Cupiennius salei and *Achaearanea tepidariorum*: spider models for investigating evolution and development

Alistair P. McGregor,[1]* Maarten Hilbrant,[1] Matthias Pechmann,[1,2] Evelyn E. Schwager,[1] Nikola-Michael Prpic,[2] and Wim G.M. Damen[1]

Summary

The spiders *Cupiennius salei* and *Achaearanea tepidariorum* are firmly established laboratory models that have already contributed greatly to answering evolutionary developmental questions. Here we appraise why these animals are such useful models from phylogeny, natural history and embryogenesis to the tools available for their manipulation. We then review recent studies of axis formation, segmentation, appendage development and neurogenesis in these spiders and how this has contributed to understanding the evolution of these processes. Furthermore, we discuss the potential of comparisons of silk production between *Cupiennius* and *Achaearanea* to investigate the origins and diversification of this evolutionary innovation. We suggest that further comparisons between these two spiders and other chelicerates will prove useful for understanding the evolution of development in metazoans.   *BioEssays* 30:487–498, 2008. © 2008 Wiley Periodicals, Inc.

Introduction

In the last few years, there has been a relative explosion in the range of model organisms used to study development and its evolution. This increased sampling has already provided some insight into which features of the genetic regulation of development are ancestral and which are derived.[1–3] It has

been proposed, however, that further sampling of better chosen taxa is required to overcome biases from current models and give a more representative picture.[4,5] While further studies of relatively distantly related animals, such as chelicerates and insects, will be extremely important for understanding the evolution of development better,[1,3] it has been recognised that comparing a single example of each has its limitations.[4,5] After all, many features of *Drosophila* development are not representative of insects, but comparisons between *Nasonia*, *Tribolium* and *Drosophila* have been rewarding, for example, in understanding how the regulation of anteroposterior (AP) polarity has evolved in this class.[6,7] In addition, more considered sampling among animals can also allow studies of important evolutionary innovations that are not necessarily found in standard model organisms, such as wing spots in butterflies[8] and the production of silk in spiders.[9,10]

Outside insects, new models are emerging in the other arthropod classes, such as the crustaceans *Parhyale*,[11] *Artemia*[12] and *Daphnia*,[13] and the myriapods *Lithobius*,[14] *Strigamia*[15] and *Glomeris*.[16] Our favourite arthropod class is the chelicerates, which includes mites and ticks (Acari), scorpions (Scorpiones), horseshoe crabs (Xiphosura) and spiders (Araneae) (Fig. 1). Chelicerates are basally branching arthropods[17,18] and therefore their phylogenetic position is especially good for understanding the evolution of development in this phylum (Fig. 1).

A number of chelicerates are emerging as models for studying development. For example, the two-spotted spider mite, *Tetranychus urticae*, has some manipulative tools and the promise of a sequenced genome,[19,20] and the genome of the deer tick, *Ixodes scapularis* will be very useful for comparative purposes.[21] Interesting studies of the evolution of development have also been carried out in scorpions[22] and horseshoe crabs.[23]

Our favourite chelicerates are the spiders, in particular the common house spider, or greenhouse spider, *Achaearanea tepidariorum* and the Central American wandering spider *Cupiennius salei* (Fig. 2). We do not regard either as *the* model chelicerate, but rather suggest they are both good models for
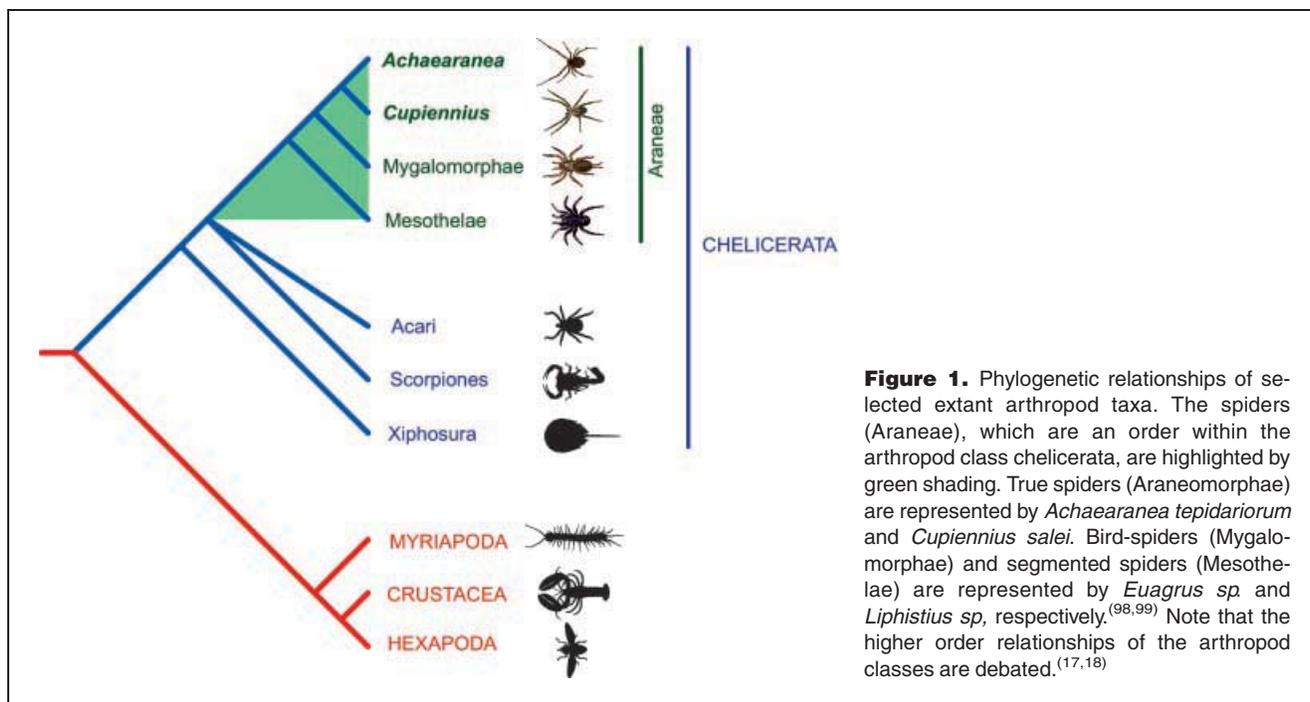
Figure 1. Phylogenetic relationships of selected extant arthropod taxa. The spiders (Araneae), which are an order within the arthropod class chelicerata, are highlighted by green shading. True spiders (Araneomorphae) are represented by *Achaearanea tepidariorum* and *Cupiennius salei*. Bird-spiders (Mygalomorphae) and segmented spiders (Mesothelae) are represented by *Euagrus sp.* and *Liphistius sp,* respectively.[98,99] Note that the higher order relationships of the arthropod classes are debated.[17,18]

