Evolutionary crossroads in developmental biology: the spider *Parasteatoda tepidariorum*

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Summary

Spiders belong to the chelicerates, which is an arthropod group that branches basally from myriapods, crustaceans and insects. Spiders are thus useful models with which to investigate whether aspects of development are ancestral or derived with respect to the arthropod common ancestor. Moreover, they serve as an important reference point for comparison with the development of other metazoans. Therefore, studies of spider development have made a major contribution to advancing our understanding of the evolution of development. Much of this knowledge has come from studies of the common house spider, *Parasteatoda tepidariorum*. Here, we describe how the growing number of experimental tools and resources available to study *Parasteatoda* development have provided novel insights into the evolution of developmental regulation and have furthered our understanding of metazoan body plan evolution.

Key words: Axis formation, Evolution, Gene regulation, Segmentation, Spiders

Introduction

The common house spider *Parasteatoda tepidariorum* (formerly known as *Achaearanea tepidariorum*) (Saaristo, 2006) is an excellent model organism with which to investigate developmental mechanisms and their evolution. Spiders belong to the chelicerates (see Glossary, Box 1), which branch at the base of the arthropods (Fig. 1), and therefore represent a sister group to the other extant arthropod phyla, myriapods, crustaceans and insects (see Glossary, Box 1) (Regier et al., 2010; Rota-Stabelli et al., 2010). Given the phylogenetic position of *Parasteatoda*, comparing its development with that of other arthropods and ecdysozoa (see Glossary, Box 1), can be useful in helping to identify which aspects of development are possibly ancestral or derived among these animals, and even other metazoans. Studies of spiders have also been insightful for understanding general developmental concepts, ranging from cell migration to differentiation (e.g. Akiyama-Oda and Oda, 2010; Stollewerk and Seyfarth, 2008), and they have proven to be particularly good models for investigating the regulation of segmentation (Damen, 2007; Minelli and Fusco, 2004). Moreover, spider embryogenesis exhibits features, for example a structure called the cumulus (see Glossary, Box 1) (Oda and Akiyama-Oda, 2008), that are not known in other arthropod models, and studies of these animals can therefore also enrich our knowledge of the diversity of developmental mechanisms used among animals.

Studies of other chelicerates, including the Central American wandering spider *Cupiennius salei*, have made a valuable contribution to understanding developmental evolution (e.g.

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Stollewerk et al., 2003; Telford and Thomas, 1998) and are likely to continue to do so. However, *Parasteatoda* is now the most commonly used chelicerate model for developmental studies, particularly with respect to embryogenesis (McGregor et al., 2008a; Oda and Akiyama-Oda, 2008). This is in part owing to the platform provided by classical embryological studies of spider development (reviewed in Anderson, 1973), the ease of culturing *Parasteatoda*, its short life cycle, year-round access to all embryonic stages, and an expanding range of genetic resources and functional tools (McGregor et al., 2008a).

We reasoned previously that further studies of the spiders *Parasteatoda* and *Cupiennius* could provide new insights into developmental mechanisms and their evolution (McGregor et al., 2008a). In this Primer, we now review the advances made in these areas using *Parasteatoda* as a model. Specifically, we describe the development of *Parasteatoda* and summarise the range of tools and resources available that have facilitated the identification of several fundamental aspects of developmental regulation. We also highlight the potential for gaining further insights into the evolution of developmental mechanisms by the continuing development of this model, which is used by a growing community of researchers.

Life cycle and development

Female *Parasteatoda* lay clutches of up to 400 eggs (Miyashita, 1987; McGregor et al., 2008a) of ~0.5 mm in diameter, and wrap these in a protective silk egg sack or cocoon (Montgomery, 1903). Eggs are fertilised during oviposition (Montgomery, 1907), which takes ~3-4 minutes (Montgomery, 1903), resulting in the embryos in each cocoon developing in concert. The first divisions of the energids (see Glossary, Box 1) occur in the centre of the spherical centrolecithal eggs (see Glossary, Box 1). Although precisely when cellularisation takes place remains to be determined, it appears to occur before the fifth division cycle while the nuclei within the energids are migrating from the centre to the periphery of the yolk mass (Kanayama et al., 2010). At the 64-cell stage, at ~11 hours after egg laying (hAEL) at 25°C, the cells are visible on the surface of the embryo and the blastoderm forms (Kanayama et al., 2010; Montgomery, 1909) (Fig. 2A, stage 2). The cells continue to divide and gradually converge towards one pole of the egg. At 25-30 hAEL, this leads to the formation of a cell-dense, dome-shaped germ disc (see Glossary, Box 1) and a region that contains a few scattered, putatively extra-embryonic, cells (Akiyama-Oda and Oda, 2003; Montgomery, 1909) (Fig. 2A, stage 4).

Between 55 and 65 hAEL, gastrulation and a series of complex cell movements take place, and the onset of segmentation is observed (described in more detail below). This transforms the initial radially symmetrical germ disc into a multilayered short germ band (see Glossary, Box 1) with bilateral symmetry (Akiyama-Oda and Oda, 2003; Kanayama et al., 2011) (Fig. 2A, stage 8). Between 65 and ~90 hAEL, during a period of development comparable to *Cupiennius* stages 9 to 12 (Wolff and Hilbrant, 2011), most segments of the opisthosoma (see Glossary,

Box 1. Glossary

Centrolecithal eggs. Eggs in which the yolk is concentrated in the centre of the cytoplasm.

