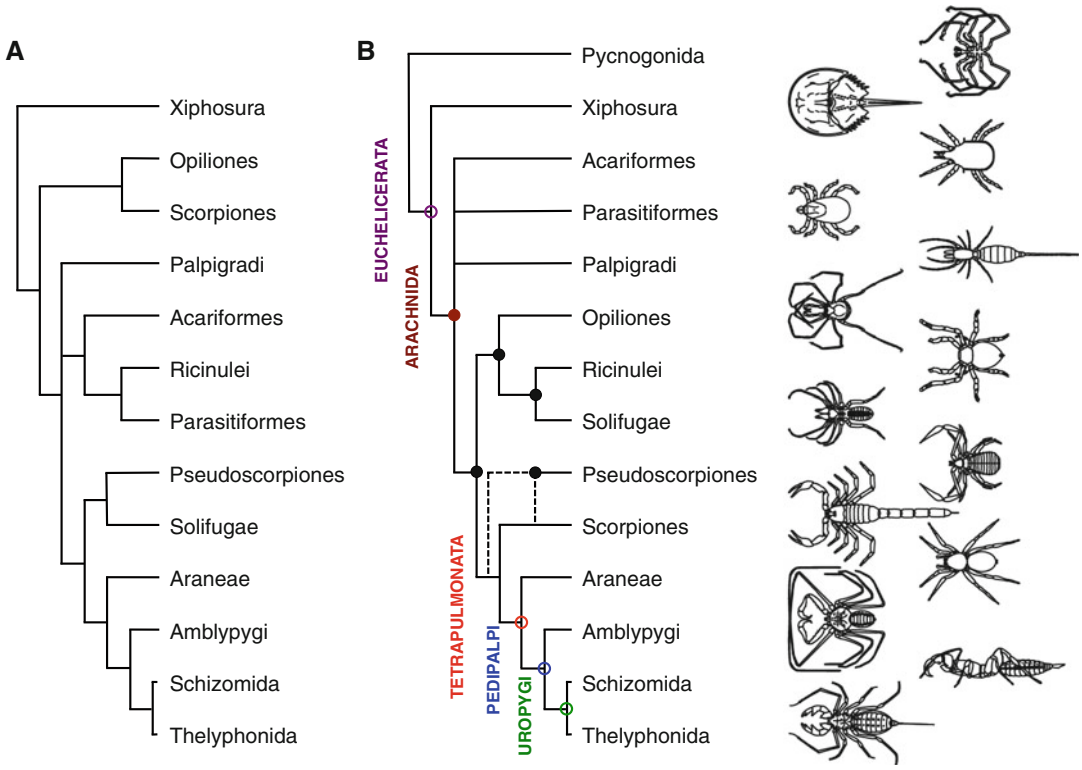

Fig. 5.1 Chelicerate phylogenies. (**A**) Phylogeny based on analysis of morphological characters by Shultz (2007). Note that pycnogonids were not included in this study. (**B**) Phylogeny based on the phylogenomic approach of Sharma et al. (2014a) using transcriptomic and genomic data. *Filled circles* indicate nodes that were supported only by subsets of the slowest-evolving loci used by Sharma et al. (2014a). The *broken lines* indicate two alternative relationships of Pseudoscorpiones to Scorpiones suggested by Sharma et al. (2014a) (© Prashant P. Sharma, 2015. All Rights Reserved)

reviewed by Dunlop 2010). The monophyly of the euchelicerates is very well supported by both molecular and morphological data (Fig. 5.1; Weygoldt and Paulus 1979; Dunlop 2010). While the consensus is that Arachnida (all terrestrial chelicerates) is also monophyletic, mainly based on morphological data (Wheeler and Hayashi 1998; Shultz 2007; Dunlop 2010; but see Giribet et al. 2002), molecular sequence data infrequently recover the monophyly of arachnids (Regier et al. 2010; Börner et al. 2014; Sharma et al. 2014a). A recent phylogenomic study by Sharma et al. (2014a), which used extensive molecular data, including transcriptomes and whole genomes, recovered a nested position of Xiphosura (horseshoe crabs) within arachnids, due to the placement of Pseudoscorpiones, Parasitiformes, and Acariformes. Indeed, the position of the Xiphosura was also found to be inconsistent with the monophyly of the arachnids by Roeding et al. (2009), Meusemann et al. (2010), and Börner et al. (2014). However, upon analysing only a subset of the most slowly evolving genes, Sharma et al. recovered maximal phylogenetic support for arachnid monophyly, suggesting that arachnid non-monophyly is attributable to systematic bias resulting from accelerated rates of evolution in certain "problematic" chelicerate orders (Fig. 5.1; Sharma et al. 2014a).

Among arachnids, the clades Tetrapulmonata, Pedipalpi, and Uropygi are strongly and consistently supported by both morphological and molecular data (Fig. 5.1; Wheeler and Hayashi 1998; Giribet et al. 2002; Shultz 2007; Dunlop 2010; Edgecombe 2010; Regier et al. 2010; Börner et al. 2014; Sharma et al. 2014a). However, the precise phylogenetic relationships of other arachnid orders have been much debated (Dunlop 2010). For example, there are conflicting views on whether Acari (Acariformes [mites] + Parasitiformes [ticks]) is monophyletic, although the most recent evidence supports the view that it is paraphyletic (Dunlop and Arango 2005; Pepato et al. 2010; Sharma et al. 2014a). The position of Opiliones (harvestmen) in the chelicerate tree has also proven to be enigmatic, but recent molecular data suggest that harvestmen form a group with Ricinulei (hooded tick spiders) and Solifugae (camel spiders) (Fig. 5.1B; Sharma et al. 2014a), although this relationship has not emerged from any previous studies (Fig. 5.1A; Wheeler and Hayashi 1998; Giribet et al. 2002; Shultz 2007; Dunlop 2010; Regier et al. 2010).

Resolving chelicerate and arachnid relationships is critical to our understanding of key evolutionary transitions, including many important open questions in evolutionary developmental biology. In this respect the continual expansion of chelicerate genomic resources holds great promise for resolving outstanding issues in the phylogeny of these animals, a necessary framework to explore their evolution and development.

Chelicerate Genome Biology

As with other organisms, the development of new sequencing technologies has allowed transcriptome and whole-genome sequencing of chelicerates that build on classical studies, mainly among spiders, of genome size and cytogenetics (Tsurusaki and Cokendolpher 1990; Chen 1999; Gregory and Shorthouse 2003).

The first chelicerate genome to be published was that of the two-spotted spider mite, *Tetranychus urticae* (Grbic et al. 2011). This was soon followed by the scorpion, *Mesobuthus martensii* (Cao et al. 2013), and two spiders (the social velvet spider, *Stegodyphus mimosarum*, and the Brazilian white-knee tarantula, *Acanthoscurria geniculata*) (Sanggaard et al. 2014) and the Atlantic horseshoe crab *Limulus polyphemus* (Nossa et al. 2014). In addition, the genome of the tick *Ixodes scapularis* has also been sequenced (www.vectorbase.org). Together, these genome sequencing projects corroborate the great variation in genome size among chelicerates and show that there are large differences in the predicted numbers of genes among these animals (Table 5.1). These genomes are only the tip of the iceberg, with several other chelicerate genomes likely to be available soon through initiatives such as i5K (http://www.arthropodgenomes.org/wiki/i5K).

Table 5.1 Chelicerate genome sizes

Order	Species	Genome size (Mb)	Predicted gene number	Reference
Xiphosura	*Limulus polyphemus*	2,740	>34,000	Nossa et al. (2014)
Acariformes	*Tetranychus urticae*	90	18,414	Grbic et al. (2011)
Parasitiformes	*Ixodes scapularis*	2,100	24,925	www.vectorbase.org
Scorpiones	*Mesobuthus martensii*	1,323	32,016	Cao et al. (2013)
Araneae	*Acanthoscurria geniculata*	6,500	73,821[a]	Sanggaard et al. (2014)
Araneae	*Stegodyphus mimosarum*	2,550	27,235	Sanggaard et al. (2014)
Araneae	*Parasteatoda tepidariorum*	1,200	up to 40,000	Posnien et al. (2014)

[a]For *Acanthoscurria geniculate* this is the predicted number of transcripts rather than genes

As well as whole-genome sequencing, there is already a large and growing number of transcriptome projects in various chelicerates to describe the general expression profiles of genes or to decipher tissue- or stage-specific expression (e.g., Croucher et al. 2013; Clarke et al. 2014; Posnien et al. 2014). Transcriptomics can tell us much about the gene content and expression profiles of the genomes of chelicerates, even for species for which the whole genome has not yet been sequenced.

Genomic sequencing of chelicerates has already provided considerable insights into the evolution of many important genes and gene families, from developmental genes to silk and venom genes. Intriguingly, it appears that there have been at least one and perhaps two whole-genome duplications in a horseshoe crab (Nossa et al. 2014). Even excluding the horseshoe crab, chelicerate genomes exhibit marked variability in genome size and content, with miniaturised genomes associated with gene loss in mites (*Tetranychus urticae*; Grbic et al. 2011) and genomes bearing among the largest known numbers of genes in arthropods (Table 5.1). Pinpointing gene family expansion and/or whole-genome duplication events has immediate downstream implications for understanding both the evolution of genomic architecture and gene regulatory networks in these animals.

The rapidly emerging genomic resources for chelicerates therefore represent new and exciting opportunities for the analysis of genome biology, gene expression, gene function, and gene regulatory evolution in existing chelicerate models and have great potential to empower investigation of evolutionary developmental biology in more enigmatic, understudied chelicerate lineages with interesting embryological and morphological features.

The Chelicerate Orders and Evolutionary Developmental Biology

The embryology of chelicerates has been studied for over 150 years (see below). Although some chelicerate models have made an important contribution to understanding animal evolution and development over the past 20 years, others remain very much understudied, as highlighted previously by Harvey (2002). Below, a short overview of the biology of each chelicerate order is given, together with a brief summary of their contribution and/or potential contribution to the field of evolution and development.

Pycnogonida (Sea Spiders)

Sea spiders are marine chelicerates that can live deep in the ocean and feed on sponges, cnidarians, and mollusks (Cobb 2010; Barreto and Avise 2011). These animals are characterised by their narrow cephalosoma, which carries the four sets of their appendages: the chelifores, palps, ovigers, and walking legs (typically four pairs; up to six pairs occur in a few lineages) (Figs. 5.1 and 5.2; Cobb 2010). The ovigers of pycnogonids

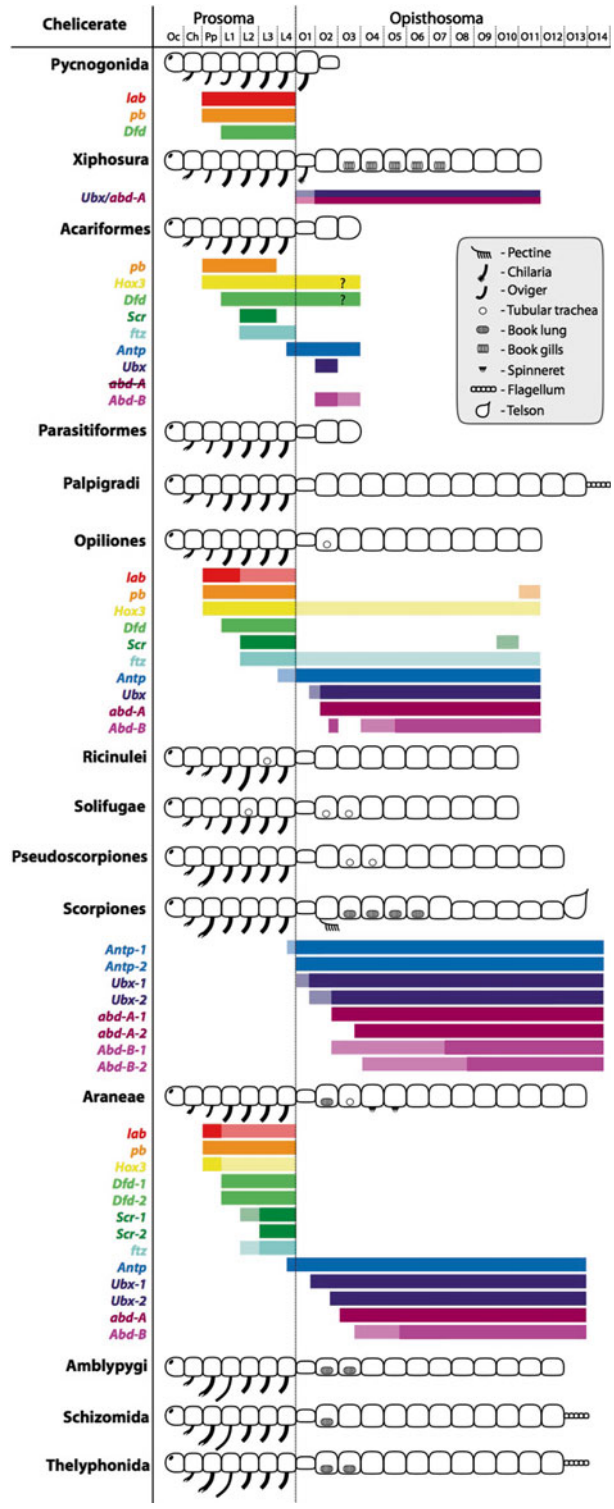

Fig. 5.2 Body plans and known Hox gene expression in chelicerate orders. The number of prosomal segments is conserved in Euchelicerata; variation in segment number of Pycnogonida is not shown. The eucheliceratе prosoma consists of six appendage-bearing segments: a pair of cheliceres, a pair of pedipalps, and four pairs of walking legs. There is considerable innovation in the function of these limbs, such as the long-range tactile first legs of Amblypygi or the muscular pedipalps of Scorpiones. In contrast to the prosoma, opisthosomal segment number is variable both between and within orders. The respiratory organs that are found in chelicerates differ in morphology as well as number and position. For instance, Xiphosura has book gills rather than the book lungs that are found in many other chelicerates. A tracheal respiratory system occurs in several arachnid orders as well as in derived spiders. The position of these is variable and can occur within the prosoma and/or opisthosoma (simplified to show typical positions of spiracles in Acariformes and Parasitiformes). Palpigrade opisthosomal "sacs" are of dubitable homology and are not shown here. Appendages shown that are specific to certain orders include pectines (scorpions), ovigers (pycnogonids), and chilaria (horseshoe crabs). In conjunction with morphological studies, expression of Hox genes in chelicerates has been characterised in Pycnogonida (Jager et al. 2006), Xiphosura (Popadic and Nagy 2001), Acariformes (Telford and Thomas 1998b; Barnett and Thomas 2013a), Opiliones (Sharma et al. 2012b), Scorpiones (Sharma et al. 2014b), and Araneae (Damen et al. 1998; Damen and Tautz 1999; Schwager et al. 2007). The variable anterior expression boundaries of posterior Hox genes are strongly consistent with involvement in patterning opisthosomal segment identity. Note that *abd-A* has been lost in mites. The conserved expression domains of *lab*, *pb*, and *Dfd* in the prosoma of Pycnogonida has contributed significantly to understanding segmental homology of arthropod head segments. In both Araneae and Scorpiones, Hox paralogs have been observed to have both spatial and temporal expression differences. *Oc* ocular segment, *Ch* cheliceres, *Pp* pedipalps, *L1–L4* walking legs, *O1*–O14 opisthosomal segments (© Alistar P. McGregor 2015. All Rights Reserved)

are unique to this group and in many species are typically used by males to carry masses of eggs deposited by females. Interestingly, given the narrow cephalosoma (anterior tagma) of sea spiders and the loss of the opisthosoma ("abdomen" or posterior tagma), the gonads and other organs are found in their appendages (Cobb 2010). However, the fossil record of pycnogonids, which extends to the Cambrian (Waloszek and Dunlop 2002), includes an extinct lineage with a long, completely segmented, limbless posterior region, indicating that stem pycnogonids once bore an opisthosoma (Bergström et al. 1980).

