

## REVIEW

# The dynamic evolution of panarthropod germ cell specification mechanisms

Jonchee A. Kao<sup>1,\*</sup>, Emily L. Rivard<sup>1,†,§</sup>, Rishabh R. Kapoor<sup>2</sup> and Cassandra G. Extavour<sup>1,3,4,¶</sup>

## ABSTRACT

Germ cells enable the reproduction of an organism and the continuity of its lineage. Across animals, these crucial cells are segregated from the soma at different times and places and via distinct mechanisms. Understanding the evolution of germ cell specification across animals is complicated by the difficulty of making meaningful comparisons of embryonic development between diverse animal species. Here, we characterize germ cell specification in Panarthropoda, an ancient clade that encompasses massive animal biodiversity, within which we can conduct meaningful comparative embryology. We amass data from centuries of studies describing the timing and mechanisms of germ cell formation, and apply ancestral state reconstruction to these data to propose hypotheses about the trajectory of evolution in this process. Furthermore, we speculate about the mechanisms underlying these evolutionary dynamics by considering the relationships among germ cell specification, concurrent developmental processes and the germ line gene network. Collectively, this Review derives new insights from a rich historical database of embryological observations, offering broad implications for understanding the evolution of metazoan germ cells.

**KEY WORDS:** Germ plasm, Pole cells, Inductive signaling, BMP signaling pathway, Cytoplasmic determinant

## Introduction

During animal development, the germ line comprises a collection of cells specified early in embryonic development that later give rise to the next generation via eggs or sperm. Whereas historical researchers relied primarily on observation of unperturbed embryos to understand germ cell specification and development (e.g. histological analysis of fixed, sectioned embryos; Bounoure, 1939; Wolff, 1962), we can now investigate these processes using functional genetic analysis. This allows us not only to identify germ cells with more certainty, but also to gain more insight into the molecular mechanisms of germ cell specification. In this Review,

we have used ‘specification’ to mean the process that imbues embryonic cells with the ultimate fate of generating eggs and sperm when the animal is sexually mature, and ‘differentiation’ to mean the processes that make germ cells distinct from somatic cells in one or more of their morphology, behavior or gene expression. Several hypotheses have been proposed to explain evolutionary transitions between the mechanisms underlying germ line specification and differentiation across animals, including potential changes in cis-regulatory mechanisms, changes in evolvability or body plan diversity, or impacts on germ line mutation rate and, ultimately, fitness (Buss, 1982, 1983; Crother et al., 2007, 2016; Evans et al., 2014; Extavour, 2007; Johnson et al., 2011; Whittle and Extavour, 2016, 2017). However, such hypotheses are difficult to test because embryonic development of animals is too different across vast evolutionary time scales to be meaningfully compared. Moreover, there is disagreement in the field about whether existing well-studied mechanisms of germ cell specification (discussed below) are truly the only mechanisms, and whether they are discrete, mutually exclusive mechanisms. Furthermore, there are conflicting reports about the impact of germ cell specification mode on the evolution of genomes, body plans and fitness (Evans et al., 2014; Otto and Hastings, 1998; Queller, 2000; Whittle and Extavour, 2016, 2017). Here, we examine variation in germ cell specification across the most diverse clade of animals, the Panarthropoda, wherein the general features of embryogenesis can nevertheless be meaningfully compared. This clade displays striking diversity in the temporal and spatial origin of germ cells, as well as multiple transitions between specification mechanisms, offering an ideal system in which to study evolution and regulation of germ cell specification.

Here, we first review the existing observational data regarding embryonic germ line development across panarthropods. We are primarily interested in the first embryonic appearance of germ cells before their arrival at the gonad, during which time we refer to them as primordial germ cells (PGCs). We identify three categories of temporally distinct developmental context in which PGCs are first detectable during embryogenesis, and we analyze the phylogenetic distribution of these three categories. We then summarize perturbation-based experimental studies aimed at uncovering the mechanisms of germ cell specification. Finally, we discuss the implications of these data for our understanding of the evolution of germ cell specification in panarthropods, and more broadly across Metazoa.

## Identification of primordial germ cells

Historically, embryologists studied early development using histological preparations of sectioned embryos (His, 1868). With light microscopy, PGCs can be readily distinguished from surrounding somatic cells by their rounder and larger nuclei (Nieuwkoop and Sutasurya, 1979). The chromatin of germ cells often appears more diffuse than that of somatic cells, and the cytoplasm, especially cortical or perinuclear cytoplasm, may contain electron-dense, non-membrane-bound, granular material (Eddy, 1975). However, tracking these cells

<sup>1</sup>Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA. <sup>2</sup>Program for Systems, Synthetic and Quantitative Biology, Harvard University, Boston, MA 02138, USA. <sup>3</sup>Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA. <sup>4</sup>Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA.

\*Present address: Laboratory of Insect Physiology, School of Agriculture, Kyoto University, Kyoto 606-8502, Japan. †Present address: Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853, USA.

§These authors contributed equally to this work

¶Author for correspondence (extavour@oeb.harvard.edu)

© J.A.K., 0000-0003-1701-3265; E.L.R., 0000-0001-6675-9886; R.R.K., 0000-0003-3082-1507; C.G.E., 0000-0003-2922-5855

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

throughout development using fixed samples and morphological features alone is not always technically possible.

Since the late 20th century, studies assessing gene expression patterns unique to or diagnostic of germ cells (henceforth referred to as molecular markers) have been instrumental in identifying germ cells at earlier stages than previously recognized. The most commonly used markers are the mRNA transcript or protein products (detectable by *in situ* hybridization or antibody staining, respectively) of the genes *vasa*, *nanos*, *tudor* and *piwi* (Ewen-Campen et al., 2010). The use of molecular markers can enable germ cell identification at earlier developmental stages, since some germ cells express the genes that encode these molecules before their morphological differentiation becomes unambiguously detectable (e.g. Ewen-Campen et al., 2013a). Additionally, these markers facilitate tracking of germ cells over the course of development. However, using molecular markers is a biased approach, as it relies on previous knowledge of germ line gene expression from other species. Furthermore, interpretation of molecular marker data can be complicated by pleiotropy, as the genes that encode these molecules may also be expressed by somatic cells (e.g. Yajima and Wessel, 2011).

### Germ cell formation in panarthropods

In our review of the embryological literature from 1860 to the present, we identified three categories of PGC specification in panarthropods, based on the timing of specification during embryogenesis (Fig. 1). We describe these categories below, along with relevant details of embryogenesis for context.

#### Category 1: PGC specification at early cleavage and blastoderm stages

After fertilization, embryogenesis begins with cleavage, defined as a series of mitotic divisions of the zygote (Gilbert, 1997). In most arthropods, cleavage leads to the formation of the blastoderm, a relatively uniform layer of cells at the egg cortex, surrounding a central yolk mass populated by a small number of yolk cells (Anderson, 1973; Scholtz and Wolff, 2013). The first category of PGC specification in panarthropods occurs during or before the formation of the blastoderm (Fig. 1A).

##### In insects

In most insects, the blastoderm is initially a syncytium, which is a single cell containing multiple nuclei generated by incomplete cleavage division cycles that do not include cytokinesis (Anderson, 1973; Johannsen and Butt, 1941). In some insects, the PGCs are the first cells to form, appearing at the posterior pole before the rest of the blastoderm is cellularized (Fig. 1Ai), and therefore often referred to as pole cells. While pole cells are most commonly seen in species of Diptera (flies, midges and mosquitoes), Coleoptera (beetles) and Hymenoptera (bees, ants and wasps), they have also been reported in Thysanoptera (thrips) and Dermaptera (earwigs) (references in Table S1).

Pole cells were first reported in a species of the midge *Chironomus* by Weismann (1863), although Metschnikow (1866) was the first to report their migration into the gonad. In many insects, the cytoplasm at the posterior pole of the embryo is morphologically distinct from that of the rest of the embryo, often with visible granules (Hegner, 1914; Zissler, 1992). The close association of this granular cytoplasm with pole cells led researchers to speculate that the granular material contains germ line determinants (e.g. Hegner, 1909a; Zissler, 1992). It was long believed that pole cells gave rise to somatic cell types in addition to the germ cells (Anderson, 1962a,b; Poulson, 1947, 1950). However, careful lineage tracing in the fruit fly

*Drosophila melanogaster* showed that all pole cells are determined as germ cells, and any that become lost during their migration die (Underwood et al., 1980).

While the function of pole cells as PGCs appears to be conserved across insects, the specific details of their formation can vary considerably across species. For example, pole cells differ in which cleavage cycle they cellularize: they form during the ninth mitotic cycle of *D. melanogaster* (Huettnner, 1923) and during the sixth mitotic cycle in the parasitic wasp *Nasonia vitripennis* (Buchta et al., 2013). To our knowledge, the earliest observed formation of pole cells is during the third cleavage cycle in the midges *Miastor metraloas* and *Chironomus* sp. (Hegner, 1914; Weismann, 1863). The number of pole cells formed is also variable: a single pole cell forms in *Chironomus* embryos (Hegner, 1914; Weismann, 1863), whereas four appear in the fruit fly *Bactrocera tryoni* (Anderson, 1962a) and 16 pole cells form in the beetle *Calligrapha multipunctata* (Hegner, 1909b).

After the pole cells form, they enter the egg and migrate to the location of the future embryonic gonad, which is located far from the posterior pole (Santos and Lehmann, 2004). Several different migration pathways have been described. In *D. melanogaster* and many other Diptera, the pole cells are carried into the egg during posterior midgut invagination, then migrate through the epithelium of the posterior midgut to enter the abdominal mesoderm and associate with the developing somatic gonad (Metschnikow, 1866; Sonnenblick, 1941). In Hymenoptera, pole cells are internalized with the rest of the mesoderm during gastrulation (Gatenby, 1917). Yet another variation is observed in Chrysomelidae (leaf beetles) (Hegner, 1909a), in which germ cells are internalized with the rest of the germ band as the extra-embryonic membranes fold over it.

##### In other arthropods

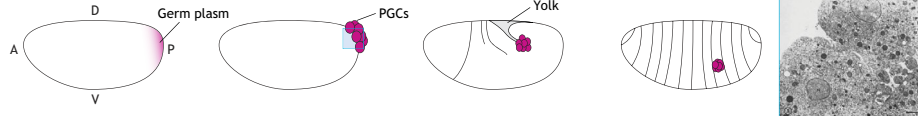
PGCs are also specified early in Collembola (springtails), a clade of hexapods that are sister to insects (Du et al., 2024). Embryogenesis in these animals begins with a period of holoblastic cleavage, and certain blastomeres on the basal (yolk) side of the blastoderm can be recognized as likely PGCs very early in development (Jura, 1967; Klag, 1982). The cytoplasm of these cells contains granular material visible by light and electron microscopy (Klag, 1982), resembling the cytoplasm containing germ line determinants observed in insects (Hegner, 1914; Zissler, 1992). Rather than maintaining contact with the germ band as in insects, collembolan germ cells migrate into the center of the yolk after their specification until eventually migrating to associate with the abdominal mesoderm, from which the embryonic gonad develops (Jura, 1967).

Among non-hexapod Pancrustacea, embryos of Copepoda (copepods), Amphipoda (beach hoppers), Decapoda (crabs, lobsters and shrimp) and some Cladocera (water fleas) undergo stereotypical holoblastic cleavage, allowing precise tracking of cell lineages and the identification of a specific cleavage blastomere as the progenitor of PGCs (Gerberding et al., 2002; Hertzler, 2005; Kühn, 1913; McClendon, 1907). In amphipods and decapods, a putative germ plasm has been identified in the freshly laid egg, termed the RNA-containing body (Gupta and Extavour, 2013) or intracellular body (Biffis et al., 2009; Chen et al., 2021). During cleavage, this region of cytoplasm is inherited asymmetrically by the blastomeres that give rise to the PGCs (Gupta and Extavour, 2013) (Fig. 1Aii).

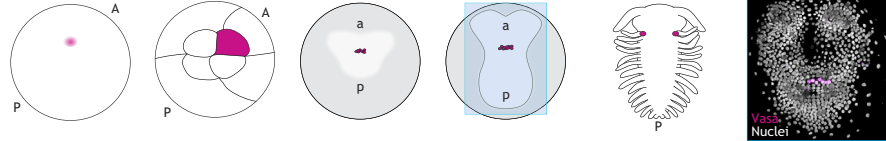
In chelicerates (spiders, scorpions, ticks and mites), candidate PGCs are first detectable with molecular markers in the yolk of blastoderm-stage embryos in the mite *Tetranychus urticae* (Dearden et al., 2003). As the germ band forms, the PGCs associate with the

**A Category 1: PGC specification at blastoderm and early cleavage stages**

i *Drosophila melanogaster* (Diptera)

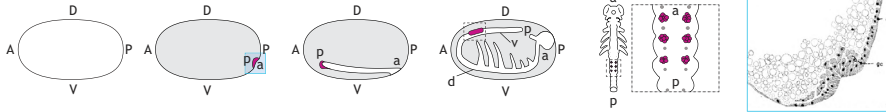


ii *Parhyale hawaiiensis* (Amphipoda)

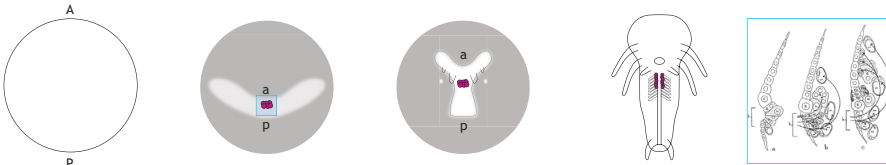


**B Category 2: PGC specification at gastrulation**

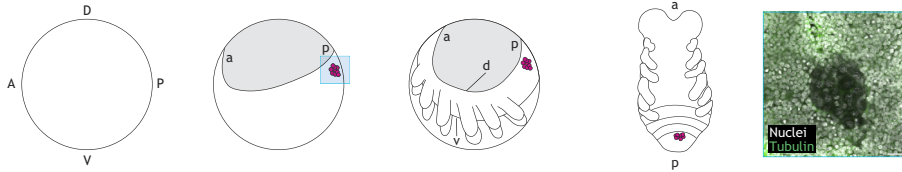
i *Oncopeltus fasciatus* (Hemiptera)



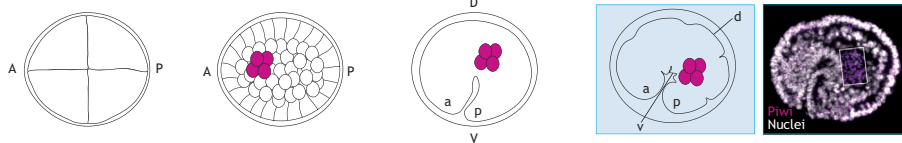
ii *Hemimysis lamornae* (Mysida)



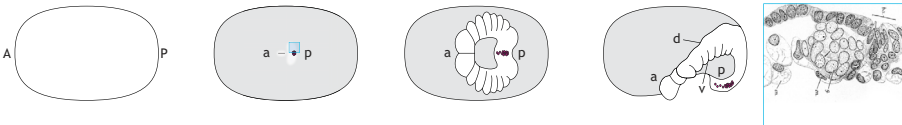
iii *Phalangium opilio* (Opiliones)



iv *Hypsibius exemplaris* (Tardigrada)

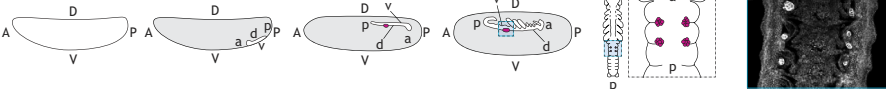


v *Peripatopsis moseleyi* (Onychophora)

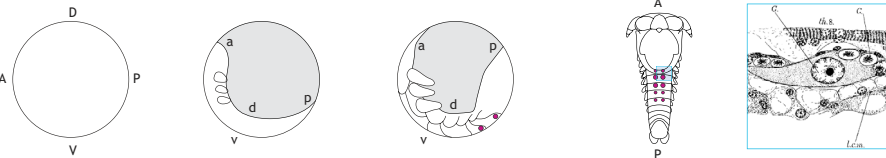


**C Category 3: PGC specification from mesoderm**

i *Gryllus bimaculatus* (Orthoptera)



ii *Nebalia bipes* (Leptostraca)



iii *Parasteatoda tepidariorum* (Araneae)

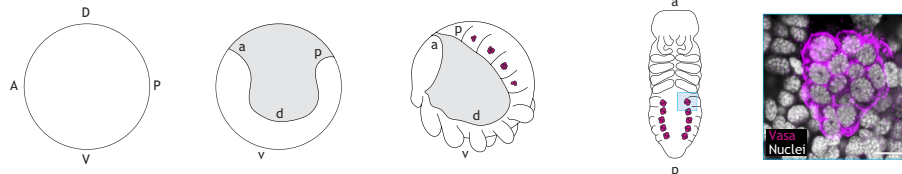


Fig. 1. See next page for legend.

**Fig. 1. Primordial germ cell (PGC) origin in the context of panarthropod embryogenesis.** In all schematics, A/a, anterior; D/d, dorsal; P/p, posterior; V/v, ventral; lower case letters are shown in cases where egg and embryonic axes are not the same, and/or indicate embryonic axes in embryos that do not occupy the entire egg volume; if D/V is not indicated, schematics show ventral view; magenta, approximate location of germ plasm or PGCs; gray, yolk or extra-embryonic tissue; blue box in schematic, stage/location where germ cells are detectable in the micrographs and drawings on the right. (A) Description of Category 1: PGC specification at early cleavage and blastoderm stages. (Ai) In the fruit fly *Drosophila melanogaster*, germ plasm accumulates at the posterior of syncytial embryos and is inherited by pole cells that migrate to the ultimate site of the embryonic gonad (Illmensee and Mahowald, 1974). Right: Transmission electron microscopy image displaying pole cells budding in *D. melanogaster* embryos. The pole cell nuclei (N) have characteristically diffuse chromatin, and polar granules (P) are distributed throughout the cytoplasm. Image reproduced with permission (Mahowald, 1968). M, mitochondria. Scale bar: 1  $\mu$ m. (Aii) In the amphipod *Parhyale hawaiiensis*, germ plasm accumulates in a region of the embryo called the RNA-containing body, which is inherited by the *g* micromere at the 8-cell stage (Extavour, 2005; Gupta and Extavour, 2013). All descendants of *g* are PGCs. Right: PGCs visualized by immunohistochemistry with a cross-reactive antibody raised against recombinant Vasa protein from both *D. melanogaster* and the grasshopper *Schistocerca americana* (Chang et al., 2002). (B) Description of Category 2: PGC specification at gastrulation. (Bi) In the milkweed bug *Oncopeltus fasciatus*, PGCs first appear in the ventral posterior blastoderm in an invaginating structure called the posterior pit (Ewen-Campen et al., 2013a). PGCs remain at the posterior tip of the germ band as it invaginates into the yolk. The PGCs migrate along the germ band toward the anterior to segments A4–A6. Right: Hand drawing of a light micrograph of a sectioned, late blastoderm-stage *O. fasciatus* embryo showing germ cells in the posterior pit. Image reproduced with permission (Butt, 1949). gb, germ band; gc, germ cell. (Bii) In the mysid *Hemimysis lamornæ*, PGCs differentiate in the center of the blastopore area and are internalized at gastrulation (Manton, 1928). Right: Drawing of sagittal view of an *H. lamornæ* embryo at gastrulation. Image reproduced with permission (Manton, 1928). (Biii) In the harvestman *Phalangium opilio*, PGCs are first identifiable at the posterior end of the germ band soon after the germ band forms (Faussek, 1888; Gainett et al., 2022). PGCs remain at the posterior end of the germ band at least until the fourth opisthosomal segment is formed, after which the germ cells can be found in that segment. Right: Chromatin (visualized with the nucleic acid dye Hoechst 33342) is more diffuse in PGCs than in surrounding cells. Image kindly provided by Prashant Sharma (University of Wisconsin–Madison, WI, USA). Green, alpha-tubulin. Scale bar: 20  $\mu$ m. (Biv) In the tardigrade *Hypsibius exemplaris*, PGCs are first identifiable at the 32-cell stage and are the first cells to be internalized during gastrulation. Right: PGCs visualized by *in situ* hybridization against *He-vasa*. Image kindly provided by Kira Heikes (Duke University, NC, USA). (Bv) In the velvet worm *Peripatopsis moseleyi*, PGCs are first identifiable near the blastopore at the beginning of gastrulation. As the embryo elongates and more segments form, the PGCs migrate to more anterior segments. Right: Hand drawing of a light micrograph of a *P. moseleyi* embryo showing primordial germ cells soon after their internalization during gastrulation, distinguished by their lighter-staining nuclei and cytoplasm. Image reproduced with permission (Manton, 1949). (C) Description of Category 3: PGC specification from mesoderm. (Ci) In the cricket *Gryllus bimaculatus*, PGCs are first detectable among the mesoderm in segments A2–A4 at embryonic stage 6, after most abdominal segments have been specified (Ewen-Campen et al., 2013b). Right: PGCs visualized by immunohistochemistry with an antibody against *Gb-Vasa*. (Cii) In the leptostracan *Nebalia bipes*, PGCs are first identifiable in the mesoderm in the thorax and abdomen during the extended germ band stage (Manton, 1934). Right: Hand drawing of a light micrograph of a sectioned *N. bipes* embryo showing PGCs among the mesoderm in the extended germ band, distinguished by their lighter-staining cytoplasm and larger nuclei. Image reproduced with permission (Manton, 1934). G, genital rudiment; l.c.m., circular muscle round liver lobe; th.8, eighth thoracic segment. (Ciii) In the house spider *Parasteatoda tepidariorum*, PGCs are first identifiable in segmental clusters in the opisthosomal mesoderm after all segments have formed (Schwager et al., 2015). Right: PGCs visualized by immunohistochemistry using an antibody raised against *Pt-Vasa*. Scale bar: 10  $\mu$ m.

posterior germ band near the primordia of the fourth walking legs (Dearden et al., 2003). We are not aware of any other chelicerate or myriapod (centipedes and millipedes) species in which PGC specification has been proposed to occur before gastrulation.

## Category 2: PGC specification at gastrulation

After formation of the blastoderm, a portion of the blastoderm is specified as the presumptive embryonic region, variably referred to as the germ band, germ rudiment, embryonic rudiment or germ disc depending on its initial form (Anderson, 1973; Johannsen and Butt, 1941). The rest of the blastoderm differentiates into one or more extra-embryonic membranes (Johannsen and Butt, 1941). Soon after this specification, gastrulation occurs, whereby some of the blastoderm cells are internalized and become the mesoderm (Stern, 2004). In insects, mesoderm internalization primarily occurs along the ventral midline of the germ band (Leptin, 2004; Roth, 2004). In other arthropods, mesoderm may be internalized through a blastopore near the germ rudiment (Chaw et al., 2007; Gerberding and Patel, 2004), or by epiboly (Edgar et al., 2015). The second category of PGC differentiation occurs at or very close to the time of gastrulation (Fig. 1B).

### In insects

Among insects, PGC specification during gastrulation is the most commonly reported timing in members of Hemiptera (true bugs), Psocodea (lice) and Lepidoptera (moths and butterflies), although it is also reported in species of Dermoptera, Blattodea (cockroaches), Hymenoptera, Megaloptera (alderflies), Strepsiptera (twisted-wing flies), Coleoptera, Mecoptera (scorpion flies) and Diptera. Three different spatial patterns of germ cell specification have been reported around the time of gastrulation: before germ band formation, at the posterior of the germ band after its formation, or at the ventral midline of the blastoderm just before gastrulation. In Hemiptera, Psocodea and Tenebrionidae (darkling beetles; Coleoptera), germ cells are first detectable at the ventral posterior region of the cellular blastoderm in an invaginating structure called the posterior pit (Fig. 1Bi) (Butt, 1949; Goss, 1952; Mellanby, 1935; Schröder, 2006; Shinji, 1919). These cells appear to form on the side of the blastoderm epithelium facing the yolk, rather than on the outside of the blastoderm, such as the pole cells described in Category 1. In Hemiptera and Psocodea, the invagination continues into the egg towards the anterior, forming a definitive germ band with germ cells at its posterior tip (Fig. 1Bi). Germ cells in tenebrionid beetles move further inside the embryo once the amnion (ventral extra-embryonic membrane) encloses the entire ventral surface of the germ band, similar to the internalization seen in coleopteran species that specify their germ cells as pole cells [e.g. the weevils *Callosobruchus maculatus* (Brauer, 1925, 1949; Quan, 2018) and *Acanthoscelides obtectus* (Jung, 1966; Lynch et al., 2011)]. The newly formed germ cells then migrate along the dorsal side of the germ band toward the anterior, eventually settling into the somatic gonad in the abdominal mesoderm.

In several species that appear to specify germ cells around the time of gastrulation, PGCs were first identified at the posterior tip of the germ band after its detachment from the blastoderm [e.g. *Periplaneta orientalis* (Blattodea) (Heymons, 1895), *Sialis mitsuhashii* (Megaloptera) (Suzuki et al., 1981), *Nematus ribesii* (Hymenoptera) (Singh, 1967) and Mecoptera (Suzuki, 1990)]. Early studies of tenebrionid beetles described this pattern of germ cell differentiation as well (Rempel and Church, 1969; Ullmann, 1964). However, later work using molecular markers suggests that

PGCs in at least one such beetle, *Tribolium castaneum*, appear earlier, during the cellular blastoderm stage in a posterior pit much like the Hemiptera (Schröder, 2006). This raises the possibility that in some other tenebrionid species, PGCs may likewise differentiate from the blastoderm, with the reported later appearance in the germ band simply reflecting limitations in detection methods.

In contrast to the posterior pit, germ cells differentiate along the ventral midline of the blastoderm in Lepidoptera, separating from the blastoderm before gastrulation to lie on the dorsal side of the presumptive germ band in the presumptive abdominal region (Ando and Tanaka, 1980; Miya, 1958a; Presser and Rutschky, 1957; Tanaka, 1987; Woodworth, 1889). Unlike when germ cells form at the posterior pit or at the posterior tip of the germ band, in Lepidoptera there does not appear to be long-range germ cell migration, since the germ cells differentiate in the same abdominal location as the gonad.

#### In other panarthropods

Among non-insect Pancrustacea, PGCs are the first cells to be internalized from the blastopore lip in Mysida (opossum shrimp) (Manton, 1928), Anomopoda (water fleas) (Cannon, 1921), and the copepod orders Siphonostomatoidea (sea lice) (McClendon, 1907), Harpacticoida (Witschi, 1934) and Cyclopoida (Schimkewitsch, 1896). In Isopoda (woodlice and pill bugs), PGCs differentiate from the mesoderm soon after internalization (Fig. 1Bii) (Goodrich, 1939; Needham, 1942; Strömberg, 1965, 1968; Wolff, 2009). Thus, PGC differentiation seems to be tied to gastrulation in these crustacean orders.

In chelicerates, PGCs have been identified based on morphological criteria at the blastopore in Opiliones (harvestmen) (Fig. 1Biii) (Faussek, 1888, 1892; Holm, 1947) and Scorpiones (Brauer, 1894), and a recent study using molecular markers supported this interpretation (Gainett et al., 2022). Putative PGCs have also been identified at the blastopore in the centipedes *Scolopendra cingulata* and *Strigamia maritima* (Myriapoda) (Green and Akam, 2014; Heymons, 1901).

In Tardigrada (water bears), germ cells are the first cells to enter the blastopore during gastrulation, based on data from both morphological characteristics (Hejnlol and Schnabel, 2005) and molecular markers (Heikes et al., 2023) (Fig. 1Biv). Lineage tracing in these embryos suggests that the germ cells are derived from both blastomeres of the two-cell embryo (Gabriel et al., 2007; Hejnlol and Schnabel, 2005).

Finally, onychophoran (velvet worm) germ cells have been described only with classic histological methods, to our knowledge (Fig. 1Bv). They emerge either at the blastopore lip during gastrulation (Manton, 1949) or at the posterior end of the germ band during elongation (Manton, 1949; Mayer and Tait, 2009; Sedgwick, 1887).

#### Category 3: PGC specification from mesoderm

After gastrulation, the germ band elongates and becomes segmented, and the mesoderm becomes organized into pairs of somites in each segment. In most living arthropods, each somite forms a small coelom enclosing a coelomic cavity (Anderson, 1973; Johannsen and Butt, 1941). The coelomic cavities are eventually lost as the mesoderm differentiates into different cell types and reorganizes into the primordia of the internal organ systems, including the somatic gonad (Anderson, 1973; Johannsen and Butt, 1941). The final category of PGC differentiation timing encompasses species in which PGCs differentiate from the mesoderm at this comparatively late stage of embryogenesis (Fig. 1C).

#### In insects

In insects, PGC specification from the mesoderm has been reported in members of Archaeognatha (jumping bristletails), Zygentoma (silverfish and firebrats), Orthoptera (crickets and grasshoppers) (Fig. 1Ci), Mantodea (praying mantises), Blattodea, Trichoptera (caddisflies), Coleoptera and Lepidoptera. PGCs may differentiate before formation of the coelomic cavities [e.g. Orthoptera (Ewen-Campen et al., 2013b), Mantodea (Görg, 1959)], after formation of the coelomic cavities [e.g. Archaeognatha (Larink, 1969), Zygentoma (Woodland, 1957)] or after the coelomic cavities are no longer recognizable [e.g. Trichoptera (Miyakawa, 1974), *Odontotaenius disjunctus* (Coleoptera) (Krause, 1947), *Apis mellifera* (Hymenoptera) (Dearden, 2006; Nelson, 1915)]. In this developmental timing category, PGCs are usually specified in the same abdominal segments where the somatic gonad forms, so there is no long-range movement of PGCs after their specification.