Chelicerates. A group of arthropods that includes spiders, mites, scorpions and harvestmen, and also horseshoe crabs, for which the oldest known fossil dates back to the Upper Ordovician (circa 445 Ma) (Redkin et al., 2008).

Crustaceans. A group of arthropods that includes lobsters, crabs, shrimps and woodlice.

Cumulus mesenchyme (CM) and cumulus. The CM is a group of endodermal cells that migrates away from the primary thickening towards the periphery of the germ disc. CM cells closely associate with the germ disc epithelium on their trajectory, and this interaction induces DV axis formation. Together, CM and epithelium form a clump that bulges out from the surface, conventionally called the cumulus.

Cytoneme. A long and thin plasma-membrane projection oriented towards a morphogen-producing cell (Ramírez-Weber and Kornberg, 1999).

Dorsal field. The extra-embryonic tissue that appears after the cumulus has migrated through the germ disc.

Ecdysozoa. The name given to all moulting animals (including arthropods and nematodes), based on an influential phylogenetic study that used ribosomal RNA sequences (Aguinaldo et al., 1997) and resulted in new views on the phylogenetic relationships of animals (Adoutte et al., 2000).

Energid. A nucleus and the cytoplasm with which it interacts during the early superficial cleavages.

Germ disc. An aggregation of blastoderm cells forming the embryonic primordium. Note that the word 'disc' is slightly misleading as the primordium is dome shaped and not flat.

Insects. A group of hexapod arthropods that includes beetles, flies, grasshoppers and dragonflies. The body of insects is made up of a head, thorax and abdomen.

Myriapods. A group of arthropods that includes centipedes and millipedes.

Opisthosoma. The posterior part of the body of spiders and other chelicerates (similar in appearance to the insect abdomen).

Primary thickening. An agglomerate of the first internalising cells at the centre of the germ disc.

Prosoma. The anterior part of the body of spiders and other chelicerates, which is sometimes referred to as cephalothorax. The spider prosoma bears the walking legs, but also the brain and feeding appendages.

Segment addition zone (SAZ). The unsegmented tissue at the posterior of the germ band from which new segments are formed.

Short germ band. In the short germ band mode of development, only the anterior-most segments are initially specified and subsequently the posterior segments are added from the SAZ.

Fig. 1. Phylogenetic relationships between arthropoda and related phyla. For each of the phyla, the genera of two selected model organisms are shown in parentheses. The red circle indicates the branch point of the Chelicerata and the Mandibulata (Myriapoda, Hexapoda, Crustacea). Outgroups to the Arthropoda, represented by velvet worm *Euperipatoides* and nematode *Caenorhabditis* genera, are shown in grey (Regier et al., 2010; Rota-Stabelli et al., 2010).

Embryonic development takes about eight days at 25°C. The largely immotile postembryo (Downes, 1987) then ecloses but remains within the protection of the cocoon (Fig. 2A, postembryo). The first moult then takes place after about two days, and gives rise to the first instar (Fig. 2A), which leaves the cocoon. The period from leaving the cocoon to reaching adulthood takes a minimum of 30 days with four to six moults for males, and 40 days with five to seven moults for females (Miyashita, 1987) (Fig. 2A). After the females reach adulthood they become receptive to mating with male spiders (Fig. 2B). In parallel, vitellogenesis begins and a female can produce her first clutch of eggs as soon as one week after reaching adulthood. The generation time at 25°C is therefore ~50-60 days.

Over the course of ~80 days, a well-fed adult female can make ten cocoons on average (Miyashita, 1987), which is an unusually large number compared with other spider species (Marshall and Gittleman, 1994), and makes *Parasteatoda* particularly amenable to embryological studies.

Experimental tools and genomic resources

A range of excellent descriptive and manipulative tools to analyse gene expression and function have been established in *Parasteatoda* (Box 2). Gene expression patterns can be assayed readily from the early germ disc stages onwards using whole-mount in situ hybridisation (Akiyama-Oda and Oda, 2003), and both embryonic RNA interference (eRNAi) and parental RNAi (pRNAi) allow gene function to be investigated during different stages of embryogenesis.

pRNAi in *Parasteatoda* is very efficient for investigating gene function for several reasons. First, a single injected female will produce several cocoons, each containing up to 400 eggs. The injection of a limited number of females is thus sufficient to generate large numbers of embryos with the expression of the focal gene(s) knocked down. Second, the effect of pRNAi typically becomes progressively stronger through the first few clutches before wearing off; therefore, a range of phenotypic effects from the knockdown of gene expression can be obtained. Third, because all embryos within a cocoon are at the same or very similar stages of development, the effects of gene knockdown can be assessed over the course of development by analysing embryos from the same cocoon at progressively later stages.

Box 1) are added sequentially from the posterior segment addition zone (SAZ) (see Glossary, Box 1). Concomitantly, the appendages begin to form and the central nervous system starts to differentiate, as also observed in other spiders (Liu et al., 2009; Wolff and Hilbrant, 2011) (Fig. 2A, stage 12).