Since sea spiders are widely regarded as the sister group to the euchelicerates (Fig. 5.1; see above), knowledge of their development has great potential to inform our understanding of chelicerate evolution and development more generally. To date, many studies of sea spiders have had a phylogenetic focus, informed by characterisation of their neuroanatomy and Hox gene expression (see above; Arango 2002; Jager et al. 2006; Manuel et al. 2006; Arango and Wheeler 2007; Brenneis et al. 2008). Classical descriptions of pycnogonid development are rare and incomplete (Brenneis et al. 2011a, b). However, modern methodological approaches have recently been applied to describe the embryonic and post-embryonic development of sea spiders and to generate staging systems for *Pseudopallene* sp. and *Pycnogonum litorale* (Vilpoux and Waloszek 2003; Ungerer and Scholtz 2009; Machner and Scholtz 2010; Brenneis et al. 2011a, b). This work can serve as a platform for further studies of gene expression and possibly gene function in these animals and to help resolve questions regarding the evolution and development of chelicerates.

Xiphosura (Horseshoe Crabs)

Horseshoe crabs are the largest existing euchelicerates, although there are only four extant species (Obst et al. 2012). These chelicerates are marine and feed on other invertebrates and algae on the bed of shallow coastal waters (Ruppert et al. 2004).

Horseshoe crabs have a distinctive carapace that covers the dorsum of the cephalothorax and is joined by a hinge to the dorsal exoskeleton that covers the abdomen (Fig. 5.1). Posterior to the three-segmented cheliceres, horseshoe crabs have a sexually dimorphic pair of pedipalps and four pairs of walking legs (Fig. 5.2). Whereas in female horseshoe crabs the pedipalp is nearly identical to a walking leg, the pedipalps of mature males are modified to form terminally swollen, non-chelate "claspers" used to grasp females during mating. The last pair of walking legs, which is used for pushing on the substrate, is argued to be biramous because it exhibits a putative exopod called a flabellum that is sensory (Fig. 5.2; Snodgrass 1938). This putative homology is supported by the discovery of fossil synziphosurines with *bona fide* exopods on the pedipalps and all walking leg segments, suggesting that the flabellum is a vestige of the posterior-most exopod pair (Briggs et al. 2012). Other sensory organs include two ocelli on the carapace and two lateral compound eyes. Horseshoe crabs respire through five pairs of book gills located on abdominal segments three to seven (Fig. 5.2).

Female horseshoe crabs can lay thousands of eggs on beaches at high tide that are then fertilised by the males and covered in sand. Upon hatching the larvae then enter the sea. This has allowed researchers access to the embryos of these animals and their development has been described in some detail, as well as studied through embryonic manipulations (Kingsley 1892; Kishinouye 1893; Iwanoff 1933; Itow and Sekiguchi 1979, 1980; Sekiguchi et al. 1982; Itow 1990, 2005; Itow et al. 1991). Furthermore, there is a growing number of studies that have examined gene or protein expression during horseshoe crab embryogenesis and other aspects of development that have provided some valuable insights into evolutionary developmental biology (Popadic and Nagy 2001; Damen et al. 2002; Mittmann 2002; Blackburn et al. 2008). In addition, the recent sequencing of the genome of *Limulus polyphemus* (Table 5.1; Nossa et al. 2014) is anticipated to fuel further studies of gene expression in this species.

Scorpiones (Scorpions)

Scorpions are found in a range of habitats, from deserts to tropical rainforests. There are nearly

2,000 described species of scorpions. These chelicerates exhibit a familiar body plan that includes the characteristic pincers of the pedipalpal segment and the aculeus, or stinger, that harbours a barb coupled to a pair of venom-producing glands at the posterior end of the segmented metasoma (post-abdomen or tail) (Fig. 5.2; Beccaloni 2009). These structures are used in combination by these predators for defence and subduing their prey.

The cephalothorax of scorpions has a pair of median eyes and a variable number of lateral eyes (one to five pairs; Gromov 1998; Yang et al. 2013). Scorpions respire through four pairs of book lungs found on abdominal segments three to six (Fig. 5.2; Hjelle 1990), which correspond to embryonic abdominal segments four to seven (the first opisthosomal segment disappears during development). The second abdominal segment (or third embryonic abdominal segment) bears a pair of sensory pectines that is involved in chemoreception and detecting the substrate (Fig. 5.2; Hjelle 1990).

Scorpions are viviparous with embryogenesis taking place inside the females, which subsequently give birth to juveniles. Two distinct modes of development occur in scorpions. Apoikogenic development is characterised by large yolky eggs, surrounded by extra-embryonic membranes, and development occurs in the oviduct. In katoikogenic development, the eggs bear little or no yolk, and the embryos are nourished through connections of the ovariuterus that facilitate trophic exchange from the female's hepatopancreas (Hjelle 1990; Lourenço 2000); development occurs in modified, blind outgrowths of the ovariuterus. Development in either case can be prolonged, with a gestation period lasting 2–18 months in various species.

Despite the ensuing issue with access to embryos, protein and mRNA expressions have been studied during embryogenesis in species such as *Smeringurus mesaensis*, *Euscorpius flavicaudis*, and *Centruroides sculpturatus* (Table 5.2; Popadic and Nagy 2001; Simonnet et al. 2004, 2006; Sharma et al. 2014b, c). This means that it is possible to study the development of several aspects of scorpion morphology to provide new evolutionary insights due to the probable phylogenetic placement of these chelicerates as sister group to Tetrapulmonata (Regier et al. 2010; Sharma et al. 2014a). This includes the developmental patterning of the arachnid book lungs in spiders and scorpions, the serial homology of opisthosomal appendage types, and the sub- or neofunctionalisation of paralogous genes in both spider and scorpion genomes (Schwager et al. 2007; Cao et al. 2013; Sharma et al. 2014b).

Opiliones (Harvestmen)

Harvestmen live in a wide variety of temperate and tropical habitats worldwide, and they can be predators, scavengers, or even herbivores. More than 6,500 species of harvestmen have already been described, and there are estimated to be 10,000 extant species (Machado et al. 2007). These chelicerates are readily recognisable from the four pairs of elongated walking appendages of most species and are hence commonly known as "daddy longlegs" in some parts of the world. The long pedipalps of some harvestmen resemble legs, but in the suborder Laniatores, the pedipalps are raptorial and used to seize prey (Shultz and Pinto-da-Rocha 2007). The second pair of legs is usually longer than the other three pairs in phalangid (i.e., non-Cyphophthalmi) harvestmen, whereas the first pair is generally the longest in the primitive suborder Cyphophthalmi; the longest pair of legs is tactile and/or chemoreceptive throughout the order (Willemart et al. 2009).

Harvestmen respire through tracheal tubes with the spiracles (openings) located on the second opisthosomal segment (Fig. 5.2). These chelicerates do not synthesise silk or venom, but have evolved repugnatorial glands, which secrete acrid compounds including phenols (Raspotnig et al. 2012). The cephalothorax of phalangid harvestmen bears a pair of median eyes, but lacks lateral eyes. By contrast, cyphophthalmid harvestmen bear a single pair of eyes on the sides of the cephalothorax that are believed to be homologous to lateral eyes (Garwood et al. 2014).

Like some mites, male and female Phalangida have a penis and ovipositor, respectively, on the ventral cephalothorax, and thus, fertilisation is internal in these chelicerates; the plesiomorphic

Table 5.2 Expression of key developmental genes in chelicerates

Gene name	Species	Expression domain	Reference
Orthologs of *Drosophila* segmentation cascade genes			
engrailed	*Cupiennius salei*	Expression in segmental stripes at the anterior border of each parasegment (posterior border of each segment), six prosomal stripes, and 12 opisthosomal stripes; additional ocular spots in the pre-cheliceral lobe and expression anterior to the labrum	Damen et al. (1998), Damen (2002)
	Parasteatoda tepidariorum	Segmental stripe expression appears in nonsequential order in prosomal segments; subsequently, stripes in opisthosomal segments are sequentially added	Schwager et al. (2009)
	Phalangium opilio	Segmental stripes at the posterior border of each segment, seven opisthosomal stripes	Sharma et al. (2012b)
	Archegozetes longisetosus	Segmental stripes at the posterior border of each segment, cheliceral, pedipalpal and L1–L3 appear first, then O1, then L4 and lastly O2	Telford and Thomas (1998a), Barnett and Thomas (2012)
	Tetranychus urticae	Expression in pedipalp and four walking leg segments and two opisthosomal segments	Grbic et al. (2011)
runt	*Cupiennius salei*	Early: anteriorly moving stripes in the SAZ Late: neuroectoderm, head, and legs	Damen et al. (2000)
	Tetranychus urticae	Five pairs of ventral ring-shaped expression domains that later form stripes, segmental expression in the nervous system, later in the head lobes, legs	Dearden et al. (2002)
hedgehog	*Parasteatoda tepidariorum*	Early: expression at the rim of the germ disc that then forms a posteriorly moving stripe that will eventually split into three stripes in the pre-cheliceral region, the cheliceral and pedipalpal segment Late: segmental stripes (all segments, including a stripe in cheliceral lobe), SAZ	Pechmann et al. (2009), Akiyama-Oda and Oda (2010), Kanayama et al. (2011)
	Euscorpius flavicaudis	Posterior borders of segments, stripes in the SAZ before segment formation, labrum	Simonnet et al. (2004)
	Archegozetes longisetosus	Stripes at the posterior borders of segments, Ch, Pp, L1–L3 form first, then O1, then L4, and lastly O2. Expression is also found in stomodeum and proctodeum	Barnett and Thomas (2012)
fushi tarazu	*Cupiennius salei*	Groups of cells in the ventral neural ectoderm extending from the posterior portion of the first walking leg to the border between the fourth walking leg and the first opisthosomal segment, ring-shaped expression domains in the distal tips of the developing 3rd and 4th walking leg and weakly in the 2nd walking leg	Damen et al. (2005)
	Phalangium opilio	Distal tips of 3rd and 4th walking legs, ventral ectoderm expression expanding from the 2nd walking leg to the SAZ, later extending only to O4	Sharma et al. (2012b)

Pax group III	*Tetranychus urticae*	*Pax 3/7*: Initially three ventral stripes are expressed (Pp, L2, L4), a fourth stripe emerges between the two most anterior stripes (L1), and a fifth stripe (L3) appears between the two most posterior stripes, one small opisthosomal stripe Pax 3/7 protein expression: Segmental stripes in Ch, Pp, L1–L4, O1, O2. Pre-cheliceral CNS	Dearden et al. (2002), Davis et al. (2005)
	Cupiennius salei	*pairberry 1–3*: Dynamic SAZ expression, segmental expression in prosomal and opisthosomal segments Pax 3/7 protein expression: Ventral segmental stripes at the posterior segmental borders. O1 stripe is extended dorsally. Pre-cheliceral CNS, segmental neural cell clusters	Schoppmeier and Damen (2005), Damen et al. (2005), Davis et al. (2005)
	Limulus polyphemus	Pax 3/7 protein expression: Expressed as concentric rings from the posterior that form into stripes. Segmental neural cell clusters	Davis et al. (2005)
patched	*Parasteatoda tepidariorum*	Early: circular expression domain in the blastopore area, which expands to ubiquitous expression in the entire germ disc epithelium; followed by a reduction of expression to the rim of the germ disc Late: expression in the SAZ and in a stripe at the anterior border of the germband	Akiyama-Oda and Oda (2010), Barnett and Thomas (2013a)
	Archegozetes longisetosus	Early: expression commences as double stripes in prosomal segments corresponding to L1, L2, chelicerae, and pedipalps and in a broad expression domain in the growth zone Late: pronounced expression of a medial stripe in prosomal segments (excluding L3), followed by expression in a broad stripe in O1 and subsequently O3 and O4, which also eventually split into double stripes, in the two latter segments	
hairy	*Cupiennius salei*	Dynamic stripe formation in the SAZ before morphologically visible segmentation	Damen et al. (2000)
	Parasteatoda tepidariorum	Ring at the rim of the germ disc that will form a broad anterior stripe, which will later form stripes in the Ch and Pp segments. Circular expression in the centre of the germ disc that will clear from the centre to form a broad stripe that will later split into stripes in L2–L4. Opisthosomal stripes are sequentially added from the SAZ	Pechmann et al. (2009)
Head patterning genes			
orthodenticle	*Parasteatoda tepidariorum*	Early: ring around edge of germ disc, migrating wave of expression in the future head region Late: pre-cheliceral lobes and ventral midline	Akiyama-Oda and Oda (2003), Pechmann et al. (2009)
	Tegenaria saeva	Stripe in pre-cheliceral lobes, anterior to limb buds on O2–O5	Simonnet et al. (2006)
	Euscorpius flavicaudis	Early: pre-cheliceral stripe Late: pre-cheliceral lobes, ventral midline, lateral expression anterior to opisthosomal limb buds, and lateral dots in all metasomal segments	Simonnet et al. (2006)
	Archegozetes longisetosus	Pre-cheliceral lobes, ventral midline, possibly in labrum	Telford and Thomas (1998)
	Phalangium opilio	Eye fields, pre-cheliceral lobes, ventral midline, labrum	Garwood et al. (2014)

(continued)

Table 5.2 (continued)

Gene name	Species	Expression domain	Reference
empty spiracles	*Tegenaria saeva*	Posterior pre-cheliceral region, segmental patches neuroectoderm, walking legs, lateral stripes in O2–O5	Simonnet et al. (2006)
	Euscorpius flavicaudis	Posterior pre-cheliceral region, lateral segmental stripes in all segments, prosomal appendages	Simonnet et al. (2006)
	Phalangium opilio	Posterior pre-cheliceral region, lateral segmental stripes in all segments, prosomal appendages	Garwood et al. (2014)
cap-n-collar	*Phalangium opilio*	Weak ubiquitous expression	Sharma et al. (2014c)
	Centruroides sculpturatus	Weak ubiquitous expression	Sharma et al. (2014c)
Pax6	*Limulus polyphemus*	Head lobes and developing brain, paired clusters of cells in the ventral neuroectoderm and developing nervous system	Blackburn et al. (2008)
	Phalangium opilio	Head lobes, paired stripes in all prosomal and opisthosomal segments	Garwood et al. (2014)

Expression patterns of genes characterised in at least one chelicerate other than spiders, with the exception of Hox gene expression patterns and leg patterning genes, which are treated in Figs. 5.2 and 5.8, respectively

condition of fertilisation by spermatophores (indirect sperm transfer) occurs in Cyphophthalmi (Karaman 2005). Fertilised eggs are deposited singly or in batches that can number into the hundreds (Juberthie 1964). This means that some species of harvestmen can readily provide large numbers of embryos that can be collected at different stages to study the embryogenesis of these animals (Moritz 1957; Juberthie 1964; Muñoz-Cuevas 1971; Gnaspini and Lerche 2010). Indeed, RNA in situ hybridisation to visualise gene expression patterns (e.g., see Table 5.2) and RNAi to characterise gene function have already been established in *Phalangium opilio*. This has facilitated studying the regulation of development in this species compared to other animals, including analysis of Hox and leg gap genes (Fig. 5.2; Sharma et al. 2012a, b, 2013, 2014c; Garwood et al. 2014).