comparison with other chelicerates with the larger aim of understanding the evolution of arthropods and beyond.
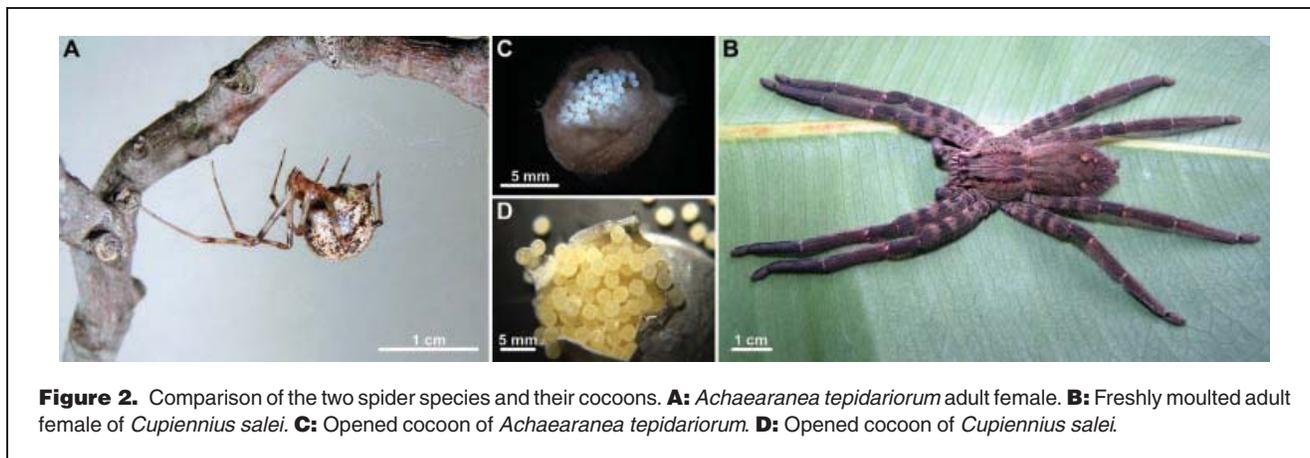
The spiders

Achaearanea and *Cupiennius* are both members of the Araneomorphae (as opposed to the Mygalomorphae, which includes bird-spiders) (Fig. 1).[24−26] *Achaearanea* is a theridiid, a group of spiders with diverse morphology, ecology and behaviour, which includes the black widows, and some social spiders. *Cupiennius* is a ctenid, a large wandering spider related to wolf spiders (Fig. 1).

Achaearanea tepidariorum (Fig. 2A) is a cosmopolitan species, but it is thought to have originated in the neotropics.[25,26] This spider makes theridiid webs or 'cobwebs'

in secluded corners inside and outside of buildings, and predominantly feeds on insects that have become caught in these webs. The females range in body length from 7 to 10 mm, while the males are smaller averaging about 4 mm. The females produce pear-shaped silk cocoons (egg sacs), containing between 100 and 400 synchronized embryos of about 0.5 mm in diameter (Fig. 2C). In the laboratory, female *Achaearanea* make a new cocoon every 5 or 6 days throughout the year. The complete lifecycle of *Achaearanea* is approximately 12 weeks at 25°C.

Cupiennius salei (Fig. 2B) is native to Central America.[24] This spider does not make a web, but rather ambushes or catches its prey by taking advantage of its considerable speed over short distances (up to 4 m/s).[24] *Cupiennius* eats a wide

Figure 2. Comparison of the two spider species and their cocoons. **A:** *Achaearanea tepidariorum* adult female. **B:** Freshly moulted adult female of *Cupiennius salei*. **C:** Opened cocoon of *Achaearanea tepidariorum*. **D:** Opened cocoon of *Cupiennius salei*.

range of animals from insects and spiders to small birds and mice. Females are found with body lengths up to 45 mm, while males are again smaller growing to a maximum of about 30 mm. *Cupiennius* females make cocoons every three to four weeks in the lab, and these contain up to 1500 embryos of 1.3 mm in diameter (Fig. 2D). The lifecycle of *Cupiennius* takes between 9 and 12 months from fertilized egg to mature adult.

Colonies of both *Achaearanea* and *Cupiennius* are very easy to maintain in the laboratory. After hatching from the cocoon, larvae are separated into individual vials and fed first with fruit flies and later with crickets. After the final moult, virgin females are mated with males and cocoons are used to maintain the culture or provide embryos for analysis.

Spider embryogenesis
The first ten stages of embryogenesis in *Achaearanea* take approximately 100 hours at 25°C (Fig. 3).[27,28] In the closely related species, *Achaearanea japonica,* the early cleavage patterns have been very well described and are similar to those of *Achaearanea tepidariorum*.[29,30] The early cleavages take place in the centre of the embryo and cellularisation occurs at the 16-nuclei stage (Fig. 3A). At stage 2, the cleavage energids start to migrate to the surface of the embryo (Fig. 3B,C). The first five cleavage cycles are synchronous, but

this breaks down after the 32-nuclei stage (see Supplementary material at www.genetik.uni-koeln.de/groups/Damen/McGregor_et_al_Movicmov). During stage 3, the cells continue to divide on the surface of the embryo around the yolk mass. This is a type of meroblastic cleavage called superficial cleavage, which is widespread in arthropods.[31]

Beginning at stage 3, the cells accumulate in one hemisphere of the embryo (Fig. 3D) forming the germ disc by stage 4 (Fig. 3E; supp. movie 1), which represents all prosomal (cephalothoracic) segments (Fig. 4J,K), although the cephalic lobe only becomes visible slightly later (Fig. 4E). While the embryo has AP polarity at this stage (the peripheral ring of the germ disc is anterior and the centre is posterior), the actual anterior pole is not specified until radial symmetry is broken later (Fig. 4B).