During an intricate sequence of tissue movements called inversion (Wallstabe, 1908; Wolff and Hilbrant, 2011), the embryo then envelops the yolk (Fig. 2A, stage 17). The final stages of embryonic development involve most of the yolk migrating into the opisthosoma while internal organs, such as the heart and digestive tract, develop (Rempel, 1957) and the major brain compartments form (Doeffinger et al., 2010). The embryo gains a typical spider-like appearance as the tissue between the prosoma (see Glossary, Box 1) and opisthosoma constricts (Fig. 2A, stage 20).
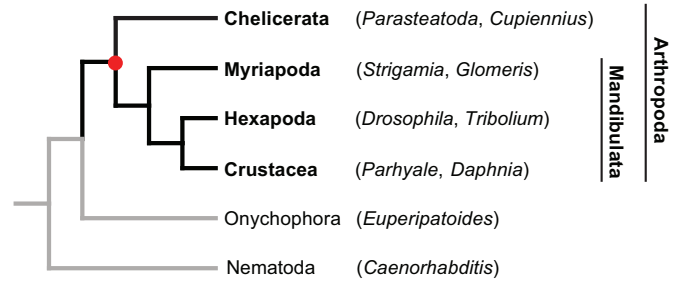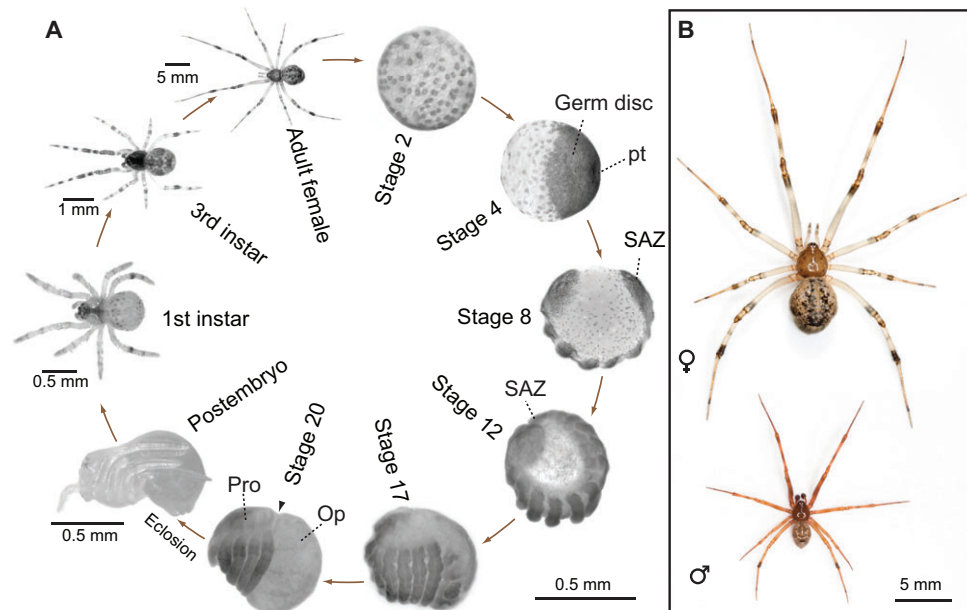
Fig. 2. *Parasteatoda tepidariorum* life cycle and morphology. (A) A selection of embryonic and post-embryonic stages: stage 2, blastoderm; stage 4, showing germ disc with primary thickening (pt); stage 8, showing the early segmented germ band with segment addition zone (SAZ); stage 12, showing an elongated germ band with extended legs; stage 17, the end of inversion, at which point the embryo has enveloped the yolk; stage 20, the prosoma (Pro) and opisthosoma (Op) become pronounced when the tissue between them (arrowhead) constricts; postembryo, the stage directly after eclosion; 1st instar, the stage that leaves the cocoon; 3rd instar, one of a series of immature free-foraging instars; adult female. Stages 2, 4 and 8 were defined by Akiyama-Oda and Oda (Akiyama-Oda and Oda, 2003) and Yamazaki et al. (Yamazaki et al., 2005), Stages 12, 17 and 20 are inferred from corresponding *Cupiennius salei* stages (Wolff and Hilbrant, 2011). Anterior is to the left in all images. Scale bar in lower right corner applies to the embryonic stages 2 to 20. **(B)** Adult female (upper picture) and male (lower picture). Anterior is to the top.

eRNAi complements the global affects of pRNAi by facilitating gene knockdown in specific cells, in the context of other cells in the tissue, and at later developmental stages. Two different approaches have been developed to knock down gene expression using eRNAi in *Parasteatoda*. Pechmann and colleagues (Pechmann et al., 2011) have established the injection of double-stranded RNA into the perivitelin fluid that surrounds the embryo (typically at stage 4). This technique results in global knockdown of gene expression in subsequent stages and can therefore be used in conjunction with pRNAi to distinguish between the early and late functions of a gene of interest (Pechmann et al., 2011). This global eRNAi approach is complemented by the application of eRNAi to subsets of cells, which was facilitated by the development of single-cell microinjection of early cleavage stage *Parasteatoda* embryos (Kanayama et al., 2011; Kanayama et al., 2010) (Fig. 3). Injection during the period when cellularisation takes place can label single cells with markers, which facilitates the tracing of clones of labelled cells until the germ band stage. Furthermore, co-injection of double-stranded RNA results in downregulation of endogenous mRNA specifically in the labelled descendants of the injected cell. Conversely, this technique can also be used to induce the production of exogenous proteins in clones by injecting mRNA, thus allowing overexpression or ectopic expression of genes of interest (Kanayama et al., 2011; Kanayama et al., 2010).