Solifugae (Camel Spiders)

Solifuges or camel spiders predominantly inhabit arid environments where they mainly predate on other arthropods, taking advantage of their speed and large powerful cheliceres (Punzo 1998). Anatomically, these arachnids are distinguished from others by their malleoli (sometimes called racquet organs). These are fan-shaped chemoreceptory organs that detect changes in the substrate, analogously to the pectines of scorpions (Brownell and Farley 1974). Being apulmonate arachnids, camel spiders lack book lungs, but have among the most densely branching tracheal system for respiration among arachnids (Fig. 5.2; Lighton and Fielden 1996). Although recent work has been carried out on the functional morphology of these arachnids (van der Meijden et al. 2012), camel spiders represent a rather understudied order of chelicerates, and there is a dearth of EvoDevo studies on the group. Although solifuges are difficult to collect and produce only one brood per year, culturing camel spiders in the laboratory is possible, albeit challenging, and females can lay clutches of up to 200 embryos (Punzo 1998). Therefore, there is potential that gene expression and gene function could be studied in camel spiders. The development of the malleoli and the genetic basis for lateral eye loss in many species of solifuges are opportune targets for evolutionary developmental study, particularly with reference to phalangid harvestmen, which also lack lateral eyes (Garwood et al. 2014).

Pseudoscorpiones (False or Book Scorpions)

There are over 3,200 species of pseudoscorpions, which occupy a wide range of habitats worldwide (Harvey 2011). These chelicerates prey on other invertebrates or are scavengers, and some have even adopted a commensal or phoretic (hitchhiking) strategy, living on and being dispersed by mammals, birds, and larger arthropods (Weygoldt 1970; Harvey 2002, 2011).

Pseudoscorpions have long modified pedipalps that terminate with chelae like scorpions, but they are distinguished from the latter in lacking the characteristic tail and stinger of scorpions, as well as median ocelli and pectines (Figs. 5.1 and 5.2). Like camel spiders and harvestmen, pseudoscorpions also lack book lungs and instead use spiracles and a tracheal system for respiration (Fig. 5.2; Weygoldt 1970; Lighton and Joos 2002; Harvey 2011). Like other chelicerates, most notably spiders, pseudoscorpions can also make silk, which is produced from prosomal glands and used for a variety of purposes, including sperm transfer and burrowing (Weygoldt 1970; Harvey 2011). Members of the suborder Iocheirata also synthesise venoms (Weygoldt 1970; Harvey 1992).

A multilocus phylogeny, which remains rare for several minor arachnid orders (Harvey 2002), has been proposed for pseudoscorpions and indicates that it is likely that venom only evolved once within this group and independently of scorpion and spider venom (Murienne et al. 2008). Although the morphology of these animals has been characterised in some detail for taxonomic purposes and aspects of their courtship behaviour described, these chelicerates have only recently been studied in the context of EvoDevo research (Jędrzejowska et al. 2013). Elucidating the genetic mechanism whereby chelate pedipalps are patterned in pseudoscorpions and scorpions may shed much needed light on how these groups are related (Fig. 5.1).

Acariformes (Mites)

To date nearly 50,000 species of mites have been described, although it is thought that there could be over a million species, and they therefore represent the most diverse group of chelicerates. These miniaturised arachnids can be free-living or parasitic and live in a wide range of habitats including aquatic environments (Beccaloni 2009).

The cephalothorax and truncated abdomen of mites are fused, but the body is divided into two autapomorphic tagmata—the anterior gnathosoma and the posterior idiosoma (Fig. 5.2)—although these can be covered by a single carapace in some species. The cheliceres and pedipalps of mites can vary in morphology between species and they have four pairs of walking legs as adults (Fig. 5.2). Most mites respire using a tracheal system and have up to four pairs of anteriorly positioned spiracles (Fig. 5.2). Sperm transfer is indirect in most mites with the males producing a spermatophore manipulated with their appendages. Females usually lay their eggs in soil or humus from which the larvae hatch up to 6 weeks later (Ruppert et al. 2004).

Research on mites has provided several important insights into evolutionary developmental biology (see below) through studying gene expression and gene function in species such as *Tetranychus urticae* and *Archegozetes longisetosus* (Table 5.2; Telford and Thomas 1998a, b; Dearden et al. 2000, 2002, 2003; Grbic et al. 2007; Khila and Grbic 2007; Barnett and Thomas 2012, 2013a). Furthermore, the genome of *T. urticae* has also been sequenced (Table 5.1; Grbic et al. 2011), which greatly complements the other tools and resources available for this species.

Parasitiformes (Ticks)

Ticks are highly speciose parasitic chelicerates that live on a range of hosts, including humans and domestic animals (Beccaloni 2009). The body plan of ticks is similar to that of mites (Fig. 5.2), although these two chelicerate lineages may not form a clade (the traditionally defined Acari; Fig. 5.1). The biology of ticks is highly relevant to health-related and agricultural interests, and the genome of *Ixodes scapularis* has thus been sequenced (Table 5.1). This has allowed comparisons of the sequences of important developmental genes to be made between this tick and other metazoans (e.g., Janssen et al. 2010). Furthermore, embryonic development has been described for *Rhipicephalus* (*Boophilus*) *microplus*, which involved using antibody stainings (Santos et al. 2013b). However, gene expression and function during tick development has not been studied to the best of our knowledge, although reports of the successful application of parental RNAi (e.g., la Fuente et al. 2007) might change this in the future.

Ricinulei (Hooded Tick Spiders)

Ricinulei represent a small (3 genera and only about 60 recognised species) and understudied order of chelicerates (Fig. 5.1; Harvey 2002; Botero-Trujillo 2014). These animals are small arachnids that live in leaf litter and caves, and most species lack eyes, although some species have basic lateral eyes (Beccaloni 2009).

Ricinulei exhibit two tagmata and also respire via a tracheal system (Fig. 5.2). Ricinulei are distinguished by a cucullus or cuticular hood that can be used to cover the cheliceres and mouthparts (Beccaloni 2009). The second pair of walking legs is longer than the others and is also sensory (Beccaloni 2009). The third walking legs of male Ricinulei are used for sperm transfer and exhibit species-specific modifications like the pedipalps of spiders (Legg 1977; Harvey 2002). Although several aspects of the morphology of Ricinulei have recently been described in great detail (Talarico et al. 2006, 2008a, b, 2011), there are no embryological or EvoDevo studies of these animals of which we are aware. Opportune targets for study of EvoDevo in this group include the differentiation of the sexually dimorphic third leg pair in males. In addition, a potential shared mechanism for the inhibition of L4 limb bud growth in first instars of Ricinulei, mites, and ticks may shed light on the phylogenetic affinities of the "acaromorph orders" (Fig. 5.1; Shultz 2007).

Palpigradi (Microwhip Scorpions)

There are approximately 80 species of microwhip scorpions (Fig. 5.1; Harvey 2002). These arachnids are widespread in tropical and subtropical regions and live in caves and damp soils (e.g., Smrz et al. 2013 and references therein).

Microwhip scorpions are very small (at most 3 mm in length), are eyeless, and exhibit a segmented flagellum at the end of their abdomen (Fig. 5.2; Beccaloni 2009). It was recently found that the species *Eukoenenia spelaea* feeds on cyanobacteria in caves, although very little else is known about the natural history of microwhip scorpions (Smrz et al. 2013). To the best of our knowledge, these rather enigmatic chelicerates have not been the subject of any embryological research. Microwhip scorpions only lay a few (one to three) embryos at a given time, and ovules of different developmental stages have been observed within the opisthosoma (Condé 1996).

Amblypygi (Whip Spiders)

Whip spiders are mostly found in tropical rainforests, and there are many cave-dwelling species. Only about 150 species of whip spiders have been described (Harvey 2003), and what is known of their biology has been previously reviewed in detail by Weygoldt (2000).

Whip spiders are similar in appearance to spiders, but are somewhat flattened in comparison. Furthermore, the cheliceres of whip spiders do not produce venom and they use modified pedipalps to capture prey (Fig. 5.2). These chelicerates can also be distinguished by their first pair of walking legs, which is elongated and tactile, and therefore considered to be antenniform (Weygoldt 2000). Whip spiders also have two sets of opisthosomal book lungs, but they do not have any other appendages on this tagma (Fig. 5.2) and they lack the ability to make silk. Like scorpions and thelyphonids (see below), parental care in this order consists of a female carrying hatchlings on her back until they reach a certain developmental stage and disperse. Unlike scorpions, only in amplypygids, thelyphonids, and pseudoscorpions do females carry eggs on the underside of the opisthosoma until hatching.

The embryology and morphology of whip spiders has been described in detail, although very little contemporary EvoDevo research has been carried out on these animals (Weygoldt 2000). However, such research would offer an interesting comparison to spiders due to the phylogenetic proximity of these two orders. The regulation of the development of the large, raptorial pedipalps and the elongate, antenniform first walking legs—in contrast to their shorter counterparts in spiders—constitute promising areas of future study (Weygoldt 2000; Harvey 2002).

Thelyphonida (Whip Scorpions)

There are over 100 described species of whip scorpions (Harvey 2002). These predators live in tropical climates and employ their enlarged raptorial pedipalps to grab prey (Fig. 5.2; Ruppert et al. 2004). To a lesser degree than in whip spiders, the first pair of legs of whip scorpions is elongated and tactile (Fig. 5.2). Whip scorpions also have a segmented opisthosoma that ends in an annulated flagellum, superficially resembling scorpions and conferring their common name (Ruppert et al. 2004). The abdomen of whip scorpions also carries two pairs of book lungs (Fig. 5.2) and two anal glands that are used to repel predators by spraying them with a mixture of acetic acid, caprylic acid, and other substances (hence, these animals are sometimes referred to as vinegaroons) (Eisner et al. 1961; Haupt and Müller 2004).

To date, whip scorpions have not been the subject of EvoDevo research. Like in Amblypygi, the embryos are carried in an external sac by the females, meaning that embryos of different developmental stages can be collected for analysis of gene expression and gene function (Ruppert et al. 2004). The miniaturisation of particular opisthosomal sternites in thelyphonids is of particular interest from the perspective of segmentation (Shultz 2007).

Schizomida (Short-Tailed Whip Scorpions, Microwhip Scorpions)

Short-tailed whip scorpions are close relatives of whip scorpions (Figs. 5.1 and 5.2) that live in tropical leaf litter (Santos et al. 2013a). Harvey (2002) estimated that there are over 500 extant species worldwide. Short-tailed whip scorpions resemble miniaturized whip scorpions. However, they are much smaller and have only one pair of book lungs (Fig. 5.2; Ruppert et al. 2004). The flagellum of short-tailed whip scorpions is also shorter than that of whip scorpions and confers their common name (Fig. 5.2). Interestingly, the flagellum is sexually dimorphic and is used during courtship, and it has been suggested that this structure may be involved

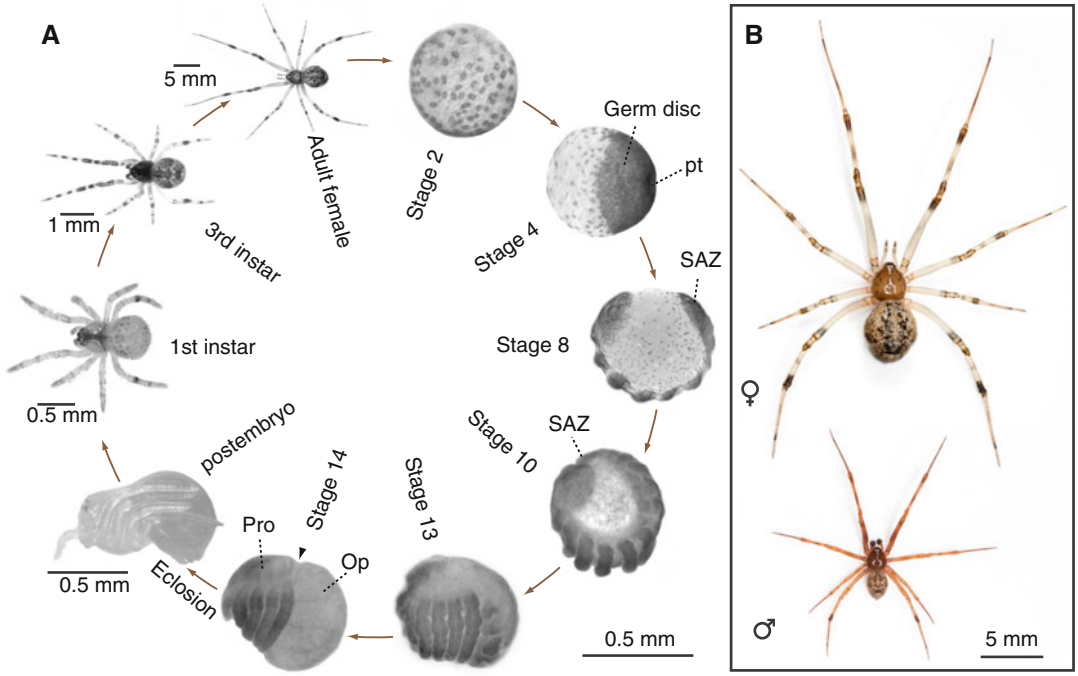

Fig. 5.3 The development and morphology of the spider *Parasteatoda tepidariorum.* (**A**) Stages of embryonic and postembryonic development: stage 2, cellularisation is complete, blastoderm formation; stage 4, germ disc including the primary thickening (*pt*) in the centre; stage 8, early germband with the segment addition zone (*SAZ*); stage 10, elongated germband with limbs; stage 13, end of inversion; stage 14, with distinct prosoma (*Pro*) and opisthosoma (*Op*) with constriction between them (*arrowhead*); postembryo; 1st instar, which exits the cocoon; 3rd instar, a free-foraging instar stage; female adult. Staging after Mittmann and Wolff (2012). In all images anterior is to the left. Scale bar is given with respect to the stage 2–14 embryos. (**B**) Adult female (*top*) and male (*bottom*). Anterior is to the top (Figure slightly modified and reproduced with permission from Hilbrant et al. (2012))

in species recognition (Harvey 2002). Like whip scorpions, the evolution and development of short-tailed whip scorpions is understudied, but the parallel evolution of a single pair of book lungs in schizomids and derived spiders from the ancestral condition of two pairs in Tetrapulmonata would be very interesting to explore further.