#### In other arthropods

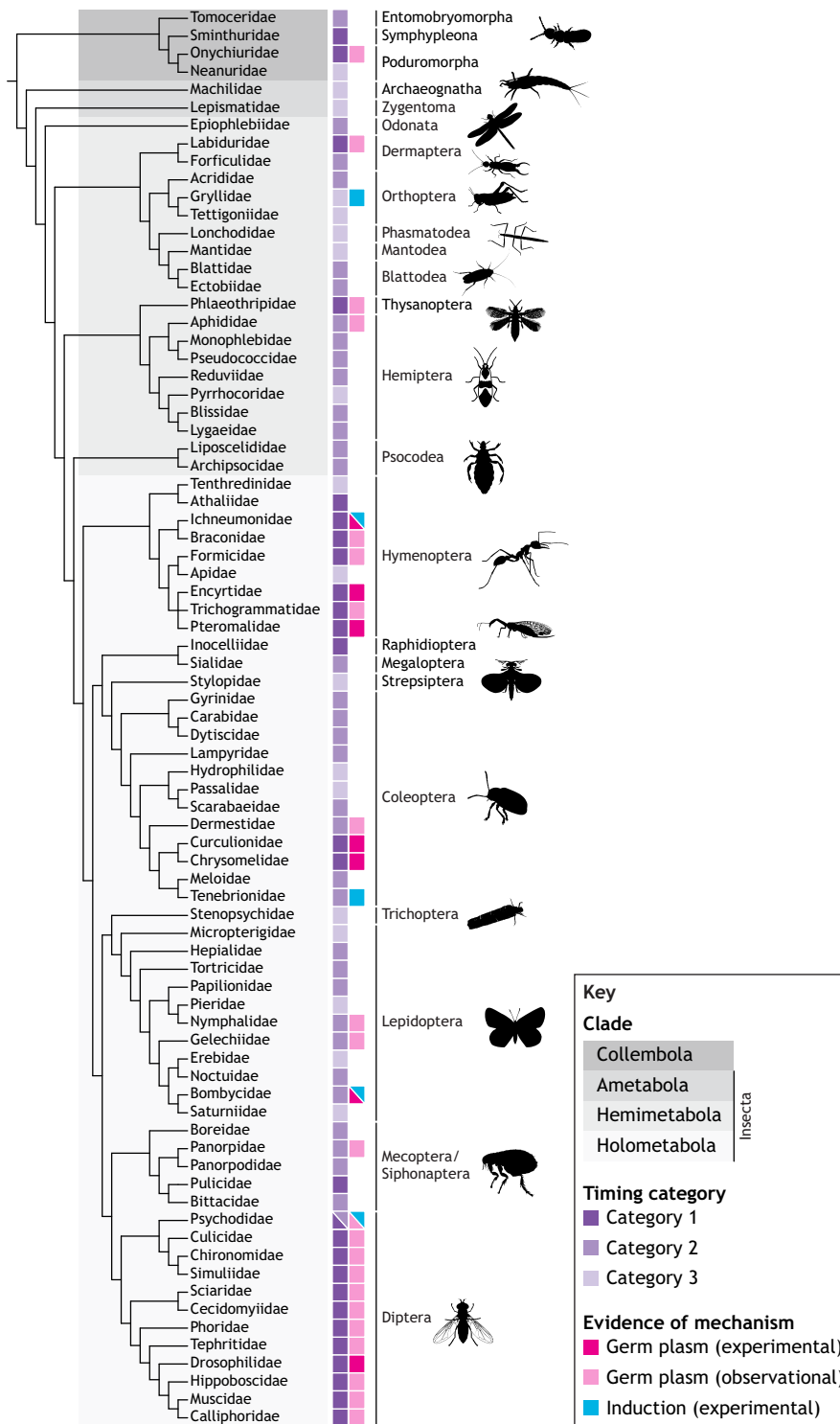
Differentiation of PGCs from the mesoderm has also been described in Leptostraca (mud shrimp) (Fig. 1Cii) (Manton, 1934), Anaspidacea (mountain shrimp) (Hickman, 1936) and Branchiopoda (fairy, clam and tadpole shrimp, and water fleas) (Anderson, 1967; Mitsumoto and Makioka, 2002; Sudler, 1899). However, data for stages of crustacean development late enough to include differentiation of the trunk mesoderm from which primordial germ cells might arise are sparse. This relative scarcity of early embryonic descriptions may be in part because many crustaceans hatch as a nauplius, comprising only the head segments (Scholtz, 2000). In these animals, germ cells might not be specified until later larval stages, after formation of the trunk. Many studies of naupliar and larval development in crustaceans are focused on organogenesis of the gonad as a whole, without specific reference to the origin of PGCs (Cannon, 1924; Ikuta and Makioka, 1994; Mitsumoto and Makioka, 2002).

Classical studies on myriapods suggest a late differentiation of PGCs from the mesoderm in three of the four myriapod classes [Pauropoda (Tiegs, 1947), Symphyla (pseudocentipedes) (Tiegs, 1940) and Diplopoda (millipedes) (Dohle, 1964)], although only one species was reported from each class in these studies. To our knowledge, germ cells in these myriapod classes have not been investigated with molecular markers.

Among chelicerates, PGCs differentiate from the mesoderm of the opisthosomal segments in spiders (Fig. 1Ciii) (Gough, 1902; Kautzsch, 1910; Schimkewitsch, 1903), consistent with data from molecular markers (Schwager et al., 2015). In pycnogonids (sea spiders), PGCs are first detectable in the mesoderm of the prosoma (Alexeeva and Tamberg, 2021; Alexeeva et al., 2018; Miyazaki and Makioka, 2012).

#### Summary: evolutionary trends from late to early germ cell specification

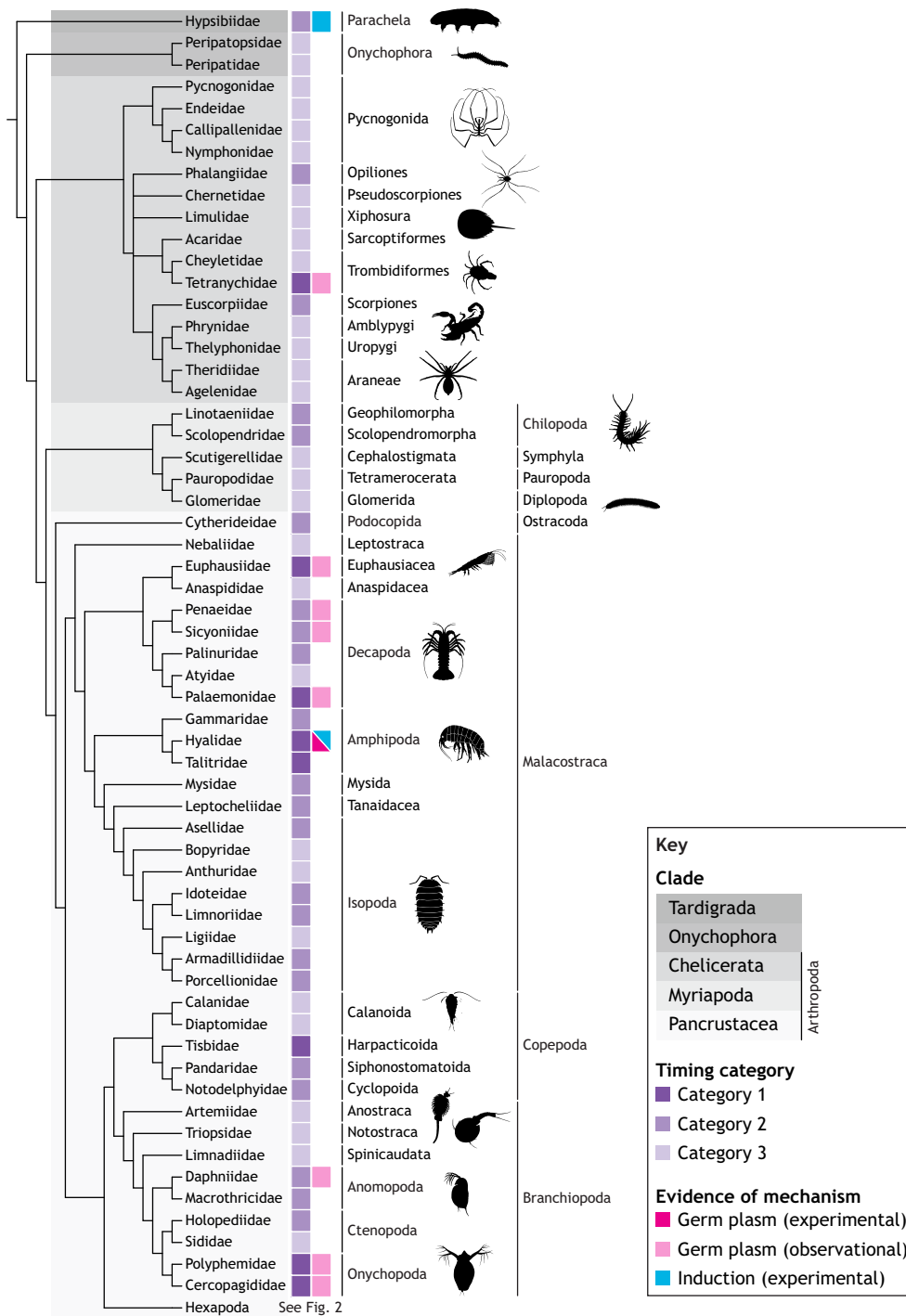
In his monograph on embryogenesis in Dermaptera and Orthoptera, Richard Heymons (1895) proposed that ‘the formation of sex cells at the rear end of the embryonic primordium, which has now been clearly demonstrated for representatives of very different groups, is probably generalizable for all insects’. Rather than making broad statements such as this, we summarize the data regarding the timing of germ cell specification (Figs 2, 3; Table S1) in the context of currently accepted panarthropod phylogeny (Ballesteros et al., 2022; Fernández et al., 2018; Giribet and Edgecombe, 2019; Misof et al., 2014; Schwentner et al., 2017). This shows that in euarthropod orders that are close to the base of the tree, PGCs tend to be first observed later in embryogenesis (discussed above as Category 3). This pattern



**Fig. 2. Germ cell specification across Hexapoda.** Phylogeny of studied hexapod lineages indicating the PGC origin timing category and the inferred mechanism of PGC specification. Phylogenetic relationships in the cladogram are derived from published relationships listed in Table S2; branch lengths are not to scale. Data on the timing of germ cell specification are shown in the first column as follows: dark purple, Category 1 (cleavage/blastoderm); medium purple, Category 2 (gastrulation); light purple, Category 3 (mesoderm differentiation). Data on the mechanism of germ cell specification, inferred as described in the supplementary information, are shown in the second column: magenta, experimental evidence supports the use of germ plasm; pink, morphological evidence of germ plasm; blue, experimental evidence of induction. Data and data sources are described in more detail in Table S1.

suggests that the timing of first germ cell appearance evolved to be earlier in development in multiple lineages independently, as seen in Dermaptera, Condylgnatha (true bugs and thrips) and Holometabola (undergoing complete metamorphosis) within insects; Copepoda, Malacostraca (the largest class of crustaceans) and Cladocera within non-hexapod Pancrustacea; Chilopoda (centipedes) within myriapods; and Opiliones, scorpions and mites within chelicerates. These data suggest the possibility that differentiation from the mesoderm is the ancestral mode of PGC specification in Euarthropoda.

Conflicting interpretations in the literature about the timing of germ cell specification have sometimes resulted from technical advances enabling identification of germ cells at stages earlier than previously detected. For example, early work on the silk moth *Bombyx mori* (Lepidoptera) reported PGCs emerging from the mesoderm of coelomic cavities in late embryogenesis (discussed above as Category 3) (Toyama, 1902), while later studies identified PGCs on the posterior side of the ventral midline of the early germ band (as in Category 2) (Miya, 1953). The onset of PGC specification before gastrulation is further supported by data from



**Fig. 3. Panarthropod germ cell specification outside of Hexapoda.** Phylogeny of studied non-hexapod panarthropod lineages indicating the PGC origin timing category and the inferred mechanism of PGC specification. Phylogenetic relationships in the cladogram are derived from published relationships listed in Table S2; branch lengths are not to scale. Data on the timing of germ cell specification are shown in the first column as follows: dark purple, Category 1 (cleavage/blastoderm); medium purple, Category 2 (gastrulation); light purple, Category 3 (mesoderm differentiation). Data on the mechanism of germ cell specification, inferred as described in the supplementary information, are shown in the second column: magenta, experimental evidence supports the use of germ plasm; pink, morphological evidence of germ plasm; blue, experimental evidence of induction. Data and data sources are described in more detail in Table S1.

molecular markers, which showed accumulation of *vasa* mRNA in a cluster of presumptive PGCs at the ventral midline of the early germ band (Nakao, 1999; Nakao et al., 2006). Therefore, it is possible that species which we classified here in Category 3, may be reclassified in Categories 1 or 2 by future work deploying molecular markers. This caveat notwithstanding, we note that molecular markers and functional genetic studies often corroborate late germ cell specification [see for example *Gryllus bimaculatus* (Orthoptera, Insecta) (Ewen-Campen et al., 2013b), *A. mellifera* (Hymenoptera, Insecta) (Dearden, 2006), *Parasteatoda tepidariorum* (Araneae, Chelicerata) (Schwager et al., 2015)]. We, therefore, do not assume

that the hypothesis of ancestral late PGC specification is an artifact of exclusive use of morphological identification methods.

Going forward, data from key understudied lineages should be gathered to further test our hypothesis about the ancestral timing of PGC specification. For example, among the hexapods, data from members of the Palaeoptera (mayflies, dragonflies and damselflies), the ametabolous Archaeognatha and Zygentoma, or the non-insect hexapod Diplura (two-pronged bristletails) could be especially useful. Similarly, data about PGC specification are missing from Oligostraca, an understudied clade of Pancrustacea that includes Ostracoda (seed shrimp) and Branchiura (fish lice).

Outside of Pancrustacea, the field has yet to achieve clear consensus on the phylogenetic relationships of the groups within Chelicerata (Sharma and Gavish-Regev, 2025), and there is too little embryological data available for us to suggest evolutionary trends in PGC specification within this subphylum. Data regarding PGC specification are even sparser for myriapods. Specifically, we are aware of relevant data from only five species across the four myriapod classes (Dohle, 1964; Green and Akam, 2014; Heymons, 1901; Tiegs, 1940, 1947). Studies of PGCs in a broader range of both chelicerates and myriapods, as well as detailed embryological studies in unexplored taxa, will therefore be needed to fully understand the developmental context and evolutionary trends of germ cell specification in these subphyla.

### Molecular and genetic mechanisms of germ cell specification in panarthropods

Beyond studying where and when germ cells first appear during arthropod embryogenesis, researchers have sought to understand how they are specified. Germ cell specification in animals has historically been classified into two mechanisms, often referred to as maternal inheritance and zygotic induction (Extavour and Akam, 2003; Nieuwkoop and Sutasurya, 1981). In maternal inheritance, germ cell determinants accumulate in a specific region of the oocyte cytoplasm, forming a germ plasm. Cells that inherit sufficient germ plasm components after the embryo divides become PGCs. In embryos that undergo zygotic induction, all cells are in principle competent to become germ cells at the beginning of embryogenesis. Certain cells later receive signals from neighboring cells, produced by the activity of the zygotic genome, which induce them to take on germ cell identity. In the following section, we describe how historical observational data and perturbational experimental approaches have offered increasingly robust evidence to distinguish between these mechanisms in several panarthropod species.

#### Germ cell specification through maternal inheritance

In the early 20th century, researchers speculated that the electron-dense, membraneless granules frequently found in the cytoplasm of PGCs were responsible for their specification. Experiments in chrysolimid beetles provided early evidence that the posterior cytoplasm of the oocyte or early embryo contains germ cell determinants. Burning or puncturing the posterior poles of beetle eggs with needles to damage or remove posterior cytoplasm reduced or eliminated PGCs (Hegner, 1908, 1911), demonstrating that this cytoplasm does not simply correlate with PGC specification, but rather plays a causal role in PGC specification. Studies in *D. melanogaster* offered further evidence for the necessity of the germ plasm in PGC specification. Removing or destroying the posterior cytoplasm of *D. melanogaster* eggs by ultraviolet irradiation demonstrated that this region is required for germ cell formation (Geigy, 1931). Subsequent centrifugation experiments in *D. melanogaster* embryos revealed that shifting the granular structures in posterior cytoplasm away from the posterior pole prevented germ cell formation or led to germ cells forming in a different part of the embryo (Jazdowska-Zagrodzińska, 1966).

Experiments transplanting specific regions of cytoplasm between *D. melanogaster* embryos showed that the contents of the posterior cytoplasm were sufficient to specify germ cells. Transplanting posterior cytoplasm from undamaged *D. melanogaster* embryos into sterile ones in which the posterior pole had been damaged with ultraviolet irradiation rescued fertility, while transplanting anterior cytoplasm did not (Okada et al., 1974). Further transplantation

experiments demonstrated that posterior pole plasm introduced into the anterior of another embryo caused formation of ectopic pole cells that gave rise to functional germ cells when transplanted again to the posterior of a third, sterile embryo (Illmensee and Mahowald, 1974). Similar experiments transferring the posterior cytoplasm from late-stage oocytes into embryos revealed that the oocyte pole plasm could specify functional PGCs (Illmensee et al., 1976), demonstrating that the necessary germ cell determinants in *D. melanogaster* are deposited during oogenesis. These foundational studies showed that maternally derived cytoplasmic granules are both necessary and sufficient for germ cell formation in some species.

Contemporary studies testing for maternal inheritance often ask whether known germ line gene products accumulate during late oogenesis or early embryogenesis in the region where PGCs first arise. For example, antibody staining or *in situ* hybridization experiments to detect germ line molecules including Vasa protein or *nanos* mRNA have suggested the presence of germ plasm in mosquitoes (Juhn and James, 2006; Juhn et al., 2008), scuttle flies (Wotton et al., 2014), ants (Khila and Abouheif, 2008; Lynch et al., 2011; Rafiqi et al., 2020), wasps (Kharel et al., 2024; Lynch et al., 2011), weevils (Lynch et al., 2011), aphids (Chang et al., 2006, 2007; Chen et al., 2009; Lin et al., 2014), amphipods (Gupta and Extavour, 2013) and prawns (Chen et al., 2021). Transcriptomic analyses of different portions of embryos and oocytes (Carter et al., 2015; Klomp et al., 2015; Quan and Lynch, 2016; Yoon et al., 2019) have also revealed asymmetric localization of molecules associated with PGC specification.

However, expression data showing asymmetric localization of putative germ plasm components are not sufficient to demonstrate that these molecules function in PGC specification. To support such claims, functional genetic techniques are needed to manipulate gene activity and test the effect on germ plasm assembly and germ line development. The function of germ plasm components in panarthropods has been most thoroughly studied in *D. melanogaster* (reviewed by Lehmann, 2016; Mahowald, 2001; Treck and Lehmann, 2019). The membraneless ribonucleoprotein granules at the posterior of *D. melanogaster* oocytes and early embryos are nucleated by the protein Oskar, which is both necessary and sufficient for germ plasm assembly (Ephrussi and Lehmann, 1992; Kistler et al., 2018). Oskar-mediated localization of core germline molecules such as Vasa, Tudor, Aubergine and *nanos* to germ granules is essential for PGC specification and maintenance of germ cell identity during cell migration and gonad formation in these fruit flies (Lehmann, 2016; Mahowald, 2001).

Analysis of germ plasm composition and localization in species beyond *D. melanogaster* has revealed both conserved and rapidly evolving features. Within the *Drosophila* genus, germ granule size, morphology (Counce, 1963; Mahowald, 1968; Rivard et al., 2025 preprint), and the relative proportions of different granule components vary widely (Doyle et al., 2023). The wasp *N. vitripennis* also has a well-characterized germ plasm in the form of a single large structure called the oosome (Bull, 1982). Akin to *D. melanogaster*, *N. vitripennis* requires *oskar* to assemble a germ plasm containing many of the same molecules (e.g. Vasa, Tudor and *nanos*) (Lynch and Desplan, 2010; Lynch et al., 2011). However, while *D. melanogaster* has many small germ granules (Mahowald, 1962), the *N. vitripennis* oosome has a distinct protein organization and transcript composition compared to that observed in *D. melanogaster* (Kempth et al., 2025; Kharel et al., 2024; Lynch et al., 2011). In several crustacean species, the germ plasm appears as a large, round granule in the cortex of embryos and contains

conserved germ plasm markers (Chen et al., 2021; Grattan et al., 2013; Gupta and Extavour, 2013). Studying germ plasm in additional species is needed to clarify whether and how variation in germ plasm makeup and organization influences the post-transcriptional regulation and function of germ line determinants.

### Germ cell specification through zygotic induction

Experimentally demonstrating inductive germ cell specification, requiring physical, molecular or genetic perturbation methods, is more technically challenging than detecting germ plasm accumulation, for which *in situ* hybridization techniques or protein detection may suffice. Consequently, most studies have inferred or hypothesized zygotic induction based on absence of evidence of germ plasm. For example, *in situ* hybridization experiments fail to reveal asymmetric localization of putative germ line gene transcripts in oocytes and early embryos of various species, including milkweed bugs (Ewen-Campen et al., 2013a), crickets (Ewen-Campen et al., 2013b), honeybees (Dearden, 2006), mites (Dearden et al., 2003), spiders (Schwager et al., 2015), centipedes (Green and Akam, 2014) and tardigrades (Heikes et al., 2023). Instead, key germ line genes are expressed in a subset of cells later in embryogenesis, coincident with reports of the first PGCs based on morphological criteria (Table S1). However, we note that even in the well-accepted case of mouse PGC induction (Saitou and Yamaji, 2010), it remains formally possible that some unknown, and therefore untested, germ line determinant or factor that makes cells competent to respond to later germ line induction signals, is asymmetrically localized and preferentially inherited by germ cells.

This caveat notwithstanding, there are multiple examples of experimental support for induction rather than inheritance across panarthropods. Maternal knockdown of putative germ plasm components in milkweed bugs and crickets reveals that the maternal products of these genes are dispensable for PGC formation (Ewen-Campen et al., 2013a,b). Laser ablation of single cells in 2- and 4-cell stage embryos of the tardigrade *Thuliana stephaniae* does not prevent PGC specification (Hejnol and Schnabel, 2005). This observation suggests that this species lacks a cytoplasmic region with inherited germ line determinants, and that it might instead specify PGCs through inductive pathways.

Germ cell formation in embryos with tissue duplications can also be interpreted as evidence against germ plasm and consistent with induction. For example, in the drain fly *Clogmia albipunctata* and the flour beetle *T. castaneum*, genetic manipulations producing double-abdomen embryos lead to germ cell formation at both anterior and posterior ends (Ansari et al., 2018; Yoon et al., 2019). Similarly, naturally occurring ‘twins’ of the stick insect *Carausius morosus* are duplicated embryos fused along their anterior–posterior (A–P) axis, which both possess germ cells (Cavallin, 1971). In contrast, double-abdomen embryos of *D. melanogaster* only produce germ cells at the true posterior end, since germ plasm is absent at the ectopic posterior end (Lasko and Ashburner, 1990).

Among panarthropods, experiments in the cricket *G. bimaculatus* have provided the most direct evidence for zygotic induction of PGCs. Knockdown of the mesoderm specification factor *twist* leads to loss of PGCs, suggesting that the mesoderm contributes to germ cell specification and/or maintenance (Ewen-Campen et al., 2013b) and consistent with morphological evidence that orthopteran PGCs first arise from the embryonic mesoderm (Wheeler, 1893). The bone morphogenetic protein (BMP) signaling pathway, which induces germ cells in mice (Hopf et al., 2011; Lawson et al., 1999; Ying and Zhao, 2001; Ying et al., 2000), is also required for cricket germ cell specification. Specifically, BMP signaling and the downstream

transcription factor Blimp-1 induce a subset of mesodermal cells to become PGCs in crickets (Donoughe et al., 2014; Nakamura and Extavour, 2016).

The shared use of both BMP and Blimp-1 in mice and crickets may have convergently evolved, or could suggest that this signaling mechanism is ancestral in bilaterian inductive PGC specification (Lochab and Extavour, 2017). Determining whether BMP and its downstream target genes are required for germ line specification in more species will be necessary to distinguish between these possibilities. Only a small number of signaling pathways contribute to developmental fate decisions in animals (Pires-daSilva and Sommer, 2003), increasing the likelihood of evolutionary convergence and limiting the number of candidates to evaluate for roles in PGC induction in different species.

### Intermediate and flexible strategies of germ cell specification

Evidence from some animal species suggests that there may not be a strict dichotomy between inheritance and induction (discussed by Seervai and Wessel, 2013). These two mechanisms might instead represent ends of a spectrum that are not necessarily mutually exclusive. Below, we discuss cases of panarthropod species for which data suggest that their mechanisms of germ cell specification may fall somewhere along this continuum.

In the silk moth *B. mori*, historical studies have generated conflicting evidence about how germ cells are specified. Germ granules were not detected in early light microscopy experiments, but germ cells failed to form after cauterization of the ventral side of cleavage-stage embryos, suggesting this region could contain germ cell determinants (Miya, 1958a). Subsequent studies found that Vasa protein is not asymmetrically localized before cellularization, again suggesting a lack of germ plasm, or at least a germ plasm lacking Vasa protein (Nakao et al., 2006). However, the mRNA of one of the four *B. mori nanos* homologs, *nanosO*, localizes along the ventral midline of syncytial embryos immediately following egg laying and is inherited by the PGCs (Nakao et al., 2008), suggesting the possibility that a germ plasm containing *nanosO* transcript specifies PGCs. Intriguingly, researchers found that maternal and zygotic *nanosO* act redundantly in PGC specification; loss of one or the other reduces but does not completely eliminate germ cells, whereas loss of both prevents germ cell formation (Nakao and Takasu, 2019). The possibility that both inheritance and induction mechanisms contribute to *B. mori* PGC specification suggests that this species could offer insight into transitions in germ cell specification mechanisms within Lepidoptera or across insects more broadly.

Although in *D. melanogaster* PGC specification is traditionally characterized as driven by germ plasm, zygotic signaling is also suggested to contribute to germ cell specification in this species (Colonna et al., 2022). Loss-of-function mutations in the BMP family morphogen Dpp lead to partial loss of transcriptional quiescence and mislocalization of germ plasm in the newly formed pole cells (Colonna et al., 2022). Furthermore, loss of BMP signaling leads to activation of the normally suppressed terminal signaling pathway in pole cells, which in turn disrupts their migration (Colonna et al., 2022). These observations illustrate a universal conceptual challenge in the field of developmental biology: distinguishing between, or even consistently defining, cell type specification and maintenance. Although pole cells can form upon disruption of BMP signaling, they do not contain the proper molecular components to act as PGCs (Colonna et al., 2022). We suggest that it may be most useful to define PGC specification mechanisms as those that ensure both the physical formation of germ

cell precursors (including adoption of specific morphologies and activation of characteristic gene regulatory states), as well as the competence of those cells to take the next developmental steps toward joining the gonad. Under this definition, we would indeed concede that both germ plasm and zygotic inductive mechanisms play roles in *D. melanogaster* germ cell specification.

The ability to post-embryonically regenerate a germ line initially specified by maternal inheritance has also been reported in multiple invertebrate species, including the sea squirt *Ciona intestinalis* (Takamura et al., 2002), the earthworm *Enchytraeus japonensis* (Tadokoro et al., 2006) and the marine worm *Capitella teleta* (Dannenberg and Seaver, 2018) (see also Özpölat, 2024 for a review of this phenomenon in Annelida). The wasp *Pimpla turionellae* (Hymenoptera) has an oosome (Meng, 1968) that is required for germ cell specification. Damaging the oosome results in no pole cells, which are the PGCs of this species (Achtelig and Krause, 1971). However, oosome-deficient embryos lacking pole cells develop into larvae with germ cells in their gonad, indicating that an additional mechanism must regenerate germ cells later in development (Achtelig and Krause, 1971). Evidence of germline regeneration has also been reported in the amphipod *Parhyale hawaiiensis* (Kaczmarczyk, 2014), which has a germ plasm that is asymmetrically segregated to the *g* micromere and its lineage (Extavour, 2005; Gerberding et al., 2002; Gupta and Extavour, 2013). Ablation of the *g* micromere eliminates PGCs in the embryo (Extavour, 2005), but the germ line is replaced post-embryonically with germ cells that appear to be derived from the mesoderm (Kaczmarczyk, 2014). The authors of this study speculated that these replacement germ cells specifically derive from cells of the somatic gonad (Kaczmarczyk, 2014), reminiscent of a previously popular theory that primordial germ cells in most animals derived from the inner epithelium of the somatic gonad (Everett, 1944; Hargitt, 1919; Heys, 1931). Species capable of regenerating PGCs present the opportunity to investigate the relative contributions of and potential interactions between inheritance- and induction-based mechanisms within a single species. Furthermore, these species may represent living examples of the intermediates that are hypothesized to exist in evolutionary transitions between modes of germline specification (Extavour, 2007).