During stage 5, the cumulus, which consists of a group of mesenchymal cells, arises and migrates under the epithelial cell layer from the primary thickening at the centre of the germ disc to the periphery[27] (Figs. 3F, 4A–C; see below). Where the cumulus meets the periphery, the germ disc begins to open (Figs. 3G, 4D,E). This opening in the germ disc expands to become the dorsal of the embryo, while the diametrically opposite point of the opening on the periphery of the germ disc becomes the anterior pole (Fig. 4C,D,E).
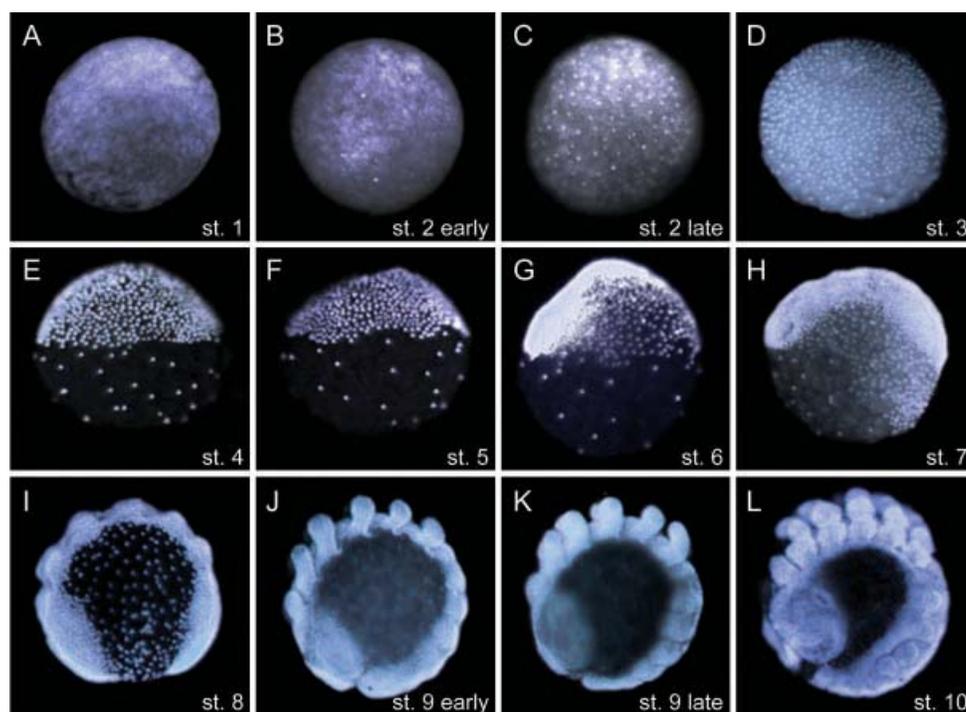

Figure 3. The first ten stages of embryogenesis of *Achaearanea tepidariorum*. **A–C:** Cleavage stages. **D,E:** Formation of the germ disc. **F–I:** Transition from radial to axial symmetry. **I–L:** Growth of the germ-band and appendages. Embryos in H–L are shown anterior to the left and ventral up. Embryos are stained with DAPI for visualisation of the nuclei. See text and Fig. 4 for detailed explanation of transitions. Staging is after Akiyama-Oda and Oda (2003), and Yamazaki et al. (2005).[27,28]
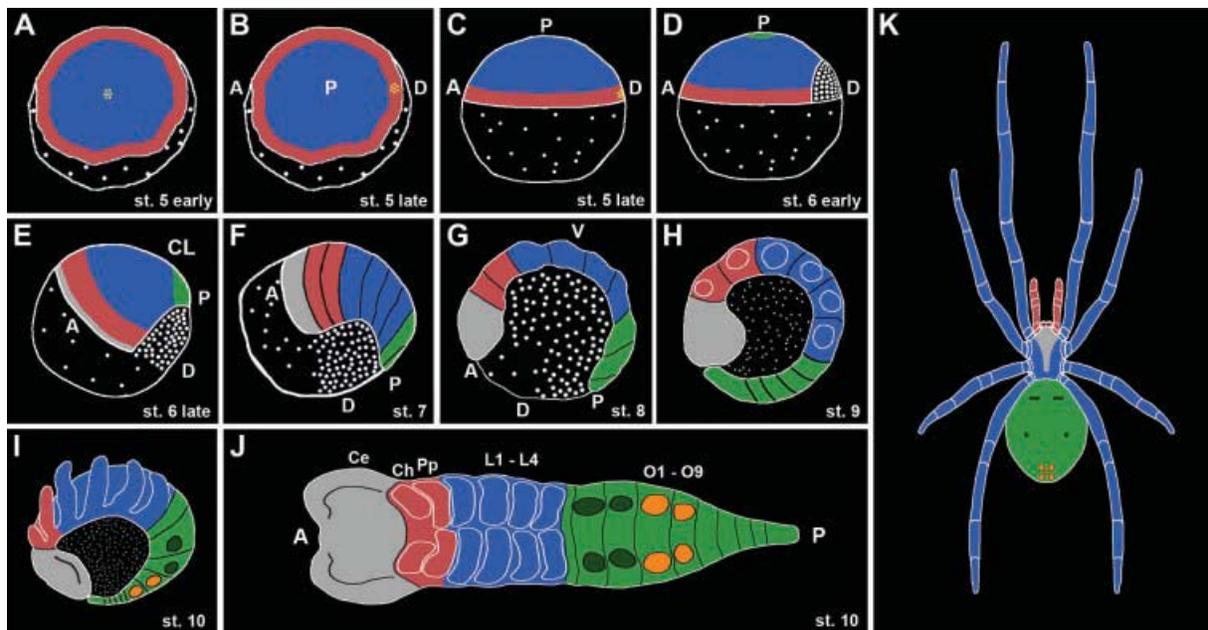
Figure 4. Segment formation during the embryonic development of *Achaearanea tepidariorum*. **A,B:** Views from the top of the germ disc. **C–I:** Lateral views. **J,K:** Ventral views. **A–C:** In stage 5, the cumulus (highlighted in yellow) moves from the centre of the germ disc to the periphery and initiates the break of the radial symmetry. **D,E:** Beginning of the transition from radial to axial symmetry. A second type of extraembryonic cells arise in the future dorsal region. **F,G:** Segments start to form during stage 7 and are well defined from stage 8 onwards. **H:** Elongation of the embryo is complete and the appendages start to grow. **I:** Further opisthosomal segments are added from the posterior differentiation zone. Opisthosomal buds appear and grow. **J:** Flattened view of stage 10. **K:** Schematic drawing of a fully developed spider. A, anterior; P, posterior; D, dorsal; V, ventral; H, head region; Ch, cheliceral segment; Pp, pedipalpal segment; L1–L4, leg bearing segments 1–4; O1–O9, opisthosomal segments; Ce, cephalic lobe; CL, caudal lobe. Head parts of the prosoma are shown in grey, cheliceral and pedipalpal-bearing segments in red, leg-bearing segments in blue and opisthosomal segments in light green. The opisthosomal buds that develop into book lungs (O2) and tubular trachea (O3) are shown in dark green, and opisthosomal buds that develop into spinnerets (O4 and O5) in orange. White dots represent extraembryonic cells.