These tools have been used to investigate the function of particular developmental genes and have led to new insights into developmental evolution in arthropods. Moreover, the combination of these functional tools with genomic resources that include various transcriptomes, together with the imminent sequencing of

the *P. tepidariorum* genome (Table 1), opens the possibility of investigating developmental processes in this spider on a genome-wide scale.

Key recent findings and their impact on the field
Insights into anteroposterior axis formation and gastrulation

In *Drosophila*, anteroposterior (AP) axis formation and patterning are regulated by maternal gene products that are deposited and localised in the eggs. However, the specific factors and regulatory mechanisms involved in defining the AP axis in insects and other arthropods have evolved in different lineages: for example, the transcription factor encoded by *bicoid* (*bcd*) is only found in higher flies like *Drosophila* (McGregor, 2005). Investigating *Parasteatoda* development could eventually help to determine how the AP axis might have been determined ancestrally in arthropods, and recent studies of early embryogenesis in this spider represent an excellent platform to address this question.

In *Parasteatoda*, AP axis determination is concomitant with the formation of the germ disc during stage 3. The periphery or rim of the disc represents the anterior, whereas the centre represents the posterior, which subsequently develops into the caudal lobe during stages 4-7 (Fig. 4A). The embryo at the early germ disc stage is thus radially symmetrical. After the formation of the germ disc, gastrulation begins at the posterior end of the embryo, beginning with the formation of the blastopore in the centre of the germ disc (Montgomery, 1909) (Fig. 4). As also observed in other spiders, this posterior region of internalising cells leads to the formation of a multilayered primary thickening (see Glossary, Box 1; Fig. 4) (Anderson, 1973). Interestingly, a second region of internalising

DEVELOPMENT

Box 2. Available experimental techniques
Antibody staining. Several antibodies are available for detecting proteins in spider embryos, e.g. Forkhead (Oda et al., 2007), pMAD (Persson et al., 1998), Pax3/7 (Davis et al., 2005), Cadherin (Oda et al., 2005), Prospero and Snail (Weller and Tautz, 2003).
Chromosomal fluorescence in situ hybridisation (FISH). This technique was used for the first time in spiders to map the genomic locations of several silk protein-coding genes in *Latrodectus* (Zhao et al., 2010), a spider genus closely related to *Parasteatoda*.
Embryonic RNAi (eRNAi). Genes can be knocked down in a clone of cells by injecting double-stranded RNA into a single cell during early cleavage stages (see Fig. 3) (Kanayama et al., 2011) or in whole embryos by injecting into the perivitelin fluid surrounding the embryo (Pechmann et al., 2011).
Exogenous protein expression. Injection of capped mRNA encoding a fusion of nuclear localisation signal (NLS) and the GFP coding region (NLSeGFP) results in clones of cells expressing nuclear GFP at later developmental stages (Kanayama et al., 2010).
Lineage tracing. The injection of a cell lineage marker into *Parasteatoda* embryos at the preblastoderm stage makes it possible to follow the progeny of this cell up to the germ band stage (see Fig. 3) (Kanayama et al., 2010).
Microarrays. The first spider microarray published used 13,655 sequences from a large expressed sequence tag (EST) data set (see Table 1), and led to the discovery of the involvement of *odd-paired* in Hh signalling-mediated head segmentation (Kanayama et al., 2011).
Parental RNAi (pRNAi). Genes can be knocked down in all progeny of a single adult female by injecting double-stranded RNA into her opisthosoma around the time of mating (Akiyama-Oda and Oda, 2006).
Whole-mount in situ hybridisation (WMISH). Fixed *Parasteatoda* embryos can be assayed for the expression of one or two different transcripts as early as stage 3 (Akiyama-Oda and Oda, 2010).

Fig. 3. Cell labelling and embryonic RNAi. (**A,B**) Schematic of the microinjection technique (Kanayama et al., 2010). At the 16-cell preblastula stage (A), the energids (light brown) lie inside the yolk mass (grey), about midway between the centre and the surface of the embryo. A cell lineage marker (pink) injected at this stage remains confined to a single cell. At the 64 cell stage (B) a cellular blastoderm (dark brown) forms. Subsequent division of the injected cell results in a clone of cells that have all inherited the marker (pink). (**C,D**) Flat-mounted stage 8 embryos that have been injected with a mixture of *otd* double-stranded RNA and the marker FITC-dextran at the preblastoderm stage, showing the localisation of *hh* transcript (purple) and FITC-dextran (i.e. labelled cells; pink). *Otd* eRNAi gives rise to disturbed segment formation if the injected cell clone (pink) is located at the anterior (C) (Kanayama et al., 2011). By contrast, *otd* dsRNA cell clones in the opisthosomal segments (D) do not disrupt segment formation. Arrows indicate the *hh* stripes in the head region, and circles and squares indicate those in the leg-bearing prosomal and the opisthosomal regions, respectively. Scale bar: 100 μm. Images in C and D were kindly provided by Hiroki Oda.

cells located at the rim of the stage 5 germ disc (Fig. 4A), has also been observed in *Parasteatoda* (Kanayama et al., 2011; Montgomery, 1909; Oda et al., 2007).