### Evolutionary drivers of panarthropod germ cell specification

In the following section, we discuss the evolutionary dynamics of germ cell specification mechanisms across Panarthropoda, and propose potential developmental and genetic factors that might contribute to these dynamics.

### Convergent evolution of panarthropod germ plasm

The phylogenetic distribution of germ cell specification mechanisms across panarthropods reveals a complex evolutionary history. Data for tardigrades, chelicerates and myriapods are consistent with induction as the ancestral mechanism of panarthropod primordial germ cell specification (Fig. 3), and this hypothesis is strongly supported by statistical tests described below (Figs S1, S2). In Pancrustacea, the most heavily represented group in our dataset (Table S1), the late origin of primordial germ cells in several species suggests induction may be widely used. However, there appear to be independent origins of germ plasm in Malacostraca and Branchiopoda, a hypothesis that is also supported by formal statistical testing described below (Figs S1, S2; Table S3). The use of induction and germ plasm in different decapod species suggests that further sampling could reveal patterns in the evolution of germ cell formation within this order (Biffis et al., 2009; Chen et al., 2021; Terao, 1929). However,

functional experiments and broader taxonomic sampling will be necessary to confirm mechanisms and reconstruct their evolutionary trajectories across Panarthropoda as a whole.

Reminiscent of a broader pattern that we and others previously reported across animals (Extavour, 2007; Extavour and Akam, 2003; Johnson et al., 2003; Whittle and Extavour, 2017), in some insect orders we noted that lineages branching closer to the base of a clade deployed induction, while later branching lineages appeared to use germ plasm (Figs 2, 3). This pattern is well exemplified in Coleoptera (Kobayashi et al., 2006; Komatsu and Kobayashi, 2012; Krause, 1947; Niikura et al., 2017; Schröder, 2006; Ullmann, 1964), with pole cells forming only in Chrysomeloidea (including long-horned and leaf beetles) and Curculionidae (Brauer, 1925; Hegner, 1908; Lynch et al., 2011). These observations raise the possibility that germ plasm may not be ancestral to Coleoptera. This phylogenetic distribution, when considered alongside the absence of germ plasm in most Lepidoptera, the reports of germ cells that first arise in the mesoderm (Category 3) in Trichoptera (Miyakawa, 1974) and of germ cells specified at gastrulation (Category 2) in Mecoptera (Suzuki, 1990), and the distinct morphologies of *N. vitripennis* and *D. melanogaster* germ plasm (see ‘Molecular and genetic mechanisms of germ cell specification in panarthropods’ section above), is consistent with a novel hypothesis: germ plasm evolved independently multiple times across insects, rather than arising only once in the Holometabola as we and others have previously proposed (Ewen-Campen et al., 2012, 2013b; Lynch et al., 2011). Our previous work suggested the independent evolution of germ plasm at the phylum level (Extavour and Akam, 2003), but here we hypothesize widespread convergent evolution of maternal inheritance-based germ cell specification within a phylum.

To test this hypothesis, we performed ancestral character reconstruction using both maximum parsimony and maximum likelihood approaches (see Supplementary Materials and Methods and details in Tables S2-S7 and Figs S1, S2). Both a parsimony analysis (Fig. S1) and marginal ancestral reconstructions of a maximum likelihood approach (Fig. S2; see Table S7 for marginal probabilities) provided strong statistical support for induction as the ancestral specification mechanism in Panarthropoda, Arthropoda and Insecta, with multiple independent instances of germ plasm evolution and reversion to induction (see Table S3 for a comparison of the two approaches).

We further find strong evidence that induction was the ancestral specification mechanism in Holometabola (probability 80.6%), followed by independent transitions to germ plasm in the common ancestors of Diptera and Mecoptera [or independently within Mecoptera, Siphonaptera (fleas) and Diptera]; derived lineages of Lepidoptera; in the common ancestor of Chrysomelidae and Curculionidae (Coleoptera); and in the common ancestor of Hymenoptera. Outside of Holometabola, we infer independent transitions to germ plasm in Thysanoptera, Hemiptera, Dermaptera, Collembola (which may have undergone multiple independent transitions within the class), Decapoda, the last common ancestor of Hyalidae and Talitridae (Amphipoda), and in the chelicerate *T. urticae*. Collectively, these qualitative and quantitative analyses suggest a previously unappreciated dynamism in the evolutionary history of germ cell specification within Panarthropoda.

### The relationship between germ cells and somatic germ layers

In arthropods with late formation of PGCs (Category 3), these cells appear to differentiate directly from the mesoderm (Evans, 1901; Hickman, 1936; Nelson, 1915). Consistent with this hypothesis, abolishing mesoderm prevents germ cell specification in the cricket *G. bimaculatus* (Ewen-Campen et al., 2013b). Germ cell

differentiation occurs concurrently with the differentiation of many other mesodermal cell types, including the somatic gonad, musculature, heart and fat body (Johannsen and Butt, 1941). We suggest that the types of mechanisms that specify germ cells are not distinct in principle from those specifying any other mesodermal cell type in these animals.

The germ layer origin of germ cells is less clear in arthropods in which germ cells appear before or very close to the time of gastrulation (Category 2). For example, PGCs in many crustaceans are described as being the first to enter the blastopore during gastrulation, and are usually considered by researchers to be derived from the mesendoderm (e.g. Cannon, 1921; Goodrich, 1939; Manton, 1928). In contrast, germ cells in Category 2 insects arise before gastrulation (Mellanby, 1935; Ullmann, 1964), consistent with the hypothesis that germ cells are distinct from the mesoderm and may not derive from any germ layer.

Crustaceans with putative germ plasm typically have stereotypical embryonic cleavage patterns, and embryonic cell lineages show that the germ cell lineage is sister to the mesoderm or the mesendoderm [e.g. Amphipoda (Extavour, 2005; Gerberding et al., 2002; Scholtz and Wolff, 2002; Wolff and Scholtz, 2002), Euphausiacea (krill) (Alwes and Scholtz, 2014), Decapoda (Hertzler, 2005)], but never to the ectoderm. In addition, removing the putative germ plasm from embryos of the amphipod *P. hawaiiensis* makes the *g* micromere morphologically indistinguishable from the other micromeres, which give rise to mesoderm or endoderm in unperturbed embryos (Gupta and Extavour, 2013). This result suggests that in the absence of germ cell determinants, the *g* micromere, normally the germline progenitor, could instead adopt mesodermal fate. This hypothesis has not been directly tested in this amphipod because gastrulation fails after ablation of the putative germ plasm (Gupta and Extavour, 2013). Together, these data suggest a close association between the germ cell and mesodermal lineages in these crustaceans. This relationship is apparent beyond Pancrustacea, since germ cells induced late in embryogenesis arise from the mesoderm (Category 3). We thus speculate that germ cells in panarthropods may be ancestrally derived from the mesodermal lineage, consistent with our inference that inductive germ cell specification is ancestral to panarthropods (also discussed earlier).

#### Evolutionary shifts in the spatial and temporal origin of PGCs

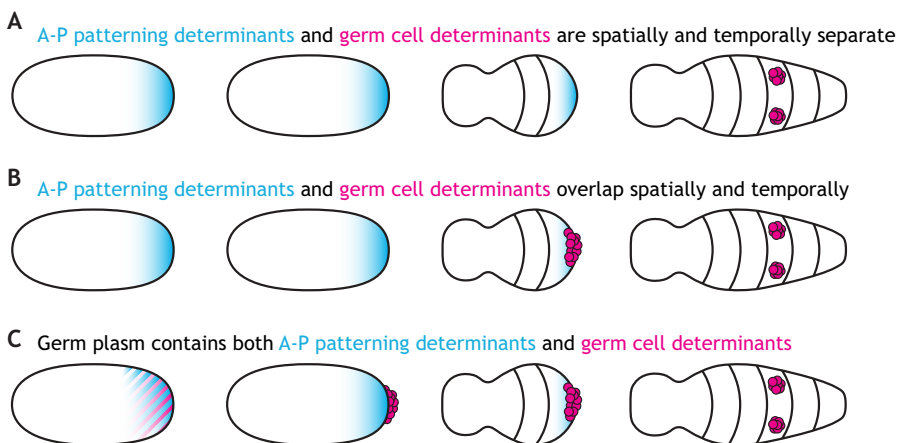
We have seen significant variation in the spatial and temporal origin of PGCs across panarthropods (Figs 2, 3; Table S1). However, regardless of when PGCs first appear, we find a common stage across many panarthropods in which PGCs are situated in bilateral segmental clusters across multiple trunk segments (Fig. 1Bi,C). Soon after this

stage, the segmental clusters on each side fuse together into bilaterally symmetrical genital ridges, corresponding to the primordial embryonic gonad (Miya, 1958b; Nelsen, 1934; Seidel, 1924; Ullmann, 1964).

Most arthropods undergo ‘short germ’ development, wherein the blastoderm tissue generates only anterior segments, with posterior segments being added sequentially to the posterior end of the germ band later in development (Krause, 1939). In contrast, the ‘long germ’ mode of development seen in *D. melanogaster*, wherein the blastoderm tissue immediately generates all body segments, is an evolutionary novelty found only in some insects (Clark et al., 2019; Krause, 1939). In Category 1 or 2 arthropods with short germ development, germ cells are often specified before the formation of the segments that include the future embryonic gonad. In Category 2 insects, germ cells often associate with the posterior end of the germ band and then migrate toward the anterior as the germ band elongates (Goss, 1953; Hegner, 1909b; Heming and Huebner, 1994; Paz, 1958; Suzuki, 1990; Ullmann, 1964). This migratory path is so common among insects that Johannsen and Butt included it in their description of embryogenesis in a stereotypical insect (Johannsen and Butt, 1941).

The association of germ cells with the posterior end of the embryo may have had other evolutionary consequences in insects. Many insects, both long and short germ, specify the body plan early in development via maternal deposition of axial patterning determinants, independent of the mechanism of germ cell specification (reviewed by Lynch, 2019; Rosenberg et al., 2009). For example, the short germ grasshopper *Schistocerca americana* exhibits asymmetric localization of the posterior patterning determinant *nanos* in the absence of a germ plasm (Lall et al., 2003). However, insects with germ plasm that includes axial patterning determinants, such as *D. melanogaster*, often exhibit long germ development. Based on these observations, we employed phylogenetic logistic regression and Pagel’s test of evolutionary rate covariation (Pagel, 1994) to test the hypothesis that the acquisition of long germ band development influences germ cell mechanism transitions or vice versa, and found statistically significant evidence of this association using both methods (see [Supplementary Materials and Methods, Figs S3, S4; Table S8](#)). Overall, this suggests that long germ band development may drive the evolution of germ plasm, which in turn may also drive transitions to long germ band development.

We further hypothesize that a spatial shift in germ cell specification to the posterior end of the embryo in Category 2 insects created a condition in which germ line determinants are localized to the same embryonic region as posterior patterning determinants (Fig. 4B). Subsequently, germ line determinants may



**Fig. 4. Hypothesized links between spatial and temporal dynamics of PGC origination and the mechanism of germ cell specification.** (A) In species that induce PGCs later in embryogenesis (Category 3), the asymmetrically localized anterior–posterior (A–P) patterning determinants (blue) and germ cell determination factors (magenta) are spatially and temporally separate. (B) Proposed shift to earlier germ cell specification at the embryonic posterior in Category 2 insects enabled overlap between the patterning and PGC determinants. (C) Subsequent hitchhiking of the asymmetric localization machinery by germ line determinants (indicated by mixture of blue and magenta at the embryo posterior) could have coupled PGC specification and posterior patterning and thus promoted the evolution of germ plasm. Anterior is left and ventral is facing the reader in all schematics.

have hitchhiked onto the asymmetric localization machinery, coupling germ cell specification to posterior patterning and thus promoting the evolution of germ plasm (Fig. 4C). Under this hypothesis, the mechanism that specifies the germ plasm of *D. melanogaster* would represent an advanced stage of this evolutionary scenario, as its germ granules include both germ line and abdominal patterning determinants (Ephrussi and Lehmann, 1992; Trcek et al., 2015; Wang and Lehmann, 1991).

*N. vitripennis* is an intriguing case in which to study the relationship between A–P patterning and germ cell specification. The posterior cytoplasm of this wasp has two populations of *nanos* mRNA: one that is incorporated into the oosome and eventually into pole cells, and another that functions in axial patterning (Lynch and Desplan, 2010). The localization of *nanos* to the oocyte posterior in this wasp depends on actin-dependent anchoring (Olesnick and Desplan, 2007), as seen in *D. melanogaster* (Forrest and Gavis, 2003), suggesting a conserved localization mechanism between these two species. We speculate that the PGC specification mechanism used in *N. vitripennis* might be an evolutionary intermediate between the mechanism used in species in which A–P patterning is entirely independent of germ cell formation (Fig. 4A), and that used in species in which these two processes are fully linked (Fig. 4C). Our evolutionary hypothesis could be tested further by examining the mechanisms of localization of posterior patterning determinants and germ line determinants in more insect species to understand how existing cytoskeletal machinery for one developmental process may have been co-opted for use in another.

#### Potential genetic basis of evolutionary shifts in the molecular mechanism of PGC specification

Beyond the timing and position of germ cell origin, evolutionary changes in the localization of gene products and timing of gene expression may also underlie transitions in the mechanism of specification (Extavour, 2007; Srouji and Extavour, 2010). Many of the genes important for specifying germ cells are conserved across metazoans (Ewen-Campen et al., 2010), but they exhibit distinct temporal and spatial expression patterns across species. In species that use inheritance, the germ line gene products are expressed during oogenesis and persist in the early embryonic cytoplasm to be inherited by the PGCs. In species that induce germ cells, these genes are either not expressed in oocytes, or the gene products are neither asymmetrically nor preferentially inherited by PGCs. As we have suggested previously (Extavour, 2007; Srouji and Extavour, 2010), relatively simple regulatory modifications affecting post-transcriptional regulation, the cytoskeleton, or RNA or protein localization could cause germ line gene product retention in oocytes, thereby enabling the transition from induction to inheritance.

The insect-specific gene *oskar* offers a potential example of gene network evolution driving shifts in germ cell specification mechanism. Since *oskar* is likely a nucleator of germ plasm in multiple insects (Ephrussi et al., 1991; Jones and Macdonald, 2007; Kempf and Lynch, 2022; Lynch et al., 2011; Rivard et al., 2025 preprint) and has been lost multiple times since its origin before the divergence of Pterogyta from Ametabola (Blondel et al., 2021), we hypothesized that *oskar* gene presence in the genome might be correlated with germ line specification mode in a given taxon (Fig. S3). A related hypothesis, that the evolutionary advent of the *oskar* gene facilitated the evolution of holometabolous germ plasm, was previously proposed based on the co-occurrence of *oskar* transcript and germ plasm in *N. vitripennis* and *D. melanogaster*, as well as the absence of both *oskar* homologs and germ plasm in *T. castaneum*, *A. mellifera*, *C. albipunctata*, most Lepidoptera, and the

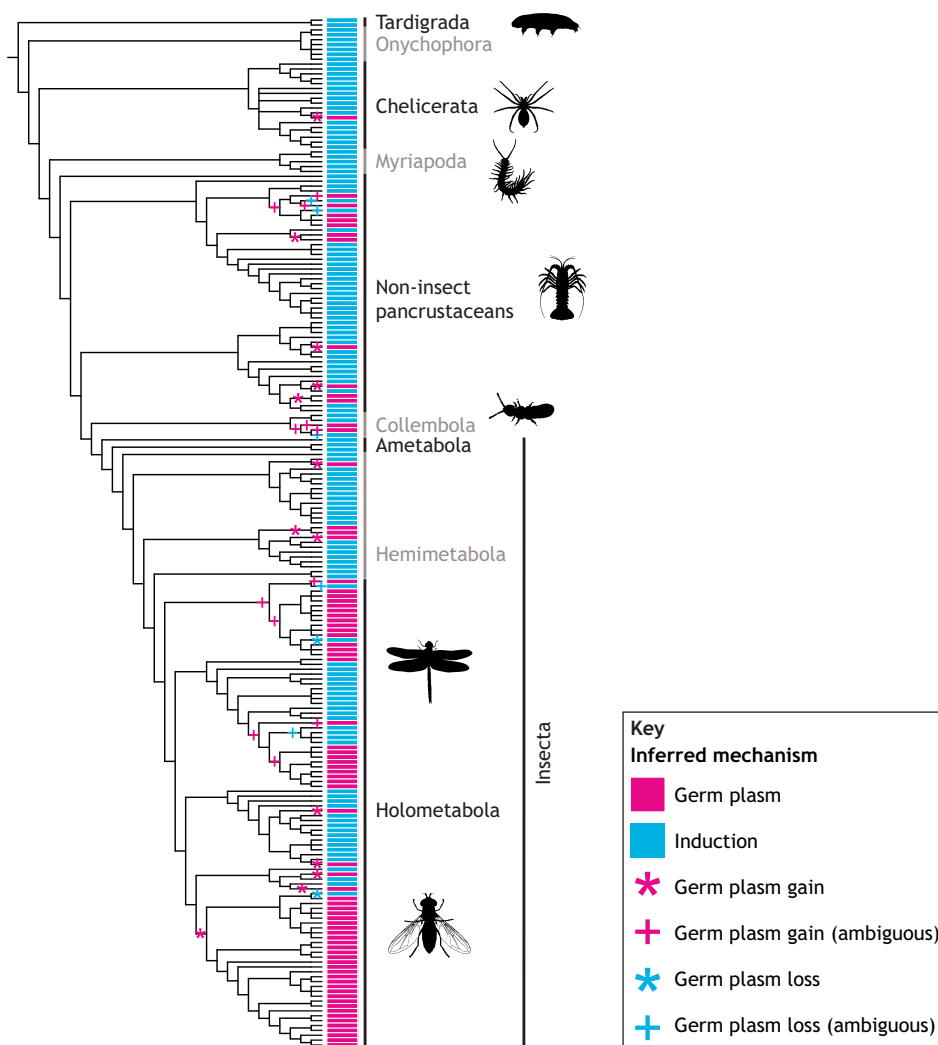
hemimetabolous genomic resources available at the time (Lynch et al., 2011). The results of applying phylogenetic logistic regression to this hypothesis suggest a strong and statistically significant association between *oskar* presence and maternal inheritance (Supplementary Materials and Methods, Figs S3, S5; Table S9). We emphasize that this observed association does not imply that *oskar* is necessary or sufficient for germ plasm formation; indeed, there are insect species without *oskar* that nevertheless have germ plasm, and species with *oskar* that lack germ plasm (Fig. S3), as we discuss below.

In contrast to prior hypotheses from our group and others of a single origin of germ plasm at the base of the Holometabola (Ewen-Campen et al., 2012, 2013b; Kempf and Lynch, 2022; Lynch et al., 2011), our current likelihood and parsimony reconstruction of induction as ancestral to Holometabola (Fig. 5; Figs S1, S2) implies that *oskar* has been independently recruited as a germ plasm nucleator at least twice, in Hymenoptera (Lynch et al., 2011) and in Diptera (Ephrussi and Lehmann, 1992). The strong association between *oskar* presence and germ plasm (Fig. S3) implies that it may have been co-opted for germ plasm nucleation even more times across insects. We note that such ‘gene reuse’ is a common feature of convergent evolution across multiple biological processes (Chan et al., 2010; Martin and Orgogozo, 2013; Morris, 2009; Reed et al., 2011; Satterlee et al., 2024). Since *oskar* is co-expressed with germ plasm components in nervous systems of hemimetabolous and holometabolous insects (Ewen-Campen et al., 2012; Kulkarni et al., 2023; Xu et al., 2013), we posit that independent acquisitions of germ plasm in distinct lineages may have proceeded by parallel co-option of a pre-existing gene interaction network. Future studies should aim to determine whether each independent origin of germ plasm in the Holometabola arose by co-option of *oskar* to the germ line, or by *oskar*-independent mechanisms of germ plasm assembly.

The results of our analysis (Fig. S3; Table S9) suggest that the functional evolution of *oskar* may have contributed to a transition from induction to inheritance, but precisely when and how *oskar* acquired a germ line role remains unclear. In the genomes of species of Carabidae (ground beetles), a beetle family that lacks pole cells, *oskar* orthologs have been identified, decoupling the presence of *oskar* from germ plasm in Holometabola (Blondel et al., 2021; Kobayashi et al., 2013). Morphological data suggest that thrips use germ plasm (Haga, 1985; Heming, 1979), and *oskar* homologs have been found in this order (Blondel et al., 2021). If *oskar* plays a role in thrips germ cell specification, it could support the entirely novel hypothesis that *oskar* evolved its germ line determinant properties before the evolution of complete insect metamorphosis.

#### The case for novel panarthropod germ plasm nucleators

Recruitment of *oskar* to the germ line is not the only mechanism explaining the evolution of germ plasm in arthropods. The genome of the hemimetabolous pea aphid *Acyrtosiphon pisum* lacks an *oskar* ortholog but their embryos have a germ plasm containing Vasa and Nanos proteins (Chang et al., 2006; Chen et al., 2009; Lin et al., 2014), which are asymmetrically localized by unknown mechanisms. Vasa is also asymmetrically localized to a putative germ plasm in amphipod and decapod species (Chen et al., 2021; Gupta and Extavour, 2013), which is likely to have evolved via distinct mechanisms since *oskar* is an insect-specific gene (Blondel et al., 2021). Outside of arthropods, germ plasm nucleators have convergently evolved in diverse animal phyla (Aoki et al., 2016; Bontems et al., 2009; Kulkarni and Extavour, 2017; Lee et al., 2020; Marlow and Mullins, 2008; Scholl et al., 2024). Similarly, we posit that germ plasm nucleators other than *oskar* likely also exist across the panarthropods, and are awaiting discovery.



**Fig. 5. Evolutionary dynamics of panarthropod germ cell specification.** Phylogenetic relationships among all study species (Table S1) are derived from published relationships listed in Table S2; branch lengths are not to scale. Additional taxonomic information is provided in Fig. S1. Colored lines to the right of each branch in the cladogram designate the mechanism of germ cell specification (inferred as described in the supplementary information) as follows: magenta, germ plasm; blue, induction. Magenta asterisks denote proposed instances of germ plasm evolution (based on maximum parsimony) and magenta plus signs mark ambiguous cases of germ plasm gain. Blue asterisks denote proposed instances of germ plasm loss and blue plus signs mark ambiguous cases of germ plasm loss.

### Concluding remarks

By re-interpreting the meticulous work of generations of classical embryologists through the lens of modern developmental genetics and an improved panarthropod phylogeny, we have generated four major, novel and testable evolutionary hypotheses about the mechanisms that specify the germ line. First, inductive mechanisms likely specified PGCs ancestrally in Panarthropoda, and differentiation from the mesoderm is the likely ancestral mode of PGC specification in Euarthropoda. Second, germ plasm arose independently multiple times across Panarthropoda, in many different crustacean and insect orders. Third, in contrast to the *Drosophila* paradigm of PGC specification via germ plasm, induction was likely the ancestral PGC specification mechanism in the Holometabola, and germ plasm likely arose independently in some lineages of flies, fleas, beetles, wasps, ants and moths. Finally, the *oskar* gene was likely co-opted as a germ plasm nucleator at least twice independently in flies and wasps, and many panarthropods are likely to use something other than *oskar* to nucleate their germ plasm. Together, these hypotheses have implications for the genetic basis of convergence in evolution that extend beyond the specific problem of germ cell formation. The advent of new genetic tools in an increasing number of panarthropod model species places us in an exciting era to test the new hypotheses we propose here, tackle age-old questions about the evolution of panarthropod germ cell specification, and understand the impact of these mechanisms

of genome evolution and body patterning in the most successful group of animals on earth.

### Acknowledgements

We thank members of the Extavour Lab for helpful discussions, Kira Heikes for the image of tardigrade germ cells (Fig. 1Biv), and Prashant Sharma for the image of harvestmen germ cells (Fig. 1Biii).

### Competing interests

C.G.E. is an Academic Editor of *Development*. C.G.E. was not involved in the editorial assessment of this submission. The authors declare no other competing interests.

### Funding

This study was supported by a National Science Foundation (NSF) award (IOS-2220747), funds from Harvard University, and from the Howard Hughes Medical Institute (C.G.E.). Additional support was provided by the NSF-Simons Center for Mathematical and Statistical Analysis of Biology at Harvard University (award number 1764269), the Harvard Quantitative Biology Initiative, a Herchel Smith Graduate Fellowship (E.L.R.), and an NSF Graduate Research Training Fellowship (to R.R.K.). C.G.E. is an Investigator of the Howard Hughes Medical Institute. Open Access funding provided by the National Science Foundation. Deposited in PMC for immediate release.

### Data availability

All data sources are listed in the accompanying supplementary information and/or are available at [https://github.com/rishabhraj Kapoor/panarthropoda\\_gc\\_specification\\_evolution](https://github.com/rishabhraj Kapoor/panarthropoda_gc_specification_evolution), commit ID ff945c.