The transition from radial to axial symmetry begins at stage 6, continues through stage 7 and is complete by stage 8 (Figs. 3H, 4F,G). During stage 7, the first opisthosomal (abdominal) segments appear from a swelling called the caudal lobe or growth zone, and further segments are sequentially added through stages 8 and 9 in concert with the appearance and extension of the limb buds (Figs. 3I–K, 4G,H). At early stage 10, the legs become segmented and approximately 9 out of 12 opisthosomal segments have been generated (Figs. 3L, 4I,J).

The development of *Cupiennius* is very similar to that of *Achaearanea,* albeit slower; the equivalent of the first 10 developmental stages takes approximately 200 hours.[32] One major difference is that, at stages 4 and 5, in contrast to *Achaearanea* embryos, the entire surface of *Cupiennius* embryos is covered with blastoderm cells. Therefore, in *Cupiennius*, the density of extraembryonic cells is higher than in *Achaearanea* and so initially the germ disc cannot be distinguished from the extraembryonic hemisphere in the former.[32] However, as the cumulus forms, the edge of the germ disc at the equator of the embryo becomes visible because the embryonic cells are now smaller than the extraembryonic cells, presumably as a result of continued cell division. The cumulus then migrates to the periphery of the germ disc and axis formation and segmentation proceed in *Cupiennius*[32] as described above for *Achaearanea.*

A process called inversion is initiated when the germ band reaches its full length. During inversion, the germ band splits along the ventral midline and each half moves laterally, eventually merging at the dorsal midline to enclose the yolk.[10,33] At the end of inversion, the embryo has developed into a pre-larva and the characteristic spider body-shape can be recognized.[10] The nymphs of *Achaearanea* and *Cupiennius* then go through approximately 6 and 10 moults, respectively, before they are fully mature adults.

Existing spider tools

The development of an animal as a model organism requires two general criteria to be met. (1) A regular supply of tissue for analysis, particularly embryos is required. As described above

both *Achaearanea* and *Cupiennius* are straightforward to culture in the laboratory and supply large numbers of embryos.

(2) Model organisms require manipulative tools and, in this respect, both spiders are well equipped. The embryos of both *Achaearanea* and *Cupiennius* can be readily fixed (although *Cupiennius* embryos are quite fragile until the germband has formed), which means that in situ hybridizations and antibody stainings can be carried out to investigate gene expression.

While analysis of gene expression patterns can give some insights, the major advances in comparative developmental biology over the last few years would not have been possible without the development of functional tools, namely RNAi, in a range of different animals such as beetles,[34] crustaceans[12] and nematodes.[35] The development of parental-RNAi (pRNAi)-mediated knockdown of gene expression in *Achaearanea*,[36] in combination with embryonic-RNAi (eRNAi) in *Cupiennius*[37] has opened the development of these chelicerates to functional analysis. As in other animals, the penetrance of RNAi knockdown of gene expression is variable between individual spider embryos.[34,36−38] This effectively gives a phenotypic series of knockdown effects that are extremely valuable in understanding the role of a given gene. pRNAi in *Achaearanea* has the added dimension that the phenotypic effects normally get gradually stronger with each cocoon providing hundreds of synchronized embryos with different strengths of knockdown.[36,39] eRNAi in *Cupiennius* compliments this by facilitating the investigation of the function of genes later in development, which would otherwise be masked by disrupting critical early functions.

Spiders as models for understanding axis formation and segmentation

A picture of the conservation and divergence of a current paradigm for the regulation of development, the *Drosophila* segmentation gene cascade, is gradually emerging from the numerous studies of the development of different arthropods and other protostomes.[1,3] Spiders have made an important contribution to this body of knowledge; for example, studies of *Cupiennius* have helped to show that the role of segment polarity genes is a conserved feature of arthropod development.[40] However, the regulation of earlier developmental steps is poorly understood outside insects, and therefore here we discuss recent functional analysis of early spider development and outline further studies in these animals that will improve our understanding of the regulation and evolution of these processes.

Axis formation

Manipulation of the cumulus by Holm[41] suggested that this structure represents an organizing centre for axis formation in spiders. In *Agelena labyrinthica* embryos, removal of the cumulus caused the loss of dorsal regions of the embryo and transplantation could cause the formation of a second axis.[41]

50 years later, elegant experiments in the laboratory of Hiroki Oda on *Achaearanea* embryos have begun to dissect the molecular mechanisms involved.[27,36,39]

The cumulus expresses *decapentaplegic* (*dpp*) and while pRNAi against this gene does not prevent the migration of the cumulus, it does inhibit the development of the extraembryonic tissue during stage 6 (Figs. 3G, 4D,E). This blocks the transition from radial to axial symmetry and results in ventralised embryos.[36] Therefore dorsoventral (DV) axis formation in spiders relies on a *dpp* signalling centre represented by the cumulus. While the involvement of *dpp* in DV axis formation in spiders is relatively unsurprising, as it is now apparent that *dpp* has an ancient role in axis formation,[42,43] the evolutionary history of the cumulus is unknown. However, a cumulus-like structure has been observed in myriapods[16,44] and, although the function of this structure has not been investigated, this suggests that the cumulus could be an ancestral feature of arthropod development.