In these regions, the earliest cells that ingress at the blastopore and at the anterior of the germ disc express the transcription factor Forkhead (Fkh) (Fig. 4A,B), and have been respectively called the central and peripheral endoderm (Akiyama-Oda and Oda, 2003; Oda et al., 2007). The ingressing endodermal cells at both locations are followed soon after by ingressing mesodermal cells that express *twist* (*twi*), which encodes another transcription factor (Fig. 4B; see below). Thus, in *Parasteatoda* embryos, the earliest morphological events along the AP axis are the formation of a germ disc followed by specification of posterior and anterior regions in the germ disc that differentiate endodermal and mesodermal cells with seemingly similar, germ layer-specific, expression profiles.

One question highlighted by this work is what factor or factors initially define AP polarity in *Parasteatoda*? It has been proposed that Hedgehog (Hh) signalling regulates formation of the germ disc and the AP axis (Akiyama-Oda and Oda, 2010). During stage 3, when the germ disc forms, *hh* is expressed in cells in the presumptive extra-embryonic region. However, during stages 4 and 5, the expression of this gene is confined to the rim of the germ disc. Therefore, Hh might form a gradient from the rim to the centre of the germ disc (Akiyama-Oda and Oda, 2010).

Interestingly, although pRNAi against several components of Hh signalling does inhibit migration of the cumulus (see below), these embryos still form a germ disc, with both a blastopore in the centre
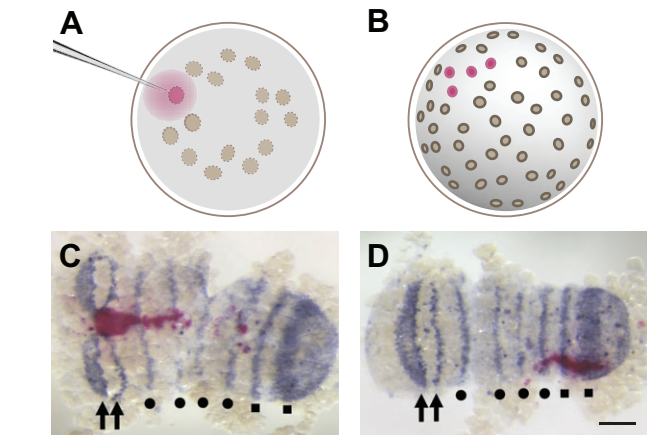
and an anterior region of gastrulation at the rim. Therefore, although the subsequent patterning of the germ disc is affected when Hh signalling is disrupted, this suggests that there must be other factors responsible for regulating the initial formation of the germ disc and the AP axis.

Insights into dorsoventral axis formation: the cumulus and the conserved role of the BMP pathway

Investigation of dorsoventral (DV) axis formation in *Parasteatoda* has revealed that this spider uses a novel developmental mechanism involving the migration of an organising centre, and has also highlighted the evolutionarily conserved regulation of DV axis specification by the bone morphogenetic protein (BMP) pathway (Akiyama-Oda and Oda, 2003; Akiyama-Oda and Oda, 2006; Lynch and Roth, 2011).

The migration of the cumulus plays a crucial role in breaking the radial symmetry of the germ disc in *Parasteatoda* to establish DV polarity and formation of the germ band. Moreover, although the evolutionary origin of the cumulus is debated (Box 3), its importance in the formation of the DV body axis in spiders has actually been known for many decades from transplantation and ablation experiments by Holm (Holm, 1952), who originally proposed that the cumulus forms an organising centre.

In *Parasteatoda*, the cumulus forms during stage 4 when a subpopulation of the Fkh-expressing endodermal cells at the primary thickening can be distinguished by the expression of a *fascin*-related gene (Akiyama-Oda and Oda, 2010). At the

Table 1. Current and imminent genomic resources for spiders

Species	Resource	Tissue source	Technical details and availability
Parasteatoda tepidariorum *Latrodectus hesperus* *Loxosceles reclusa*	Genome	*P. tepidariorum*: adult male and female (pooled)	BCM-HGSC websites*,‡ Robinson et al., 2011 Release of first assemblies expected in 2013
Parasteatoda tepidariorum	cDNA library	Various embryonic stages	22,812 ESTs (Kanayama et al., 2011)
	Transcriptomes	Early embryonic (pooled stages)	Roche 454 and Illumina HiSeq2000 Assembly and release expected in 2012
		Late embryonic, post-embryonic, adult male and female (pooled stages)	Illumina HiSeq2000 Assembly and release expected in 2012
Latrodectus hesperus	Genomic fosmid and cDNA libraries	Adult female and silk glands	Ayoub et al., 2007

BCM-HGSC, Baylor College of Medicine Human Genome Sequencing Center.
*https://hgsc.bcm.edu/content/i5k-project.
‡https://hgsc.bcm.edu/content/species-table.

beginning of stage 5, these cumulus mesenchyme (CM; see Glossary, Box 1) cells begin to express *decapentaplegic* (*dpp*), which encodes the homologue of vertebrate BMP2/4, and subsequently detach from the primary thickening to migrate anteriorly towards the rim of the germ disc.