## References

- Abbassy, M. M., Helmy, N., Osman, M., Cope, S. E. and Presley, S. M.** (1995a). Embryogenesis of the sand fly *Phlebotomus papatasi* (Diptera: Psychodidae): cell cleavage, blastoderm formation, and gastrulation. *Ann. Entomol. Soc. Am.* **88**, 809-814. doi:10.1093/aesa/88.6.809
- Abbassy, M. M., Helmy, N., Osman, M., Cope, S. E. and Presley, S. M.** (1995b). Embryogenesis of the sand fly *Phlebotomus papatasi* (Diptera: Psychodidae): Organogenesis including segmentation, blastokinesis, mouthparts, and alimentary canal. *Ann. Entomol. Soc. Am.* **88**, 815-820. doi:10.1093/aesa/88.6.815
- Abbassy, M. M., Helmy, N., Osman, M., Cope, S. E. and Presley, S. M.** (1995c). Embryogenesis of the sand fly *Phlebotomus papatasi* (Diptera: Psychodidae): Organogenesis of the nervous system, tracheal system, muscular system, heart, and gonad rudiments. *Ann. Entomol. Soc. Am.* **88**, 821-826. doi:10.1093/aesa/88.6.821
- Achtelig, M. and Krause, G.** (1971). Experimente am ungeführten Ei von *Pimpla turionellae* L. (Hymenoptera) zur Funktionsanalyse des Oosombereichs. *Wilhelm Roux Arch. Dev. Biol.* **167**, 164-182. doi:10.1007/BF00577038
- Ajidagba, P., Pitts, C. W. and Bay, D. E.** (1983). Early embryogenesis in the stable fly (Diptera: Muscidae). *Ann. Entomol. Soc. Am.* **76**, 616-623. doi:10.1093/aesa/76.4.616
- Alexeeva, N. and Tamberg, Y.** (2021). Anatomical changes in postembryonic development of *Pycnogonum litorale*. *J. Morphol.* **282**, 329-354. doi:10.1002/jmor.21308
- Alexeeva, N., Tamberg, Y. and Shunatova, N.** (2018). Postembryonic development of pycnogonids: a deeper look inside. *Arthropod Struct. Dev.* **47**, 299-317. doi:10.1016/j.asd.2018.03.002
- Alwes, F. and Scholtz, G.** (2004). Cleavage and gastrulation of the euphausiacean *Meganactiphanes norvegica* (Crustacea, Malacostraca). *Zoomorphology* **123**, 125-137. doi:10.1007/s00435-004-0095-6
- Alwes, F. and Scholtz, G.** (2014). The early development of the onychopod cladoceran *Bythotrephes longimanus* (Crustacea, Branchiopoda). *Front. Zool.* **11**, 10. doi:10.1186/1742-9994-11-10
- Amy, R. L.** (1961). The embryology of *Habrobracon juglandis* (Ashmead). *J. Morphol.* **109**, 199-217. doi:10.1002/jmor.1051090207
- Anderson, D. T.** (1962a). The embryology of *Dacus tryoni* (Frogg.) [Diptera, Trypetidae (= Tephritidae)], the Queensland fruit-fly. *Development* **10**, 248-292. doi:10.1242/dev.10.3.248
- Anderson, D. T.** (1962b). The epigenetics of the larva in Diptera. *Acta Zool.* **43**, 221-228. doi:10.1111/j.1463-6395.1962.tb00072.x
- Anderson, D. T.** (1967). Larval development and segment formation in the branchiopod crustaceans *Limnadia stanleyana* King (Conchostraca) and *Artemia salina* (L.) (Anostraca). *Aust. J. Zool.* **15**, 47-91. doi:10.1071/ZO9670047
- Anderson, D. T.** (1973). *Embryology and Phylogeny in Annelids and Arthropods*. Oxford, UK: Pergamon Press.
- Anderson, D. T. and Wood, E. C.** (1968). The morphological basis of embryonic movements in the light brown apple moth, *Epiphyas postvittana* (Walk.) (Lepidoptera: Tortricidae). *Aust. J. Zool.* **16**, 763-793. doi:10.1071/ZO9680763
- Ando, H.** (1962). *The Comparative Embryology of Odonata with Special Reference to a Relic Dragonfly, Epiphlebia superstes Selys*. Tokyo: Japan Society for the Promotion of Science.
- Ando, H. and Tanaka, M.** (1980). Early embryonic development of the primitive moths, *Endoclyta signifier* Walker and *E. excrecens* Butler (Lepidoptera: Hepialidae). *Int. J. Ins. Morph. Embryol.* **9**, 67-77.
- Ansari, S., Troelenberg, N., Dao, V. A., Richter, T., Bucher, G. and Klingler, M.** (2018). Double abdomen in a short-germ insect: Zygotic control of axis formation revealed in the beetle *Tribolium castaneum*. *Proc. Natl. Acad. Sci. USA* **115**, 1819-1824. doi:10.1073/pnas.1716512115
- Aoki, S. T., Kershner, A. M., Bingman, C. A., Wickens, M. and Kimble, J.** (2016). PGL germ granule assembly protein is a base-specific, single-stranded RNase. *Proc. Natl. Acad. Sci. USA* **113**, 1279-1284. doi:10.1073/pnas.1524400113
- Auten, M.** (1934). The early embryological development of *Phormia regina*: Diptera (Calliphoridae). *Ann. Entomol. Soc. Am.* **27**, 481-506. doi:10.1093/aesa/27.3.481
- Ayers, H.** (1884). On the development of *Oecanthus niveus*, and its parasite, *Teles.* *Mem. Boston Soc. Nat. Hist.* **3**, 225-282.
- Baker, C. M., Buckman-Young, R. S., Costa, C. S. and Giribet, G.** (2021). Phylogenomic analysis of velvet worms (Onychophora) uncovers an evolutionary radiation in the neotropics. *Mol. Biol. Evol.* **38**, 5391-5404. doi:10.1093/molbev/msab251
- Ballesteros, J. A., Santibáñez-López, C. E., Baker, C. M., Benavides, L. R., Cunha, T. J., Gainett, G., Ontano, A. Z., Setton, E. V. W., Arango, C. P., Gavish-Regev, E. et al.** (2022). Comprehensive species sampling and sophisticated algorithmic approaches refute the monophyly of Arachnida. *Mol. Biol. Evol.* **39**, msac021. doi:10.1093/molbev/msac021
- Berg, G. J. and Gassner, G.** (1978). Fine structure of the blastoderm embryo of the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). *Int. J. Insect Morphol. Embryol.* **7**, 81-105. doi:10.1016/S0020-7322(78)80017-8
- Bernot, J. P., Owen, C. L., Wolfe, J. M., Meland, K., Olesen, J. and Crandall, K. A.** (2023). Major revisions in pancrustacean phylogeny and evidence of sensitivity to taxon sampling. *Mol. Biol. Evol.* **40**, msad175. doi:10.1093/molbev/msad175
- Biffis, C., Alwes, F. and Scholtz, G.** (2009). Cleavage and gastrulation of the dendrobranchiate shrimp *Penaeus monodon* (Crustacea, Malacostraca, Decapoda). *Arthropod Struct. Dev.* **38**, 527-540. doi:10.1016/j.asd.2009.06.003
- Blaimer, B. B., Santos, B. F., Cruaud, A., Gates, M. W., Kula, R. R., Mikó, I., Rasplu, J.-Y., Smith, D. R., Talamas, E. J., Brady, S. G. et al.** (2023). Key innovations and the diversification of Hymenoptera. *Nat. Commun.* **14**, 1212. doi:10.1038/s41467-023-36868-4
- Blondel, L., Besse, S., Rivard, E. L., Ylla, G. and Extavour, C. G.** (2021). Evolution of a cytoplasmic determinant: evidence for the biochemical basis of functional evolution of the novel germ line regulator Oskar. *Mol. Biol. Evol.* **38**, 5491-5513. doi:10.1093/molbev/msab284
- Bontems, F., Stein, A., Marlow, F., Lyautey, J., Gupta, T., Mullins, M. C. and Dösch, R.** (2009). Bucky ball organizes germ plasm assembly in zebrafish. *Curr. Biol.* **19**, 414-422. doi:10.1016/j.cub.2009.01.038
- Bounoure, L.** (1939). *L'origine des cellules reproductrices et le problème de la lignée germinale*. Paris, France: Gauthier-Villars.
- Brauer, A.** (1894). Beiträge zur Kenntnis der Entwicklungsgeschichte des Skorpions. *Z. wiss. Zool.* **57**, 402-432.
- Brauer, A.** (1925). Studies on the embryology of *Bruchus quadrimaculatus*, Fabr. *Ann. Entomol. Soc. Am.* **18**, 283-312. doi:10.1093/aesa/18.3.283
- Brauer, A.** (1949). Localization of presumptive areas in the blastoderm of the pea beetle *Callosobruchus maculatus* Fabr., as determined by ultraviolet (2537 Å) irradiation injury. *J. Exp. Zool.* **112**, 165-193. doi:10.1002/jez.1401120202
- Bronskill, J. F.** (1959). Embryology of *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae). *Can. J. Zool.* **37**, 655-688. doi:10.1139/z59-068
- Buchta, T., Özüak, O., Stappert, D., Roth, S. and Lynch, J. A.** (2013). Patterning the dorsal-ventral axis of the wasp *Nasonia vitripennis*. *Dev. Biol.* **381**, 189-202. doi:10.1016/j.ydbio.2013.05.026
- Bull, A. L.** (1982). Stages of Living Embryos in the Jewel Wasp *Mormoniella* (*Nasonia*) *vitripennis* (Walker) (Hymenoptera: Pteromalidae). *Int. J. Insect Morphol. Embryol.* **11**, 1-23. doi:10.1016/0020-7322(82)90034-4
- Buss, L. W.** (1982). Somatic cell parasitism and the evolution of somatic tissue compatibility. *Proc. Natl. Acad. Sci. USA* **79**, 5337-5341. doi:10.1073/pnas.79.17.5337
- Buss, L. W.** (1983). Evolution, development, and the units of selection. *Proc. Natl. Acad. Sci. USA* **80**, 1387-1391. doi:10.1073/pnas.80.5.1387
- Butt, F. H.** (1934). Embryology of *Sciara* (Sciariidae: Diptera). *Ann. Entomol. Soc. Am.* **27**, 565-579. doi:10.1093/aesa/27.4.565
- Butt, F. H.** (1936). The early embryological development of the parthenogenetic alfalfa snout beetle, *Brachyrhinus ligustici* L. *Ann. Entomol. Soc. Am.* **29**, 1-14. doi:10.1093/aesa/29.1.1
- Butt, F. H.** (1949). Embryology of the milkweed bug, *Oncopeltus fasciatus* (Hemiptera). *Cornell Experiment Station Memoir* **283**, 3-43. doi:10.1093/aesa/42.2.186
- Cannon, H. G.** (1921). The Early Development of the summer egg of a Cladoceran (*Simocephalus vetulus*). *J. Cell Sci.* **s2-65**, 627-642. doi:10.1242/jcs.s2-65.260.627
- Cannon, H. G.** (1924). On the development of an estherid crustacean. *Philos. Trans. R. Soc. Lond. Ser. B* **212**, 395-430. doi:10.1098/rstb.1924.0010
- Carter, J.-M., Baker, S. C., Pink, R., Carter, D. R., Collins, A., Tomlin, J., Gibbs, M. and Breuker, C. J.** (2013). Unscrambling butterfly oogenesis. *BMC Genomics* **14**, 283. doi:10.1186/1471-2164-14-283
- Carter, J.-M., Gibbs, M. and Breuker, C. J.** (2015). Divergent RNA localisation patterns of maternal genes regulating embryonic patterning in the butterfly pararge aegeria. *PLoS ONE* **10**, e0144471. doi:10.1371/journal.pone.0144471
- Cavallin, M.** (1970). Développement embryonnaire de l'appareil génital chez le phasme, *Carausius morosus* Br. *Bull. Biol. Fr. Belg.* **104**, 343-366.
- Cavallin, M.** (1971). La 'polyembryonie substitutive' et le problème de l'origine de la lignée germinale chez le Phasme *Carausius morosus* Br. *C. R. Hebd. des seances l'Acad. des Sci. Ser. D: Sci. Nat.* **272**, 462-465.
- Chan, Y. F., Marks, M. E., Jones, F. C., Villarreal, G., Shapiro, M. D., Brady, S. D., Southwick, A. M., Absher, D. M., Grimwood, J., Schmutz, J. et al.** (2010). Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a *Pitx1* Enhancer. *Science* **327**, 302-305. doi:10.1126/science.1182213
- Chang, C., Dearden, P. and Akam, M.** (2002). Germ line development in the grasshopper *Schistocerca gregaria*: vasa as a marker. *Dev. Biol.* **252**, 100-118. doi:10.1006/dbio.2002.0840
- Chang, C., Lee, W.-C., Cook, C. E., Lin, G.-W. and Chang, T.** (2006). Germ-plasm specification and germline development in the parthenogenetic pea aphid *Acyrtosiphon pisum*: Vasa and Nanos as markers. *Int. J. Dev. Biol.* **50**, 413-421. doi:10.1387/ijdb.052100cc
- Chang, C., Lin, G., Cook, C. E., Horng, S., Lee, H. and Huang, T.** (2007). Apvasa marks germ-cell migration in the parthenogenetic pea aphid *Acyrtosiphon pisum* (Hemiptera: Aphidoidea). *Dev. Genes Evol.* **217**, 275-287. doi:10.1007/s00427-007-0142-7
- Chang, H., Qiu, Z., Yuan, H., Wang, X., Li, X., Sun, H., Guo, X., Lu, Y., Feng, X., Majid, M. et al.** (2020). Evolutionary rates of and selective constraints on the

- mitochondrial genomes of Orthoptera insects with different wing types. *Mol. Phylogenet. Evol.* **145**, 106734. doi:10.1016/j.ympev.2020.106734
- Chaw, R. C., Vance, E. and Black, S. D.** (2007). Gastrulation in the spider *Zygiella x-notata* involves three distinct phases of cell internalization. *Dev. Dynam.* **236**, 3484-3495. doi:10.1002/dvdy.21371
- Chen, R. P.-Y., Shih, C.-L., Lin, G.-W., Cook, C. E., Huang, T.-Y. and Chang, C.-C.** (2009). Developmental expression of *Apanos* during oogenesis and embryogenesis in the parthenogenetic pea aphid *Acyrtosiphon pisum*. *Int. J. Dev. Biol.* **53**, 169-176. doi:10.1387/ijdb.082570cc
- Chen, Y., Fang, X., Tian, X.-Q., Cui, Z., Feng, H.-Y. and Qiu, G.-F.** (2021). Germ plasm and the origin of the primordial germ cells in the oriental river prawn, *Macrobrachium nipponense*. *Cell Tissue Res.* **386**, 559-569. doi:10.1007/s00441-021-03534-4
- Choban, R. G. and Gupta, A. P.** (1972). Meiosis and early embryology of *Blissus leucopertus hirtus*, Montandon (Heteroptera: Lygaeidae). *Int. J. Insect Morphol. Embryol.* **1**, 301-314. doi:10.1016/0020-7322(72)90014-1
- Church, N. S. and Rempel, J. G.** (1971). The embryology of *Lytta viridana* LeConte (Coleoptera: Meloidae). VI. The appendiculate, 72-h embryo. *Can. J. Zool.* **49**, 1563-1570. doi:10.1139/z71-227
- Clark, E., Peel, A. D. and Akam, M.** (2019). Arthropod segmentation. *Development* **146**, dev170480. doi:10.1242/dev.170480
- Claypole, A. M.** (1898). The embryology and oögenesis of *Anurida maritima* (Guér.). *J. Morphol.* **14**, 219-300. doi:10.1002/jmor.1050140205
- Colonna, M. M., Goyal, Y., Johnson, H. E., Syal, S., Schedl, P. and Deshpande, G.** (2022). Preformation and epigenesis converge to specify primordial germ cell fate in the early *Drosophila* embryo. *PLoS Genet.* **18**, e1010002. doi:10.1371/journal.pgen.1010002
- Copilaş-Ciocianu, D., Borko, S. and Fişer, C.** (2020). The late blooming amphipods: Global change promoted post-Jurassic ecological radiation despite Palaeozoic origin. *Mol. Phylogenet. Evol.* **143**, 106664. doi:10.1016/j.ympev.2019.106664
- Counce, S. J.** (1963). Developmental morphology of polar granules in *Drosophila*. Including observations on pole cell behavior and distribution during embryogenesis. *J. Morphol.* **112**, 129-145. doi:10.1002/jmor.1051120203
- Crother, B. I., White, M. E. and Johnson, A. D.** (2007). Inferring developmental constraint and constraint release: primordial germ cell determination mechanisms as examples. *J. Theor. Biol.* **248**, 322-330. doi:10.1016/j.jtbi.2007.05.035
- Crother, B. I., White, M. E. and Johnson, A. D.** (2016). Diversification and germline determination revisited: linking developmental mechanism with species richness. *Front. Ecol. Evol.* **4**, 26. doi:10.3389/fevo.2016.00026
- Dannenberg, L. C. and Seaver, E. C.** (2018). Regeneration of the germline in the annelid *Capitella teleta*. *Dev. Biol.* **440**, 74-87. doi:10.1016/j.ydbio.2018.05.004
- daSilva, A. F., Machado, L. C., de Paula, M. B., Vieira, C. J. d. S. P., Bronzoni, R. V. d. M., Santos, M. A. V. d. M. and Wallau, G. L.** (2020). Culicidae evolutionary history focusing on the Culicinae subfamily based on mitochondrial phylogenomics. *Sci. Rep.* **10**, 18823. doi:10.1038/s41598-020-74883-3
- Davis, C.** (1967). A comparative study of larval embryogenesis in the mosquito *Culex fatigans* Wiedemann (Diptera: Culicidae) and the sheep-fly, *Lucilia sericata* Meigen (Diptera: Calliphoridae). *Aust. J. Zool.* **15**, 547-579. doi:10.1071/ZO9670547
- de la Paz, J.** (1958). Studies on the embryology of *Oncopeltus fasciatus* (Dallas) (Hemiptera: Lygaeidae). *PhD thesis*, Cornell University, Ithaca, NY.
- de Saint Phalle, B. and Sullivan, W.** (1996). Incomplete sister chromatid separation is the mechanism of programmed chromosome elimination during early *Sciara* coprophila embryogenesis. *Development* **122**, 3775-3784. doi:10.1242/dev.122.12.3775
- Dearden, P. K.** (2006). Germ cell development in the Honeybee (*Apis mellifera*): *Vasa* and *Nanos* expression. *BMC Dev. Biol.* **6**, 6. doi:10.1186/1471-213X-6-6
- Dearden, P., Grbic, M. and Donly, C.** (2003). *Vasa* expression and germ-cell specification in the spider mite *Tetranychus urticae*. *Dev. Genes Evol.* **212**, 599-603. doi:10.1007/s00427-002-0280-x
- Demotte, P., Panchaksaram, M., Kumarasinghe, H., Ly-Trong, N., Reis, M. dos, and Minh, B. Q.** (2025). IQ2MC: a new framework to infer phylogenetic time trees using IQ-TREE 3 and MCMCTree with mixture models. *EcoEvoRxiv*.
- Dogiel, V.** (1913). Embryologische Studien an Pantopoden. *Z. wiss. Zool.* **107**, 575-741.
- Dohle, W.** (1964). Die Embryonalentwicklung von *Glomeris marginata* (Villers) im Vergleich zur Entwicklung anderer Diplopoden. *Zool. Jahrb. Anat.* **81**, 241-310.
- Donnell, D. M., Corley, L. S., Chen, G. and Strand, M. R.** (2004). Caste determination in a polyembryonic wasp involves inheritance of germ cells. *Proc. Natl. Acad. Sci. USA* **101**, 10095-10100. doi:10.1073/pnas.0403625101
- Donoughe, S., Nakamura, T., Ewen-Campen, B., Green, D. A., Henderson, L. and Extavour, C. G.** (2014). BMP signaling is required for the generation of primordial germ cells in an insect. *Proc. Natl. Acad. Sci. USA* **111**, 4133-4138. doi:10.1073/pnas.1400525111
- Doyle, D. A., Burian, F. N., Aharoni, B., Klinder, A. J., Menzel, M. M., Nifras, G. C. C., Shabazz-Henry, A. L., Palma, B. U., Hidalgo, G. A., Sottolano, C. J. et al.** (2023). Germ granule evolution provides mechanistic insight into *Drosophila* germline development. *Mol. Biol. Evol.* **40**, msad174. doi:10.1093/molbev/msad174
- Du, S., Tihelka, E., Yu, D., Chen, W.-J., Bu, Y., Cai, C., Engel, M. S., Luan, Y.-X. and Zhang, F.** (2024). Revisiting the four Hexapoda classes: Protura as the sister group to all other hexapods. *Proc. Natl. Acad. Sci. USA* **121**, e2408775121. doi:10.1073/pnas.2408775121
- DuBois, A. M.** (1932). A contribution to the embryology of *Sciara* (Diptera). *J. Morphol.* **54**, 161-195. doi:10.1002/jmor.1050540105
- Eastham, L.** (1931). The embryology of *Pieris rapae* - Organogeny. *Phil. Trans. Roy. Soc. B* **219**, 1-50.
- Eddy, E. M.** (1975). Germ plasm and the differentiation of the germ cell line. *Int. Rev. Cytol.* **43**, 229-280. doi:10.1016/S0074-7696(08)60070-4
- Edgar, A., Bates, C., Larkin, K. and Black, S.** (2015). Gastrulation occurs in multiple phases at two distinct sites in *Latrodectus* and *Cheiracanthium* spiders. *EvoDevo* **6**, 33. doi:10.1186/s13227-015-0029-z
- Edgar, R. C.** (2004). MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinform.* **5**, 1-19. doi:10.1186/1471-2105-5-113
- Elmer, K. R. and Clobert, J.** (2025). Dollo's law of irreversibility in the post-genomic age. *Trends Ecol. Evol.* **40**, 136-146. doi:10.1016/j.tree.2024.09.010
- Ephrussi, A. and Lehmann, R.** (1992). Induction of germ cell formation by oskar. *Nature* **358**, 387-392. doi:10.1038/358387a0
- Ephrussi, A., Dickinson, L. K. and Lehmann, R.** (1991). oskar organizes the germ plasm and directs localization of the posterior determinant nanos. *Cell* **66**, 37-50. doi:10.1016/0092-8674(91)90137-N
- Evans, R.** (1901). On the Malayan species of *Onychophora*. Part II - The development of *Eoperipatus weldoni*. *Q. J. Microscop. Sci.* **45**, 41-88. doi:10.1242/jcs.s2-45.177.41
- Evans, T., Wade, C. M., Chapman, F. A., Johnson, A. D. and Loose, M.** (2014). Acquisition of germ plasm accelerates vertebrate evolution. *Science* **344**, 200-203. doi:10.1126/science.1249325
- Everett, N. B.** (1944). The present status of the germ-cell problem in vertebrates. *Biol. Rev.* **20**, 45-55. doi:10.1111/j.1469-185X.1945.tb00313.x
- Ewen-Campen, B., Donoughe, S., Clarke, D. N. and Extavour, C. G.** (2013b). Germ cell specification requires zygotic mechanisms rather than germ plasm in a basally branching insect. *Curr. Biol.* **23**, 835-842. doi:10.1016/j.cub.2013.03.063
- Ewen-Campen, B., Jones, T. E. M. and Extavour, C. G.** (2013a). Evidence against a germ plasm in the milkweed bug *Oncopeltus fasciatus*, a hemimetabolous insect. *Biol. Open* **2**, 556-568. doi:10.1242/bio.20134390
- Ewen-Campen, B., Schwager, E. E. and Extavour, C. G. M.** (2010). The molecular machinery of germ line specification. *Mol. Reprod. Dev.* **77**, 3-18. doi:10.1002/mrd.21091
- Ewen-Campen, B., Srouji, J. R., Schwager, E. E. and Extavour, C. G.** (2012). oskar Predates the Evolution of Germ Plasm in Insects. *Curr. Biol.* **22**, 2278-2283. doi:10.1016/j.cub.2012.10.019
- Extavour, C. G.** (2005). The fate of isolated blastomeres with respect to germ cell formation in the amphipod crustacean *Parhyale hawaiiensis*. *Dev. Biol.* **277**, 387-402. doi:10.1016/j.ydbio.2004.09.030
- Extavour, C. G. and Akam, M.** (2003). Mechanisms of germ cell specification across the metazoans: epigenesis and preformation. *Development* **130**, 5869-5884. doi:10.1242/dev.00804
- Extavour, C. G. M.** (2007). Evolution of the bilaterian germ line: lineage origin and modulation of specification mechanisms. *Integr. Comp. Biol.* **47**, 770-785. doi:10.1093/icb/icm027
- Eyun, S.-I.** (2017). Phylogenomic analysis of Copepoda (Arthropoda, Crustacea) reveals unexpected similarities with earlier proposed morphological phylogenies. *BMC Evol. Biol.* **17**, 23. doi:10.1186/s12862-017-0883-5
- Faussek, V.** (1888). Ueber die embryonale Entwicklung der Geschlechtsorgane bei der Afterspinne (Phalangium). *Biol. Zentralbl.* **8**, 359-363.
- Faussek, V.** (1892). Zur Anatomie und Embryologie der Phalangiden. *Biol. Zentralbl.* **12**, 1-8.
- Fernández, R., Edgecombe, G. D. and Giribet, G.** (2018). Phylogenomics illuminates the backbone of the Myriapoda Tree of Life and reconciles morphological and molecular phylogenies. *Sci. Rep.* **8**, 83. doi:10.1038/s41598-017-18562-w
- Fernando, W.** (1934). The early embryology of a viviparous psocid. *J. Cell Sci.* **s2-77**, 99-119. doi:10.1242/jcs.s2-77.305.99
- Fish, W. A.** (1947a). Embryology of *Lucilia sericata* Meigen (Diptera: Calliphoridae) Part I. Cell cleavage and early embryonic development. *Ann. Entomol. Soc. Am.* **40**, 15-28. doi:10.1093/aesa/40.1.15
- Fish, W. A.** (1947b). Embryology of *Lucilia sericata* Meigen (Diptera: Calliphoridae) Part II. The blastoderm, yolk cells and germ cells. *Ann. Entomol. Soc. Am.* **40**, 677-687. doi:10.1093/aesa/40.4.677
- Fish, W. A.** (1949). Embryology of *Phaenicia sericata* (Meigen) Diptera: Calliphoridae Part III. The gastrular tube. *Ann. Entomol. Soc. Am.* **42**, 121-133. doi:10.1093/aesa/42.2.121
- Fish, W. A.** (1952). Embryology of *Phaenicia sericata* (Meigen) (Diptera: Calliphoridae). Part IV. The inner layer and mesenteron rudiments. *Ann. Entomol. Soc. Am.* **45**, 1-22. doi:10.1093/aesa/45.1.1
- Fleig, R. and Sander, K.** (1986). Embryogenesis of the honeybee *Apis mellifera* L. (Hymenoptera: Apidae): an SEM Study. *Int. J. Insect Morphol. Embryol.* **15**, 449-462. doi:10.1016/0020-7322(86)90037-1

- Forrest, K. M. and Gavis, E. R.** (2003). Live imaging of endogenous RNA reveals a diffusion and entrapment mechanism for nanos mRNA localization in *Drosophila*. *Curr. Biol.* **13**, 1159-1168. doi:10.1016/S0960-9822(03)00451-2
- Gabriel, W. N., McNuff, R., Patel, S. K., Gregory, T. R., Jeck, W. R., Jones, C. D. and Goldstein, B.** (2007). The tardigrade *Hypsibius dujardini*, a new model for studying the evolution of development. *Dev. Biol.* **312**, 545-559. doi:10.1016/j.ydbio.2007.09.055
- Gainett, G., Crawford, A. R., Klementz, B. C., So, C., Baker, C. M., Setton, E. V. W. and Sharma, P. P.** (2022). Eggs to long-legs: embryonic staging of the harvestman *Phalangium opilio* (Opiliones), an emerging model arachnid. *Front. Zool.* **19**, 11. doi:10.1186/s12983-022-00454-z
- Gambrell, F. L.** (1933). The embryology of the black fly, *Simulium pictipes* Hagen. *Ann. Entomol. Soc. Am.* **26**, 641-471. doi:10.1093/aesa/26.4.641
- Gatenby, J. B.** (1917). The embryonic development of *Trichogramma evanescens*, Westw., monembryonic egg parasite of *Donacia simplex*, Fab. *J. Cell Sci.* **s2-62**, 149-187. doi:10.1242/jcs.s2-62.246.149
- Gatenby, J. B.** (1918). The segregation of the germ-cells in *Trichogramma evanescens*. *J. Cell Sci.* **s2-63**, 161-174. doi:10.1242/jcs.S2-63.250.161
- Geigy, R.** (1931). Action de l'ultra-violet sur le pôle germinale dans l'oeuf de *Drosophila melanogaster* (Castration et mutabilité). *Rev. Suisse Zool.* **38**, 187-285. doi:10.5962/bhl.part.117643
- Gerberding, M. and Patel, N. H.** (2004). Chapter 6. Gastrulation in Crustaceans: germ layers and cell lineages. In *Gastrulation: From Cells to Embryo* (ed. C. Stern), pp. 79-89. Cold Spring Harbor Laboratory Press.
- Gerberding, M., Browne, W. E. and Patel, N. H.** (2002). Cell lineage analysis of the amphipod crustacean *Parhyale hawaiiensis* reveals an early restriction of cell fates. *Development* **129**, 5789-5801. doi:10.1242/dev.00155
- Gilbert, S.** (1997). *Embryology: Constructing the Organism*. Sunderland, MA, USA: Sinauer Associates Inc.
- Giribet, G.** (2018). Current views on chelicerate phylogeny—A tribute to Peter Weygoldt. *Zool. Anz.* **273**, 7-13. doi:10.1016/j.jcz.2018.01.004
- Giribet, G. and Edgecombe, G. D.** (2019). The phylogeny and evolutionary history of arthropods. *Curr. Biol.* **29**, R592-R602. doi:10.1016/j.cub.2019.04.057
- Goodrich, A. L.** (1939). The origin and fate of the endoderm elements in the embryology of *Porcellio laevis* Latr. and *Armadillidium nasutum* B.L. (Isopoda). *J. Morphol.* **64**, 401-426. doi:10.1002/jmor.1050640303
- Görg, I.** (1959). Untersuchungen am Keim von *Hierodula* (*Rhombodera*) *crassa* Giglio Tos, ein Beitrag zur Embryologie der Mantiden. *Dtsch. Entomol. Z.* **6**, 389-450. doi:10.1002/mmnd.19590060501
- Goss, R. J.** (1952). The early embryology of the book louse, *Liposcelis divergens* Badonnel (Psocoptera; Liposcelidae). *J. Morphol.* **91**, 135-167. doi:10.1002/jmor.1050910107
- Goss, R. J.** (1953). The advanced embryology of the book louse, *Liposcelis divergens* Badonnel (Psocoptera; Liposcelidae). *J. Morphol.* **92**, 157-205. doi:10.1002/jmor.1050920106
- Gough, L. H.** (1902). The development of *Admetus pumilio*, Koch: a contribution to the embryology of the Pedipalps. *J. Cell Sci.* **s2-45**, 595-630. doi:10.1242/jcs.s2-45.180.595
- Grattan, R. M., McCulloch, R. J., Sellars, M. J. and Hertzler, P. L.** (2013). Ultrastructure of putative germ granules in the penaeid shrimp *Marsupenaeus japonicus*. *Arthropod Struct. Dev.* **42**, 153-164. doi:10.1016/j.asd.2012.11.002
- Grbic, M.** (2003). Polyembryony in parasitic wasps: evolution of a novel mode of development. *Int. J. Dev. Biol.* **47**, 633-642. doi:10.1387/ijdb.14756339
- Green, J. E. and Akam, M.** (2014). Germ cells of the centipede *Strigamia maritima* are specified early in embryonic development. *Dev. Biol.* **392**, 419-430. doi:10.1016/j.ydbio.2014.06.003
- Grobden, C.** (1879). Die Entwicklungsgeschichte der *Moina rectirostris*. *Arb. Zool. Ins. Univ. Wien* **2**, 203-226.
- Grobden, C.** (1881). Die Entwicklungsgeschichte von *Cetochilus septentrionalis* Goodsir. *Arb. Zool. Ins. Univ. Wien* **3**, 243-282.
- Gupta, T. and Extavour, C. G.** (2013). Identification of a putative germ plasm in the amphipod *Parhyale hawaiiensis*. *EvoDevo* **4**, 34. doi:10.1186/2041-9139-4-34
- Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., Couger, M. B., Eccles, D., Li, B., Lieber, M. et al.** (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nat. Protoc.* **8**, 1494-1512. doi:10.1038/nprot.2013.084
- Hafiz, H. A.** (1935). The embryological development of *Cheyletus eruditus* (a mite). *Proc. Roy. Soc. Lond. Ser. B* **117**, 174-201. doi:10.1098/rspb.1935.0022
- Haga, K.** (1985). Oogenesis and embryogenesis of the idiothripine thrips, *Bactrothrips brevitubus* (Thysanoptera, Phlaeothripidae). In *Recent Advances in Insect Embryology in Japan* (ed. H. Ando and K. Miya), pp. 45-106. Tsukuba: ISEBU.
- Hammerschmidt, J.** (1910). Beiträge zur Entwicklung der Phasmatiden. *Z. wiss. Zool.* **95**, 221-242.
- Harber, P. A. and Mutchmor, J. A.** (1970). The early embryonic development of *Culiseta inornata* (Diptera: Culicidae). *Ann. Entomol. Soc. Am.* **63**, 1609-1614. doi:10.1093/aesa/63.6.1609
- Hargitt, G. T.** (1919). Germ Cells of Coelenterates. VI. General considerations, discussion, conclusions. *J. Morphol.* **33**, 1-59. doi:10.1002/jmor.1050330102
- Hegner, R. W.** (1908). Effects of removing the germ-cell determinants from the eggs of some chrysomelid beetles. Preliminary report. *Biol. Bull.* **16**, 19-26. doi:10.2307/1536121
- Hegner, R. W.** (1909a). The effects of centrifugal force upon the eggs of some chrysomelid beetles. *J. Exp. Zool.* **6**, 507-552. doi:10.1002/jez.1400060403
- Hegner, R. W.** (1909b). The origin and early history of the germ-cells in some chrysomelid beetles. *J. Morphol.* **20**, 231-296. doi:10.1002/jmor.1050200204
- Hegner, R. W.** (1911). Experiments with chrysomelid beetles. III. The effects of killing parts of the eggs of *Leptinotarsa decemlineata*. *Biol. Bull.* **20**, 237-251. doi:10.2307/1536101
- Hegner, R. W.** (1914). Studies on germ cells. I. The history of the germ cells in insects with special reference to the Keimbahn-determinants. II. The origin and significance of the Keimbahn-determinants in animals. *J. Morphol.* **25**, 375-509. doi:10.1002/jmor.1050250302
- Heider, K.** (1889). *Die Embryonalentwicklung von *Hydrophilus piceus*, L.* Jena: Verlag von Gustav Fischer.
- Heikes, K. L., Game, M., Smith, F. W. and Goldstein, B.** (2023). The embryonic origin of primordial germ cells in the tardigrade *Hypsibius exemplaris*. *Dev. Biol.* **497**, 42-58. doi:10.1016/j.ydbio.2023.02.008
- Hejnal, A. and Schnabel, R.** (2005). The eutardigrade *Thulinia stephaniae* has an indeterminate development and the potential to regulate early blastomere ablations. *Development* **132**, 1349-1361. doi:10.1242/dev.01701
- Heming, B. S.** (1979). Origin and fate of germ cells in male and female embryos of *Haplothrips verbasci* (Osborn) (Insecta, Thysanoptera, Phlaeothripidae). *J. Morphol.* **160**, 323-343. doi:10.1002/jmor.1051600305
- Heming, B. S. and Huebner, E.** (1994). Development of the germ cells and reproductive primordia in male and female embryos of *Rhodnius prolixus* Stål (Hemiptera: Reduviidae). *Can. J. Zool.* **72**, 1100-1119. doi:10.1139/z94-148
- Hertzler, P. L.** (2002). Development of the mesendoderm in the dendrobranchiate shrimp *Sicyonia ingentis*. *Arthropod Struct. Dev.* **31**, 33-49. doi:10.1016/S1467-8039(02)00018-X
- Hertzler, P. L.** (2005). Cleavage and gastrulation in the shrimp *Penaeus* (*Litopenaeus*) *vannamei* (Malacostraca, Decapoda, Dendrobranchiata). *Arthropod Struct. Dev.* **34**, 455-469. doi:10.1016/j.asd.2005.01.009
- Heymons, R.** (1895). *Die Embryonalentwicklung von *Dermapteren* und *Orthopteren* unter Besonderer Berücksichtigung der Keimblätterbildung.* Jena: Verlag von Gustav Fischer.
- Heymons, R.** (1897). Entwicklungsgeschichtliche Untersuchungen an *Lepisma saccharina* L. *Z. wiss. Zool.* **62**, 583-631.
- Heymons, R.** (1901). Die Entwicklungsgeschichte der *Scolopender*. *Zoologica* **33**, 1-244. doi:10.5962/bhl.title.1587
- Heys, F.** (1931). The problem of the origin of germ cells. *Q Rev Biology* **6**, 1-45. doi:10.1086/394368
- Hickman, V.** (1936). The embryology of the Syncarid Crustacean, *Anaspides tasmaniae*. *Pap. Proc. R. Soc. Tasman.* **1936**, 1-36. doi:10.26749/ICYF9975
- His, W.** (1868). *Untersuchungen über die erste Anlage des Wirbelthierleibes: die erste Entwicklung des Hünchens im Ei.* Leipzig: F. C. W. Vogel.
- Hodson, A. C.** (1934). The origin and differentiation of the sex organs of *Tribolium confusum* Duval. *Ann. Entomol. Soc. Am.* **27**, 278-291. doi:10.1093/aesa/27.2.278
- Holm, Å.** (1947). On the development of *Opilio parietinus* Deg. *Zool. Bidr. Uppsala* **25**, 409-422.
- Hopf, C., Viebahn, C. and Püschel, B.** (2011). BMP signals and the transcriptional repressor *BLIMP1* during germline segregation in the mammalian embryo. *Dev. Genes Evol.* **221**, 209. doi:10.1007/s00427-011-0373-5
- Howard, R. J., Giacomelli, M., Lozano-Fernandez, J., Edgecombe, G. D., Fleming, J. F., Kristensen, R. M., Ma, X., Olesen, J., Sørensen, M. V., Thomsen, P. F. et al.** (2022). The Ediacaran origin of Ecdysozoa: integrating fossil and phylogenomic data. *J. Geol. Soc.* **179**, jgs2021-jgs2107. doi:10.1144/jgs2021-107
- Huerta-Cepas, J., Serra, F. and Bork, P.** (2016). ETE 3: Reconstruction, analysis, and visualization of phylogenomic data. *Mol. Biol. Evol.* **33**, 1635-1638. doi:10.1093/molbev/msw046
- Huettner, A. F.** (1923). The origin of the germ cells in *Drosophila melanogaster*. *J. Morphol.* **2**, 385-423. doi:10.1002/jmor.1050370204
- Hughes, T. E.** (1950). The embryonic development of the mite *Tyroglyphus farinae* Linnaeus 1758. *Proc. Zool. Soc. Lond.* **119**, 873-886. doi:10.1111/j.1096-3642.1950.tb00914.x
- Ikuta, K. and Makioka, T.** (1994). Notes on the postembryonic development of the ovary in *Argulus japonicus* (Crustacea: Branchiura). *Proc. Arthropod. Embryol. Soc. Jpn.* **29**, 15-17.
- Illmensee, K. and Mahowald, A. P.** (1974). Transplantation of posterior polar plasm in *Drosophila*. Induction of germ cells at the anterior pole of the egg. *Proc. Natl. Acad. Sci. USA* **71**, 1016-1020. doi:10.1073/pnas.71.4.1016
- Illmensee, K., Mahowald, A. P. and Loomis, M. R.** (1976). The ontogeny of germ plasm during oogenesis in *Drosophila*. *Dev. Biol.* **49**, 40-65. doi:10.1016/0012-1606(76)90257-8