While the above studies demonstrate how spiders are contributing to the understanding of DV axis formation, future studies of spiders may also prove useful for investigating the evolution of AP axis formation. Although the ventralised embryos resulting from *dpp* knockdown in *Achaearanea* have no specific anterior pole because the embryo remains radial, there is still AP polarity as this is determined earlier by formation of the germ disc. The expression of genes involved in AP polarity in insects, such as *orthodenticle* and *caudal*,[27] is only observed after the germ disc has formed and so these genes are unlikely to be responsible for the establishment of this axis. It is possible that there are earlier signals involved in this process, one for the establishment of the germ disc in one hemisphere of the embryo, perhaps mediated by the sperm entry point as found in nematodes,[45] and another signal to inform the cells of their position in the germ disc. Candidates for the latter signal include β-catenin (Armadillo) and Frizzled as the localization of these factors determines the AP axis in vertebrates[46] and cnidarians[47] respectively, and therefore it will be interesting to investigate the roles of these genes and other candidates in early spider embryos.

Somitogenesis and segmentation

The evolutionary relationship between somitogenesis in vertebrates and segmentation in arthropods and annelids remains one of the most intriguing questions in comparative evolutionary developmental biology.[1,3] In vertebrates, fibroblast growth factor (FGF), *Wnt* signalling and the Notch/Delta pathway are involved in regulating reiterative somite formation from the presomitic mesoderm.[48−50]

Although Delta−Notch signalling is not involved in segmentation of the body in *Drosophila,* knockdown of *Delta* (*Dl*), *Notch* (*N*) or *Suppressor of Hairless* (*Su(H)*) in *Achaearanea* embryos using pRNAi disrupted caudal lobe formation and

resulted in embryos with abnormal opisthosomal segments or no opisthosoma in the most severe cases.[39] In addition, eRNAi knockdown of *Dl, N, Su(H)* and *Presenilin* in *Cupiennius* embryos resulted in malformed segments, probably as a result of disruption of the organization of reiterative stripes of *hairy* expression.[51,52] This demonstrates that, in spiders, Delta–Notch signalling is required for the development of the caudal lobe and then for the sequential generation of segments from this tissue.[39,51,52]

These studies indicate that the formation of segments in arthropods and vertebrates may have shared a genetic programme in a common ancestor, and that somitogenesis and segmentation therefore may have a common origin,[39,51,52] a conclusion that would have been difficult to draw by comparing mice and flies. However, this hypothesis requires further testing with experiments such as investigating the roles of the spider orthologues of other vertebrate genes involved in somitogenesis. To this end, we are currently investigating the roles of *Wnt* genes in spiders.
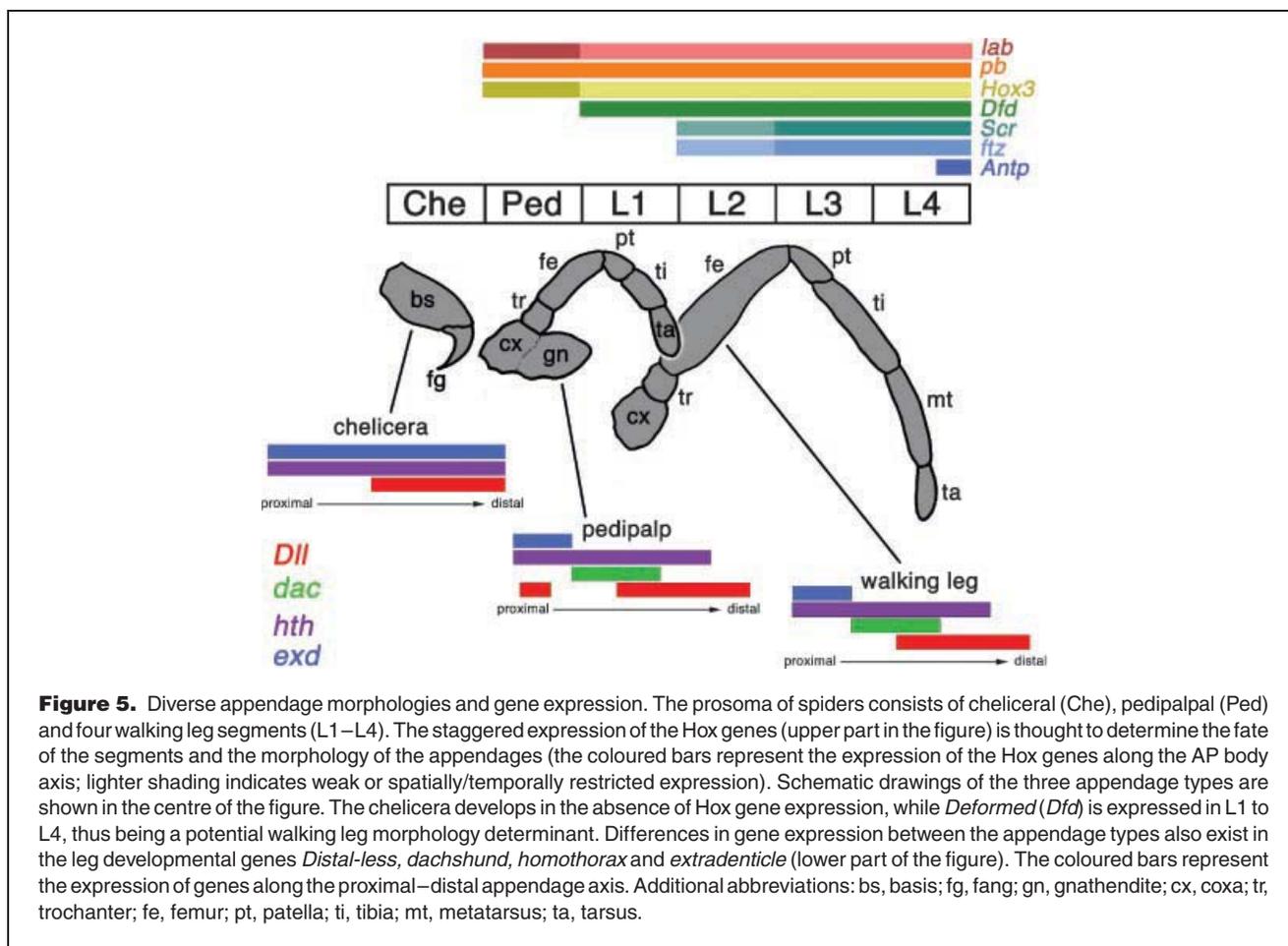
There are at least 12 subfamilies of *Wnt* genes in metazoans[53] and they are involved in many aspects of development, for example, *Wnt3a* and *Wnt8* are required for somitogenesis.[49,54,55] Interestingly, all recognized subfamilies, with the exception of *Wnt9,* are found in the sea anemone *Nematostella vectensis*, which suggests that these genes appeared early in animal evolution.[56] Therefore functional analysis of *Wnt* genes in spiders could allow a better understanding of many important developmental processes and their evolution. We are particularly interested in using this analysis to attempt to unravel the regulatory interactions between *Wnt* signalling, Delta-Notch and the fluctuating expression of the pair-rule orthologues in the spider growth zone.[57,58] As well as comparing regulation of segmentation and somitogenesis as discussed above, this could help us better understand the developmental dynamics and evolution of the arthropod 'growth zone' or 'undifferentiated zone' of which little is known.[1,3]