Araneae (Spiders)

Spiders have been intensively studied and they are the best-understood chelicerates in terms of their general biology, physiology, behaviour, development, and evolution (Fig. 5.1; Foelix 2010). Spiders are a speciose order of arachnids (over 40,000 described species) that exhibit a wide range of physiological and morphological adaptations, including silk and venom production, and morphological diversity of such appendages as the cheliceres and pedipalps (Foelix 2010).

Spiders have a prosoma and an opisthosoma with the former bearing the cheliceres, pedipalps, and four pairs of walking legs and the latter housing structures including the respiratory organs, genitalia, and spinnerets (Fig. 5.2). The group is distinguished from all other chelicerates in bearing spinnerets, modified appendages that constitute the web-spinning apparatus of spiders. The spinnerets and the webs of spiders have been argued to constitute key innovations that enabled considerable diversification in this group.

Spiders have also constituted the main model chelicerates used to address questions in evolutionary developmental biology. In particular, two Entelegynae, the central American wandering spider *Cupiennius salei* and the common house spider *Parasteatoda tepidariorum* (formerly *Achaearanea tepidariorum*; Fig. 5.3 and see boxed text), have provided great insights into

chelicerate, arthropod, and metazoan evolution and development (McGregor et al. 2008a; Hilbrant et al. 2012). More recently, the Haplogynae *Pholcus phalangioides* has been employed as a satellite model to provide a comparative perspective in spider EvoDevo within Araneomorphae (Pechmann et al. 2011), and there has also been one comparative gene expression study in a mygalomorph (Pechmann and Prpic 2009). The contribution of studies of gene expression (e.g., see Table 5.2) and gene function in spiders to our understanding of evolution and development is discussed in detail below.

The Common House Spider *Parasteatoda tepidariorum* as a Model for Evolutionary Developmental Biology

The common house spider, *Parasteatoda tepidariorum* (Koch 1841), native to South America, is synanthropic and presently distributed worldwide. *P. tepidariorum* hides in cobwebs in secluded areas. Due to the phylogenetic significance of chelicerates in arthropod phylogeny and the operational flexibility of this species, *P. tepidariorum* has become a powerful model organism in the field of evolutionary developmental biology. Females lay up to 400 embryos in silken egg sacs (cocoons) about every 5 days all year around under laboratory conditions. Due to the short fertilisation process, which takes about three minutes, embryos develop synchronously within one cocoon, which is particularly advantageous for developmental studies.

In embryos the first nuclear divisions take place in the centre of the spherical egg and cellularise when the cells start to migrate towards the periphery after about five divisions. Later, cells divide and aggregate to deploy the blastoderm at one hemisphere, where the blastopore forms in the centre upon gastrulation and invagination processes occur. After blastopore closure, the cumulus, an aggregation of mesenchymal cells in the centre of the germ disc, migrates underneath the ectodermal cell layer towards the periphery. This process specifies the DV axis and initiates the transformation from a germ disc to a germband (Fig. 5.3). The sequential addition of opisthosomal segments from the posterior segment addition zone follows, and the nervous system and appendages begin to form along the AP axis. At late stages of embryonic development, inversion processes occur where the embryo encloses the yolk and internal organs like the heart, digestive tract, and brain develop.

The whole developmental process until hatching lasts approximately 8 days and another 12 weeks for the spiderlings to develop to adulthood, including five molts for males and up to seven molts for females at 25 °C (see Fig. 5.3). Embryos of all embryonic stages can be fixed and used for in situ hybridisation and antibody staining to study mRNA and protein expression, respectively. Furthermore, gene function can be studied in *P. tepidariorum* with RNA interference: double-stranded RNA (dsRNA) injected into adult females results in several cocoons exhibiting a knockdown effect. Injecting a single cell of an embryo at the 16- or 32-cell stages with dsRNA generates clones of cells lacking gene function. The availability of transcriptomic sequences and, in the future, whole-genome sequence data will potentially allow genome-editing tools to be applied in *P. tepidariorum* to study the genetic regulation of the development of this spider in even greater detail.

In the following, a summary of the classic literature describing key aspects of the early and late development of the chelicerates is provided. Subsequently, studies that have focused on characterising gene expression and gene function in chelicerates are reviewed to highlight important insights into the evolution and development of these animals, other arthropods, and other metazoans.

EARLY DEVELOPMENT

The study of chelicerate embryos dates back to the very beginnings of invertebrate developmental biology. In 1824, Moritz Herold delivered what he claimed were the first studies of invertebrate development (Herold 1824)—and his first study subject were embryos of the European garden spider, *Araneus diadematus*. Most classical literature from the mid-late nineteenth century onwards has been extensively reviewed by Anderson (1973) and Yoshikura (1975), and to avoid duplicating these efforts, the reader may refer to their exhaustive listing of chelicerate embryological studies prior to 1975. In the following section, the focus is on describing key steps of chelicerate development that have been the focus of modern evolutionary developmental biology.

Cleavage

Most chelicerate eggs (with the exception of mites, ticks, and viviparous scorpions as well as sea spiders) are round or ovoid in shape, fairly large (0.5–3.5 mm), and rich in yolk. These types of eggs predominantly show superficial early cleavages (i.e., without cytokinesis/formation of membranes between the cleavage energids) that occur in the centre of the egg within the yolk (intralecithal) (Schimkewitsch 1887, 1898, 1906; Kingsley 1892; Iwanoff 1933; Moritz 1957; Juberthie 1964; Kondo 1969; Yoshikura 1969; Anderson 1973; Weygoldt 1975; Suzuki and Kondo 1995, 1994; Kimble et al. 2002; Kanayama et al. 2010).

The best-described examples of this cleavage mode are found in spiders, owing to the application of more sophisticated imaging techniques such as transmission electron microscopy (TEM) and, more recently, single-cell injection. In a close relative of *Parasteatoda tepidariorum*, *P. japonica*, the first four cleavages are synchronous and syncytial. The perinuclear cytoplasm is connected with the periplasm at the egg surface by thin strands that form along yolk columns, and the cell membrane invaginates from the surface, also along these yolk columns. At the 16-cell stage, cell membranes fuse and form the blastomeres, which then migrate to the embryo's surface (Suzuki and Kondo 1995, 1994). Kanayama et al. (2010) have confirmed these findings in *P. tepidariorum* by showing that fluorescent dyes injected into the surface periplasm at the 16-cell stage do not diffuse into neighbouring areas and subsequently will only be found in daughter cells of the injected cell. It has been argued that this type of superficial cleavage might be the ancestral cleavage mode in Chelicerata and that the cases of total cleavage seen in some mites, some ticks, pseudoscorpions, and viviparous scorpions are possibly derived and linked to the production of smaller, less yolky eggs (Anderson 1973; Wolff and Scholtz 2013).

In the case of mites and ticks, Laumann et al. (2010b) have argued that classical studies of their embryos might have wrongly attested to these chelicerates possessing superficial cleavage due to the techniques used to examine the embryos. Laumann et al. (2010b) base this judgment on the re-examination of the cleavage mode of *Archegozetes longisetosus* by traditional light microscopy techniques that failed to detect the total cleavage mode of this oribatid mite, which the authors previously had determined using TEM (Laumann et al. 2010a, b). The authors then conclude that since no modern studies in either ticks (Fagotto et al. 1988) or mites (Dearden et al. 2002; Walzl et al. 2004; Laumann et al. 2010a, b) have confirmed superficial cleavage, the ancestral cleavage mode within ticks as well as mites must have been total (Laumann et al. 2010a).

Most pycnogonids display total and equal cleavages that are irregular. This cleavage mode is therefore thought to be the ground pattern in pycnogonids (Ungerer and Scholtz 2009). However, there are certain groups of pycnogonids with larger, more yolk-rich eggs that display unequal, yet still total cleavages (Ungerer and Scholtz 2009). In some of these pycnogonids (*Callipallene* and *Propallene*) even the first cleavage is unequal, which is suggestive of an early cell fate determination that would make

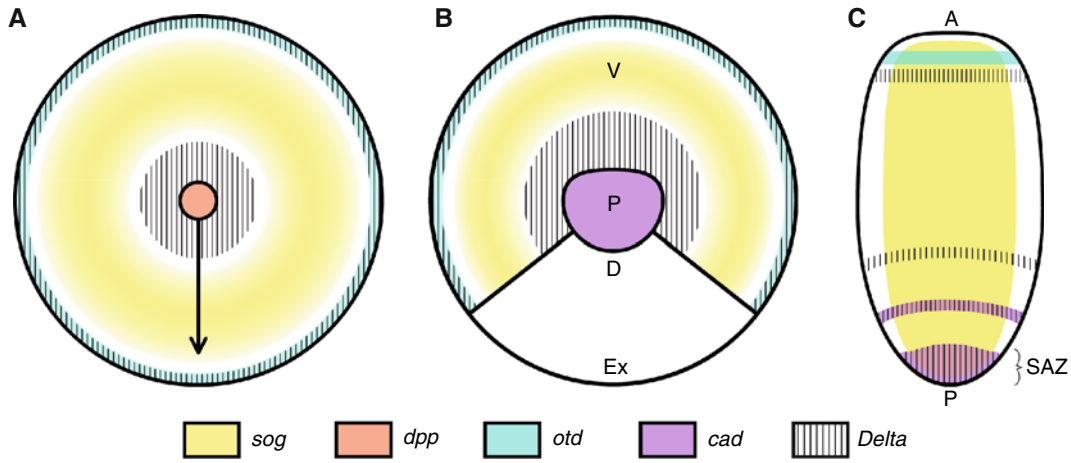

Fig. 5.4 Axis specification and formation of segments in spiders. (**A**) During early development blastomeres collect at one pole of the embryo to form a germ disc. At the centre of this is *decapentaplegic* (*dpp*) expression (*red*) from the mesenchymal cells of the cumulus. Surrounding the *dpp* expression is a circular domain of *Delta* (*Dl*) (hatched), then *short gastrulation* (*sog*) (*yellow*), with a co-expressed domain of *orthodenticle* (*otd*) (*light blue*), and a weak *Dl* signal around the periphery of the germ disc. As the *dpp*-expressing cumulus migrates, the radial symmetry is broken. (**B**) *dpp* expression then disappears when the dorsal field (*D*) starts to form. This dorsal region extends around the periphery of the germ disc forming the extra-embryonic (*Ex*) and dorsal tissues with the *sog* domain forming the ventral tissue (*V*). (**C**) Expression of *otd* and *Dl* in the periphery of the germ disc is later localised to the anterior prosomal region of the germband, with the opened central ring of *Dl* (*hatched*) moving to the approximate area where the prosoma/opisthosoma boundary develops. As the dorsal field opens up, the centre of the germ disc loses *Dl* expression and begins to express *caudal* (*cad*) in the forming caudal lobe (**B**). As the germband elongates, dynamic expression of *Dl* and *cad* in the segment addition zone (*SAZ*) buds off stripes associated with nascent opisthosomal segments (**C**). The exact spatial relationship of these genes' expression and which segments they form are still unclear. In (**B**, **C**), *A* anterior and *P* posterior ()

these embryos the only example of chelicerates showing determinate cleavage. Other chelicerates are not thought to specify cell lines early in development. However, cell lineage studies have so far only been attempted in spiders (Holm 1952; Kanayama et al. 2010) and a mite (Dearden et al. 2002).

Germ Rudiment Formation and Axis Formation

The Cumulus

The cumulus is a mesenchymal cell cluster that, in spiders, migrates from the centre of the germ disc to the rim of the germ disc and thereby breaks the radial symmetry of the embryo, establishing its dorsoventral (DV) axis (Fig. 5.4A). The cumulus has recently been shown to express *decapentaplegic* (*dpp*), and it is thought that Dpp protein is then received by germ disc epithelial cells and thereby represses ventralising *short gastrulation* (*sog*) expression (see below; Akiyama-Oda and Oda 2003, 2006).

The nomenclature of the cumulus has been confusing in classical chelicerate literature, since both the blastopore and the distinct, migrating cell group that originates from the blastopore form white, slightly elevated "cumulus-like" structures (from Latin, *cumulus*, meaning "heap" or "pile"). Therefore, classical literature has to be carefully judged for mislabeling the blastopore as a true cumulus. The cumulus' function as an organiser was first determined by Holm (1952) through cauterising and transplantation of cumulus material in embryos of the spider *Agelena labyrinthica*. Similar experiments as well as interspecific grafts have been performed on horseshoe crab embryos (Itow and Sekiguchi 1979; Itow 1990; Itow et al. 1991). Curiously, grafts of horseshoe crab "centre cells" from the blastopore region (but before actual cumulus

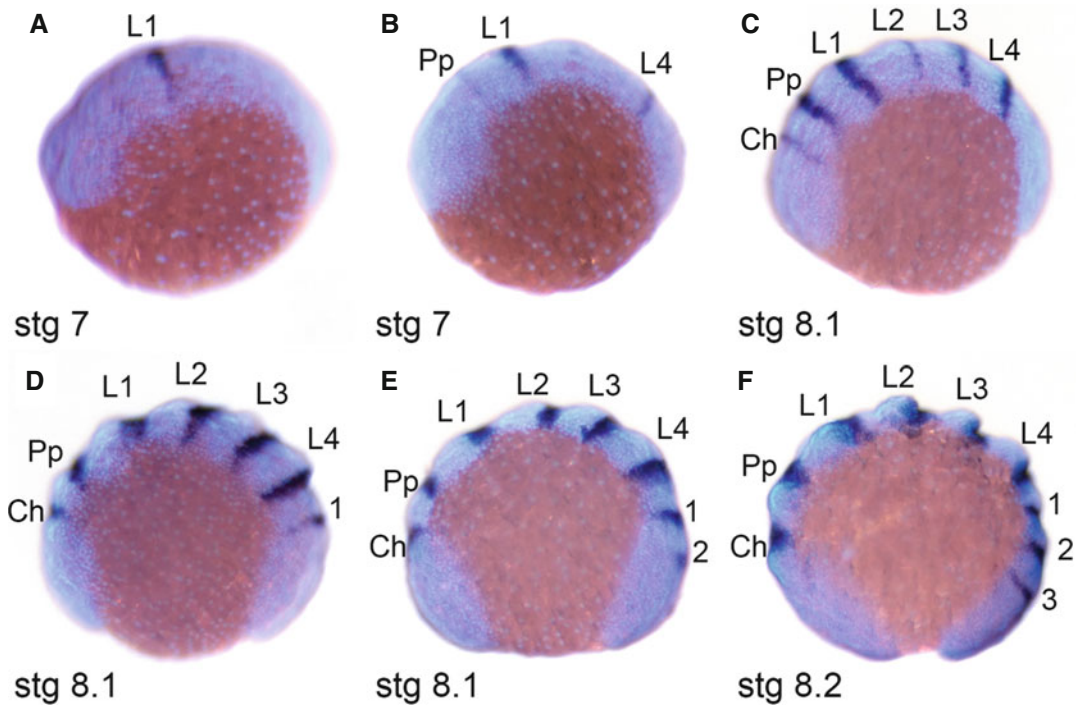

Fig. 5.5 Segmentation in spiders. Stripe appearance of the segmentation marker gene *engrailed* (*en*) in the spider *Parasteatoda tepidariorum*. The prosomal *en* stripes form nearly simultaneously, in a stereotyped order. (**A**) The first *en* stripe forms in L1. (**B**) Soon thereafter, stripes appear in L4 and the pedipalpal segment. (**C**) Subsequently stripes develop in L2, L3, and the cheliceral segment. (**D–F**) *en* stripes in the opisthosoma appear in a strict anterior to posterior order; only the first 3 of eventually 12 opisthosomal stripes are shown here. *Ch* cheliceral segment, *Pp* pedipalpal segment, *L* walking leg segments, 1, 2 and 3 opisthosomal segments (Figure slightly modified and reprinted from *Current Biology*, Schwager et al. (2009), with permission from Elsevier)

formation) are also capable of inducing a second embryonic axis in embryos of the frog *Xenopus laevis* (Itow 2005). However, Itow et al. claim that the posterior cumulus (i.e., the structure most likely homologous to the spider cumulus) has no effect on axis formation in the horseshoe crab (Itow 1990; Itow et al. 1991).