- Ives, A. R. and Garland Jr., T. (2010). Phylogenetic logistic regression for binary dependent variables. *Syst. Biol.* **59**, 9–26. doi:10.1093/sysbio/syp074
- Jazdowska-Zagrodzińska, B. (1966). Experimental studies on the role of “polar granules” in the segregation of pole cells in *Drosophila melanogaster*. *J. Embryol. Exp. Morph.* **16**, 391–399. doi:10.1242/dev.16.3.391
- Jiménez-Guri, E., Wotton, K. R., Gavilán, B. and Jaeger, J. (2014). A staging scheme for the development of the moth midge *Clogmia albipunctata*. *PLoS ONE* **9**, e84422. doi:10.1371/journal.pone.0084422
- Johannsen, O. A. (1929). Some phases in the embryonic development of *Diacrisia virginica* Fabr. (Lepidoptera). *J. Morphol.* **48**, 493–541. doi:10.1002/jmor.1050480209
- Johannsen, O. A. and Butt, F. H. (1941). *Embryology of Insects and Myriapods: The Developmental History of Insects, Centipedes, and Millepedes from Egg Deposition to Hatching*. New York: McGraw-Hill, Inc.
- Johnson, A. D., Drum, M., Bachvarova, R. F., Masi, T., White, M. E. and Crother, B. I. (2003). Evolution of predetermined germ cells in vertebrate embryos: implications for macroevolution. *Evol. Dev.* **5**, 414–431. doi:10.1046/j.1525-142X.2003.03048.x
- Johnson, A. D., Richardson, E., Bachvarova, R. F. and Crother, B. I. (2011). Evolution of the germ line–soma relationship in vertebrate embryos. *Reproduction* **141**, 291–300. doi:10.1530/REP-10-0474
- Jones, J. R. and Macdonald, P. M. (2007). Oskar controls morphology of polar granules and nuclear bodies in *Drosophila*. *Development* **134**, 233–236. doi:10.1242/dev.02729
- Juhn, J. and James, A. A. (2006). oskar gene expression in the vector mosquitoes, *Anopheles gambiae* and *Aedes aegypti*. *Insect Mol. Biol.* **15**, 363–372. doi:10.1111/j.1365-2583.2006.00655.x
- Juhn, J., Marinotti, O., Calvo, E. and James, A. A. (2008). Gene structure and expression of nanos (nos) and oskar (osk) orthologues of the vector mosquito, *Culex quinquefasciatus*. *Insect Mol. Biol.* **17**, 545–552. doi:10.1111/j.1365-2583.2008.00823.x
- Jung, E. (1966). Untersuchungen am Ei des Speisebohnenkäfers *Bruchidius obtectus* Say (Coleoptera). *Z. Morphol. Ökol. Tiere* **56**, 444–480. doi:10.1007/BF00442293
- Jura, C. (1967). Origin of germ cells and gonads formation in embryogenesis of *Tetradontophora bielienensis* (Collembola). *Acta Biol. Crac. Zool.* **10**, 97–103.
- Kaczmarczyk, A. N. (2014). Germline maintenance and regeneration in the amphipod crustacean, *Parhyale hawaiiensis*. *PhD thesis*, University of California, Berkeley, CA.
- Kao, J. A., Ewen-Campen, B. and Extavour, C. G. (2025). Divergence of germ cell-less roles in germ line development across insect species. *Dev. Biol.* **525**, 93–101. doi:10.1016/j.ydbio.2025.05.004
- Kautzsch, G. (1910). Über die Entwicklung von *Agelena labyrinthica* Clerck. II. Teil. *Zool. Jahrb. Anat.* **30**, 535–602. doi:10.1007/BF02263817
- Kawahara, A. Y., Plotkin, D., Espeland, M., Meusemann, K., Toussaint, E. F. A., Donath, A., Ginnich, F., Frandsen, P. B., Zwick, A., dos Reis, M. et al. (2019). Phylogenomics reveals the evolutionary timing and pattern of butterflies and moths. *Proc. Natl. Acad. Sci. USA* **116**, 22657–22663. doi:10.1073/pnas.1907847116
- Kelly, G. M. and Huebner, E. (1989). Embryonic development of the hemipteran insect *Rhodnius prolixus*. *J. Morphol.* **199**, 175–196. doi:10.1002/jmor.1051990205
- Kemph, A. and Lynch, J. A. (2022). Evolution of germ plasm assembly and function among the insects. *Curr. Opin. Insect Sci.* **50**, 100883. doi:10.1016/j.cois.2022.100883
- Kemph, A., Kharel, K., Tindell, S. J., Arkov, A. L. and Lynch, J. A. (2025). Novel structure and composition of the unusually large germline determinant of the wasp *Nasonia vitripennis*. *Mol. Biol. Cell* **36**, ar55. doi:10.1091/mbc.E24-11-0499
- Kessel, E. L. (1939). *The Embryology of Fleas*. Washington: Smithsonian Institution.
- Kharel, K., Tindell, S. J., Kemph, A., Schmidtke, R., Alexander, E., Lynch, J. A. and Arkov, A. L. (2024). Dynamic protein assembly and architecture of the large solitary membraneless organelle during germline development in the wasp *Nasonia vitripennis*. *Development* **151**, dev202877. doi:10.1242/dev.202877
- Khila, A. and Abouheif, E. (2008). Reproductive constraint is a developmental mechanism that maintains social harmony in advanced ant societies. *Proc. Natl. Acad. Sci. USA* **105**, 17884–17889. doi:10.1073/pnas.0807351105
- Khila, A. and Abouheif, E. (2010). Evaluating the role of reproductive constraints in ant social evolution. *Philos. Trans. R. Soc. B: Biol. Sci.* **365**, 617–630. doi:10.1098/rstb.2009.0257
- Kingsley, J. S. (1893). The embryology of *Limulus*. — part II. *J. Morphol.* **8**, 195–268. doi:10.1002/jmor.1050080202
- Kistler, K. E., Trcek, T., Hurd, T. R., Chen, R., Liang, F.-X., Sall, J., Kato, M. and Lehmann, R. (2018). Phase transitioned nuclear Oskar promotes cell division of *Drosophila* primordial germ cells. *eLife* **7**, e37949. doi:10.7554/eLife.37949
- Klag, J. (1982). Germ line of *Tetradontophora bielienensis* (Insecta, Collembola). Ultrastructural study on the origin of primordial germ cells. *Development* **72**, 183–195. doi:10.1242/dev.72.1.183
- Klag, J. and Bilinski, S. (1993). Oosome formation in two ichneumonid wasps. *Tissue Cell* **25**, 121–128. doi:10.1016/0040-8166(93)90069-W
- Klag, J. and Świątek, P. (1999). Differentiation of primordial germ cells during embryogenesis of *Allacma fusca* (L.) (Collembola: Symphyleona). *Int. J. Insect Morphol. Embryol.* **28**, 161–168. doi:10.1016/S0020-7322(99)00021-5
- Klomp, J., Athy, D., Kwan, C. W., Bloch, N. I., Sandmann, T., Lemke, S. and Schmidt-Ott, U. (2015). A cysteine-clamp gene drives embryo polarity in the midge *Chironomus*. *Science* **348**, 1040–1042. doi:10.1126/science.aaa7105
- Kobayashi, Y. and Ando, H. (1984). Mesodermal organogenesis in the embryo of the primitive moth, *Neomicropteryx nipponensis* Issiki (Lepidoptera, Micropterygidae). *J. Morphol.* **181**, 29–47. doi:10.1002/jmor.1051810105
- Kobayashi, H. and Ando, H. (1985). Early embryogenesis of fireflies, *Luciola cruciata*, *L. lateralis* and *Hotaria parvula* (Coleoptera, Lampyridae). In *Recent Advances in Insect Embryology in Japan* (ed. H. Ando and K. Miya), 157–169. Tsukuba: ISEBU.
- Kobayashi, Y., Niikura, K., Oosawa, Y. and Takami, Y. (2013). Embryonic development of *Carabus insulicola* (Insecta, Coleoptera, Carabidae) with special reference to external morphology and tangible evidence for the subcoxal theory. *J. Morphol.* **274**, 1323–1352. doi:10.1002/jmor.20181
- Kobayashi, Y., Watanabe, T. and Suzuki, H. (2006). Embryonic development of the firefly *Pyrocoelia rufa* Olivier (Insecta: Coleoptera, Lampyridae), with special reference to its hibernal diapause. *Proc. Arthropod. Embryol. Soc. Jpn.* **41**, 47–53.
- Komatsu, S. and Kobayashi, Y. (2012). Embryonic development of a whirligig beetle, *Dineutus mellyi*, with special reference to external morphology (Insecta: Coleoptera, Gyrrinidae). *J. Morphol.* **273**, 541–560. doi:10.1002/jmor.20005
- Krause, G. (1939). Die Eitypen der Insekten. *Biol. Zentralbl.* **59**, 495–536.
- Krause, J. B. (1947). The development of the gonads of the wood-eating beetle, *Passalus cornutus* Fabricius. *Ann. Entomol. Soc. Am.* **40**, 172–202. doi:10.1093/aesa/40.2.172
- Kühn, A. (1913). Die Sonderung der Keimesbezirke in der Entwicklung der Sommereier von *Polyphemus pediculus* de Geer. *Zool. Jahrb. Anat.* **35**, 243–340.
- Kulkarni, A. and Extavour, C. G. (2017). Convergent evolution of germ granule nucleators: A hypothesis. *Stem Cell Res.* **24**, 188–194. doi:10.1016/j.scr.2017.07.018
- Kulkarni, A., Ewen-Campen, B., Terao, K., Matsumoto, Y., Li, Y., Watanabe, T., Kao, J. A., Parhad, S. S., Ylla, G., Mizunami, M. et al. (2023). oskar acts with the transcription factor *Creb* to regulate long-term memory in crickets. *Proc. Natl. Acad. Sci. USA* **120**, e2218506120. doi:10.1073/pnas.2218506120
- Kumar, S., Suleski, M., Craig, J. M., Kasprovicz, A. E., Sanderford, M., Li, M., Stecher, G. and Hedges, S. B. (2022). TimeTree 5: An expanded resource for species divergence times. *Mol. Biol. Evol.* **39**, msac174. doi:10.1093/molbev/msac174
- Kütke, H.-W. (1966). Das Differenzierungszentrum als selbstregulierendes Faktorensystem für den Aufbau der Keimanlage im Ei von *Dermestes frischii* (Coleoptera). *Wilhelm Roux Arch. Entwickl. Mech. Org.* **157**, 212–302. doi:10.1007/BF00576947
- Lall, S., Ludwig, M. Z. and Patel, N. H. (2003). Nanos plays a conserved role in axial patterning outside of the Diptera. *Curr. Biol.* **13**, 224–229. doi:10.1016/S0960-9822(03)00045-9
- Lane, R. L. (1977). A development investigation of the reproductive systems of *Armadillidium vulgare* (Latreille) and *Porcellionides pruinosus* (Brandt) (Isopoda). *Crustaceana* **33**, 237–248. doi:10.1163/156854077X00377
- Larink, O. (1969). Zur Entwicklungsgeschichte von *Petrobius brevistylis* (Thysanura, Insecta). *Helgoländer Wiss. Meeresunters.* **19**, 111–155. doi:10.1007/BF01625861
- Lasko, P. F. and Ashburner, M. (1990). Posterior localization of vasa protein correlates with, but is not sufficient for, pole cell development. *Genes Dev.* **4**, 905–921. doi:10.1101/gad.4.6.905
- Lassmann, G. W. P. (1936). The early embryological development of *Melophagus ovinus* L., with special reference to the development of the germ cells. *Ann. Entomol. Soc. Am.* **29**, 397–414. doi:10.1093/aesa/29.3.397
- Lawson, K. A., Dunn, N. R., Roelen, B. A. J., Zeinstra, L. M., Davis, A. M., Wright, C. V. E., Korving, J. P. W. F. M. and Hogan, B. L. M. (1999). *Brmp4* is required for the generation of primordial germ cells in the mouse embryo. *Genes Dev.* **13**, 424–436. doi:10.1101/gad.13.4.424
- Lee, C.-Y. S., Putnam, A., Lu, T., He, S., Ouyang, J. P. T. and Seydoux, G. (2020). Recruitment of mRNAs to P granules by condensation with intrinsically-disordered proteins. *eLife* **9**, e52896. doi:10.7554/eLife.52896
- Lehmann, R. (2016). Germ plasm biogenesis - An oskar-centric perspective. *Curr. Top. Dev. Biol.* **116**, 679–707. doi:10.1016/bs.ctdb.2015.11.024
- Leptin, M. (2004). Chapter 7: Gastrulation in *Drosophila*. In *Gastrulation: From Cells to Embryo* (ed. C. Stern), pp. 91–104. New York, NY, USA: Cold Spring Harbor Laboratory Press.
- Letunic, I. and Bork, P. (2024). Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Res.* **52**, W78–W82. doi:10.1093/nar/gkac268
- Lin, G., Cook, C. E., Miura, T. and Chang, C. (2014). Posterior localization of *ApVas1* positions the preformed germ plasm in the sexual oviparous pea aphid *Acyrthosiphon pisum*. *EvoDevo* **5**, 18. doi:10.1186/2041-9139-5-18

- Lochab, A. K. and Extavour, C. G.** (2017). Bone Morphogenetic Protein (BMP) signaling in animal reproductive system development and function. *Dev. Biol.* **427**, 258-269. doi:10.1016/j.ydbio.2017.03.002
- Luginbill, P.** (1953). A contribution to the embryology of the May beetle. *Ann. Entomol. Soc. Am.* **46**, 505-528. doi:10.1093/aesa/46.4.505
- Lynch, J. A.** (2019). Evolution of maternal control of axial patterning in insects. *Curr. Opin. Insect Sci.* **31**, 37-42. doi:10.1016/j.cois.2018.07.011
- Lynch, J. A. and Desplan, C.** (2010). Novel modes of localization and function of nanos in the wasp *Nasonia*. *Development* **137**, 3813-3821. doi:10.1242/dev.054213
- Lynch, J. A., Özüak, O., Khila, A., Abouheif, E., Desplan, C. and Roth, S.** (2011). The Phylogenetic Origin of oskar Coincided with the Origin of Maternally Provisioned Germ Plasm and Pole Cells at the Base of the Holometabola. *PLoS Genet.* **7**, e1002029. doi:10.1371/journal.pgen.1002029
- Ma, K.-Y., Tian, X.-Q., Liu, Z.-Q. and Qiu, G.-F.** (2019). Observations on the embryonic development of the oriental river prawn *Macrobrachium nipponense* (De Haan, 1849) (Decapoda: Caridea: Palaemonidae). *J. Crustac. Biol.* **39**, 261-266. doi:10.1093/jcbl/ruz006
- Mahowald, A. P.** (1962). Fine structure of pole cells and polar granules in *Drosophila melanogaster*. *J. Exp. Zool.* **151**, 201-215. doi:10.1002/jez.1401510302
- Mahowald, A. P.** (1968). Polar granules of *Drosophila*. II. Ultrastructural changes during early embryogenesis. *J. Exp. Zool.* **167**, 237-261. doi:10.1002/jez.1401670211
- Mahowald, A. P.** (1975). Ultrastructural changes in the germ plasm during the life cycle of *Miastor* (Cecidomyiidae, Diptera). *Wilhelm Roux Arch. Entwickl. Mech. Org.* **176**, 223-240. doi:10.1007/BF00576803
- Mahowald, A. P.** (2001). Assembly of the *Drosophila* germ plasm. *Int. Rev. Cytol.* **203**, 187-213. doi:10.1016/S0074-7696(01)03007-8
- Mahowald, A. P., Illmensee, K. and Turner, F. R.** (1976). Interspecific transplantation of polar plasm between *Drosophila* embryos. *J. Cell Biol.* **70**, 358-373. doi:10.1083/jcb.70.2.358
- Manni, M., Berkeley, M. R., Seppely, M., Simão, F. A. and Zdobnov, E. M.** (2021). BUSCO update: Novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. *Mol. Biol. Evol.* **38**, 4647-4654. doi:10.1093/molbev/msab199
- Manton, S. M.** (1928). On the embryology of a mysid crustacean, *Hemimysis lamornae*. *Philos. Trans. R. Soc. B.* **216**, 363-463. doi:10.1098/rstb.1928.0008
- Manton, S. M.** (1934). On the embryology of the crustacean *Nebalia bipes*. *Philos. Trans. R. Soc. B.* **223**, 163-238. doi:10.1098/rstb.1934.0005
- Manton, S. M.** (1949). Studies on the Onychophora VII. The early embryonic stages of *Peripatopsis*, and some general considerations concerning the morphology and phylogeny of the Arthropoda. *Philos. Trans. R. Soc. B.* **233**, 483-580. doi:10.1098/rstb.1949.0003
- Marlow, F. L. and Mullins, M. C.** (2008). Bucky ball functions in Balbiani body assembly and animal-vegetal polarity in the oocyte and follicle cell layer in zebrafish. *Dev. Biol.* **321**, 40-50. doi:10.1016/j.ydbio.2008.05.557
- Martin, A. and Orgogozo, V.** (2013). The loci of repeated evolution: a catalog of genetic hotspots of phenotypic variation. *Evolution* **67**, 1235-1250. doi:10.1111/evo.12081
- Mayer, G. and Tait, N. N.** (2009). Position and development of oocytes in velvet worms shed light on the evolution of the ovary in Onychophora and Arthropoda. *Zool. J. Linn. Soc. Lond.* **157**, 17-33. doi:10.1111/j.1096-3642.2009.00523.x
- McClendon, J. F.** (1907). On the development of parasitic copepods. Part II. *Biol. Bull.* **12**, 53-88. doi:10.2307/1535771
- Mellanby, H.** (1935). The Early Embryonic Development of *Rhodnius prolixus* (Hemiptera, Heteroptera). *Q. J. Microscop. Sci.* **309**, 71-90. doi:10.1242/jcs.s2-78.309.71
- Meng, C.** (1968). Strukturwandel und histochemische Befunde insbesondere am Oosom während der Oogenese und nach der Ablage des Eies von *Pimpla turionellae* L. (Hymenoptera, Ichneumonidae). *Wilhelm Roux Arch. Entwickl. Mech. Org.* **161**, 162-208. doi:10.1007/BF00585970
- Metcalfe, M. E.** (1935). The germ-cell cycle in *Phytophaga destructor* Say. *J. Cell Sci.* **s2-77**, 585-604. doi:10.1242/jcs.s2-77.308.585
- Metschnikow, E.** (1866). *Embryologische Studien an Insecten*. Leipzig, Germany: Verlag von Wilhelm Engelmann.
- Meusemann, K., Trautwein, M., Friedrich, F., Beutel, R. G., Wiegmann, B. M., Donath, A., Podsiadlowski, L., Petersen, M., Niehuis, O., Mayer, C. et al.** (2020). Are fleas highly modified Mecoptera? Phylogenomic resolution of Antliophora (Insecta: Holometabola). *bioRxiv*, 2020.11.19.390666.
- Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A. and Lanfear, R.** (2020). IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* **37**, 1530-1534. doi:10.1093/molbev/msaa015
- Misof, B., Liu, S., Meusemann, K., Peters, R. S., Donath, A., Mayer, C., Frandsen, P. B., Ware, J., Flouri, T., Beutel, R. G. et al.** (2014). Phylogenomics resolves the timing and pattern of insect evolution. *Science* **346**, 763-767. doi:10.1126/science.1257570
- Mitsumoto, H. and Makioka, T.** (2002). Preliminary notes on the structure and postembryonic development of gonads in two tadpole shrimps *Triops longicaudatus* and *T. numidicus* (Crustacea: Branchiopoda, Notostraca). *Proc. Arthropod. Embryol. Soc. Jpn.* **37**, 13-17.
- Mitsumoto, H. and Makioka, T.** (2003). Postembryonic development of gonads in *Triops numidicus* (Grube) (Crustacea: Branchiopoda, Notostraca). *Proc. Arthropod. Embryol. Soc. Jpn.* **38**, 9-12.
- Miura, T., Braendle, C., Shingleton, A., Sisk, G., Kambhampati, S. and Stern, D. L.** (2003). A comparison of parthenogenetic and sexual embryogenesis of the pea aphid *Acyrtosiphon pisum* (Hemiptera: Aphidoidea). *J. Exp. Zool. B* **295B**, 59-81.
- Miya, K.** (1953). The presumptive genital region at the blastoderm stage of the silkworm egg. *J. Fac. Agr. Iwate Univ.* **1**, 223-227.
- Miya, K.** (1958a). Studies on the embryonic development of the gonad in the silkworm, *Bombyx mori* L. Part I. Differentiation of germ cells. *J. Fac. Agr. Iwate Univ.* **3**, 436-467.
- Miya, K.** (1958b). Studies on the embryonic development of the gonad in the silkworm, *Bombyx mori* L. Part II. Formation of the gonad. *J. Fac. Agr. Iwate Univ.* **4**, 126-151.
- Miyakawa, K.** (1974). The embryology of the caddisfly *Stenopsyche griseipennis* MacLachlan (Trichoptera, Stenopsychidae): IV. Organogenesis: mesodermal derivatives. *Kontyû* **42**, 451-466.
- Miyazaki, K. and Makioka, T.** (2012). Postembryonic development of the female reproductive system in the pycnogonid, *Propallene longiceps* (Pycnogonida, Callipallenidae). *Invertebr. Reprod. Dev.* **56**, 287-292. doi:10.1080/07924259.2011.613167
- Morris, S. C.** (2009). The predictability of evolution: glimpses into a post-Darwinian world. *Naturwissenschaften* **96**, 1313-1337. doi:10.1007/s00114-009-0607-9
- Nair, K. B.** (1939). The reproduction, oogenesis and development of *Mesopodopsis orientalis* Tatt. *Proc. Indian Acad. Sci. Sect. B* **9**, 175-223. doi:10.1007/BF03049623
- Nair, K. B.** (1949). The embryology of *Caridina laevis* Heller. *Proc. Indian Acad. Sci. Sect. B* **29**, 211-288. doi:10.1007/BF03049969
- Nakamura, T. and Extavour, C. G.** (2016). The transcriptional repressor *Blimp-1* acts downstream of BMP signaling to generate primordial germ cells in the cricket *Gryllus bimaculatus*. *Development* **143**, 255-263. doi:10.1242/dev.127563
- Nakao, H.** (1999). Isolation and characterization of a *Bombyx vasa*-like gene. *Dev. Genes Evol.* **209**, 312-316. doi:10.1007/s004270050257
- Nakao, H. and Takasu, Y.** (2019). Complexities in *Bombyx* germ cell formation process revealed by *Bm-nosO* (a *Bombyx* homolog of *nanos*) knockout. *Dev. Biol.* **445**, 29-36. doi:10.1016/j.ydbio.2018.10.012
- Nakao, H., Hatakeyama, M., Lee, J. M., Shimoda, M. and Kanda, T.** (2006). Expression pattern of *Bombyx vasa*-like (*BmVGL*) protein and its implications in germ cell development. *Dev. Genes Evol.* **216**, 94-99. doi:10.1007/s00427-005-0033-8
- Nakao, H., Matsumoto, T., Oba, Y., Niimi, T. and Yaginuma, T.** (2008). Germ cell specification and early embryonic patterning in *Bombyx mori* as revealed by *nanos* orthologues. *Evol. Dev.* **10**, 546-554. doi:10.1111/j.1525-142X.2008.00270.x
- Needham, A. E.** (1937). Some points in the development of *Neomysis vulgaris*. *J. Cell Sci.* **s2-79**, 559-588. doi:10.1242/jcs.s2-79.316.559
- Needham, A. E.** (1942). The structure and development of the segmental excretory organs of *Asellus aquaticus* (Linné). *J. Cell Sci.* **s2-83**, 205-243. doi:10.1242/jcs.s2-83.330.205
- Nelsen, O. E.** (1934). The segregation of the germ cells in the grasshopper, *Melanoplus differentialis* (Acrididae: Orthoptera). *J. Morphol.* **55**, 545-575. doi:10.1002/jmor.1050550306
- Nelson, J. A.** (1915). *The Embryology of the Honey Bee*. Princeton: Princeton University Press.
- Nie, R.-E., Andújar, C., Gómez-Rodríguez, C., Bai, M., Xue, H.-J., Tang, M., Yang, C.-T., Tang, P., Yang, X.-K., Vogler, A. P.** (2020). The phylogeny of leaf beetles (Chrysomelidae) inferred from mitochondrial genomes. *Syst. Entomol.* **45**, 188-204. doi:10.1111/syen.12387
- Nieuwkoop, P. D. and Sutasurya, L. A.** (1979). *Primordial Germ Cells in the Chordates: Embryogenesis and Phylogenesis*. Cambridge: Cambridge University Press.
- Nieuwkoop, P. D. and Sutasurya, L. A.** (1981). *Primordial Germ Cells in the Invertebrates: From Epigenesis to Preformation*. Cambridge: Cambridge University Press.
- Niikura, K., Hirasawa, K., Inoda, T. and Kobayashi, Y.** (2017). Embryonic Development of a Diving Beetle, *Hydaticus pacificus* Aubé (Insecta: Coleoptera; Dytiscidae): External Morphology and Phylogenetic Implications. *Proc. Arthropod. Embryol. Soc. Jpn.* **48**, 19-32.
- Noskiewicz, J. and Poluszyński, G.** (1927). Embryologische Untersuchungen an Strepsipteren. 1. Teil. Embryogenese der Gattung *Stylops* Kirby. *Bull. Acad. Pol. Sci. Lett. B.* **10**, 1193-1227.
- Okada, M., Kleinman, I. A. and Schneiderman, H. A.** (1974). Restoration of fertility in sterilized *Drosophila* eggs by transplantation of polar cytoplasm. *Dev. Biol.* **37**, 43-54. doi:10.1016/0012-1606(74)90168-7
- Olesnicky, E. C. and Desplan, C.** (2007). Distinct mechanisms for mRNA localization during embryonic axis specification in the wasp *Nasonia*. *Dev. Biol.* **306**, 134-142. doi:10.1016/j.ydbio.2007.03.012