Appendage development and evolution

Eight legs and more
On the prosoma, spiders have six pairs of appendages. The first pair, the chelicerae, are followed by a pair of pedipalps and four pairs of walking legs (Fig. 4J,K).

Figure 5. Diverse appendage morphologies and gene expression. The prosoma of spiders consists of cheliceral (Che), pedipalpal (Ped) and four walking leg segments (L1–L4). The staggered expression of the Hox genes (upper part in the figure) is thought to determine the fate of the segments and the morphology of the appendages (the coloured bars represent the expression of the Hox genes along the AP body axis; lighter shading indicates weak or spatially/temporally restricted expression). Schematic drawings of the three appendage types are shown in the centre of the figure. The chelicera develops in the absence of Hox gene expression, while *Deformed* (*Dfd*) is expressed in L1 to L4, thus being a potential walking leg morphology determinant. Differences in gene expression between the appendage types also exist in the leg developmental genes *Distal-less, dachshund, homothorax* and *extradenticle* (lower part of the figure). The coloured bars represent the expression of genes along the proximal–distal appendage axis. Additional abbreviations: bs, basis; fg, fang; gn, gnathendite; cx, coxa; tr, trochanter; fe, femur; pt, patella; ti, tibia; mt, metatarsus; ta, tarsus.

The walking legs consist of seven podomeres (leg segments) that are called (from proximal to distal) the coxa, trochanter, femur, patella, tibia, metatarsus and tarsus (Fig. 5). The pedipalps are morphologically similar to the walking legs, but lack one podomere, the metatarsus, and have at the base an outgrowth (gnathendite) that serves as lateral wall of the preoral cavity (Fig. 5). The pedipalps are mainly used for food manipulation but also have sensory functions in many species.[10] Male spiders also use the pedipalps to transfer sperm to the genital opening of females.[10] The chelicerae are fang-like and consist of only two podomeres (Fig. 5). These appendages are used for prey capture and defence, and, in most species, contain venom glands.[10]

Spiders also have four pairs of appendages on the opisthosoma (Fig. 4J,K). The paired appendages on the second and third opisthosomal segment develop as typical limb buds, but then invaginate to become breathing organs (book lungs, tubular tracheae). The paired appendages on the fourth and fifth segment develop into the spinnerets. The spinnerets of most spiders are short and consist of only two podomeres, but some basal spiders have leg-like spinnerets of several segments that even move in step with the walking legs.[10]

A special type of appendage is the labrum. It is an unpaired outgrowth in front of the chelicerae that nevertheless develops similarly to the paired appendages of the prosoma.[59] However, its appendicular nature is disputed.[60]

Investigating the evolution of appendages
Arthropod appendages are a superb model to study the principles of adaptive evolution and the origin of morphological diversity; from a primitive limb in the ancestral arthropod, the morphology of arthropod appendages has been selected for numerous new functions. An advantage of arthropods is that they combine appendages of different morphologies and functions in one animal. What are the mechanisms distinguishing one appendage type from the other and how did these mechanisms evolve?

Based on studies of *Drosophila*, a number of leg developmental genes have been isolated from several spider species. The best studied of these genes is *Distal-less* (*Dll*). In *Drosophila, Dll* is expressed in the distal portion of the legs and these parts are missing in *Dll* mutants.[61] Studies in *Cupiennius* have shown this role of *Dll* is conserved.[37] Other leg developmental genes known from *Drosophila* include *dachshund, homothorax* and *extradenticle*.[62] The function of these genes has not yet been investigated in spiders, but their expression has been studied in *Steatoda triangulosa* and *Cupiennius*.[63–65] While these studies revealed small differences in the expression of these genes between spiders and *Drosophila*, in general the patterns suggest that the function of these genes and this part of the network regulating leg development is conserved. Other parts of the network, however, seem to be less conserved. For example, the role of *wingless* and *dpp* in spider leg development is unclear since their expression patterns are only partially similar in flies and spiders.[63,66] Virtually nothing is known about the development of leg segmentation in spiders. In *Drosophila*, leg segmentation is controlled by the Notch-signalling pathway and the leg segments seem to arise through a complex temporal arrangement, i.e. not in a tip-to-base (or vice versa) manner.[67] Nothing is yet known about Notch signalling in spider leg development, but the complex temporal appearance of a putative Notch target gene, *nubbin* (*nub*), in segmental rings in the legs of *Cupiennius*[68] suggests there are some similarities between Drosophila and *Cupiennius*.

Research into what makes the appendage types in spiders different from each other is still very much at the beginning. The expression patterns of most leg developmental genes in *Cupiennius* are virtually identical in the walking leg and the pedipalp,[65] which is consistent with the morphological similarities of these two appendage types. A significant difference, however, exists in the pattern of *Dll*; the gnathendite of the pedipalp expresses a second domain that is absent in the walking legs. This makes *Dll* a potential candidate for a gene that leads to the morphological differences between walking leg and pedipalp. Another candidate is *nub*, which is expressed in several rings in walking legs. The strongest ring of expression is found in the metatarsus and, intriguingly, both the ring of *nub* expression and the metatarsus are missing in the pedipalps.[68]

In contrast to the very similar gene expression patterns in legs and pedipalps, the patterns in the chelicera and opisthosomal appendages are quite different,[65,69] which is consistent with the disparate morphologies of these appendages. However, the connection between these morphologies and the changes in gene regulation is presently unclear. In *Drosophila*, a key role is played by the staggered expression domains of the Hox genes that results in a different ''Hox code'' and thus in a different identity for each body segment.[70] These genes also have staggered expression patterns in spiders[71–73] (Fig. 5), but their relation to the morphology of the appendages has not yet been tested.