Migrating cumuli have also been noted in Amblypygi (Weygoldt 1975), Opiliones (Holm 1947; Juberthie 1964), possibly in a solifuge (Heymons 1904; Holm 1947) and most recently in a tick (Santos et al. 2013b). The cumulus has therefore been suggested as belonging to the ground pattern in Chelicerata (Hilbrant et al. 2012). However, the tick cumulus seems not to express Dpp, but instead, it appears to receive Dpp (Santos et al. 2013a, b). Accordingly, more evidence, especially molecular data, is required from chelicerate orders in which cumuli have not been described so far, to address the origin of the cumulus (with reference to the *dpp*-expressing structure observed in spiders) and perhaps ultimately to define this structure with respect to form, migration, developmental function, and gene interactions.

Segmentation

Most chelicerate embryos are of the short germ type, where a number of anterior segments is patterned by subdivision of the initial germ anlage and posterior segments are added sequentially from a posterior segment addition zone (SAZ). The initial germ anlage commonly forms all prosomal segments (pre-cheliceral lobe, cheliceral, pedipalpal, and the four walking leg segments—Pl, Ch, Pp, L1–L4), and a differing number of opisthosomal segments are added sequentially (Figs. 5.2, 5.3, and 5.5). This generalised form of

segmentation is found in spiders, harvestmen (Juberthie 1964), whip scorpions (Anderson 1973), Amblypygi (Weygoldt 1975), ticks (Anderson 1973; Santos et al. 2013b), and pseudoscorpions (Yoshikura 1975).

However, horseshoe crabs, scorpions and pycnogonids form only pre-cheliceral lobes and cheliceral and pedipalpal segments as well as a SAZ from their embryonic primordium, while walking leg segments and opisthosomal segments are then sequentially added from the SAZ (Anderson 1973; Itow and Sekiguchi 1980; Farley 2001; Brenneis et al. 2011b).

It is interesting to note that the anterior segments do not appear simultaneously, but instead are formed in a specific order that varies between groups. Where the timing of segment appearance has been observed (such as in spiders, Amblypygi and Xiphosura), the first segment to appear and the first segmental border to be established is usually the L1 segment or the Pp/L1 border (Anderson 1973; Weygoldt 1975; Itow and Sekiguchi 1980), and the last segment to be defined is most commonly the cheliceral segment. For example, in the spider *Parasteatoda tepidariorum*, *engrailed* (*en*) stripes appear first in L1, then Pp and L4 stripes emerge, then L2 and L3, and lastly Ch (Fig. 5.5; Schwager et al. 2009).

In contrast to the other chelicerates, the L4 segment of mites, ticks, and Ricinulei also derives from the SAZ. In case of the mite *Archegozetes longisetosus*, the remaining segments do not appear in sequential order from the SAZ, but, as evidenced by appearance of *en* and *hedgehog* (*hh*) stripes, first O1 is segmented, then L4, and finally O2 (Barnett and Thomas 2012).

As stated above, in almost all other chelicerates, opisthosomal segments are added sequentially from a SAZ. Despite the recent advances in our understanding of the genetic pathways involved in segmentation in spiders (McGregor et al. 2009; Hilbrant et al. 2012), we still lack insight into how exactly the SAZ of spiders and other chelicerates is organised, especially as cell division patterns have not been studied in detail, nor have cell movements been characterised. Generally, about 12 opisthosomal segments are formed from the SAZ. The first of these is later reduced to form the pedicel, linking the prosoma and opisthosoma in spiders and their close relatives. In other orders, O1 becomes greatly diminished (e.g., Opiliones) or almost completely removed (e.g., scorpions) in the course of embryonic development. In a few groups, such as opisthothele (non-mesothele) spiders, ticks, and mites, external opisthosomal segmentation is lost after embryogenesis (Anderson 1973; Yoshikura 1975).

Mites can also display a severe reduction in the number of opisthosomal segments: while most chelicerates develop around 12 embryonic opisthosomal segments (Fig. 5.2; Yoshikura 1975), in embryos of both *Tetranychus urticae* and *Archegozetes longisetosus*, only two *en* stripes are formed in the opisthosomal region (Grbic et al. 2011; Barnett and Thomas 2012). Ticks can show reduced opisthosomal segment numbers, but segments are clearly visible in embryos (Anderson 1973; Santos et al. 2013a, b). In addition, derived groups of mites (e.g., the gall mite family Eriophyidae) are even more segmentally aberrant, bearing only two legs and a worm-like body as adults.

Two groups that diverge from the general chelicerate segmentation pattern are (1) the katoikogenic scorpions, in which the mesosoma (the first eight embryonic segments of the opisthosoma in scorpions) is precociously segmented, with each segment bearing a pair of dorsolateral protrusions that supplement exchange surfaces with the mother, whereas the prosoma is segmented much later, and (2) the pycnogonids, which form a free-swimming larva that usually only possesses the cheliforal and two larval appendages (Vilpoux and Waloszek 2003; Machner and Scholtz 2010; Brenneis et al. 2013).

Development of the Nervous System

While the development of the nervous system in most Chelicerata has been described in classical literature (Anderson 1973), recent advances in imaging techniques as well as the use of molecular markers have allowed a more detailed look at chelicerate neurogenesis. However, these detailed

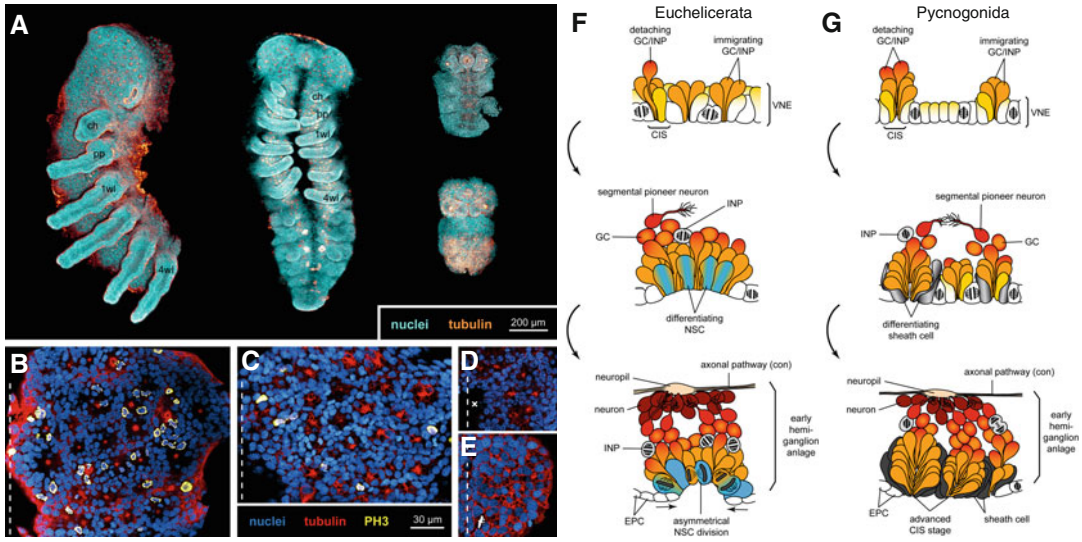

Fig. 5.6 Neurogenesis in euchelicerates and pycnogonids. (**A**) Confocal images of germbands of spiders and pycnogonids stained for tubulin and counterstained with a nuclear dye to illustrate cell internalisation sites (*CIS*) that are organised in a grid-like pattern in each hemisegment. From left to right: *Cupiennius salei* (left half of prosoma only), *Parasteatoda tepidariorum*, *Callipallene* sp. (*top right*), and *Pseudopallene* sp. (*bottom right*). (**B**–**E**) Apical horizontal sections of the ventral neuroectoderm (*VNE*) of a walking leg segment of (**B**) *C. salei* and (**C**) *P. tepidariorum*. Mitotic cells (stained by Phosphorylated Histone 3 antibody) are not found near CIS but are scattered throughout the hemi-neuromere. In the two pycnogonids (**D**) *Callipallene* and (**E**) *Pseudopallene*, the hemi-neuromeres are much smaller than found in the spiders. (**F**, **G**) Schematic sagittal sections through single hemi-neuromeres. Colours indicate different neuronal precursor (*NP*) cell types. In Eucelicerata (**G**, based mainly on *C. salei*), CIS form sequentially in the VNE, which shows unordered, mostly tangential cell divisions. Immature ganglion cells (*GCs*) delaminate and start differentiating basally to the CIS. CIS can also form cell-rich units, enclosed by glial-like sheath cells. Close to the forming neuropil, scattered symmetrically dividing intermediate neural precursors (*INPs*) can be found. Apically, the epidermis overgrows the hemi-neuromeres. In Pycnogonida (**F**, based mainly on *Pseudopallene* sp.), only few CIS and unordered, tangential cell divisions are found in the VNE. Basally, single GCs and INPs detach from the VNE and form a loose layer, in which neurons start differentiating. Apically, a central invagination forms and continues to deepen. Neural stem cells (*NSCs*), large, spindle-shaped cells, become discernible and start dividing tangentially or slightly obliquely, forming smaller daughter cells that invaginate independently. INPs also divide sub-apically. Epidermis overgrows the invagination site. *ch* cheliceral/cheliforal segment, *EPC* epidermis cell, *pp* pedipalpal segment, *wl* walking leg segment (Figure slightly modified and reproduced with permission from Brenneis et al. (2013))

studies of neurogenesis have only been performed on spiders, a horseshoe crab, and some pycnogonids.

Neurogenesis in spiders and horseshoe crabs takes place in the central ventral ectoderm. In each hemisegment about 30 groups of bottle-shaped neuronal precursors are specified and form cell internalisation sites (previously termed invagination sites). These sites are organised in a grid-like pattern and subsequently simultaneously delaminate from the neuroectoderm when early neurogenesis is complete (Fig. 5.6; Stollewerk et al. 2001; Mittmann 2002; Stollewerk and Chipman 2006; Doeffinger et al. 2010). In contrast to insects (Vol. 5) and crustaceans (Vol. 4), no neuroblasts (neural stem cells) are involved in neurogenesis in these chelicerates. Due to these differences with respect to Tetraconata and marked similarities with Myriapoda, it has been suggested that this mode of neurogenesis might be ancestral within arthropods (reviewed by Stollewerk and Chipman 2006).

Conversely, it has recently been shown that neurogenesis in pycnogonids surprisingly does involve neural stem cells (Fig. 5.6; Brenneis et al. 2013). Like the euchelicerates, neurogenesis in pycnogonids is initiated by formation of

post-mitotic neuronal precursor groups that form cell internalisation sites. In a second step, in pycnogonids, however, larger neural stem cells with high mitotic activity differentiate from the precursor groups and form ganglion cells by asymmetric cell divisions (Fig. 5.6; Brenneis et al. 2013).

Whether the pycnogonid neural stem cells have evolved convergently or, alternatively, chelicerates and myriapods have lost this cell type will, according to Brenneis et al. (2013), require studies of the molecular mechanisms of neurogenesis in pycnogonids, as well as detailed reinvestigation of neurogenesis in other arthropod groups. It is interesting to note that some authors of classical literature have noted the existence of neural stem cells within a few chelicerates (Anderson 1973). Because the study of neurogenesis with modern techniques is limited to just two chelicerate orders, re-examination of neurogenetic processes with advanced techniques is imperative in non-spider arachnids.

LATE DEVELOPMENT

Inversion

In most chelicerates the germband forms on the surface of the round or ovoid yolky egg. Hence, at some point during the development of the chelicerate embryo, the yolk has to be transferred into the embryo proper, specifically into the opisthosoma, where it will later be ingested by the hatchlings via the midgut. This problem is solved in two distinctive ways among the different chelicerate orders. The embryo either simply grows around the yolk dorsally until dorsal closure commences, or it undergoes a process termed "inversion". This process is most pronounced in entelegyne spiders, less so in more basally branching groups such as mygalomorph and haplogyne spiders, and is almost absent in mesothele spiders (Yoshikura 1975).

During inversion, the germband splits in half along the ventral midline and forms the ventral sulcus, which is only covered by a single layer of cells. The two halves, still connected at least at the anterior and posterior ends, move dorsally around the yolk, widening the ventral sulcus, until their dorsal sides converge at the dorsal midline. Only after dorsal closure do the two halves of the germband reconnect ventrally for ventral closure. During this process, most of the yolk is transferred into the opisthosoma (Anderson 1973).

The amount by which the ventral sulcus widens differs across spiders, and as mentioned before, is less pronounced in more basally branching groups. However, other chelicerates display inversion processes similar to spiders, most notably in Thelyphonida, Amblypygi (Weygoldt 1975), and possibly Solifugae, and others such as Opiliones and some mites show only a very slight widening of the ventral sulcus during the dorsal closure process (Anderson 1973). The process of inversion inherently has consequences for the behaviour of the midline and also for neurogenesis in spiders (Linne et al. 2012).

Development of Germ Cells

Characterisation of the developmental origin of germ cells of chelicerates is limited to classical, mainly histological, studies of only a few groups (spiders, scorpions, mites, ticks, harvestmen, and solifuges) (Anderson 1973).

In these studies, germ cells have mostly been reported to originate from the mesoderm later in embryogenesis, for example, at the posterior end of the germband in ticks (Aeschlimann 1958) or in spiders, where germ cells appear as segmental clusters close to the coelomic pouches in the opisthosoma (Kautzsch 1909; Strand 1906). In the early embryos of some spiders, harvestmen and solifuges primordial germ cells (PGCs) have also been described to originate in or near the blastopore (Faussek 1891; Brauer 1894; Heymons 1904; Montgomery 1909). Recently however, by assaying the mRNA and protein expression of two molecular germ cell markers, *piwi and vasa*, Schwager et al. (2014) did not find any evidence of germ cells near the blastopore in early spider embryos. Instead, in *Parasteatoda*, PGCs arise as segmental clusters in opisthosomal segments O2–O6.