- OpenTreeOfLife, Redelings, B., Reyes, L. L. S., Cranston, K. A., Allman, J., Holder, M. T. and McTavish, E. J.** (2019). Open tree of life synthetic tree (12.3). *Zenodo*. doi:10.5281/zenodo.3937742
- Otto, S. P. and Hastings, I. M.** (1998). Mutation and selection within the individual. *Genetica* **102-103**, 507-524. doi:10.1007/978-94-011-5210-5\_38
- Özhan-Kizil, G., Havemann, J. and Gerberding, M.** (2009). Germ cells in the crustacean *Parhyale hawaiiensis* depend on Vasa protein for their maintenance but not for their formation. *Dev. Biol.* **327**, 230-239. doi:10.1016/j.ydbio.2008.10.028
- Özpolat, B. D.** (2024). Annelids as models of germ cell and gonad regeneration. *J. Exp. Zool. Part B* **342**, 126-143. doi:10.1002/jez.b.23233
- Pagel, M.** (1994). Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc. Roy. Soc. Lond. Ser. B Biol. Sci.* **255**, 37-45. doi:10.1098/rspb.1994.0006
- Paterson, N. F.** (1931). A contribution to the embryological development of *Euryope terminalis* Baly. *S. Afr. J. Sci.* **28**, 344-371.
- Paterson, N. F.** (1935). Observations on the Embryology of *Corynodes pusis* (Coleoptera, Chrysomelidae). *J. Cell Sci.* **s2-78**, 91-131. doi:10.1242/jcs.s2-78.309.91
- Pawliak, J. B., Sellars, M. J., Wood, A. and Hertzler, P. L.** (2010). Cleavage and gastrulation in the Kuruma shrimp *Penaeus (Marsupenaeus) japonicus* (Bate): a revised cell lineage and identification of a presumptive germ cell marker. *Dev. Growth Differ.* **52**, 677-692. doi:10.1111/j.1440-169X.2010.01205.x
- Pires-daSilva, A. and Sommer, R. J.** (2003). The evolution of signalling pathways in animal development. *Nat. Rev. Genet.* **4**, 39-49. doi:10.1038/nrg977
- Poulson, D. F.** (1947). The pole cells of Diptera, their fate and significance. *Proc. Natl. Acad. Sci. USA* **33**, 182-184. doi:10.1073/pnas.33.6.182
- Poulson, D. F.** (1950). Histogenesis, organogenesis, and differentiation in the embryo of *Drosophila melanogaster* Meigen. In *Biology of Drosophila* (ed. M. Demerec), pp. 168-274. New York: Hafner Pub. Co.
- Presser, B. D. and Rutschky, C. W.** (1957). The embryonic development of the corn earworm, *Heliothis zea* (Boddie) (Lepidoptera, Phalaenidae). *Ann. Entomol. Soc. Am.* **50**, 133-164. doi:10.1093/aesa/50.2.133
- Qiu, G.-F., Chen, Y., Cui, Z. and Zhu, X.-L.** (2013). Localization of germline marker vasa homolog RNA to a single blastomere at early cleavage stages in the oriental river prawn *Macrobrachium nipponense*: evidence for germ cell specification by preformation. *Gene* **513**, 53-62. doi:10.1016/j.gene.2012.10.079
- Quan, H.** (2018). Evolution of insect germline specification. *PhD thesis*, University of Illinois at Chicago, Chicago, IL.
- Quan, H. and Lynch, J. A.** (2016). The evolution of insect germline specification strategies. *Curr. Opin. Insect Sci.* **13**, 99-105. doi:10.1016/j.cois.2016.02.013
- Quan, H., Arsal, D. and Lynch, J. A.** (2019). Transcriptomic and functional analysis of the oosome, a unique form of germ plasm in the wasp *Nasonia vitripennis*. *BMC Biol.* **17**, 78. doi:10.1186/s12915-019-0696-7
- Queller, D. C.** (2000). Relatedness and the fraternal major transitions. *Philos. Trans. R. Soc. Lond. Ser. B: Biol. Sci.* **355**, 1647-1655. doi:10.1098/rstb.2000.0727
- Rafiqi, A. M., Rajakumar, A. and Abouheif, E.** (2020). Origin and elaboration of a major evolutionary transition in individuality. *Nature* **585**, 239-244. doi:10.1038/s41586-020-2653-6
- Raminani, L. N. and Cupp, E. W.** (1975). Early embryology of *Aedes aegypti* (L.) (Diptera: Culicidae). *Int. J. Insect Morphol. Embryol.* **4**, 517-528. doi:10.1016/0020-7322(75)90028-8
- Reed, R. D., Papa, R., Martin, A., Hines, H. M., Counterman, B. A., Pardo-Diaz, C., Jiggins, C. D., Chamberlain, N. L., Kronforst, M. R., Chen, R. et al.** (2011). Optix drives the repeated convergent evolution of butterfly wing pattern mimicry. *Science* **333**, 1137-1141. doi:10.1126/science.1208227
- Rempel, J. G. and Church, N. S.** (1969). The embryology of *Lytta viridana* Le Conte (Coleoptera: Meloidae). V. The blastoderm, germ layers, and body segments. *Can. J. Zool.* **47**, 1157-1171. doi:10.1139/z69-180
- Revell, L. J.** (2024). phytools 2.0: an updated R ecosystem for phylogenetic comparative methods (and other things). *PeerJ* **12**, e16505. doi:10.7717/peerj.16505
- Revell, L. J. and Harmon, L. J.** (2024). A discrete character evolution model for phylogenetic comparative biology with  $\Gamma$ -distributed rate heterogeneity among branches of the tree. *bioRxiv*, 2024.05.25.595896.
- Rivard, E. L., Srouji, J., Repouliou, A. and Extavour, C. G.** (2025). Protein sequence evolution underlies interspecies incompatibility of a cell fate determinant. *bioRxiv*, 2025.08.02.668269.
- Roonwal, M. L.** (1937). Studies on the embryology of the African migratory locust, *Locusta migratoria migratorioides* Reiche and Frm. (Orthoptera, Acrididae) II - Organogeny. *Philos. Trans. R. Soc. Lond. B* **227**, 175-244. doi:10.1098/rstb.1937.0003
- Rosenberg, M. I., Lynch, J. A. and Desplan, C.** (2009). Heads and tails: evolution of antero-posterior patterning in insects. *BBA* **1789**, 333-342. doi:10.1016/j.bbarm.2008.09.007
- Roth, S.** (2004). Chapter 8: Gastrulation in other insects. In *Gastrulation: From Cells to Embryo* (ed. C. Stern), pp. 105-121. New York, NY, USA: Cold Spring Harbor Laboratory Press.
- Ryan, R. B.** (1963). Contribution to the embryology of *Coeloides brunneri* (Hymenoptera: Braconidae). *Ann. Entomol. Soc. Am.* **56**, 639-648. doi:10.1093/aesa/56.5.639
- Sabroux, R., Corbari, L. and Hassanin, A.** (2023). Phylogeny of sea spiders (Arthropoda: Pycnogonida) inferred from mitochondrial genome and 18S ribosomal RNA gene sequences. *Mol. Phylogenet. Evol.* **182**, 107726. doi:10.1016/j.ympev.2023.107726
- Sagawa, K., Yamagata, H. and Shiga, Y.** (2005). Exploring embryonic germ line development in the water flea, *Daphnia magna*, by zinc-finger-containing VASA as a marker. *Gene Expr. Patterns* **5**, 669-678. doi:10.1016/j.modgep.2005.02.007
- Saito, S.** (1937). On the development of the tusser, *Antheraea pernyi* Guérin-Meneville, with special reference to the comparative embryology of insects. *J. Fac. Agr. Hokkaido Imp. Univ.* **40**, 35-109.
- Saitou, M. and Yamaji, M.** (2010). Germ cell specification in mice: signaling, transcription regulation, and epigenetic consequences. *Reproduction* **139**, 931-942. doi:10.1530/REP-10-0043
- Santos, A. C. and Lehmann, R.** (2004). Germ cell specification and migration in *Drosophila* and beyond. *Curr. Biol.* **14**, R578-R589. doi:10.1016/j.cub.2004.07.018
- Satterlee, J. W., Alonso, D., Gramazio, P., Jenike, K. M., He, J., Arrones, A., Villanueva, G., Plazas, M., Ramakrishnan, S., Benoit, M. et al.** (2024). Convergent evolution of plant prickles by repeated gene co-option over deep time. *Science* **385**, eado1663-eado1663. doi:10.1126/science.ado1663
- Schimkewitsch, V. M.** (1903). Über die Entwicklung von *Thelyphonus caudatus* (L.). *Zool. Anz.* **26**, 665-685.
- Schimkewitsch, W.** (1896). Studien über parasitische Copepoden. *Z. wiss. Zool.* **61**, 339-362.
- Scholl, A., Liu, Y. and Seydoux, G.** (2024). *Caenorhabditis elegans* germ granules accumulate hundreds of low translation mRNAs with no systematic preference for germ cell fate regulators. *Development* **151**, dev202575. doi:10.1242/dev.202575
- Scholl, G.** (1963). Embryologische Untersuchungen an Tanaidaceen (Heterotanaidaceen oerstedii Kröyer). *Zool. Jahrb. Anat.* **80**, 500-554.
- Scholtz, G.** (1990). The formation, differentiation and segmentation of the post-naupliar germ band of the amphipod *Gammarus pulex* L. (Crustacea, Malacostraca, Peracarida). *Proc. R. Soc. Lond. B* **239**, 163-211. doi:10.1098/rspb.1990.0013
- Scholtz, G.** (2000). Evolution of the nauplius stage in malacostracan crustaceans. *J. Zool. Syst. Evol. Res.* **38**, 175-187. doi:10.1046/j.1439-0469.2000.383151.x
- Scholtz, G. and Wolff, C.** (2002). Cleavage, gastrulation, and germ disc formation of the amphipod *Orchestia cavimana* (Crustacea, Malacostraca, Peracarida). *Contrib. Zool.* **71**, 9-28. doi:10.1163/18759866-0710103002
- Scholtz, G. and Wolff, C.** (2013). Chapter 4: Arthropod embryology: cleavage and germ band development. In *Arthropod Biology and Evolution* (ed. A. Minelli, G. Boxshall and G. Fusco), pp. 63-89. Heidelberg: Springer. doi:10.1007/978-3-642-36160-9\_4
- Schrader, F.** (1922). The sex ratio and oogenesis of *Pseudococcus citri*. *Z. für Indukt. Abstamm.- Vererbungslehre* **30**, 163-182.
- Schröder, R.** (2006). vasa mRNA accumulates at the posterior pole during blastoderm formation in the flour beetle *Tribolium castaneum*. *Dev. Genes Evol.* **216**, 277-283. doi:10.1007/s00427-005-0054-3
- Schwager, E. E., Meng, Y. and Extavour, C. G.** (2015). vasa and piwi are required for mitotic integrity in early embryogenesis in the spider *Parasteatoda tepidariorum*. *Dev. Biol.* **402**, 276-290. doi:10.1016/j.ydbio.2014.08.032
- Schwentner, M., Combosch, D. J., Nelson, J. P. and Giribet, G.** (2017). A phylogenomic solution to the origin of insects by resolving crustacean-hexapod relationships. *Curr. Biol.* **27**, 1818-1824.e5. doi:10.1016/j.cub.2017.05.040
- Sedgwick, A.** (1887). The development of the Cape species of *Peripatus*: Part III. On the changes from stage A to stage F. *J. Cell Sci.* **s2-27**, 467-550. doi:10.1242/jcs.s2-27.108.467
- Seervai, R. N. H. and Wessel, G. M.** (2013). Lessons for inductive germline determination. *Mol. Reprod. Dev.* **80**, 590-609. doi:10.1002/mrd.22151
- Seidel, F.** (1924). Die geschlechtsorgane in der Embryonalen Entwicklung von *Pyrhocoris apterus* L. *Z. Morphol. Ökol. Tiere* **1**, 429-506. doi:10.1007/BF00407470
- Shafiq, S. A.** (1954). A Study of the Embryonic development of the Gooseberry Sawfly, *Pteronidea ribesii*. *Q. J. Microscop. Sci.* **95**, 93-113.
- Sharma, P. P. and Gavish-Regev, E.** (2025). The evolutionary biology of chelicerata. *Annu. Rev. Entomol.* **70**, 143-163. doi:10.1146/annurev-ento-022024-011250
- Shinji, G. O.** (1919). Embryology of coccids, with especial reference to the formation of the ovary, origin and differentiation of the germ cells, germ layers, rudiments of the midgut, and the intracellular symbiotic organisms. *J. Morphol.* **33**, 72-167. doi:10.1002/jmor.1050330104
- Singh, J. P.** (1967). Early embryonic development of gonads in *Labidura riparia* (Pallas) (Labiduridae: Dermaptera). *Agra Univ. J. Res.* **1621**, 67-76.
- Song, N., Wang, M.-M., Huang, W.-C., Wu, Z.-Y., Shao, R. and Yin, X.-M.** (2024). Phylogeny and evolution of hemipteran insects based on expanded genomic and transcriptomic data. *BMC Biol.* **22**, 190. doi:10.1186/s12915-024-01991-1

- Sonnenblick, B. P.** (1941). Germ cell movements and sex differentiation of the gonads in the *Drosophila* embryo. *Proc. Natl. Acad. Sci. USA* **27**, 484-489. doi:10.1073/pnas.27.10.484
- Srouji, J. and Extavour, C. G.** (2010). Redefining stem cells and assembling germ plasm: key transitions in the evolution of the germ line. In *Key Innovations in Animal Evolution* (ed. R. DeSalle and B. Schierwater), pp. 360-397. Enfield, NH: CRC Press.
- Stanke, M., Steinkamp, R., Waack, S. and Morgenstern, B.** (2004). AUGUSTUS: a web server for gene finding in eukaryotes. *Nucleic Acids Res.* **32**, W309-W312. doi:10.1093/nar/gkh379
- Stanley, M. S. M. and Grundmann, A. W.** (1970). The embryonic development of *Tribolium confusum*. *Ann. Entomol. Soc. Am.* **63**, 1248-1256. doi:10.1093/aesa/63.5.1248
- Stern, C. D.** (2004). *Gastrulation: From Cells to Embryo* (ed. C. D. Stern). New York, USA: Cold Spring Harbor Laboratory Press.
- Strömberg, J.-O.** (1965). On the embryology of the isopod *Idotea*. *Ark. Zool.* **2**, 421-473.
- Strömberg, J.-O.** (1968). Segmentation and organogenesis in *Limnoria lignorum* (Rathke) (Isopoda). *Ark. Zool.* **20**, 91.
- Strömberg, J.-O.** (1971). Contribution to the embryology of bopyrid isopods with special reference to Bopyroides, Hemiarthrus, and Peseudione (Isopoda, Epicaridea). *Sarsia* **47**, 1-46. doi:10.1080/00364827.1971.10411191
- Strömberg, J.-O.** (1972). *Cyathura polita* (Crustacea, Isopoda), some embryological notes. *Bull. Mar. Sci.* **22**, 463-482.
- Sucena, É., Vanderberghe, K., Zhurov, V. and Grbić, M.** (2014). Reversion of developmental mode in insects: evolution from long germband to short germband in the polyembryonic wasp *Macrocentrus cingulum* Brischke. *Evol. Dev.* **16**, 233-246. doi:10.1111/ede.12086
- Sudler, M. T.** (1899). The development of *Penilia schmackeri* Richard. *Proc. Boston Soc. Nat. Hist.* **29**, 109-131.
- Sun, X. and Cheng, J.** (2023). Conflicts in mitochondrial phylogenomics of Branchiopoda, with the first complete mitogenome of *Laevicaudata* (Crustacea: Branchiopoda). *Curr. Issues Mol. Biol.* **45**, 820-837. doi:10.3390/cimb45020054
- Suvorov, A., Kim, B. Y., Wang, J., Armstrong, E. E., Peede, D., D'Agostino, E. R. R., Price, D. K., Waddell, P. J., Lang, M., Courtier-Orgogozo, V. et al.** (2022). Widespread introgression across a phylogeny of 155 *Drosophila* genomes. *Curr. Biol.* **32**, 111-123.e5. doi:10.1016/j.cub.2021.10.052
- Suzuki, N.** (1990). Embryology of the Mecoptera (Panorpidae, Panorpididae, Bittacidae and Boreidae). *Bull. Sugadaira Montane Res. Cent.* **11**, 1-87.
- Suzuki, N., Shimizu, S. and Ando, H.** (1981). Early embryology of the alderfly, *Sialis mitsuhashii* Okamoto (Megaloptera: Sialidae). *Int. J. Insect Morphol. Embryol.* **10**, 409-418. doi:10.1016/0020-7322(81)90021-0
- Tadokoro, R., Sugio, M., Kutsuna, J., Tochinali, S. and Takahashi, Y.** (2006). Early segregation of germ and somatic lineages during gonadal regeneration in the annelid *Enchytraeus japonensis*. *Curr. Biol.* **16**, 1012-1017. doi:10.1016/j.cub.2006.04.036
- Takamura, K., Fujimura, M. and Yamaguchi, Y.** (2002). Primordial germ cells originate from the endodermal strand cells in the ascidian *Ciona intestinalis*. *Dev. Genes Evol.* **212**, 11-18. doi:10.1007/s00427-001-0204-1
- Tanaka, M.** (1985). Early embryonic development of *Amata fortunei* (Lepidoptera, Arctidae). In *Recent Advances in Insect Embryology in Japan* (ed. H. Ando and K. Miya), pp. 139-155. Tsukuba: ISEBU.
- Tanaka, M.** (1987). Differentiation and behaviour of primordial germ cells during the early embryonic development of *Parnassius glacialis* Butler, *Luehdorfia japonica* Leech and *Byasa (Atrophaneura) alcinous* alcinous Klug (Lepidoptera: Papilionidae). In *Recent Advances in Insect Embryology in Japan and Poland* (ed. H. Ando and C. Jura), pp. 255-266. Tsukuba: ISEBU.
- Taube, E.** (1909). Beiträge zur Entwicklungsgeschichte der Euphausiden. I. Die Furchung des Eies bis zur Gastrulation. *Z. Wiss. Zool.* **92**, 427-464.
- Taube, E.** (1915). Beiträge zur Entwicklungsgeschichte der Euphausiden II. Von der Gastrula bis zum Furciliastadium. *Z. Wiss. Zool.* **114**, 577-656.
- Terao, A.** (1929). On the embryonic development of the spiny lobster, *Panulirus japonicus* (v. Siebold). *Jpn. J. Zool.* **2**, 387-449.
- Terao, A. and Cheng, T. Z.** (1926). On the rudiments of the gonad in an isopod, *Ligyda exotica* (Roux). *Annot. Zool. Japon.* **11**, 29-32.
- Thorpe, J. A. T.** (2024). Phylogenomics supports a single origin of terrestriality in isopods. *Proc. Roy. Soc. B* **291**, 20241042. doi:10.1098/rspb.2024.1042
- Tiegs, O. W.** (1940). The embryology and affinities of the Symphyla, based on a study of *Hanseniella agilis*. *J. Cell Sci.* **s2-82**, 1-208. doi:10.1242/jcs.s2-82.325.1
- Tiegs, O. W.** (1947). The development and affinities of the Paupoda, based on a study of *Paupopus silvaticus*, Part I. *J. Cell Sci.* **s3-88**, 165-267. doi:10.1242/jcs.s3-88.2.165
- Tiegs, O. W. and Murray, F. V.** (1938). The embryonic development of *Calandra oryzae*. *J. Cell Sci.* **s2-80**, 159-273. doi:10.1242/jcs.s2-80.318.159
- Thielka, E., Giacometti, M., Huang, D.-Y., Pisani, D., Donoghue, P. C. J. and Cai, C.-Y.** (2020). Fleas are parasitic scorpionflies. *Palaeoentomology* **3**, 641-653. doi:10.11646/palaeoentomology.3.6.16
- Tomizuka, S. and Machida, R.** (2015). Embryonic development of a collembolan, *Tomocerus cuspidatus* Börner, 1909: With special reference to the development and developmental potential of serosa (Hexapoda: Collembola, Tomoceridae). *Arthropod Struct. Dev.* **44**, 157-172. doi:10.1016/j.asd.2014.12.004
- Toyama, K.** (1902). Contributions to the study of silk-worms. I. On the embryology of the silk-worms. *Bull. Coll. Agric. Tokyo Imp. Univ.* **5**, 73-118.
- Trcek, T. and Lehmann, R.** (2019). Germ granules in *Drosophila*. *Traffic* **20**, 650-660. doi:10.1111/tra.12674
- Trcek, T., Grosch, M., York, A., Shroff, H., Lionnet, T. and Lehmann, R.** (2015). *Drosophila* germ granules are structured and contain homotypic mRNA clusters. *Nat. Commun.* **6**, 7962. doi:10.1038/ncomms8962
- Tsutsumi, K. and Machida, R.** (2006). Embryonic development of a snakefly, *Inocellia japonica* Okamoto: an outline (Insecta: Neuroptera, Raphidioidea). *Proc. Arthropod. Embryol. Soc. Jpn.* **41**, 37-45.
- Uemiyama, H. and Ando, H.** (1991). Mesoderm formation in a springtail, *Tomocerus ishishashii* Yosii (Collembola: Tomoceridae). *Int. J. Insect Morphol. Embryol.* **20**, 283-290. doi:10.1016/0020-7322(91)90017-4
- Ullmann, S. L.** (1964). The origin and structure of the mesoderm and the formation of the coelomic sacs in *Tenebrio molitor* L. [Insecta, Coleoptera]. *Philos. Trans. R. Soc. Lond. B* **248**, 245-277. doi:10.1098/rstb.1964.0012
- Underwood, E. M., Caulton, J. H., Allis, C. D. and Mahowald, A. P.** (1980). Developmental fate of pole cells in *Drosophila melanogaster*. *Dev. Biol.* **77**, 303-314. doi:10.1016/0012-1606(80)90476-5
- Vincent, K. A. and Hertzler, P. L.** (2018). Putative germ granular material is inherited by the primordial germ cell in *Marsupenaeus japonicus* (Spence Bate, 1888) (Decapoda: Dendrobranchiata: Penaeidae). *J. Crustac. Biol.* **38**, 267-277. doi:10.1093/jcbl/ryy001
- von Baldass, and F.** (1937). Entwicklung von *Holopedium gibberum*. *Zool. Jahrb. Anat.* **63**, 399-454.
- Wang, C. and Lehmann, R.** (1991). Nanos is the localized posterior determinant in *Drosophila*. *Cell* **66**, 637-647. doi:10.1016/0092-8674(91)90110-K
- Weismann, A.** (1863). Die Entwicklung der Dipteren im Ei, nach Beobachtungen an *Chironomus spec.*, *Musca vomitoria* und *Pulex canis*. *Zeit. wiss. Zool.* **13**, 107-220.
- Wenck, W.** (1914). Entwicklungsgeschichtliche Untersuchungen an Tardigraden (*Macrobiotus lacustris* Duj.). *Zool. Jahrb. Anat.* **37**, 465-514.
- West, J. A., Cantwell, G. E. and Shortino, T. J.** (1968). Embryology of the house fly, *Musca domestica* (Diptera: Muscidae), to the blastoderm stage. *Ann. Entomol. Soc. Am.* **61**, 13-17. doi:10.1093/aesa/61.1.13
- Weygoldt, P.** (1960). Embryologische Untersuchungen an Ostrakoden: Die Entwicklung von *Cyprideis litoralis* (G.S. Brady) (Ostracoda, Podocopa, Cytheridae). *Zool. Jahrb. Anat.* **78**, 369-426.
- Weygoldt, P.** (1964). Vergleichend-embryologische Untersuchungen an Pseudoscorpionien (Chelonethi). *Z. Morphol. Ökol. Tiere* **54**, 1-106. doi:10.1007/BF00404408
- Weygoldt, P.** (1975). Untersuchungen zur Embryologie und Morphologie der Geißelspinne, *Tarantula marginemaculata* C. L. Koch (Arachnida, Amblypygi, Tarantulidae). *Zoomorphologie* **82**, 137-199. doi:10.1007/BF00993587
- Wheeler, W. M.** (1893). A contribution to insect embryology. *J. Morphol.* **8**, 1-160. doi:10.1002/jmor.1050080102
- Whittle, C. A. and Extavour, C. G.** (2016). Refuting the hypothesis that the acquisition of germ plasm accelerates animal evolution. *Nat. Commun.* **7**, 12637. doi:10.1038/ncomms12637
- Whittle, C. A. and Extavour, C. G.** (2017). Causes and evolutionary consequences of primordial germ-cell specification mode in metazoans. *Proc. Natl. Acad. Sci. USA* **114**, 5784-5791. doi:10.1073/pnas.1610600114
- Wiegmann, B. M., Trautwein, M. D., Winkler, I. S., Barr, N. B., Kim, J.-W., Lambkin, C., Bertone, M. A., Cassel, B. K., Bayless, K. M., Heimberg, A. M. et al.** (2011). Episodic radiations in the fly tree of life. *Proc. Natl. Acad. Sci. USA* **108**, 5690-5695. doi:10.1073/pnas.1012675108
- Wiesmann, R.** (1926). Entwicklung und Organogenese der Cölomblasen. In *Zur Kenntnis der Anatomie und Entwicklungsgeschichte der Stabheuschrecke *Carausius morosus* Br.* (ed. H. Leuzinger, R. Wiesmann and F. E. Lehmann), pp. 124-321. Jena: Verlag von Gustav Fischer.
- Witschi, E.** (1934). On determinative cleavage and yolk formation in the harpacticoid copepod *Tisbe furcata* (Baird). *Biol. Bull.* **67**, 335-340. doi:10.2307/1537515
- Wolfe, J. M., Breinholt, J. W., Crandall, K. A., Lemmon, A. R., Lemmon, E. M., Timm, L. E., Siddall, M. E. and Bracken-Grissom, H. D.** (2019). A phylogenomic framework, evolutionary timeline and genomic resources for comparative studies of decapod crustaceans. *Proc. R. Soc. B* **286**, 20190079. doi:10.1098/rspb.2019.0079
- Wolff, C.** (2009). The embryonic development of the malacostracan crustacean *Porcellio scaber* (Isopoda, Oniscidea). *Dev. Genes Evol.* **219**, 545-564. doi:10.1007/s00427-010-0316-6
- Wolff, C. and Scholtz, G.** (2002). Cell lineage, axis formation, and the origin of germ layers in the amphipod crustacean *Orchestia cavimana*. *Dev. Biol.* **250**, 44-58. doi:10.1006/dbio.2002.0789
- Wolff, E.** (1962). *L'Origine De La Lignée Germinale Chez Les Vertébrés et chez Quelques Groupes D'invertébrés*. Paris, France: Éditions Hermann.
- Wong, T. K. F., Ly-Trong, N., Ren, H., Baños, H., Roger, A. J., Susko, E., Bielow, C., Maio, N. D., Goldman, N., Hahn, M. W. et al.** (2025). IQ-TREE