The present data suggest that the set of genes expressed in different appendage types is similar, but their regulation is not. A major topic for future research is, therefore, the differential regulation of genes in the different appendage types. Apart from differential regulation of genes that are present in all appendages, the expression of appendage-type-specific genes (genes that are expressed in a single appendage type only) is another possibility to explain specific morphologies. To our knowledge (except for *Deformed*) no appendage-type-specific genes have as yet been identified in spiders. We anticipate that functional analyses in *Cupiennius* and *Achaearanea* of known genes and new candidates from the ongoing

EST sequencing projects in spiders and other arthropods will help us to address these questions.

Spider neurogenesis

Evolutionary implications of spider neurogenesis
In the last few years, morphological, molecular and functional studies of the *Cupiennius* developing nervous system have improved our knowledge of comparative arthropod neurogenesis. This research has revealed that spider neurogenesis is more similar to that of myriapods than to insect or crustacean neurogenesis.[74–76]

The comparison of neurogenesis between chelicerates, myriapods, insects and crustaceans, recently reviewed by Stollewerk and Simpson[75] and Stollewerk and Chipman,[76] provided an important piece of morphological evidence for the pancrustacean clade (insects arising within or as a sister group to the crustaceans) and therefore against an insect-myriapod sister group relationship as proposed by the Atelocerata hypothesis. But whether the similarities seen in spider, horseshoe crab and myriapod neurogenesis are true synapomorphies that would point to a spider-myriapod sister group relationship (myriochelata/paradoxopoda) or plesiomorphies shared with other near relatives of the arthropods cannot yet be ruled out.[75] To confirm any of the above theories, more data from additional chelicerate and myriapod species and most importantly from outgroups such as onychophorans or tardigrades are required.

Genetic networks underlying neurogenesis in arthropods
Despite the morphological differences between spider and insect neurogenesis, some genes that regulate the recruitment of neuronal precursors appear to be functionally conserved, as shown by eRNAi in *Cupiennius*. Initially, proneural genes of the *achaete-scute* family prefigure the regions in which invaginating clusters/neuroblasts form.[74] The number of neural precursors is then limited by lateral inhibition mediated by the neurogenic genes *N* and *Dl*, like in *Drosophila* and also vertebrates.[77]

Less is known, however, about the genetic networks underlying the specification of neural precursors in the spider and also other arthropods. In *Drosophila*, this diversification is mediated by both spatial and temporal patterning mechanisms. First the expression of segment polarity genes (AP) and columnar genes (DV), subdivide the neuroectoderm into a grid-like pattern.[78] Expression patterns of the segment polarity gene *engrailed* in invaginating neural precursor groups in *Cupiennius* suggest that this spatial patterning mechanism might be conserved in spiders.[76] The spatial patterning genes interact with temporal patterning genes in *Drosophila* to specify the fate of each descendant of the neuroblasts. For example a clock-like mechanism of sequential expression of *Krüppel* (*Kr*), *hunchback* (*hb*), *Pdm* and *castor* has been proposed to act in *Drosophila* neuroblasts.[79] Expression of the *Kr* and *hb* orthologues in a myriapod suggest that this mechanism might be conserved in other arthropods.[80] However, data from spiders are missing and thus it cannot be determined if the regulatory interactions underlying neural precursor determination evolved at the base of the arthropods or only within insects.

Interestingly, the nervous system re-uses many of the genes that earlier in the ontogeny (at least in *Drosophila*) were used for segmentation. Whether any of the genes of the *Drosophila* segmentation gene cascade, apart from the segment polarity genes, have a function in segmentation in arthropods other than insects is still debated.[1,3] Since many orthologues of these genes are even expressed in neural precursors of non-arthropods, it is possible that the genetic networks acting in neurogenesis could have been co-opted for segmentation in the insect lineage.[3,81] The spiders *Cupiennius* and *Achaearanea* can be used to answer this question by testing the function of these genes in both the nervous system and in segmentation.
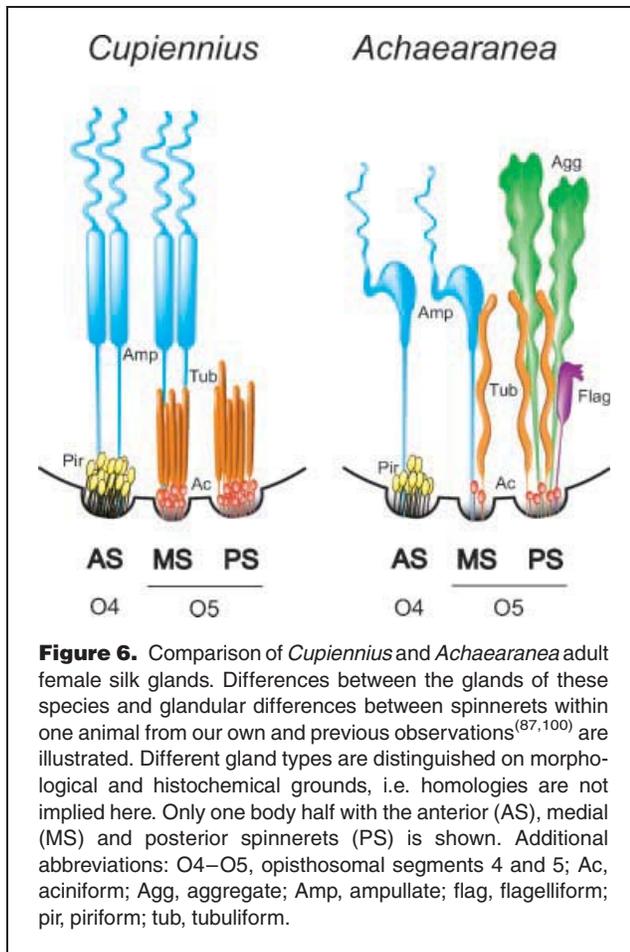
Spiders as models to investigate evolutionary innovations