The CM cells closely associate with the epithelial cells above their path and induce them, possibly through cytonemes (see Glossary, Box 1), to express phosphorylated Mothers against dpp (pMad). pMad then antagonises the Dpp inhibitor Short gastrulation (Sog) to set up the DV axis (Akiyama-Oda and Oda, 2003; Akiyama-Oda and Oda, 2006). Thus, *Parasteatoda* uses a similar set of factors to those employed in other metazoans to regulate DV axis formation (Lynch and Roth, 2011).

At the morphological level, the migration of the cumulus breaks radial symmetry of the germ disc progressively from posterior to anterior, specifying the dorsal area of the embryo as it migrates whereas the opposite region of the germ disc becomes ventral (Fig. 4, stage 5). This also involves epithelial cells expressing *fkh* (Akiyama-Oda and Oda, 2003) and adopting a presumptive extra-embryonic fate known as the dorsal field (see Glossary, Box 1; Fig. 4).

Several recent studies have also provided insights into the molecular mechanisms underlying the migration of the cumulus and the morphogenetic properties of this structure. For example, in *dpp*-depleted embryos, even though the cumulus migrates normally, radial symmetry is not broken and the dorsal field does not develop (Akiyama-Oda and Oda, 2006). Furthermore, knockdown of several components of the Hh pathway, including the Hh receptor encoded by *patched*, blocks the migration of CM cells, which suggests that this movement depends on the source of Hh localised at the rim of the germ disc. If this model is true, and assuming that Hh signalling is equally intense from around the circumference of the germ disc, this implies that the initial direction of travel of the cumulus is stochastically determined (Akiyama-Oda and Oda, 2010).

The segment addition zone: insights into segmentation

Like most arthropods, spiders add posterior segments sequentially from the SAZ, although the relative contribution of cell division and cell rearrangements to the production of new segments is not yet known in *Parasteatoda*. Indeed, despite the importance of the SAZ, the development of this structure and the subsequent production of segments are still not well understood generally among arthropods. However, studies in *Parasteatoda* have provided new and important information regarding how the formation of the SAZ and the generation of segments are regulated.

Oda and colleagues have shown that the Notch signalling pathway regulates germ layer specification at the embryonic posterior, and that this is crucial for the correct formation of the SAZ (Oda et al., 2007). The gene *delta* (*dl*), which encodes a ligand for the Notch signalling pathway, is first expressed in an evenly dispersed 'salt-and-pepper' pattern among surface cells in the region of the blastopore that then co-express *twi* and internalise as mesodermal precursor cells (Fig. 4B). The adjacent cells do not express *dl* or *twi* but instead express *caudal* (*cad*) and adopt an ectodermal fate. It is thought that this pattern is specified through a process of lateral inhibition, with the original *dl*-expressing cells inhibiting adjacent cells from adopting a mesodermal fate possibly by directly or indirectly repressing *twi* expression (Oda et al., 2007). This is supported by the fact that

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Box 3. The evolution of the cumulus