- 3: Phylogenomic inference software using complex evolutionary models. *EcoEvoRxiv*. doi:10.32942/x2p62n
- Woodland, J. T.** (1957). A contribution to our knowledge of lepidematid development. *J. Morphol.* **101**, 523-577. doi:10.1002/jmor.1051010306
- Woodworth, C. W.** (1889). Studies on the embryological development of *Euvanessa antiopa*. In *The Butterflies of The Eastern United States and Canada, With Special Reference to New England* (ed. S. H. Scudder), pp. 95-104.
- Wotton, K. R., Jiménez-Guri, E., Matheu, B. G. and Jaeger, J.** (2014). A staging scheme for the development of the scuttle fly *Megaselia abdita*. *PLoS ONE* **9**, e84421. doi:10.1371/journal.pone.0084421
- Wray, D. L.** (1937). The embryology of *Calendra callosa* Olivier; the southern corn billbug (Coleoptera, Rhynchophoridae). *Ann. Entomol. Soc. Am.* **30**, 361-409. doi:10.1093/aesa/30.2.361
- Xu, X., Brechbiel, J. L. and Gavis, E. R.** (2013). Dynein-dependent transport of nanos RNA in *Drosophila* sensory neurons requires Rumpelstiltskin and the germ plasm organizer oskar. *J. Neurosci.* **33**, 14791-14800. doi:10.1523/JNEUROSCI.5864-12.2013
- Yajima, M. and Wessel, G. M.** (2011). The DEAD-box RNA helicase Vasa functions in embryonic mitotic progression in the sea urchin. *Development* **138**, 2217-2222. doi:10.1242/dev.065052
- Yang, Z.** (1997). PAML: a program package for phylogenetic analysis by maximum likelihood. *Bioinformatics* **13**, 555-556. doi:10.1093/bioinformatics/13.5.555
- Yang, Z.** (2007). PAML 4: phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol.* **24**, 1586-1591. doi:10.1093/molbev/msm088
- Ying, Y. and Zhao, G.-Q.** (2001). Cooperation of endoderm-derived BMP2 and extraembryonic ectoderm-derived BMP4 in primordial germ cell generation in the mouse. *Dev. Biol.* **232**, 484-492. doi:10.1006/dbio.2001.0173
- Ying, Y., Liu, X. M., Marble, A., Lawson, K. A. and Zhao, G. Q.** (2000). Requirement of Bmp8b for the generation of primordial germ cells in the mouse. *Mol. Endocrinol.* **14**, 1053-1063. doi:10.1210/mend.14.7.0479
- Yoon, Y., Klomp, J., Martin-Martin, I., Criscione, F., Calvo, E., Ribeiro, J. and Schmidt-Ott, U.** (2019). Embryo polarity in moth flies and mosquitoes relies on distinct zold genes with localized transcript isoforms. *eLife* **8**, e46711. doi:10.7554/eLife.46711
- Yu, D., Du, S., Wei, X., Zhu, J., Ding, Y., Hu, F., Liu, M. and Zhang, F.** (2024). Whole-genome-based phylogenetic analyses provide new insights into the evolution of springtails (Hexapoda: Collembola). *Mol. Phylogenet. Evol.* **200**, 108169. doi:10.1016/j.ympev.2024.108169
- Zhang, S.-Q., Che, L.-H., Li, Y., Dan, L., Pang, H., Ślipiński, A. and Zhang, P.** (2018). Evolutionary history of Coleoptera revealed by extensive sampling of genes and species. *Nat. Commun.* **9**, 205. doi:10.1038/s41467-017-02644-4
- Zhurov, V., Terzin, T. and Grbic, M.** (2004). Early blastomere determines embryo proliferation and caste fate in a polyembryonic wasp. *Nature* **432**, 764-769. doi:10.1038/nature03171
- Zissler, D.** (1992). From egg to pole cells: ultrastructural aspects of early cleavage and germ cell determination in insects. *Microsc. Res. Tech.* **22**, 49-74. doi:10.1002/jemt.1070220106
- Zissler, D. and Sander, K.** (1973). The cytoplasmic architecture of the egg cell of *Smittia* spec. (Diptera, Chironomidae). I. Anterior and posterior pole regions. *Wilhelm Roux Arch. Entwickl. Mech. Org.* **172**, 175-186. doi:10.1007/BF00582073

## Supplementary Materials and Methods

Throughout these Methods, custom scripts ([blue text](#)) and output files and folders ([magenta text](#)) described are located at the github repository for this study, [https://github.com/rishabhraj Kapoor/panarthropoda\\_gc\\_specification\\_evolution](https://github.com/rishabhraj Kapoor/panarthropoda_gc_specification_evolution), commit ID ff9945c. Analysis packages used are indicated in [green text](#). Publicly available scripts used are indicated in [gold text](#).

### *Inferring germ cell specification mechanisms from published literature*

For several species, experimental manipulations have provided direct evidence for the use of either germ plasm or induction (Table S1). For other species with only observational data available in published literature, we inferred the likely mechanism of specification for the ancestral character reconstruction based on patterns in the developmental timing and location of germ cell origination. For species designated as Category 1, with germ cells forming in the early blastoderm and cleavage stages, we inferred that they were using germ plasm. For species designated as Category 3, with germ cells forming late in embryogenesis from the mesoderm, we inferred that they were using induction. For Category 2 species, we inferred the use of germ plasm for species in which granules with the characteristic morphological and spatiotemporal appearance of germ granules (e.g. Eddy, 1975) were reported; otherwise, we inferred induction. Our inferred mechanisms are available in Table S1 and in [all\\_species\\_annotated.tsv](#).

### *Phylogram construction*

To visualize the phylogenetic relationships among the 211 species in our dataset of germ cell specification data (Table S1), we concatenated published phylogenies from 23 pre-existing studies of various panarthropod lineages (Table S2) (using notebook [phylogram\\_constraints.ipynb](#), output file [constraint\\_trees/all\\_combined\\_species.nwk](#)). Newick trees were obtained directly from the published studies, from the TimeTree (Kumar et al., 2022) or from the Open Tree of Life (OpenTreeOfLife et al., 2019) websites when available, or were manually extracted from published figures. Individual Newick trees are available in the “[constraint\\_trees](#)” directory. Phylogenetic relationships were delimited up to the level of taxonomic family, except for the families Chrysomelidae, Culicidae, and Drosophilidae, which were further resolved to the subfamily, genus, and species levels, respectively, due to the greater representation of these families in our dataset. We used polytomies to represent intrafamily relationships, paraphyly in inter-family relationships, or unresolved nodes in the arthropod phylogeny. Phylograms were taxonomically annotated with the [ete3](#) package (Huerta-Cepas et al., 2016), manually concatenated, and visualized in the iTOL web server (Letunic and Bork,

2024). Branch lengths in the resulting phylograms (Figures 2-3, Figure S1) are arbitrary and do not reflect evolutionary divergence times.

#### *Ancestral character reconstruction via maximum parsimony*

Ancestral character reconstruction of inferred germ cell specification modes (GP: germ plasm; IND: induction) via parsimony was performed on the above phylogram with the GUI interface of Mesquite version 4.0 using default parameters (Figure S1; see Table S3 for hypothesized transitions; input nexus file with GP/IND annotations available at [GC\\_mech\\_parsimony\\_input.nex](https://www.mesquiteproject.org)) (<https://www.mesquiteproject.org>; version 4.0). The results inferred that the ancestral panarthropod specification mode was induction, with a minimum of 24 changes over the history of the panarthropods. When considering all equally parsimonious character histories, changes from induction to maternal inheritance (min = 18, max = 23, average = 20.5) were the most common, but reverse transitions were also inferred (min = 2, max = 7, average = 4.5). At a minimum, reversions from maternal inheritance to induction were inferred in the honeybee *A. mellifera* (Hymenoptera) and the drain fly *C. albipunctata* (Diptera). We note that all maximum parsimony reconstructions assign induction as the ancestral state of Holometabola (Figure S1, node 11), which requires many fewer total transitions within Holometabola (N = 12) than would be required if germ plasm evolved a single time in the last common ancestor of Holometabola (N = 20) as previously proposed (Ewen-Campen et al., 2013a; Lynch et al., 2011).

#### *Maximum likelihood phylogenetic tree inference*

Ancestral character reconstruction requires as an input a phylogenetic tree in which branch lengths are proportional to evolutionary divergence (executed with [time\\_calibrated\\_busco\\_phylogenomics.ipynb](#)). To obtain genomic data for phylogenetic inference, we searched NCBI for genomes or transcriptome shotgun assemblies (TSAs) corresponding to our taxa of interest, filtering by >50% BUSCO (Manni et al., 2021) single copy complete ortholog completeness (with arthropoda\_odb12, using the “Download and prep protein datasets” portion of [time\\_calibrated\\_busco\\_phylogenomics.ipynb](#)) (Table S4). We selected sequence data from exact species matches when available (N = 77 species), else substituted sequence data from a different species in the same taxonomic family (N = 45 substitutions) or order (N = 2 substitutions) when the germ cell specification mechanism was the same for all species in the family or order respectively in our dataset (Table S1). In the case of a substitution, the leaf name in the phylogenetic tree was replaced by the name of the family or order shared with the species in our dataset (Figure S2).

We used BUSCO version 5.8.3 with the arthropoda\_odb12 set (Manni et al., 2021) to extract single-copy protein ortholog sequences. Upstream of BUSCO, protein predictions were generated for TSAs using TransDecoder version 5.7.1 (using

[transdecoder.sh](#) and [run\\_busco\\_prot\\_tsa.sh](#)) (Haas et al., 2013) or extracted from .fa files for annotated genomes in NCBI, selecting the longest splice isoform per gene (using the “Download and prep protein datasets” portion of [time\\_calibrated\\_busco\\_phylogenomics.ipynb](#) and [run\\_busco\\_annotated\\_genome.sh](#)). For unannotated genes, protein sequences were extracted directly from nucleotide fasta files using BUSCO with the “-m genome” flag. BUSCO protein sequences were submitted to the BUSCO\_phylogenomics pipeline (release 20240919) ([https://github.com/jamiemcg/BUSCO\\_phylogenomics](https://github.com/jamiemcg/BUSCO_phylogenomics)) with default parameters to generate a concatenated supermatrix of trimmed MUSCLE alignments (Edgar, 2004) (output supermatrix: [BUSCO\\_py/supermatrix/SUPERMATRIX.phylip](#)).

Deep-branching panarthropod relationships have been comprehensively examined by several prior studies and are often susceptible to reconstruction artifacts (Giribet, 2018; Meusemann et al., 2020). We therefore used the same literature-derived tree topologies (Table S2) used for phylogram construction to constrain the family, order, class, and phylum-level relationships in our trees when these ranks had previously been shown to be monophyletic (constraint tree at [BUSCO\\_py/constraint\\_tree\\_iqtree\\_species.tree](#)). Polytomies in our constraint tree were resolved via maximum likelihood inference from our supermatrix with IQ-Tree version 2.4 (model: LG+G4, with per-gene partitions) (Minh et al., 2020) (executed with [run\\_iqtree\\_constrained\\_search.sh](#), outputs in [BUSCO\\_py/supermatrix with the “constrained\\_optimized” prefix](#)). The resulting maximum likelihood tree was rooted with Tardigrada as an outgroup (Howard et al., 2022).

### *Time calibration*

Approximate likelihood molecular clock calibrations of our IQ-Tree maximum likelihood tree were performed using the IQ2MC workflow (Demotte et al., 2025), which integrates IQ-Tree version 3.0.1 (Wong et al., 2025) with MCMCTree in PAML version 4.10.9 (Yang, 1997; Yang, 2007) (using [time\\_calibrated\\_busco\\_phylogenomics.ipynb](#)). The fossil calibrations were derived from references listed in Table S5, including (Wiegmann et al., 2011) for Diptera, (Misof et al., 2014) for non-dipteran insects, (Bernolet et al., 2023) for non-insect Pancrustacea, and (Howard et al., 2022) for Chelicerata and the internal nodes of the panarthropod phylogeny (rooted constraint tree available at [BUSCO\\_py/supermatrix/fossil\\_rooted\\_constrained\\_optimized.treefile](#)). Hessian matrices and gradient vectors were obtained using IQ-Tree with the “-mcmctree” flag, the pre-computed supermatrix and maximum-likelihood tree, an LG+G4 substitution model, and a single partition to promote computational efficiency (using [iqtree3\\_iq2mcmc.sh](#), outputs available at [BUSCO\\_py/supermatrix/ with prefix “mcmc\\_prep\\_no\\_partition”](#)). MCMCTree was then run with an independent rates relaxed molecular clock, burnin = 20000, sampfreq = 100 and nsample = 20000. Convergence of inferred divergence times was confirmed via two independent runs of MCMCTree

(PAML control files at [BUSCO\\_py/supermatrix/run1R\\_mcmc\\_prep\\_no\\_partition.mcmctree.ctl](#) and [BUSCO\\_py/supermatrix/run2R\\_mcmc\\_prep\\_no\\_partition.mcmctree.ctl](#), output files in the same directory with [mcmctree.out](#) suffix, selected time-calibrated Newick tree at [mcmctree.tree](#)).

#### *Maximum likelihood ancestral character reconstruction*

We used the fossil-calibrated phylogeny of 124 species to infer ancestral states via marginal maximum likelihood using the `phytools` package (Revell, 2024) (using [maximum\\_likelihood\\_ancestral\\_reconstruction.R](#)). Five *Mk* substitution models were fit to the data, including models allowing for gamma heterogeneity in rates among branches or hidden rate transitions to account for possibly divergent evolutionary regimes under the deep time scales analyzed in our data (Table S6). The best-fitting model by the Akaike information criterion was the symmetric rates model with gamma-rates heterogeneity (Table S6), suggesting that although the overall rate of transition from germ plasm to induction is equivalent to the rate of the reverse transition, the value of this single rate parameter shows variation over the phylogeny (Revell and Harmon, 2024). The equivalent rates of transition to and from germ plasm are consistent with the reversions to induction noted in the parsimony analysis above, although we note that the quantification of evolutionary rate was only possible with our time-calibrated phylogeny. The rate heterogeneity is consistent with the observation via maximum parsimony of 15/24 transitions in Insecta alone (Figure S1). Inferred transitions with support from maximum likelihood are reported in Table S3.

#### *Inferring germ band development type from published literature*

We classified species by germ band type based on embryological descriptions as follows: in short germ band embryos, the initial germ band comprises only head segments; in intermediate germ band embryos, the initial germ band comprises the head and thorax; and in long germ band embryos, the initial germ band comprises head, thorax and abdominal segments.

#### *Testing the relationship between germ band development type and germ cell specification mode*

To test the hypothesis that long vs. short germ band development (Krause, 1939) influences the evolution of germ plasm in insects, we first performed phylogenetic logistic regression (Ives and Theodore, 2010) with `phylolm` (version 2.6.5 `phylolm`, method = `logistic_MPLE`), in which the independent variable was germ band type (three-level categorical predictor with “short” as the reference level and “long” and “intermediate” as two alternatives) and germ cell specification mode was the dependent variable (induction = 0, germ plasm = 1; Figure S3) (using [germ\\_band\\_correlations.R](#),

with the phylogeny in [insect\\_only.tree](#) and data in [insect\\_germ\\_band.tsv](#)). We found a statistically significant association between long germ band development and germ plasm (coefficient = 3.02, p-value = 2.81e-05) and no such association between intermediate germ band development and germ plasm (coefficient = -0.01, p-value = 0.99).

Since Pagel's test (Pagel, 1994) is performed on binary variables, we recoded all "intermediate" germ band annotations as "short" to produce a binary trait (using `germ_band_correlations.R`). The null model assumes that the rate of evolution of germ cell development mode is independent of the transition in long vs. short germ band development. Our three alternative hypotheses assume either that germ band type and germ cell specification mode mutually influence each other's evolution ("bidirectional"), or that germ band type influences germ cell specification mode but not vice versa ("GC dependent"), or that germ cell specification mode influences germ band type but not vice versa ("GB dependent"). Maximum likelihood rate fits for the bidirectional model are shown in Figure S4.

All Pagel covariation models assuming coupled evolutionary rates among these two characters are significantly better fits to the data than the null model of independent evolution (likelihood ratio test p-value < 1e-3; Figure S4; Table S8). We note that the bidirectional and GB-dependent models perform similarly to the GC-dependent model.

### Search for oskar orthologs

We searched the genomes and transcriptomes listed in Table S4 using `hmmsearch` (<http://eddylab.org/software/hmmer/Userguide.pdf>) with the consensus profile HMMs for the LOTUS (LOTUS\_CONSENSUS.hmm) and OSK (OSK\_CONSENSUS.hmm) domains as previously defined (Blondel et al., 2021) (using [oskar\\_search.ipynb](#), `hmmsearch` outputs are in the [hmmsearch\\_results](#) folder). For this *oskar* ortholog search, a chromosome-level, unannotated genome assembly (GCA\_965637365.1) for *Clogmia albipunctata* that became available at the time of performing this analysis was substituted for the genome assembly for this species that is listed in Table S4 (GCA\_022818195.1). Following our previous approach (Blondel et al., 2021), we defined Oskar orthologs as proteins that were `hmmsearch` (E-value < 0.05) hits to both the LOTUS and OSK domains. Amino acid sequences of all detected Oskar proteins are available in [oskar\\_proteins.fa](#) file b.

For unannotated genomes, we first obtained *ab initio* protein predictions using the AUGUSTUS webserver with default parameters (Stanke et al., 2004). Pre-trained species parameters for gene model prediction were selected from close phylogenetic relatives in the AUGUSTUS web server as follows: *Drosophila melanogaster* for *C. albipunctata* (GCA\_022818195.1) and *Mayetiola destructor* (GCA\_000149185.1); *Nasonia vitripennis* for *Macrocentrus cingulum* (GCA\_045786645.1); *Heliconius melpomene* for *Epiphyas postvittana* (GCA\_964304675.1) and *Nymphalis antiopa*

(GCA\_964258955.1); *Rhodnius prolixus* for *Pyrrhocoris apterus* (GCA\_039877355.1); and *Tribolium castaneum* for *Tribolium confusum* (GCA\_029207805.1).

Due to the unique position of *C. albipunctata* (family: Psychodidae) as the sole dipteran lacking germ plasm in our dataset, and its lacking a published annotated genome, we additionally tested the hypothesis that this species' genome lacks *oskar* via tblastn (blast version 2.9.0) with the *Phlebotomus papatasi* (family: Psychodidae) Oskar protein (XP\_055701593.1) as a query (using [oskar\\_search.ipynb](#)). We found no full-length tblastn hits but did find a possible hit to the OSK domain with evidence of multiple in-frame stop codons (Figure S5), consistent with the hypothesis of pseudogenization of *oskar* in *C. albipunctata*.

### *Testing the relationship between oskar presence and germ cell specification mode*

Since gene loss in metazoans is thought to be irreversible (Elmer and Clobert, 2025) and Pagel's test (Pagel, 1994) permits bidirectional state transitions, we opted only for phylogenetic logistic regression with phylolm (method logistic\_MPLE) to test the association between germ cell specification mode (dependent variable, 1 = germ plasm, 0 = induction) and *oskar* presence (independent variable, 1 = presence, 0 = absence) within insects while accounting for phylogenetic relatedness. We analyzed all 81 insect datasets shown in Table S9, including 24 within-family substitutions when genomic or transcriptomic data were not available for a species (using [oskar\\_fitphyloglm.R](#) with [oskar\\_data.tsv](#) and [insect\\_only.tree](#) as inputs).

We found no association of germ-plasm mode with BUSCO completeness (coefficient = 0.23, p-value = 0.37). However, *oskar* presence (Table S9) was moderately (although not statistically significantly) associated with BUSCO completeness (mean BUSCO completeness for *oskar*-containing datasets = 83.29%, mean BUSCO completeness for *oskar*-absent datasets = 78.09%, Mann-Whitney U p-value = 0.08). We therefore additionally included the z-score normalized BUSCO score as a covariate in our model

The results showed a significant association between *oskar* presence in the genome and the use of germ plasm to specify PGCs (coefficient = 2.58, adjusted odds-ratio: = 13.28, p-value = 0.0006). The association between *oskar* presence and maternal inheritance mode remained strong and statistically significant even after removing 24 within-family substitutions made when neither genomic nor transcriptomic data were available for a species (coefficient = 2.56, adjusted odds ratio = 12.93, p-value = 0.0003).

### *Tree visualization*

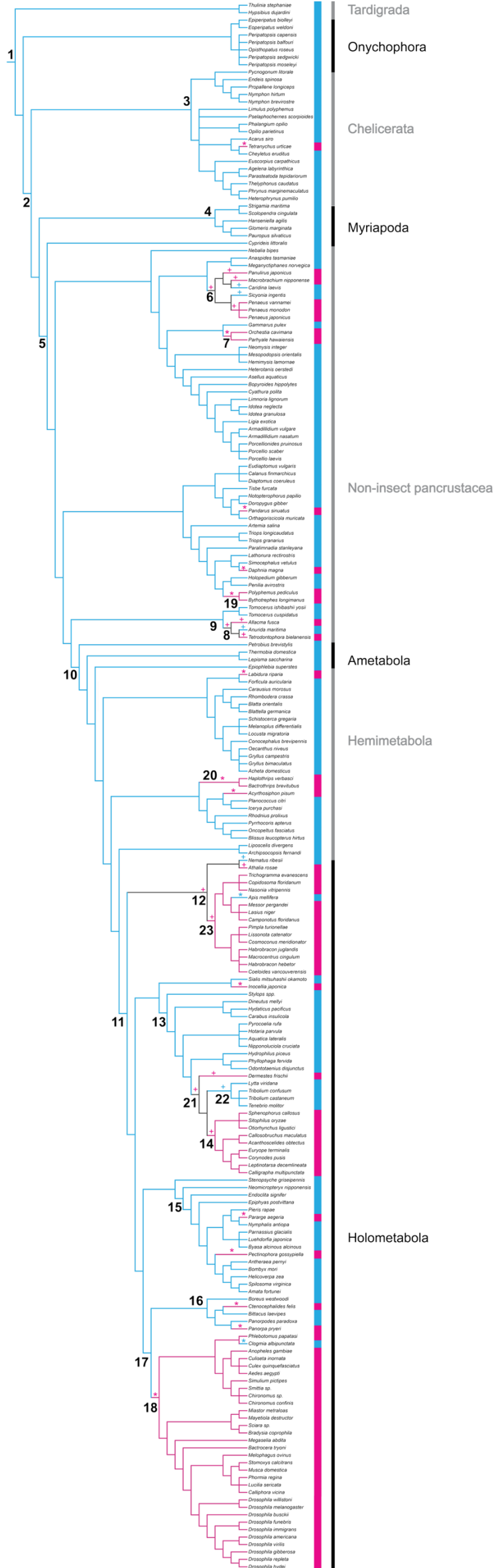
Trees were visualized and annotated using the iTOL web server v7 (Letunic and Bork, 2024), except for Figure S1 and Figure S2, which were generated as outputs of Mesquite and phytotools, respectively.

**Inferred mechanism**

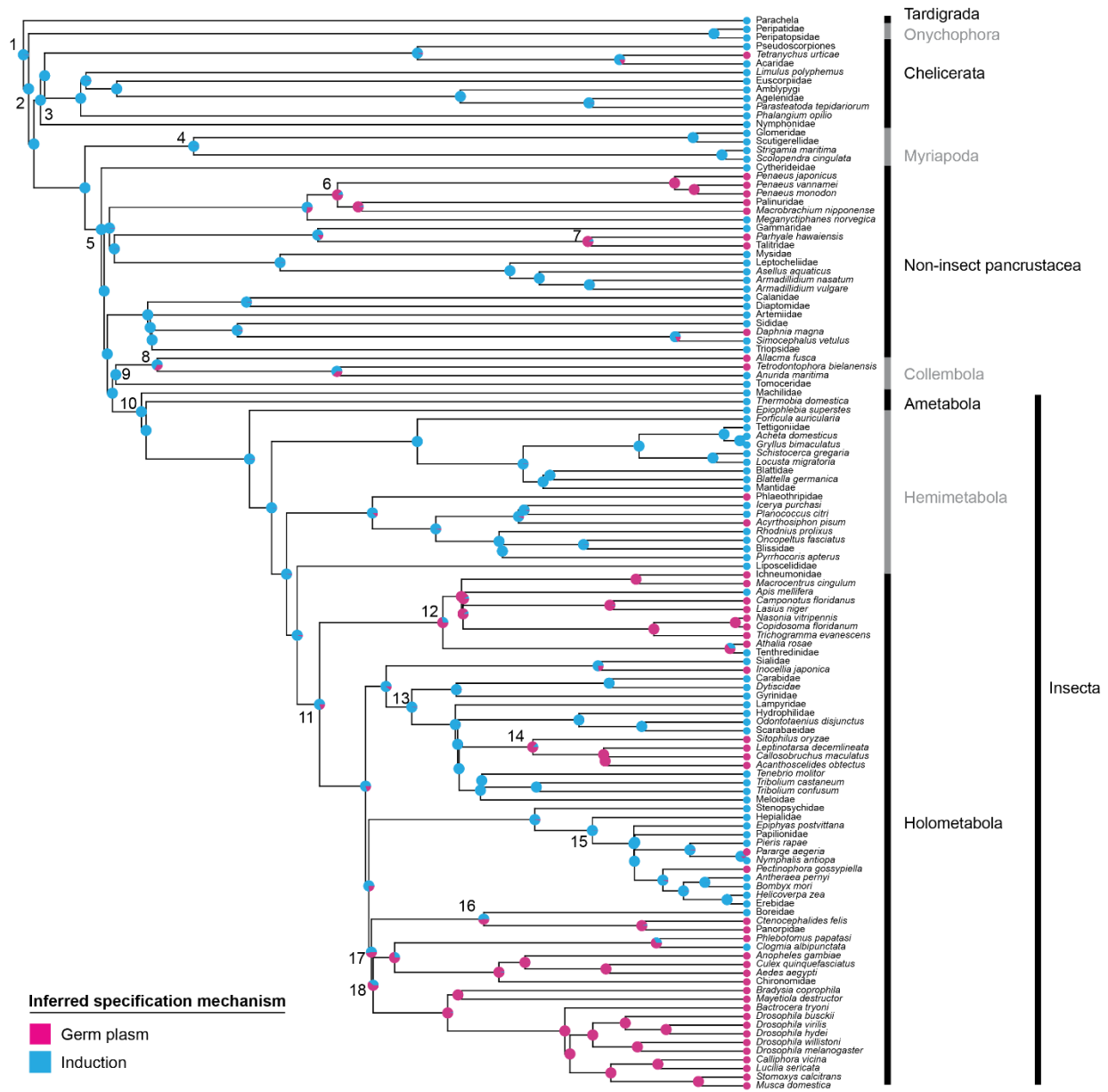
- Germ plasm
- Induction
- Ambiguous

**Inferred ancestral transition**

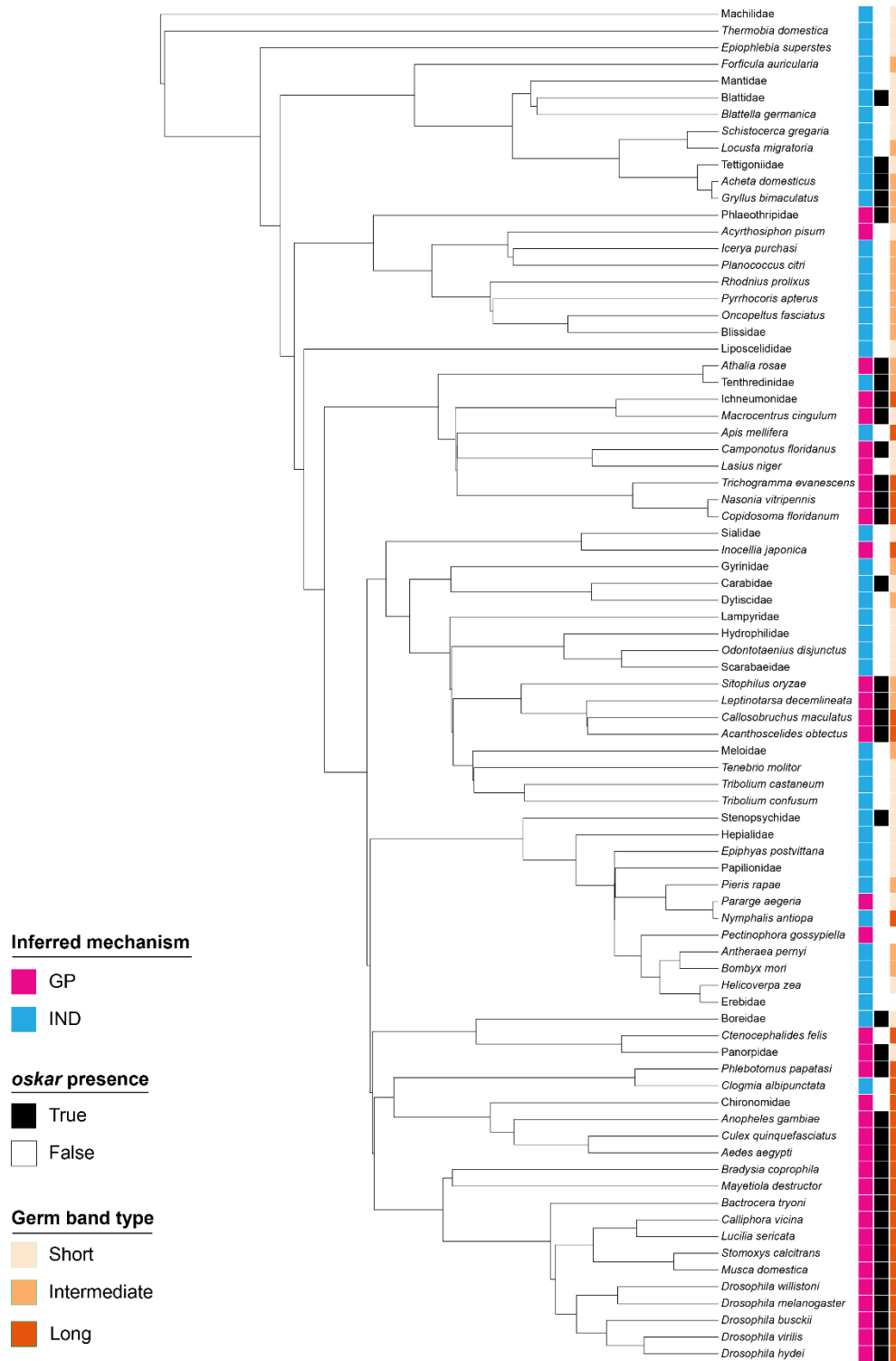
- \* Germ plasm gain
- \* Germ plasm loss
- + Germ plasm gain (ambiguous)
- + Germ plasm loss (ambiguous)