One of the most-fascinating features of spiders is their ability to produce complex silken structures. The spinning apparatus that evolved to do this includes a set of glands and appendages called spinnerets (Figs. 4, Fig. 6). These organs are a unique evolutionary innovation, and play a central role in the ecology of spiders. In no other animal group have silk glands reached such complexity.[9,82]

The spider silk spinning apparatus: innovation and diversity
After the emergence of the spinning apparatus, many morphological and physiological differences in these organs[83] and in the diversity of silk proteins,[84] evolved within and between species. This diversification allowed spiders to exploit many kinds of prey, including flying insects, and to occupy a wide range of terrestrial, and occasionally even aquatic, habitats. Silken structures range from the lining of the burrows of ground dwelling spiders to the more complex stereotypical orb webs. There are structures used for protection, migration, predation, communication and even for breathing in case of the air bells of aquatic spiders.

To build silken structures, all spiders are capable of producing more than one silk type[9] for which they use different glands (although some glands are capable of producing more than one type of silk). Gland diversity ranges from a comparatively simple set in Mygalomorph spiders,[85] to a highly complex set of up to seven different gland types in the Orbiculariae,[86] a group that includes orb weavers and cobweb spiders like *Achaearanea*. With four gland types, *Cupiennius* is intermediate in complexity[87] (Fig. 6).

Figure 6. Comparison of *Cupiennius* and *Achaearanea* adult female silk glands. Differences between the glands of these species and glandular differences between spinnerets within one animal from our own and previous observations[87,100] are illustrated. Different gland types are distinguished on morphological and histochemical grounds, i.e. homologies are not implied here. Only one body half with the anterior (AS), medial (MS) and posterior spinnerets (PS) is shown. Additional abbreviations: O4–O5, opisthosomal segments 4 and 5; Ac, aciniform; Agg, aggregate; Amp, ampullate; flag, flagelliform; pir, piriform; tub, tubuliform.

The spinnerets are also crucial for making complex silk structures and have evolved various different morphologies.[88] For example, the Orbiculariae bear three pairs of spinnerets whereas the Mygalomorphae bear only two. Also, the spinnerets of the Orbiculariae are much smaller than those of the Mygalomorphae and are rather distinct from each other, reflecting the different functions of the different types of glands attached to them. The spinnerets of *Cupiennius* are also relatively small.

The exclusivity of complex silk spinning organs to spiders and the diversity of these between different spiders raises two questions. First, how did they originally evolve? Second, how did the subsequent diversification evolve? With regard to the first question, one hypothesis is that the silk glands evolved from coxal glands, which are excretory organs associated with the legs of many arthropods including some spiders.[89] Therefore silks may have evolved from a waste product that proved to be useful, and spinnerets are modified legs. Another theory is that the silk glands evolved from glands associated with the male genitalia,[88] and that these glands became associated with appendages already present or newly

evolved. The shared implication of these hypotheses is that the opisthosomal silk glands are serially homologous to (ancestral) organs on other segments. However, it has proven very difficult to distinguish between competing theories using comparative morphology and histochemistry. With regard to the second question, the discussion has centred on which ecological differences may have led to adaptations of the spinning apparatus[90] and the developmental changes underlying these adaptations have not been studied.

Investigating the evolution and development of silk production

To better understand the origin and diversification of the spinning apparatus, it is necessary to understand its development. The primordia of the anterior and posterior spinnerets are serial homologues of the other appendages, however, the origin of the medial spinnerets remains enigmatic[69,88,91] (Figs. 4J, 6). We also know that some silk glands derive from ectodermal invaginations of the spinnerets,[92,93] but there are few molecular data available on their development.[94] To address the questions mentioned above, additional detailed comparative studies of morphological and molecular development of the spinning apparatus is needed. *Achaearanea* and *Cupiennius* are excellent models for this purpose, because they differ substantially in their ecology and in the composition and morphology of their silk glands (Fig. 6), and functional molecular tools exist.

We are developing silk-gland-specific markers to trace the development of the different glands and spinnerets. Once the primordia of the different silk glands have been localised, the early differentiation of these tissues can be investigated and compared with other organs (e.g. coxal glands, venom glands and genital associated glands) to gain insight into their evolutionary origin. Second, comparing the differentiation of different glands both within and between *Achaearanea* and *Cupiennius* will give better understanding of the genetic changes involved in the evolution of novel silk gland types. Finally, broader comparisons can be made between the genetics of the developing opisthosomal silk glands of spiders and the well-studied silk glands of animals like the lepidopteran insect *Bombyx mori* or the salivary glands of *Drosophila* (which are homologous organs[95]). This will allow us to test if parallel evolution of the same genes underlies these evolutionary innovations.

Conclusions and future directions

Here we have outlined how studies of spiders using existing tools have and may continue to help us understand the development and evolution of many traits, from axis formation to silk production. Another milestone would be the development of transgenesis, which could eventually facilitate a whole host of other applications necessary for more detailed analysis, such as insertional mutagenesis, overexpression,

temporal and tissue-specific gene knockdown, cell labelling and dissection of cis-regulatory regions of genes. The development of a suite of transgenic tools that work in a wide range of hosts,[11,96] and the fact that spider embryos are amenable to injection, means that the prospects for developing this technology are reasonable, at least for *Achaearanea.*

As a first step toward genomic analysis of spider development, *Achaearanea* and *Cupiennius* EST sequencing projects are underway both in our laboratories and in the laboratory of Hiroki Oda in Japan.[39] One obvious application for which these data can be used in the near future is microarray analysis of gene expression between wild-type embryos and those subjected to RNAi. This could allow the regulatory gene networks to be dissected in these spiders and compared to other animals. Of course this would be helped greatly by a sequenced genome. The genomes of *Achaearanea* and *Cupiennius* are approximately 1200 Mb and 2500 Mb respectively[97] and so the former would be the better candidate for a genome project. As well as the obvious benefits for studies of *Achaearanea,* a spider genome would compliment the existing and planned chelicerate genome projects in the tick and the mite, respectively.[19,21]

Acknowledgments

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