Interestingly, none of the previous studies that found PGCs in or near the blastopore in harvestmen, scorpions, and solifuges were able to trace these cells to the gonads at later stages (Faussek 1889, 1891; Brauer 1894). Therefore, to determine the germ cell origin in these three groups, it will be essential to re-examine their embryos using molecular tools where possible.

In the only other modern study of chelicerate germ cells, in the spider mite *Tetranychus urticae*, the germ cell marker gene *vasa* has been used to identify a group of dispersed cells deep in the yolk as PGCs that later are thought to migrate towards the posterior of the embryo to form a cluster of germ cells near the prosomal/opisthosomal boundary (Dearden et al. 2003). This mode of germ cell specification from non-blastodermal cells does not match any of the modes described for the other chelicerates. Indeed, since *vasa* has also been found to be expressed in numerous other tissues, including stem cell-like cells, the cells described in *T. urticae* might not actually be PGCs. Examining PGC specification in the spider mite using more germ cell markers might help to shed further light on this issue (Schwager et al. 2014).

Development of Respiratory Organs

Among chelicerates, three main types of respiratory organs can be found: book gills, book lungs, and tracheae (Fig. 5.2). Book lungs and tracheae appear alone or in combination across the chelicerate orders (Fig. 5.2). Some miniaturised species (e.g., microwhip scorpions and some mites) lack specialised respiratory organs entirely, with gas exchange occurring through the cuticle (Ax 2000; Zhang 2003; Foelix 2010). Similarly, respiration in pycnogonids occurs through direct diffusion.

Book Gills and Book Lungs

The book gills of Xiphosura are thought to represent the most ancestral respiratory organ among euchelicerates, but their relationship to scorpion and spider book lungs is not well understood. Recent phylogenomic efforts suggest a single origin of the arachnid book lung, consistent with the anatomy of these organs in spiders, amblypygids, uropygids, and scorpions (Scholtz and Kamenz 2006; Regier et al. 2010; Sharma et al. 2014b). In *Limulus polyphemus*, book gill development commences with the formation of bilateral ridges on the opisthosoma, the primordia of the genital operculum, and the branchial appendage, which will later become the gill-bearing segment (Yamasaki et al. 1988). The genital operculum and the first branchial appendage further develop into a large lateral and a small medial lobe on the ventral side of the opisthosoma (Farley 2010). Trabeculae then become apparent on these opisthosomal segments, which will later function as space holders in the haemolymph channels of the book gills (Kingsley 1892). The surface of the operculum and the branchial appendage form small pores and invaginations, which may facilitate gas exchange. Cross sections of the operculum and branchial appendage have revealed that trabeculae bridge the lumen of these lobes and seem to be connected with the invaginations on the surface of these appendages (Farley 2010). At the stage of the swimming and burrowing first instar, the first branchial segment appears as a broad but thin appendage, which carries four gill lamellae. The book gills are therefore surface outgrowths of the first branchial appendage. The lamellar structures of the book gills provide the surface for gas exchange between water and haemolymph in horseshoe crabs. The invaginations at the surface of the operculum and the branchial segment are connected to the gill lamellae through the trabeculae (Farley 2010).

Arachnopulmonata (scorpions + tetrapulmonates) exhibit variable numbers of paired book lungs (Fig. 5.2). The "primitive" spiders (mesotheles, mygalomorphs, and most paleocribellates) exhibit two pairs of book lungs, but in labidognathous spiders (i.e., derived araneomorphs), the posterior pair has been modified into tubular tracheae (Kästner 1929; Yoshikura 1975). Scorpions exhibit four pairs of book lungs, while whip scorpions and whip spiders have two pairs, and microwhip scorpions only have one pair (Fig. 5.2; Levi 1967).

The development of scorpion and spider book lungs is uniform and first becomes apparent as an

ectodermal invagination at the posterior margin of an opisthosomal segment (Laurie 1890; Purcell 1909; Farley 2008). This invagination then increases in size, forming a pulmonary sac, while the limb bud itself ingresses into the ectoderm of the segment (Farley 2011). The anterior wall of the pulmonary sac develops projecting lamellae, which extend into the pulmonary sac (Anderson 1973; Farley 2010, 2011).

The fully developed book lungs in scorpions and spiders open as stigmata on the ventral side of the opisthosoma into the atrium, which enlarges into a cuticle-lined cavity (Kamenz et al. 2005). Cuticular invaginations filled with haemolymph, interspersed by air pockets, extend horizontally from the lung sinus opposite the atrium into the cavity. The name "book lungs" is derived from the stacked structure of the lamellae, where the oxygenation of the haemolymph occurs (Reisinger et al. 1991; Kamenz et al. 2005; Foelix 2010).

Tracheae

A tracheal respiratory system is found in mites, ticks, pseudoscorpions, camel spiders, harvestmen, hooded tick spiders, and, in conjunction with one pair of book lungs, most araneomorph spiders (Fig. 5.2). Tracheae can vary in structure and are either tubular (camel spiders, harvestmen, and some spiders) or sieve tracheae (pseudoscorpions, hooded tick spiders, some spiders) (Kamenz et al. 2005; Foelix 2010). The latter are composed of a bundle of tubes, which look like a perforated membrane in cross section, hence the name. It has been proposed that the sieve tracheae are derived from lung lamellae (Foelix 2010; Nentwig 2013).

In spiders the tubular tracheae are located on the third opisthosomal segment, behind the anterior pair of book lungs, and are visible as stigmata (openings), in close vicinity to the spinnerets (Fig. 5.2). Generally, a stigma leads into an atrium whence two lateral and two median tubes arise. The lateral tubes are connected to the second pair of book lungs and the median tubes originate from muscular insertions, which become hollow and function as breathing organs (Foelix 2010). Tracheae in spiders exhibit open ends, which are in direct contact with haemolymph that transports the oxygen to the organs. The localisation and expansion of the tubular tracheae, however, is not as uniform as for book lungs and can vary significantly between species ranging from a restriction to the opisthosoma to extensive branching up to the prosoma (Foelix 2010). Within spiders, tubular tracheae are regarded as more derived than book lungs, as they are not found in basally branching spiders or non-spider tetrapulmonates, which employ only book lungs (Höfer et al. 2000; Foelix 2010). The simultaneous knockdown of multiple posterior Hox genes results in homeotic transformation of book lungs (and possibly the tubular tracheae as well) to leg-like outgrowths in the spider *Parasteatoda tepidariorum*, corroborating the serial homology of paired respiratory organs and prosomal appendages in a tetrapulmonate arachnid (Khadjeh et al. 2012). The relationship between the tubular tracheae of spiders and those of apulmonate arachnids is not understood in the context of developmental genetics.

THE GENETIC REGULATION OF CHELICERATE DEVELOPMENT

Axis Formation

In chelicerates, the regulation of the formation of the anterior-posterior (AP) and dorsoventral (DV) axes are best understood in the spider *Parasteatoda tepidariorum*. During the formation of the germ disc in this spider (Fig. 5.3; see boxed text), the cumulus develops as a cluster of mesenchymal cells under the main epithelial disc (Fig. 5.4A). Gene expression and functional analyses of orthologous genes that pattern the body axes of other arthropods have highlighted the importance of the cumulus as a key signalling centre for embryonic organisation in the spider (see above; Oda and Akiyama-Oda 2008).

During the initial formation of the germ disc, Hh signalling plays a crucial role in coordinating the cumulus and controlling its movement (Akiyama-Oda and Oda 2010). Hh ligands from around the rim of the germ disc are received by

patched (*ptc*) and *smoothened* (*smo*). It has been suggested that Hh forms a positional value gradient and thereby high levels promote the presumptive anterior, while low levels at the centre of the disc designate the posterior region where the cumulus forms (Fig. 5.4A; Akiyama-Oda and Oda 2010). The movement of the cumulus to the periphery also relies on Hh signalling because parental RNAi against *ptc* and *smo* can perturb cumulus migration (Fig. 5.4A; Akiyama-Oda and Oda 2010).

As mentioned above, the migration of the cumulus from the centre to the periphery of the germ disc breaks the radial symmetry and forms the DV axis (Fig. 5.4; Akiyama-Oda and Oda 2003). While the basal mesenchymal cells of the cumulus migrate under the germ disc, they express *dpp*, which activates the phosphorylation of mothers against dpp (pMad) in the epithelium, possibly via cytonemes (Fig. 5.4A; Akiyama-Oda and Oda 2003). When the *dpp* expression reaches the rim of the germ disc, it represses part of the circular expression domain of *sog* (Fig. 5.4B; Akiyama-Oda and Oda 2006). This event is concomitant with the opening of the dorsal field and the loss of *dpp* expression as the cumulus disappears (Fig. 5.4B). The expression of *sog* retracts ventrally between the anterior expression of *orthodenticle* (*otd*) and *caudal* (*cad*) expression in the caudal lobe (Fig. 5.4B, C; Akiyama-Oda and Oda 2003; Pechmann et al. 2009). *sog* expression progressively narrows to the ectoderm of the ventral midline, surrounded by pMad in the dorsal region (Fig. 5.4C; Akiyama-Oda and Oda 2006).

Segmentation

Formation of the Caudal Lobe and Posterior Segmentation

Studying the genetic regulation of segmentation in chelicerates, especially spiders, has provided key insights into the evolution of segment formation among arthropods and even other metazoans with segmented bodies (Damen 2007; McGregor et al. 2008a, 2009; Oda and Akiyama-Oda 2008; Hilbrant et al. 2012). Before the appearance of segments, the DV and AP axes are defined, as well as the first regulatory steps that specify the germ layers (see above). The genetic regulation of these processes, again, has been most fully characterised in *Parasteatoda tepidariorum*. During early embryogenesis in this spider, the Delta-Notch pathway is involved in allocating cells to the ectoderm, mesoderm, and endoderm as well as specifying the caudal lobe that gives rise to the SAZ, from which subsequently the posterior segments are generated (Oda et al. 2007).

Concurrent with the formation of the cumulus, the centre of the germ disc begins to express *Delta* (*Dl*) (Fig. 5.4). Cells that express *forkhead* and *twist* (*twi*) near these *Dl*-expressing cells internalise beneath the epithelia and become endoderm and mesoderm cells, respectively (Oda et al. 2007). Subsequently, expression of *Dl* and *twi* clears from the centre of the germ disc and *cad* is expressed in the caudal lobe (Fig. 5.4B; Oda et al. 2007). Furthermore, these dynamic changes in gene expression that specify the caudal lobe and subsequently the SAZ all require *Wnt8* (McGregor et al. 2008b).

During the formation of the germband from the germ disc (Figs. 5.3 and 5.4), the posterior domain of *Dl* expression forms a stripe. Expression of *Dl* then reappears in the SAZ and subsequently dynamic stripes of *Dl* expression in the SAZ are associated with the formation of nascent segments from this tissue. Previously, it was also shown that such stripes of *Dl* expression in the SAZ are required for segmentation in *Cupiennius salei*, another spider (Stollewerk et al. 2003). Since *Dl* is also necessary for segmentation in the cockroach *Periplaneta americana* (Pueyo et al. 2008), this suggests that Delta-Notch, Wnt, and Cad organiser was used ancestrally for segmentation at least in arthropods and was subsequently lost in some lineages (McGregor et al. 2009; Wilson et al. 2010; Kainz et al. 2011; Chesebro et al. 2013). This work has also contributed to the debate about the evolution of segmentation in metazoans more generally (Couso 2009; Chipman 2010).

After the initial cues from Delta-Notch and Wnt have activated segmentation from the

posterior SAZ, it has been shown in both *Cupiennius salei* and *Parasteatoda tepidariorum* that the orthologs of the pair rule genes are then differentially activated across the AP axis. In *P. tepidariorum*, *Wnt8* may help to regulate the transcription of the primary pair rule gene *hairy* in the SAZ (McGregor et al. 2008b). In *C. salei*, dynamic stripes of *even skipped* and *runt-1* progress from the SAZ during the formation of nascent posterior segments (Damen et al. 2005). The secondary pair rule gene *pairberry-3* also exhibits dynamic expression in the SAZ but forms stable stripes in nascent segments (Damen et al. 2005). However, the other secondary pair rule genes, *odd-skipped-related-1*, *odd-paired* (*opa*), and *sloppy paired*, are not expressed in the SAZ but are observed in stripes anterior to this structure in the nascent segments (Damen et al. 2005). The primary pair rule genes therefore appear to initially define segments from the SAZ and then the secondary pair rule gene orthologs maintain segment positioning. Subsequently, the parasegmental boundaries are defined by *Wnt* and *en* expression (Damen 2002), which is now known to be a conserved feature of arthropod segmentation (Vols. 4, 5; Damen 2007).

Prosomal Segmentation

It has been shown in spiders that the mechanism and underlying genetic regulation of prosomal segmentation differ from that described above for the opisthosomal segments. In the presumptive prosoma, segmentation is achieved by subdividing a pre-existing field of cells into segments, and *engrailed* stripes do not appear sequentially in this region (see above and Fig. 5.5). This prosomal segmentation mechanism is similar to *Drosophila melanogaster* segmentation. Indeed, in *Parasteatoda tepidariorum* this process requires the ortholog of the *D. melanogaster* gap gene *hunchback*, and knockdown of this gene in *P. tepidariorum* also produces a gap gene phenotype with multiple missing adjacent segments (Schwager et al. 2009). Interestingly, in both *P. tepidariorum* and the haplogyne spider *Pholcus phalangioides*, *Distal-less* (*Dll*), a gene normally known for its involvement in appendage patterning (see below), is expressed in the presumptive prosoma (Pechmann et al. 2011). Even more surprisingly, *Dll* is required for formation of prosomal segments because inhibition of *Dll* expression in *P. tepidariorum* results in a gap-like phenotype (Pechmann et al. 2011).

In *Parasteatoda tepidariorum* embryos, the most anterior prosomal region, however, yet again uses a different segmentation mechanism that Kanayama et al. (2011) have termed "split-type segmentation". Here, first a wave of *otd* expression, in conjunction with a travelling wave of *hh* expression, is thought to specify the head segments (Pechmann et al. 2009; Kanayama et al. 2011). Then, the *hh* stripe splits to generate the cheliceral and pedipalpal segments, which also involves convergent extension movements and depends on an autoregulatory signalling network of *otd*, *hh*, *opa,* and *cubitus interruptus* (*ci*) (Kanayama et al. 2011).