During *Parasteatoda* embryogenesis, migration of the cumulus mesenchyme sets up the DV axis (reviewed by Oda and Akiyama-Oda, 2008), which is an intriguing example of a migrating embryonic signalling centre (Akiyama-Oda and Oda, 2010). Furthermore, this means that gastrulation precedes DV axis formation in *Parasteatoda* (Fig. 4A). The reverse is true for many insect species in which gastrulation is localised to the part of the blastoderm that has already been specified as ventral tissue without the involvement of a cumulus (Roth, 2004). Therefore, considering the phylogenetic position of chelicerates at the base of the arthropod tree, it has been proposed that DV axis formation via a cumulus-like mechanism might represent the arthropod ancestral state (McGregor et al., 2008a). Evidence against this hypothesis comes from a recent study of onychophorans, the closest living relatives to arthropods (Fig. 1), in which it was shown that gastrulation takes place along a ventral gastral groove, without the formation of a cumulus (Mayer and Whitington, 2009). Therefore, although the existence of a cumulus in myriapods continues to be unresolved (Brena and Akam, 2011), currently the most parsimonious explanation is that the cumulus evolved after the chelicerate-mandibulate split. However, as a migrating cumulus has also been described in a horseshoe crab (a marine group of chelicerates) (Sekiguchi, 1973), it is likely that this mode of development evolved very early in the chelicerate lineage (Redkin et al., 2008).
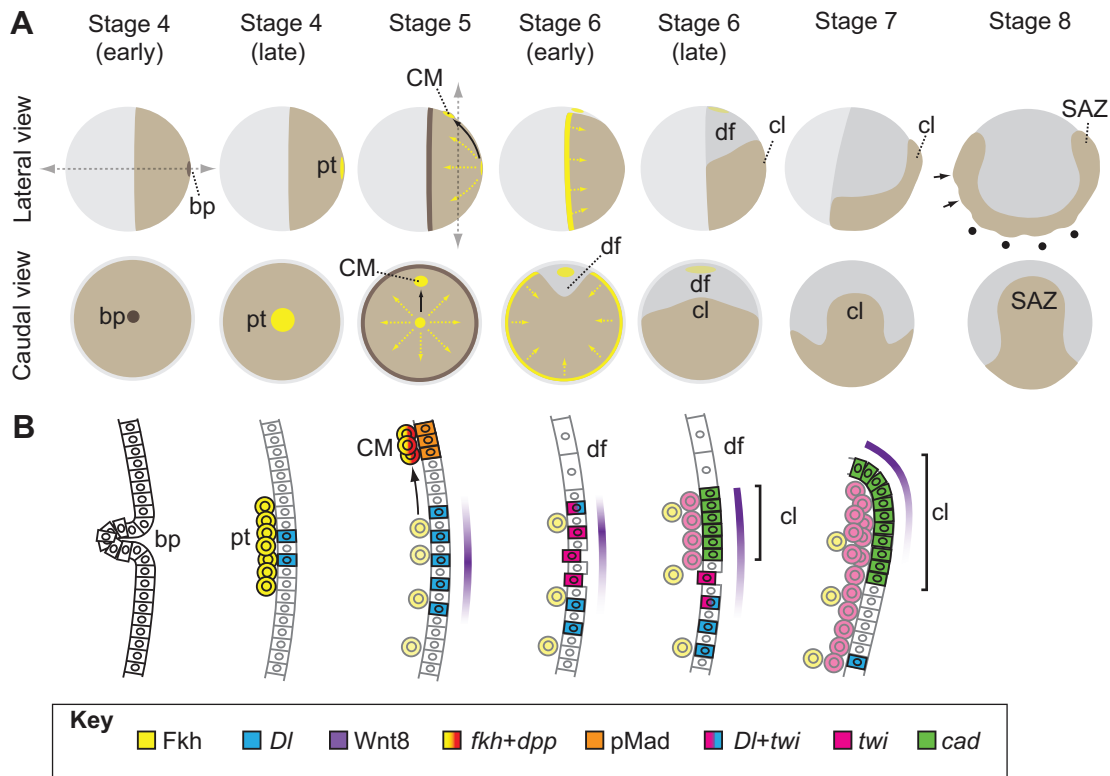
Fig. 4. Development of *Parasteatoda* from germ disc to germ band. (**A**) Schematic overview of stages 4 to 8 from a lateral (upper) and caudal (lower) view. In all lateral views anterior is to the left (as indicated by dashed arrow in stage 4, early). Dorsal is up from stage 5 onwards in both views (as indicated by dashed arrow in stage 5). The developing embryonic tissue in all stages is coloured light brown whereas light grey shading indicates the extra-embryonic region. Dark grey represents the dorsal field (df). Dark brown shading indicates the two regions in which gastrulation takes place: the closing blastopore (bp) at stage 4 and the rim of the germ disc at stage 5. Yellow structures and arrows indicate the initial location, after internalisation and subsequent movements, of endodermal cells (for clarity, the mesoderm is not shown). A special group of endoderm cells form the cumulus mesenchyme (CM) that migrates anteriorly as a cluster during stage 5 (black arrow) and degenerates during stage 6. At stage 8, arrows indicate segments in the head region, and circles indicate segments in the leg-bearing prosomal region. (**B**) Schematic cross-section of the centre of the germ disc [based on a similar scheme in Oda et al. (Oda et al., 2007)]. Stages correspond to the stages represented directly above in A, and dorsal is up from stage 5 onwards. Cellular expression of given genes and proteins is indicated by different colours as shown in the key. A putative gradient of the Wnt8 ligand is indicated in purple. See main text for details. cl, caudal lobe; pt, primary thickening; SAZ, segment addition zone.

when components of the Notch signalling pathway are knocked down, the number of *twi*-expressing cells increases, the centre of the germ disc develops into a disorganised cell mass, and *cad* expression in presumptive ectodermal cells at stage 6 is lost (Oda et al., 2007).

A similar effect is observed when the *Wnt8* gene, which encodes one of the subfamilies of secreted ligands for Wnt signalling (Janssen et al., 2010), is knocked down, suggesting that Wnt and Notch signalling together regulate formation of the SAZ (McGregor et al., 2008b). Indeed, pRNAi against either *Wnt8* or components of Notch signalling results in truncated embryos without any opisthosomal segments (McGregor et al., 2008b; Oda et al., 2007). Curiously, *Wnt8* RNAi embryos exhibit an enlargement of the adjacent prosomal segments. Therefore, Wnt8 might form a posterior-to-anterior gradient (Fig. 4B), which is not only involved in the formation of the SAZ but also maintains an undifferentiated population of cells in this tissue that are used in the subsequent addition of opisthosomal segments (McGregor et al., 2009; McGregor et al., 2008b). Furthermore, the dynamic expression pattern of *dl* in the SAZ and nascent posterior segments of *Parasteatoda* embryos (Oda et al., 2007), which is blocked

when *Wnt8* is knocked down (McGregor et al., 2008b), suggests that a clock-like mechanism involving Notch and Wnt signalling, analogous to that observed in vertebrates, regulates segment addition in *Parasteatoda*.

Interestingly, it has been found that Notch signalling is involved in segmentation in cockroaches (Pueyo et al., 2008), and Wnt8 is required for the formation of posterior segments in beetles (Bolognesi et al., 2008). This suggests that these pathways, together with *cad*, were components of an ancestral network for posterior development in arthropods (McGregor et al., 2009) and possibly even other animals (Couso, 2009). Note, however, that the precise role of some genes, particularly those encoding Wnt ligands, may have evolved (Janssen et al., 2010), and Notch signalling might not be involved in segmentation in several arthropod lineages, including *Tribolium* (Aranda et al., 2008; Kainz et al., 2011).