Hox Genes and the Regulation of Segment Identity in Chelicerates

Hox genes are responsible for specifying segmental identity along the AP axis in bilaterian animals (reviewed in Carroll et al. 2005). In chelicerates, the evolution of particular Hox genes is correlated with differences among chelicerate body plans and compared to other arthropods. Generally, the spatial expression patterns of the prosomal Hox genes are well conserved, whereas those that are expressed in the opisthosoma are more divergent (Figs. 5.2 and 5.7; Abzhanov and Kaufman 1999; Schoppmeier and Damen 2001; Khila and Grbic 2007; Pechmann et al. 2011). This may correlate with the evolutionary conservation of the prosoma compared to the more variable opisthosoma.

In all chelicerate lineages studied to date (apart from mites), as well as mandibulate arthropods (Chapter 6; Vols. 4, 5) and Onychophora (Chapter 4), at least ten Hox genes have been identified (Fig. 5.2; Janssen and Damen 2006; Sharma et al. 2012a, 2013, 2014b; Barnett and Thomas 2013a; Janssen et al. 2014), which suggests that this was the ancestral number of Hox

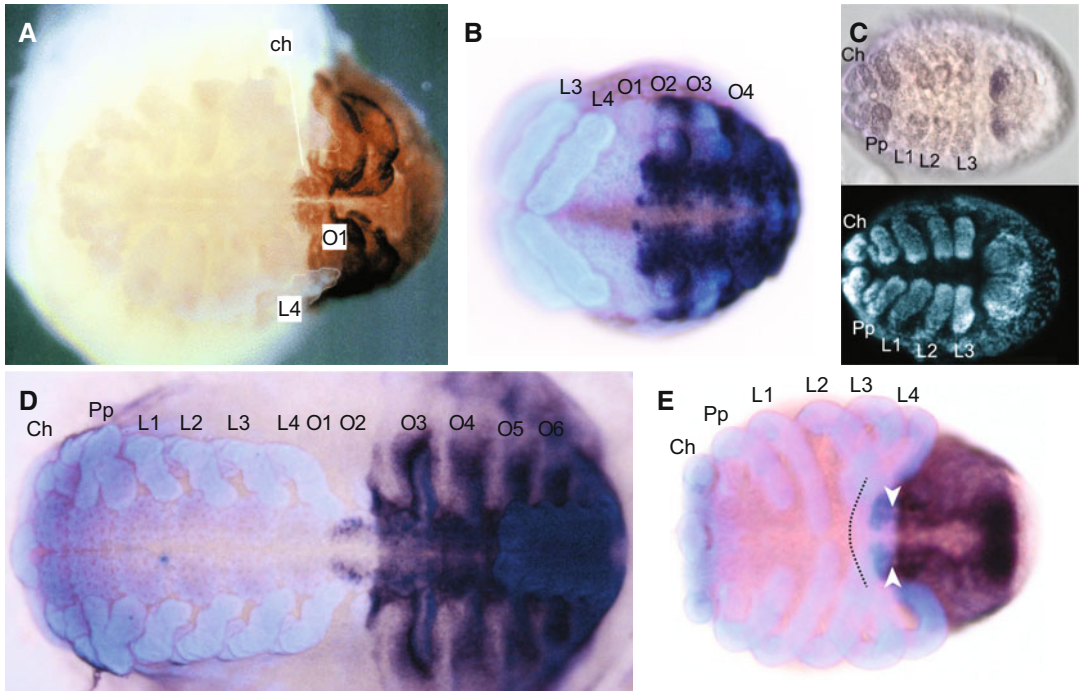

Fig. 5.7 Expression of *Ubx* in chelicerate embryos. (**A**) In the horseshoe crab *Limulus polyphemus*, Ubx/abd-A antibody staining is initially observed in O2 and all segments more posterior. In the later stage shown, it extends more anteriorly into the medial portion of O1 (the chilarial segment) (Slightly modified and reproduced from Popadic and Nagy (2001) with permission from John Wiley and Sons). (**B**) Expression of *Ubx-1* in the spider *Parasteatoda tepidariorum* extends ventrally into the posterior half of O1; otherwise, *Ubx-1* is expressed in O2 and all more posterior segments. (**C**) *Ubx* expression in the mite *Archegozetes longisetosus* (*top*, brightfield image; *bottom*, nuclear staining) is only found in O2 (Image slightly modified reproduced with permission of the authors of Barnett and Thomas (2013a)). (**D**) *Ubx-2* expression in the scorpion *Centruroides sculpturatus* is found in the ventral part of O2 and all segments more posterior. (**E**) In the harvestman *Phalangium opilio*, *Ubx* is expressed in O2 (*arrowheads* indicate the genital pores on O2, *dotted line* demarcates the prosomal/opisthosomal boundary) and all segments posterior to it. All embryos are oriented with anterior to the left. Embryos in (**B**, **D**, **E**) have also been stained with a nuclear dye. *ch* chilaria, *Ch* cheliceral segment, *Pp* pedipalpal segment, *L* walking leg segments, *O* opisthosomal segments

genes in arthropods. However, in *Cupiennius salei*, *proboscipedia*, *Deformed*, *Sex combs reduced*, and *Ultrabithorax* (*Ubx*) have all been found to be duplicated (Damen et al. 1998; Schwager et al. 2007). Furthermore, the paralogs have different spatiotemporal expression patterns, which suggests that there could have been significant duplication and divergence of Hox genes during the evolution of chelicerate body plans (Figs. 5.2; Schwager et al. 2007). Similarly, 19 Hox genes have been reported in the scorpion *Centruroides sculpturatus*, with two copies of each gene except for *Hox3* (Sharma et al. 2014b). Furthermore, different spatiotemporal gene expression patterns were observed for all four paralogous pairs of the opisthosomal Hox genes (*Antennapedia* (*Antp*), *Ubx*, *abdominal-A* (*abd-A*), and *Abdominal-B* (*Abd-B*)) (Figs. 5.2 and 5.7; Sharma et al. 2014b). Intriguingly, shifts in anterior boundaries of opisthosomal Hox group paralogs are tightly correlated with shifts in segmental identity in the scorpion mesosoma and metasoma, consistent with the involvement of the paralogs in canonical Hox patterning (Sharma et al. 2014b).

Evolutionary changes to the Hox cluster are also found in the mite *Tetranychus urticae* (Grbic et al. 2011). This species has lost *abd-A* from its

genome (Grbic et al. 2011), which appears to be correlated with drastic reduction of the opisthosoma to only two segments (Fig. 5.2). This possible role of *abd-A* in defining opisthosomal segment number may also be consistent with the finding of a highly divergent *abd-A* in the sea spider and the reduction in size of this tagma in these animals (Manuel et al. 2006).

The expression domains of *Ubx*, *abd-A*, and *Abd-B* have also been found to be important in determining the identity of opisthosomal segments among chelicerates (Fig. 5.2; Damen and Tautz 1999; Popadic and Nagy 2001; Sharma et al. 2012b, 2014c; Barnett and Thomas 2013a). The different anterior expression domains of these Hox genes in harvestmen, scorpions, and spiders are correlated with the position of different segment types, such as book lungs, spinnerets, and the posterior-most undifferentiated segments (Fig. 5.2; Sharma et al. 2012a, 2014b). It therefore appears that the evolution of Hox gene expression is a likely mechanism for the diversification of chelicerates via the modification of posterior segment identity, a hypothesis that is beginning to be tested with functional tools in spiders (Khadjeh et al. 2012).

In addition, analysis of Hox gene expression has played a major role in solving the question of the evolution of arthropod head segments and their associated appendages (Telford and Thomas 1998a; Budd 2002; Maxmen et al. 2005; Scholtz and Edgecombe 2006; Brenneis et al. 2008; Damen 2010). Cheliceres and pedipalps (Fig. 5.2) were thought to be analogous to the intercalary and mandible segments in insects, respectively, due to their supposed innervation from particular regions of the ganglia. It has been further postulated that the segment in chelicerates that is analogous to the first antennal segment in myriapods, crustaceans, and insects has been lost during the course of evolution (Weygoldt 1985; Bitsch and Bitsch 2007). However, studies of Hox gene expression suggest that the segments bearing the cheliceres (and chelifores of pycnogoids) and pedipalps are homologous to the first antennal and intercalary (or second antennal) segments of mandibulates, respectively. Independent corroboration of this hypothesis is provided by the segmental organisation of the tripartite arthropod brain; both the first antennal segment and chelicer al (or cheliforal) segment are innervated by the deutocerebral ganglia (Telford and Thomas 1998a; Jager et al. 2006; Brenneis et al. 2008).

Appendage Development

The prosoma of euchelicerates comprises an evolutionarily conserved tagma, as inferred from segmental distribution of appendage types (Fig. 5.2). In other arthropod subphyla, genes including *Dll*, *homothorax* (*hth*), *extradenticle* (*exd*), and *dachshund* (*dac*) are required for appendage development, and it has been shown that these genes are also necessary for appendage development in chelicerates (Fig. 5.8; Prpic et al. 2001, 2003; Prpic and Damen 2004; Pechmann and Prpic 2009; Barnett and Thomas 2013b; Sharma et al. 2013).

The development of all the appendages requires *Dll*; knockdown of the expression of this gene inhibits outgrowth from limb primordia in, for example, spiders, mites, and harvestmen (Schoppmeier and Damen 2001; Khila and Grbic 2007; Pechmann et al. 2011; Sharma et al. 2013).

During the evolution of cheliceres, it appears that there has been a shift from primitive three-segmented cheliceres in orders like harvestmen, horseshoe crabs, and pycnogonids to the more derived two-segmented cheliceres of lineages like spiders (Sharma et al. 2012a, 2013; Barnett and Thomas 2013a; Brenneis et al. 2013; Brenneis and Scholtz 2014). Interestingly, an expression domain of *dac* in the proximal region of the harvestman *Phalangium opilio* is not found in arachnids that have cheliceres composed of two segments (Fig. 5.8; Sharma et al. 2012a), suggesting a role for this gene in the transition from three- to two-segmented cheliceres. Consistent with this hypothesis, knockdown of the expression of *dac* in *P. opilio* indicates that this gene is required for the development of the proximal cheliceral segment (Sharma et al. 2013). Further corroborating this mechanism, the proximal-most part of the cheliceres of the mite *Archegozetes longisetosus* transiently expresses

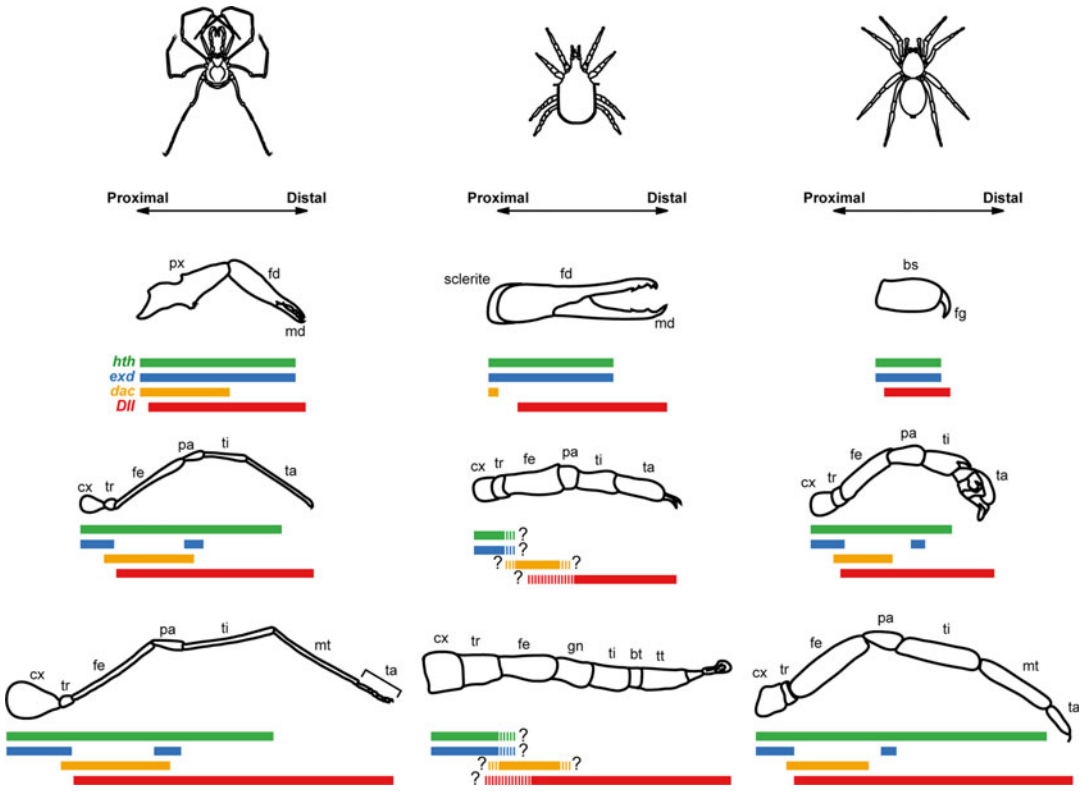

Fig. 5.8 Gene expression during appendage development. Comparative expression patterns of leg gap genes in three chelicerates. From *left to right*: Opiliones, Acariformes, and Araneae. Appendage types from *top* to *bottom* are chelicera, pedipalp, and walking leg. *Coloured bars* indicate expression domains of *homothorax* (*green*), *extradenticle* (*blue*), *dachshund* (*orange*), and *Distal-less* (*red*). Hashed bars in Acariformes indicate uncertainty of expression boundaries with respect to podomeres. *2nd* secondary article, *bs* basis, *bt* basitarsus, *cx* coxa, *fe* femur, *fg* fang, *gn* genu, *ma* mobile article, *mt* metatarsus, *pa* patella, *px* proximal segment, *ta* tarsus, *ti* tibia, *tr* trochanter, *tt* telotarsus (© Prashant P. Sharma, 2015. All Rights Reserved)

dac (Fig. 5.8; Barnett and Thomas 2013b). Accordingly, adults of many Acariformes form a sclerite in this region whose segmental nature had been debated, and *dac* expression in mite embryos suggests that this sclerite is a vestige of the fully formed proximal segment of groups like Opiliones and Xiphosura (Sharma et al. 2012a, 2013; Barnett and Thomas 2013b).

A separate aspect of the appendages that is evolutionarily labile and functionally significant to feeding in chelicerates is the gnathobases (endites), a separate ramus of the chelicerate appendage (Boxshall 2004). A variable number of gnathobases occurs across Chelicerata, and these structures have played a key role in morphological phylogenetic hypotheses of the group (Shultz 2007). For example, outgrowths of a single appendage pair, the pedipalpal gnathobases, form the "maxilla" of spiders (not homologous to the maxillae of mandibulates). Gnathobases of the same appendage pair form part of the subcapitulum of mites and ticks and putatively unite these as "Acari". In groups like Opiliones and scorpions, additional gnathobases occur on the walking leg segments; these fuse to form the preoral chamber, a structure that has putatively united harvestmen and scorpions in morphological phylogenies (Shultz 1990, 2007). In spiders (both araneomorphs and mygalomorphs), mites, and harvestmen, all outgrown gnathobases strongly express *Dll*, and knockdown of *Dll* expression results in the loss of these structures, together

with the distal telopod segments (Schoppmeier and Damen 2001; Khila and Grbic 2007; Sharma et al. 2013). These data suggest a common, but unknown, developmental patterning mechanism underlying morphogenesis of feeding structures derived from gnathobasis outgrowths.

Neurogenesis

Research on gene expression during neurogenesis in chelicerates has made an important contribution to our understanding of the evolution and development of arthropods more generally (Stollewerk and Chipman 2006). In arthropods the *achaete-scute* complex is important in the early stages of neurogenesis. It has been shown that the spider homolog *ASH1* has a similar function during the formation of neural precursor cells to that of crustaceans and insects (Doeffinger et al. 2010). High levels of *ASH1* expression induce the later invagination of regions to form the optic lobes, mushroom bodies, and arcuate body (Doeffinger et al. 2010).

Furthermore, Delta-Notch signalling determines neuronal precursor number through lateral inhibition across arthropod lineages (Stollewerk 2002), and the genes that are responsible for patterning neural fates are also somewhat conserved. For example, *en* (Doeffinger et al. 2010) and *gooseberry* (Jarvis et al. 2012) are involved in organising the neuroectoderm in chelicerates and mandibulates. Interestingly, alterations in Hox expression in the developing nervous system are also correlated with changes in Hox expression across the AP axis. These changes in both neurology and segment morphology due to Hox genes may help appendages to evolve sensory functionality (Jarvis et al. 2012).

Although some genes have conserved roles during neurogenesis, the function of others has diverged. For example, in the spiders *Cupiennius salei* and *Parasteatoda tepidariorum*, Netrins have been shown to have elements of conserved function in commissural axon guidance in the ventral midline with respect to insects and crustaceans (Linne and Stollewerk 2011). However, in *C. salei*, Netrins may also contribute to the correct differentiation of the axonal scaffold through maintaining short-range adhesive interactions between sheath cells and neural precursor cells (Linne and Stollewerk 2011).

Another gene that has diverged in function is *single-minded* (*sim*). In crustaceans and insects, *sim* functions as an important regulator of ventral midline development (Nambu et al. 1990, 1991; Vargas-Vila et al. 2010). This is in contrast to chelicerates, where *sim* is expressed in the median region of the ventral neuroectoderm and is not required for ventral midline development (Linne et al. 2012). It has been hypothesised that the midline precursors seen in crustaceans and insects evolved from an ancestral median area of ventral neuroectoderm. The modification of *sim* expression from the median to the midline tissue could be responsible for this change (Linne et al. 2012).

Therefore, while considerable progress has been made on understanding the evolution and regulation of neurogenesis in chelicerates, it is clear that further insights into the evolution of neurogenesis will be gained through investigation of gene expression and function in non-arachnid chelicerates like pycnogonids and horseshoe crabs (Brenneis et al. 2013; Brenneis and Scholtz 2014).

FUTURE RESEARCH FOCI FOR CHELICERATE EVODEVO

Many important questions in evolutionary biology can be uniquely addressed through evolutionary developmental study of Chelicerata, both via comparisons within chelicerates and between Chelicerata and other metazoans. Key processes that can only be deciphered through studies of chelicerates include the genetic basis for the synthesis of diverse and potent venoms (e.g., scorpion and spider venoms), the diversification of silk genes, and the evolution of terrestrialisation.

Newly sequenced genomes of non-developmental models have provided much needed insights as to genomic architecture and gene family diversification in notable chelicerate

groups (Table 5.1). Developmental studies using established chelicerate models, such as the spider *Parasteatoda tepidariorum*, can be expanded by availability of genomic resources (Posnien et al. 2014). This is complemented by the rapid dissemination of developmental transcriptomes and modern developmental techniques for satellite models such as the harvestman *Phalangium opilio* (Sharma et al. 2012a, 2013) and the scorpion *Centruroides sculpturatus* (Sharma et al. 2014b, c), coupled with refined understanding of phylogenetic relationships (Regier et al. 2010; Sharma et al. 2014a).

However, establishing laboratory cultures in concert with further development of gene expression and functional techniques in exemplars of other chelicerate orders would be insightful for a number of questions. For example, understanding the evolution and development of the specialised appendages (e.g., chelate pedipalps of pseudoscorpions and scorpions; antenniform legs of whip scorpions and whip spiders; sexually dimorphic appendages for sperm transfer in spiders and Ricinulei) could have a great impact on our understanding of origins of morphological novelties and diversity in arthropod appendages.

In the following, two examples of important evolutionary processes whose investigation requires the study of chelicerates are highlighted.

Terrestrialisation

Numerous selective pressures are proposed to have driven the ancestrally aquatic arthropods to adapt to terrestrial habitats (Little 2009). Modern phylogenomic assessments of arthropod relationships indicate multiple terrestrialisation events in the arthropod tree of life, particularly in Mandibulata (e.g., Hexapoda, Myriapoda, some lineages of malacostracan crustaceans). In Chelicerata, the earliest records of marine lineages are Cambrian fossil Pycnogonida, whereas horseshoe crabs and other extinct marine orders (Eurypterida and Chasmataspidida) were present by the Ordovician (Dunlop 2010). Nearly all arachnid orders are present in the fossil record by the Carboniferous (Petrunkevitch 1955; Selden et al. 1991; Dunlop 2010).

A scenario for chelicerate terrestrialisation is contentious. Some researchers have supported a single terrestrialisation event in the ancestor of a monophyletic Arachnida, based on morphology and/or the inferred improbability of terrestrialisation events (Scholtz and Kamenz 2006; Shultz 2007). Others have proposed an independent colonisation of land by scorpions, based on the interpretation of a marine (or at least aquatic) habitat of Palaeozoic scorpion fossils (Jeram 1997; Dunlop and Braddy 2001). At the core of the dispute is marked character conflict within both morphological and molecular phylogenetic datasets and the ensuing elusiveness of a robust chelicerate tree of life (Shultz 2007; Regier et al. 2010). However, there is now strong support for a single origin of the arachnid book lung due to the phylogenetic placement of scorpions as sister group to tetrapulmonates (Sharma et al. 2014a). Separately, the inference of multiple terrestrialisation events in mandibulate arthropods and concomitantly, of morphological convergence driven by terrestrial habitat (e.g., independent origins of tubular tracheae and Malpighian tubules in insects and myriapods), is now robustly supported by phylogenomic analyses. These discoveries discredit an argument for a single terrestrialisation event in the arachnid ancestor grounded on the assumption that terrestrialisation (and ensuing convergence in arthropods) is a historically rare or improbable event (reviewed by Shultz 2007; Sharma et al. 2014a).

Morphological and developmental comparison of book gills in Xiphosura and book lungs in Tetrapulmonata underlie the widespread view that book lungs developed from book gills via internalisation (Lankester 1881; Purcell 1909; Kamenz et al. 2005; Scholtz and Kamenz 2006; Farley 2010). The serial homology of the two appendage types is compelling (but see Dunlop 1997), but has yet not been demonstrated in the context of developmental genetics. Intriguingly, one previous study has suggested that book gills, both respiratory organ types of derived spiders (book lungs and tubular tracheae), as well as spider spinnerets and insect wings were all serial

homologs of crustacean gills, inasmuch as all of these originated from epipods (Damen et al. 2002). This argument, first made in support of a serial homology of insect wings and crustacean gills, was based on the differential expression of *pdm/nubbin* and *apterous* (*ap*); a solid expression domain of both genes is observed in the epipods of a fruit fly and a crustacean (wings and gills, respectively), whereas one or more rings of weak expression are observed in the distal endopods (legs) of the corresponding appendages (Averof and Cohen 1997). The similarity of expression patterns was the basis of the homology statement. Subsequently, Damen et al. (2002) showed that strong expression of *pdm/nub* and *ap* is observed in the book gills of *Limulus polyphemus*, as well as in the respiratory organs and spinnerets of the spider *Cupiennius salei*.

However, the inference that the respiratory organs of spiders originated as epipods is inconsistent with the recent functional work of Khadjeh et al. (2012), which demonstrated homeotic transformation of the book lungs to walking leg-like limb buds upon Hox gene knockdown, suggesting that book lungs (and possibly tubular tracheae) are derived from endopods. While no functional work has been conducted on spinneret development, the spinnerets of many basally branching spiders are also directly comparable to chelicerate endopods (e.g., walking legs) in that they can be segmented and leg-like in adults, and express all leg gap genes embryonically (Pechmann and Prpic 2009). One possible explanation is that *pdm/nub* is not a reliable and/or conserved marker for distinguishing endopods and epipods in chelicerates. Indeed, Damen et al. (2002) observed stronger expression of *pdm/nub* throughout the developing legs (endopods) of *Cupiennius salei* than had been observed in insect or crustacean legs, which questions the utility of this marker for discerning appendage rami in arachnids based on strength of expression level alone. While expression of one of the two spider *ap* paralogs seems to be consistent with the position of vestigial epipods (*ap-1* is expressed dorsally to the walking legs in later stages of *Cupiennius salei*), the fossil record of chelicerates reveals that biramous chelicerates bore exopods in this part of the body, not epipods (Boxshall 2004; Briggs et al. 2012). Together with documented homoplasy of certain genes' expression patterns (Janssen et al. 2011; Sharma et al. 2014c), these results indicate that the exact serial homology between the respiratory organs and prosomal appendages of chelicerates is not sufficiently clear at present.

Beyond these studies, essentially nothing is known about the genetic patterning of the book gills and book lungs, the development of chelicerate tubular tracheae, or the relationship between the tracheae of apulmonate arachnids and derived spiders. Therefore, two key experiments must be conducted towards understanding the evolution of respiratory systems in Chelicerata with existing EvoDevo resources. First, a double knockdown of the Hox genes *abd-A* and *abd-B* must be conducted in a spider and in an apulmonate arachnid (e.g., *Phalangium opilio*) to test the serial homology of the respiratory organs and the prosomal endopods (i.e., legs) of these groups, with the prediction that both respiratory organ types of these arachnids should be homeotically transformed to legs if they are serially homologous to prosomal endopods and to each other. Second, the function of *pdm/nub* and *ap* must be characterised in the spider, to assess the alternative hypothesis of an epipodal origin of respiratory organs and spinnerets. If this hypothesised homology statement was true (*sensu* Damen et al. 2002), then knockdown of *pdm/nub* should severely affect the development of the book lungs, tubular tracheae, and spinnerets, but only the segmentation of the prosomal appendages. This result would support the proposed homology to epipods, given that loss-of-function mutations of *pdm/nub* in *Drosophila melanogaster* result in loss of wing structures (Ng et al. 1995).

Evolution of the Spider Spinning Apparatus and Silk

Two minor orders of chelicerates produce silk, namely, some mites and pseudoscorpions, which utilise silks for tasks such as dispersal, protecting

eggs, and lining burrows (Beccaloni 2009). However, the most familiar silk-producing chelicerates are of course the spiders. Spiders produce diverse types of silk, which has greatly contributed to their successful adaption to different environments (Brunetta and Craig 2010). Spiders use silk to make cocoons to encase eggs and to build different types of webs (e.g., tube-, orb-, or wheel-shaped webs) as hiding places, to capture prey, and even as support for their respiration under water, as in the case of air bells of aquatic spiders (Brunetta and Craig 2010; Foelix 2010).

In the course of adapting to different environments, spiders have evolved morphological differences in their spinning apparatus and a great diversity in silk proteins within and between species (Marples 1967; Gatesy et al. 2001; Challis et al. 2006). The silk-producing organ of all spiders consists of the internal silk glands and the exterior spinnerets, but varies in number and composition between species.

Spider silk consists of fibrous proteins, which is stored in the silk glands in its liquid form and becomes solid through shearing upon excretion (Craig 1997). For various purposes, spiders can produce silks with distinct characteristics from different types of silk glands, which differ in morphology and function (Brunetta and Craig 2010). The simplest silk glands can be found in Orthognatha, whereas at least four different gland types occur in Ctenidae and up to eight distinctive types are present in Orbiculariae (Peters 1955; Mullen 1969; Palmer et al. 1982).

In most spiders, the spinnerets are located at the posterior end of the ventral side of the opisthosoma and consist of a varying number of spinneret pairs with various spatial arrangements (Marples 1967; Shultz 1987). Mesothelae exhibit four pairs of spinnerets, which is considered the "primitive" state. The more derived Orthognatha bear two to three pairs and some labidognathous spiders have two pairs of spinnerets, but additionally exhibit a specialised spinning structure, the cribellum (Shultz 1987). The spinnerets are covered with hairlike structures, the spigots, which are openings to the ducts that connect with the silk glands in the abdomen (Marples 1967).

Both the complexity of the spinning apparatus and the diverse composition of silks prompt questions regarding the evolutionary origins of the morphological and molecular apparatus underlying web spinning, with the corollary of the basis for spider web diversity. Different scenarios for the evolution of spigots and silk glands in spiders have been proposed. Some have argued that the silk glands evolved from a secretory organ, the coxal gland, on a modified leg segment and that the spigots derived from simple hair structures (Bristowe 1932; Butt and Taylor 1991). Another hypothesis proposes that spigots are modified sensory hairs, rather than simple hairs (Palmer 1991). Independently, it has been suggested that silk glands developed from epidermal invagination events, comparable to the male genital glands (epiandrous glands) (Palmer 1991; Craig 1997). Hypotheses grounded in such morphological studies are anticipated to be greatly informed by the advent of molecular and developmental genetic approaches. At present, comparatively little is understood about the genetic basis for spinneret and spigot development, whereas recent and redoubled efforts are shedding light on the characterisation of spider silk genes (Hayashi and Lewis 1998, 2000; Hayashi et al. 1999; Ayoub et al. 2007, 2013; Garb et al. 2010; Clarke et al. 2014; Sanggaard et al. 2014).

To elucidate the evolutionary rise of spinneret, silk gland, and silk protein diversity, these efforts should be complemented by comparative morphological, phylogenetic, and developmental studies, in tandem with comparative genetic and biochemical analysis of silk proteins. Such an integrative and cross-disciplinary pursuit is anticipated to inform understanding of spider diversification, as well as key innovations in evolution, more broadly.

In addition to the examples of terrestrialisation and silk production outlined above, there are several important open questions that can be addressed by future studies of chelicerates in comparison to those of other metazoans to provide new insights into evolutionary developmental biology. Some of these open questions are highlighted below, but this list is by no means exhaustive.

OPEN QUESTIONS

- How are book lungs, book gills, and tracheae patterned in the different chelicerate orders?
- What is the genetic basis for appendage diversity across Chelicerata and how is each appendage type specified?
- What is the genetic basis for sexual dimorphism in Chelicerata, and is this mechanism homologous to its mandibulate equivalent?
- How does the visual system develop, and what is the developmental genetic relationship between faceted eyes (Xiphosura only), lateral eyes (most arachnids), and median ocelli (all Chelicerata)?
- How is the development of the digestive system regulated in chelicerates?
- When during their development do chelicerates other than spiders and mites specify germ cells and which molecular mechanisms do they employ?
- How is the formation of the SAZ regulated and how are new segments generated from this tissue?
- Besides *hb* and *Dll*, which other factors are required for segmentation of the prosoma?

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