Prosomal segmentation and early patterning
In contrast to *Drosophila* and other insects, the early embryos of spiders and several other arthropods are cellularised at an earlier stage (Kanayama et al., 2010). This has important implications for

patterning because gradients of transcription factors, like the Bcd morphogen gradient in *Drosophila* embryos, would not be effective in a cellularised blastoderm, and, thus, positional information must be provided by other mechanisms. In *Parasteatoda*, it has been shown that the patterning and subsequent segmentation of the anterior prosoma are the result of both dynamic and static mechanisms that are initiated early in the germ disc stage. Furthermore, these studies have revealed that prosomal segmentation involves mechanisms that are different to those used to generate opisthosomal segments.

Patterning of the anterior prosoma (up to the pedipalpal segment) requires travelling (Pechmann et al., 2009) and splitting (Kanayama et al., 2011) of a wave of *hh* and *hairy* (*h*) expression. Initially *orthodenticle* (*otd*), *hh* and *h* are expressed at the periphery of the germ disc at stage 5, and subsequently at the anterior rim of the germ band. At stages 6 and 7, the stripes of *otd*, *hh* and *h* expression are found in a more posterior position and divide into multiple stripes. The travelling and splitting of these expression patterns depends on *otd* and *hh* function and is a prerequisite for the correct positioning of segmental gene expression in the embryo. Silencing of *otd* blocks the initial movement of the normally dynamic anterior stripes of *hh* and *h* expression, such that the expression of these genes is restricted to the anterior rim and does not split into stripes. This results in embryos that lack all tissue anterior to the pedipalpal segment (Pechmann et al., 2009). Furthermore, *otd*-eRNAi cell clones located away from the rim no longer express *hh*, which suggests that Otd is also required to maintain *hh* expression during the travelling and splitting phase (Kanayama et al., 2011). Conversely, the expression and activity of *otd* strongly depends on *hh* (Akiyama-Oda and Oda, 2010; Kanayama et al., 2011). It has therefore been proposed that spider head segmentation requires an autoregulatory signalling network in which *otd* is required to regulate dynamically the distribution of patterns of *hh* signalling sources (Kanayama et al., 2011).

By contrast, patterning of the leg-bearing segments of the spider prosoma depends on a static mechanism that resembles insect gap gene patterning. This involves the spider orthologue of the gap gene *hunchback* (*hb*) (Schwager et al., 2009) and the broadly conserved limb-patterning gene *Distal-less* (*Dll*), which is not only required for development of the appendages, as expected because this is an evolutionarily conserved function of this gene, but, surprisingly, also acts as a gap gene in *Parasteatoda* and probably in other spiders (Pechmann et al., 2011). Knockdown of *hb* or *Dll* in *Parasteatoda* entirely removes some leg-bearing segments, giving rise to phenotypes that are highly reminiscent of insect gap phenotypes. These morphological phenotypes correlate with downregulation of the segmentation genes *engrailed* (*en*) and *hh* in the affected region. Although the identity of the upstream factor(s) that regulate *hb* and *Dll* in *Parasteatoda* is not yet known, given the cellular nature of the early spider embryo, it is tempting to speculate that intercellular signalling pathways might also play a role in the activation of these gap genes.

These findings in *Parasteatoda* provide insights into the evolution of anterior segmentation mechanisms in arthropods and might help us to answer the challenging question of how diversification of developmental mechanisms is related to animal body plan evolution. In this respect, spider development might rely more on intercellular signalling mechanisms than is the case during the syncytial stages of development in *Drosophila*, and *Parasteatoda* is a good model for investigating this idea further.

Conclusions and future perspectives

Studies of *Parasteatoda* have broadened our understanding of development and have provided further insights into the evolution of developmental mechanisms. For example, a number of studies have helped to reveal developmental genetic mechanisms that are probably ancestral to arthropods, and perhaps even other animals, and are therefore useful for furthering our understanding of metazoan body plan evolution. In addition, recent studies of spiders have also allowed the identification of novel developmental mechanisms, including a role for *hh* in axis formation (Akiyama-Oda and Oda, 2010), an auto-regulatory signalling network involving *hh* and *otd* to control anterior patterning (Kanayama et al., 2011; Pechmann et al., 2009), and the recent finding of a role of *Dll* as a gap gene (Pechmann et al., 2011).

We anticipate that ongoing and future research in this field will provide us with additional knowledge of how development has evolved. This will include insight into the specific topics discussed herein, such as development of the SAZ and the genetic interactions that regulate the production of new segments, as well as insight into the development of other tissues, such as appendages, the germ line and the nervous system, that are being actively investigated by other researchers in the *Parasteatoda* community. The transcriptomic resources that have been developed will also allow analysis at a genome-wide level. Importantly, this will be greatly assisted by the imminent sequencing of the genome, which will facilitate analysis of the *Parasteatoda* genome and investigation of gene regulation, as well as allowing the potential discovery of novel mechanisms at play in the spider that might inform our understanding of development in other metazoans.

Competing interests statement
The authors declare no competing financial interests.

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