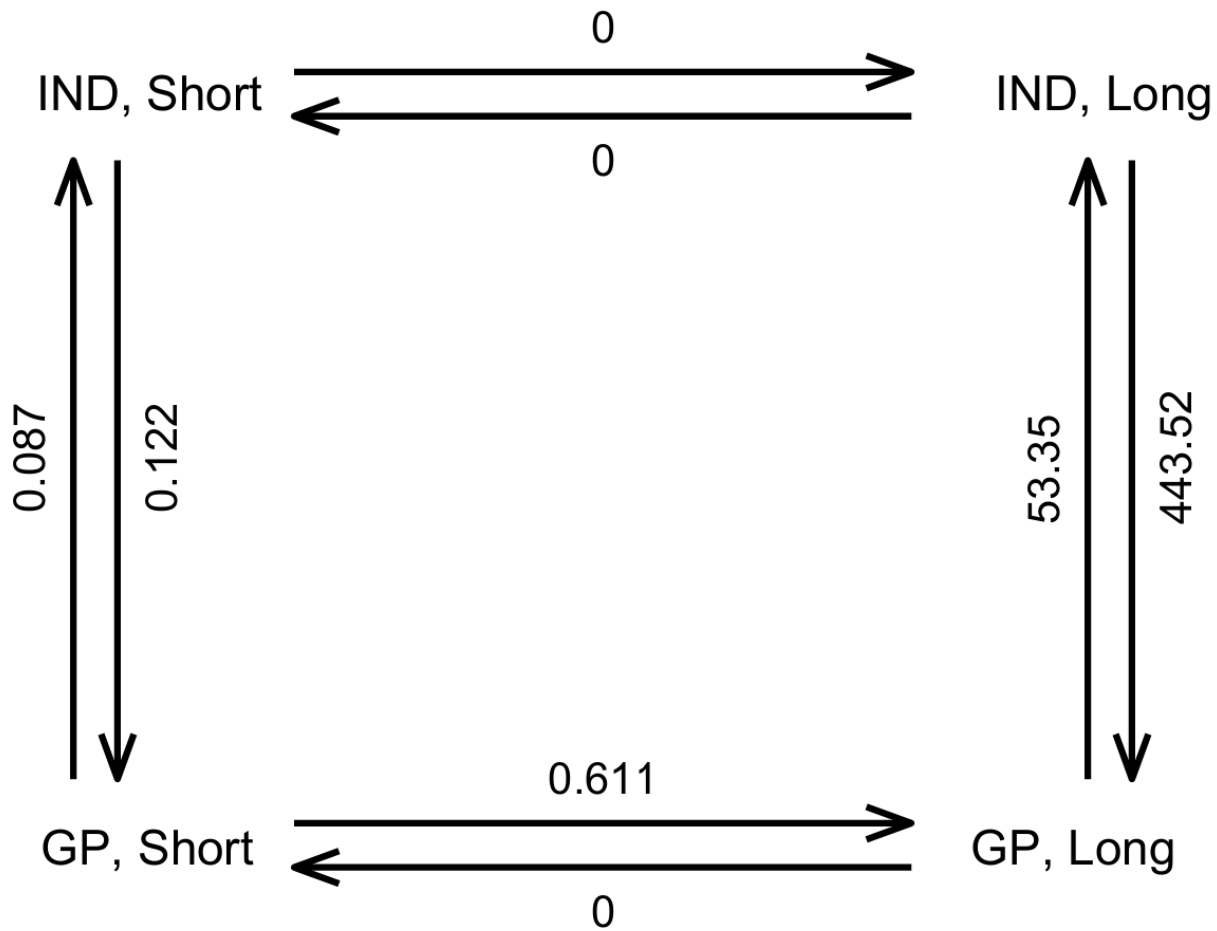
**Fig. S1. Phylogram with maximum parsimony ancestral state inference.** Phylogram of the 211 species for which we compiled germ cell specification data in this study (Table S1), with select inner nodes numbered for reference (1 = Panarthropoda; 2 = Arthropoda; 3 = Chelicerata; 4 = Myriapoda; 5 = Pancrustacea; 6 = Decapoda; 7 = Hyalidae + Talitridae; 8 = Poduromorpha + Symphypleona; 9 = Collembola; 10 = Insecta; 11 = Holometabola; 12 = Hymenoptera; 13 = Coleoptera; 14 = Chrysomelidae + Curculionidae; 15 = Lepidoptera; 16 = Mecoptera + Siphonaptera; 17 = Antliophora (Diptera + Mecoptera + Siphonaptera); 18 = Diptera; 19 = Onychopoda; 20 = Thysanoptera; 21 = Meloidae + Tenebrionidae + Dermestidae + Chrysomelidae; 22 = Meloidae + Tenebrionidae). Species are labeled with blue or pink boxes to indicate induction or germ plasm, respectively. Internal branches are colored according to inferred ancestral states, with grey indicating that multiple equally parsimonious ancestral states are possible. Asterisks indicate unambiguous transitions (gains or losses of germ plasm); i.e., no other transition at these nodes would result in a parsimonious character history. Plus (+) signs indicate possible transitions at nodes with ambiguous character states. Note that some ambiguous character histories are mutually exclusive: e.g., one parsimonious scenario for Decapoda (node 6) is a gain of germ plasm at node 6 followed by terminal losses in the branches leading to *Caridina laevis* and *Sicyonia ingentis*, while another equally parsimonious but mutually exclusive scenario would be independent gains in *Panulirus japonicus*, *Macrobrachium nipponense*, and the common ancestor of the genus *Penaeus*.



**Fig. S2. Maximum likelihood ancestral state reconstruction.** Maximum likelihood inference was performed under the symmetric transition model with gamma rate heterogeneity. Tips are labeled with colored circles according to observed induction (blue) or germ plasm (pink) mode. Pie charts at internal nodes report marginal probabilities of induction or germ plasm as the ancestral state. Select ancestral nodes are numbered with clade names and probabilities reported in Table S7. Leaf names at the family or order levels indicate a substitution for tree construction.



**Fig. S3. Relationship between germ cell specification mode (pink/blue), *oskar* presence (black/white), and germ band type (orange shades).** Time-calibrated phylogeny of the insect species with genomic/transcriptomic data available at the time of writing (N = 81; Table S9). Leaf names at the family level indicate a substitution for tree construction and inference of *oskar* presence or absence.



**Fig. S4. Pagel test result.** Maximum likelihood rate fits for Pagel’s test of a bidirectional dependency between transition rates in germ cell specification mode and germ band mode. GP = germ plasm; IND = induction; Short = short germ band; Long = long germ band. A relationship between germ plasm and long germ band modes is supported by the higher transition rate from “GP, Short” to “GP, Long” (0.611) than from “GP, Long” to “GP, Short” (0) or from “IND, Short” to “IND, Long” (0). Likewise, the transition rate from “IND, Long” to “GP, Long” (443.52) is higher than the transition rate from “GP, Long” to “IND, Long” (53.55). The dependent rates model better fits the data than an independent rates model (Table S8).

```
Query 236 TDYNRLLVLLKEMGIQAIVTTLPPIANHSYLSSMQHNWMKLNFLRRQPLCIDICEL 292
      T ++ +L+LL E+ + + P I + S++Q W +F++ Q LC D+C L
Sbjct 42289309 TKWSTILILLIEVFQSGQIFSFPYI*SSLC*SAVQRPWFPLIFVKCQKLCFDLCSL 42289479
```

**Fig. S5. tblastn results of Oskar against the *Clogmia albipunctata* genome.** The sole tblastn hit of *Phlebotomus papatasi* Oskar protein (XP\_055701593.1) against the *C. albipunctata* genome (version GCA\_965637365.1), showing a partial hit on chromosome 2 (accession OZ281279.1) to the OSK domain.

**Table S1.** Data on the timing and mechanism of germ cell origin across Panarthropoda. The “GP?” column designates whether observational (OB) or experimental (EX) data have supported the existence of germ plasm. The “IND?” column designates whether observational (OB) or experimental (EX) data have supported induction as the mechanism of germ cell specification. The “timing” column describes the timing of germ cell origination during embryogenesis and categorizes the timing as (1) blastoderm formation, (2) gastrulation, or (3) mesoderm differentiation. The “location” column describes the spatial position of germ cells when they are first reported as detectable. The “criteria” column lists the nature of the data used to identify germ cells and infer mechanism of specification: LM = light microscopy, TEM = transmission electron microscopy, SEM = scanning electron microscopy, MM = molecular markers, CL = cell lineage tracing, TS = transcriptomic data, EX = experimental manipulations.

order	suborder	family	scientific name	common name	GP?	IND?	timing	location	criteria	reference
<b>TARDIGRADA</b>										
Parachela		Hypsibiidae	<i>Thulinia stephaniae</i>			EX	(2) cleavage	blastopore	LM, CL, EX (laser ablation)	(Hejnal and Schnabel, 2005)
Parachela		Hypsibiidae	<i>Hypsibius dujardini</i>				(2) cleavage	blastopore	LM, MM ( <i>piwi</i> , <i>vasa in situ</i> ), CL	(Gabriel et al., 2007; Heikes et al., 2023; Wenck, 1914)
<b>ONYCHOPHORA</b>										
		Peripatidae	<i>Eoperipatus weldoni</i>				(3) late segmented germ band	posterior germ band (walls of splanchnic mesoderm)	LM	(Evans, 1901)
		Peripatidae	<i>Epiperipatus biolleyi</i>				(3) early segmented germ band	posterior germ band	TEM	(Mayer and Tait, 2009)
		Peripatopsidae	<i>Opisthopatus roseus</i>				(3) early segmented germ band	posterior germ band	TEM	(Mayer and Tait, 2009)
		Peripatopsidae	<i>Peripatopsis capensis</i>				(3) segmented germ band	posterior germ band	LM	(Manton, 1949; Sedgwick, 1887)
		Peripatopsidae	<i>Peripatopsis balfouri</i>				(3) segmented germ band	posterior germ band	LM	(Manton, 1949)
		Peripatopsidae	<i>Peripatopsis moseleyi</i>				(2) gastrulation	blastopore	LM	(Manton, 1949)
		Peripatopsidae	<i>Peripatopsis sedgwicki</i>				(3) segmented germ band	posterior germ band	LM	(Manton, 1949)
<b>CHELICERATA</b>										
Pantopoda	Colossendeoidea	Pycnogonidae	<i>Pycnogonum litorale</i>				(3) fifth instar	flanking the midgut	LM, TEM, SEM	(Alexeeva and Tamberg, 2021)
Pantopoda	Phoxychilidioidea	Endeidae	<i>Endeis spinosa</i>				(3) sixth instar	mesoderm surrounding midgut	LM	(Dogiel, 1913)
Pantopoda	Nymphonoidea	Nymphonidae	<i>Nymphon hirtum</i>				(3) sixth instar	mesoderm surrounding midgut	LM	(Dogiel, 1913)
Pantopoda	Nymphonoidea	Nymphonidae	<i>Nymphon brevirostre</i>				(3) third instar	flanking the midgut	LM, TEM, SEM	(Alexeeva et al., 2018)
Pantopoda	Nymphonoidea	Callipallenidae	<i>Propallene longiceps</i>				(3) third instar	flanking the midgut	LM	(Miyazaki and Makioka, 2012)
Xiphosura		Limulidae	<i>Limulus polyphemus</i>	Atlantic horseshoe crab			(3) segmented germ band	dorsal side of coelomic cavities	LM	(Kingsley, 1893)
Trombidiformes		Cheyletidae	<i>Cheyletus eruditus</i>				(3) segmented germ band	ventral mesoderm	LM	(Hafiz, 1935)
Trombidiformes		Tetranychidae	<i>Tetranychus urticae</i>	red spider mite, two-spotted spider mite	OB		(1) blastoderm	randomly distributed in yolk	LM, MM ( <i>vasa in situ</i> )	(Dearden et al., 2003)
Sarcoptiformes		Acaridae	<i>Acarus siro</i>	flour mite			(3) segmented germ band	behind third larval leg	LM	(Hughes, 1950)
Opiliones	Eupnoi	Phalangidae	<i>Opilio parietinus</i>				(2) early germ band	posterior germ band/blastopore	LM	(Holm, 1947)
Opiliones	Eupnoi	Phalangidae	<i>Phalangium opilio</i>				(2) early germ band	posterior germ band/blastopore	LM, MM ( <i>vasa in situ</i> )	(Faussek, 1888; Faussek, 1892; Gainett et al., 2022)
Scorpiones		Euscorpidae	<i>Euscorpis carpathicus</i>				(2) early germ band	posterior germ disc/blastopore	LM	(Brauer, 1894)
Pseudoscorpiones		Chernetidae	<i>Pselaphochernes scorpoides</i>				(3) segmented germ band	mesoderm surrounding posterior midgut	LM	(Weygoldt, 1964)
Amblypygi		Phryniidae	<i>Heterophrynus pumilio</i>				(3) segmented germ band	ventral walls of mesodermal coelomic sacs	LM	(Gough, 1902)
Amblypygi		Phryniidae	<i>Phrynus marginemaculatus</i>	spotted tailless whip scorpion			(3) segmented germ band	ventral walls of mesodermal coelomic sacs	LM	(Weygoldt, 1975)
Uropygi		Thelyphonidae	<i>Thelyphonus caudatus</i>				(3) segmented germ band	ventral walls of mesodermal coelomic sacs	LM	(Schimkewitsch, 1903)
Araneae	Araneomorphae	Theridiidae	<i>Parasteatoda tepidariorum</i>	common house spider			(3) segmented germ band	ventral walls of mesodermal coelomic sacs	LM, MM ( <i>vasa</i> , <i>piwi in situ</i> )	(Schwager et al., 2015)

Araneae	Araneomorphae	Agelenidae	<i>Agelena labyrinthica</i>				(3) segmented germ band	ventral walls of mesodermal coelomic sacs	LM	(Kautzsch, 1910)
<b>MYRIAPODA</b>										
Scolopendromorpha		Scolopendridae	<i>Scolopendra cingulata</i>	Mediterranean banded centipede			(2) gastrulation	blastopore	LM	(Heymons, 1901)
Geophilomorpha		Linotaeniidae	<i>Strigamia maritima</i>				(2) blastoderm	blastopore	LM, MM ( <i>vasa</i> and <i>nanos in situ</i> )	(Green and Akam, 2014)
Tetramerocerata		Pauropodidae	<i>Pauropus silvaticus</i>				(3) germ band	mesoderm	LM	(Tiegs, 1947)
Cephalostigmata		Scutigereidae	<i>Hanseniella agilis</i>				(3) germ band	mesoderm	LM	(Tiegs, 1940)
Glomerida		Glomeridae	<i>Glomeris marginata</i>	pill millipede			(3) germ band	mesoderm in what will become the seventh and eighth leg segments as they form	LM	(Dohle, 1964)
<b>NON-HEXAPOD PANCRUSTACEA</b>										
Podocopa		Cytherideidae	<i>Cyprideis torosa</i>				(2) gastrulation	from the mesoderm shortly after gastrulation	LM	(Weygoldt, 1960)
Calanoida		Calanidae	<i>Calanus finmarchicus</i>				(3) nauplius hatchling	from the mesoderm on either side of the intestine at posterior end of nauplius	LM	(Grobbs, 1881)
Calanoida		Diaptomidae	<i>Eudiaptomus vulgaris</i>				(3) nauplius hatchling	from the mesoderm on either side of the intestine at posterior end of nauplius	LM	(Grobbs, 1881)
Siphonostomatoida		Dichelethiidae	unnamed species				(1) 32-cell stage	blastopore; last cell to bud out from the yolk and first cell to enter during gastrulation	LM	(McClendon, 1907)
Siphonostomatoida		Pandaridae	<i>Orthagoriscicola muricata</i>				(1) 32-cell stage	blastopore; last cell to bud out from the yolk and first cell to enter during gastrulation	LM	(McClendon, 1907)
Siphonostomatoida		Pandaridae	<i>Pandarus sinuatus</i>				(1) 32-cell stage	blastopore; last cell to bud out from the yolk and first cell to enter during gastrulation	LM	(McClendon, 1907)
Harpacticoida		Tisbidae	<i>Tisbe furcata</i>				(1) 64-cell stage	blastopore; last cell to bud out from the yolk and first cell to enter during gastrulation	LM	(Witschi, 1934)
Cyclopoida		Notodelphyidae	<i>Pachypygus gibber</i>				(2) gastrulation	blastopore, internalized soon after endoderm	LM	(Schimkewitsch, 1896)
Cyclopoida		Notodelphyidae	<i>Notopterophorus papilio</i>				(2) gastrulation	blastopore, internalized soon after endoderm	LM	(Schimkewitsch, 1896)
Leptostraca	Nebaliacea	Nebaliidae	<i>Nebalia bipes</i>				(3) late germ band	ventral walls of coelomic sacs	LM	(Manton, 1934)
Mysida		Mysidae	<i>Hemimysis lamornae</i>				(2) before gastrulation	center of germ disc/blastopore	LM	(Manton, 1928)
Mysida		Mysidae	<i>Neomysis integer</i>				(2) before gastrulation	center of germ disc/blastopore	LM	(Needham, 1937)
Mysida		Mysidae	<i>Mesopodopsis orientalis</i>				(2) before gastrulation	along median line of germ disc behind ectoteloblasts	LM	(Nair, 1939)
Tanaidacea		Leptocheliidae	<i>Heterotanais oerstedii</i>				(2) gastrulation	blastopore, internalized soon after yolk cells	LM	(Scholl, 1963)
Isopoda	Cymothoida	Bopyridae	<i>Bopyroides hippolytes</i>				(3) early germ band	lateral clusters, associated with liver rudiment	LM	(Strömberg, 1971)
Isopoda	Cymothoida	Anthuridae	<i>Cyathura polita</i>				(3) early germ band	lateral clusters, associated with liver rudiment	LM	(Strömberg, 1972)
Isopoda	Oniscidea	Ligiidae	<i>Ligia exotica</i>	sea roach			(3) just before hatching	last thoracic segment and first two pleonic segments	LM	(Terao and Cheng, 1926)
Isopoda	Oniscidea	Armadillidiidae	<i>Armadillidium nasatum</i>				(2) gastrulation	mesendodermal cluster at beginning of gastrulation	LM	(Goodrich, 1939)
Isopoda	Oniscidea	Armadillidiidae	<i>Armadillidium vulgare</i>	common pill-bug			(2) gastrulation	mesendodermal cluster at beginning of gastrulation	LM	(Lane, 1977)
Isopoda	Oniscidea	Porcellionidae	<i>Porcellio laevis</i>	swift woodlouse			(2) gastrulation	mesendodermal cluster at beginning of gastrulation	LM	(Goodrich 1939)
Isopoda	Oniscidea	Porcellionidae	<i>Porcellionides pruinosus</i>				(2) gastrulation	mesendodermal cluster at beginning of gastrulation	LM	(Lane, 1977)
Isopoda	Oniscidea	Porcellionidae	<i>Porcellio scaber</i>	common rough woodlouse			(2) gastrulation	mesendodermal cluster at beginning of gastrulation	LM	(Wolff, 2009)
Isopoda	Valvifera	Idoteidae	<i>Idotea granulosa</i>				(2) gastrulation	mesendodermal cluster at beginning of gastrulation	LM	(Strömberg, 1965)
Isopoda	Valvifera	Idoteidae	<i>Idotea neglecta</i>				(2) gastrulation	mesendodermal cluster at beginning of gastrulation	LM	(Strömberg, 1965)
Isopoda	Limnoriidea	Limnoriidae	<i>Limnoria lignorum</i>				(2) gastrulation	mesendodermal cluster at beginning of gastrulation	LM	(Strömberg, 1968)
Isopoda	Asellota	Asellidae	<i>Asellus aquaticus</i>	pond slater, water louse			(2) gastrulation	blastopore	LM	(Needham, 1942)
Amphipoda	Senticaudata	Gammaridae	<i>Gammarus pulex</i>				(2) gastrulation	mesendodermal cluster at beginning of gastrulation	LM	(Scholtz, 1990)
Amphipoda	Senticaudata	Talitridae	<i>Orchestia cavimana</i>				(1) 8-cell stage	<i>a</i> micromere	LM, CL	(Scholtz and Wolff, 2002; Wolff and Scholtz, 2002)
Amphipoda	Senticaudata	Hyalidae	<i>Parhyale hawaiiensis</i>	sand flea	EX	EX	(1) 8-cell stage	<i>g</i> micromere (note: evidence that germ line can also regenerate)	LM, MM ( <i>vasa</i> , <i>gcl</i> , <i>orb in situ</i> ; <i>Vasa</i> antibody), EX (cell ablation)	(Extavour, 2005; Gupta and Extavour, 2013; Kaczmarczyk, 2014; Özhan-Kizil et al., 2009)
Anaspidacea		Anaspididae	<i>Anaspides tasmaniae</i>	mountain shrimp			(3) shortly before	ventral walls of mesodermal coelomic sacs in T1/T2	LM	(Hickman, 1936)

							hatching			
Euphausiacea		Euphausiidae	<i>Meganyctiphanes norvegica</i>	northern krill	OB		(1) 32-cell stage	X <sub>d</sub> and X <sub>v</sub> cells, sister to endoderm	LM, CL	(Alwes and Scholtz, 2004; Taube, 1909; Taube, 1915)
Decapoda	Dendrobranchiata	Sicyoniidae	<i>Sicyonia ingentis</i>	ridgeback prawn	OB		(2) gastrulation	MEvpp cell (medial ventral mesoderm)	LM, CL	(Hertzler, 2002; Pawlak et al., 2010)
Decapoda	Dendrobranchiata	Penaeidae	<i>Penaeus vannamei</i>	Pacific white shrimp, whiteleg shrimp	OB		(2) gastrulation	X <sub>v</sub> pp cell (ventral posterior mesendoderm)	LM, CL	(Hertzler, 2005; Pawlak et al., 2010)
Decapoda	Dendrobranchiata	Penaeidae	<i>Penaeus monodon</i>	black tiger shrimp	OB		(2) gastrulation	X <sub>v</sub> pp cell (ventral posterior mesendoderm)	LM, CL	(Biffis et al., 2009)
Decapoda	Dendrobranchiata	Penaeidae	<i>Penaeus japonicus</i>	Kuruma shrimp; Japanese tiger shrimp	OB		(2) gastrulation	X <sub>v</sub> pp cell (ventral posterior mesendoderm)	LM, CL, TEM	(Grattan et al., 2013; Pawlak et al., 2010; Vincent and Hertzler, 2018)
Decapoda	Pleocyemata (Caridea)	Atyidae	<i>Caridina laevis</i>				(3) segmented embryo	ventral mesoderm on either side of the gut	LM	(Nair, 1949)
Decapoda	Pleocyemata (Caridea)	Palaemonidae	<i>Macrobrachium nipponense</i>	oriental river prawn	OB		(1) 16-cell stage	unnamed blastomere	LM, MM ( <i>vasa in situ</i> , Vasa antibody), TEM	(Chen et al., 2021; Ma et al., 2019; Qiu et al., 2013)
Decapoda	Pleocyemata (Reptantia, Achelata)	Palinuridae	<i>Panulirus japonicus</i>	Japanese spiny lobster			(3) segmented embryo	ventral walls of mesodermal coelomic sacs in T1	LM	(Terao, 1929)
Anostraca	Artemiina	Artemiidae	<i>Artemia salina</i>				(3) nauplius hatching	mesoderm of first trunk segment	LM	(Anderson, 1967)
Notostraca		Triopsidae	<i>Triops longicaudatus</i>				(3) fourth instar larva	on either side of the gut	LM	(Mitsumoto and Makioka, 2002)
Notostraca		Triopsidae	<i>Triops granarius</i>				(3) fourth instar larva	on either side of the gut	LM	(Mitsumoto and Makioka, 2003)
Spinicaudata		Limnadiidae	<i>Paralimnadia stanleyana</i>				(3) nauplius hatching	mesoderm of first trunk segment	LM	(Anderson, 1967)
Ctenopoda		Holopediidae	<i>Holopedium gibberum</i>				(1) 16-cell stage	D <sup>IV</sup> blastomere at the vegetal pole, which becomes the site of gastrulation	LM	(Baldass, 1937)
Ctenopoda		Sididae	<i>Penilia avirostris</i>				(3) late embryo	mesoderm on either side of intestine	LM	(Sudler, 1899)
Anomopoda		Macrothricidae	<i>Lathonura rectirostris</i>				(2) gastrulation	four cells at blastopore lip	LM	(Grobbs, 1879)
Anomopoda		Daphniidae	<i>Simocephalus vetulus</i>				(2) late blastoderm	posterior “ventral mass” on inside of blastoderm, later the site of gastrulation	LM	(Cannon, 1921)
Anomopoda		Daphniidae	<i>Daphnia magna</i>		OB		(1) 16-cell stage	unnamed blastomere	LM, MM (Vasa antibody)	(Sagawa et al., 2005)
Onychopoda		Cercopagididae	<i>Bythotrephes longimanus</i>	spiny water flea	OB		(1) 16-cell stage	one daughter cell of the dI micromere	LM	(Alwes and Scholtz, 2014)
Onychopoda		Polyphemidae	<i>Polyphemus pediculus</i>		OB		(1) 16-cell stage	one daughter cell of the dI micromere	LM, CL	(Kühn, 1913)
<b>HEXAPODA</b>										
<b>Ametabola</b>										
Poduromorpha		Neanuridae	<i>Anurida maritima</i>	seashore springtail			(3) segmented germ band	abdominal mesoderm	LM	(Claypole, 1898)
Poduromorpha		Onychiuridae	<i>Tetrodontophora bielanensis</i>	giant springtail	OB		(1) cleavage stage embryo	region of cortical cytoplasm in early-stage embryo	TEM	(Klag, 1982)
Symphyleona		Sminthuridae	<i>Allacma fusca</i>				(1) blastoderm-stage embryo	scattered in yolk	TEM	(Klag and Świątek, 1999)
Entomobryomorpha		Tomoceridae	<i>Tomocerus cuspidatus</i>				(1) cleavage-stage embryo	scattered in the yolk, inner mass	LM, TEM	(Tomizuka and Machida, 2015)
Entomobryomorpha		Tomoceridae	<i>Tomocerus ishikashii</i>				(1) blastoderm-stage embryo	center of yolk after blastoderm formation	LM	(Uemiyama and Ando, 1991)
Archaeognatha		Machilidae	<i>Petrobius brevistylis</i>				(3) segmented germ band	dorsal coelomic sacs of T2–A1 mesoderm	LM	(Larink, 1969)
Zygentoma		Lepismatidae	<i>Lepisma saccharina</i>	silverfish			(2) early germ band	posterior end of germ band; author notes difficulty in following migration of putative germ cells	LM	(Heymons, 1897)
Zygentoma		Lepismatidae	<i>Thermobia domestica</i>	firebrat			(3) segmented germ band	dorsal coelomic sacs of abdominal mesoderm	LM	(Woodland, 1957)
<b>Palaeoptera</b>										
Odonata	Epiprocta	Epiophlebiidae	<i>Epiophlebia superstes</i>				(2) cellular blastoderm	posterior blastoderm; unclear whether these cells are the same cells that appear in the gonads later in embryogenesis	LM	(Ando, 1962)
<b>Polynoptera</b>										
Dermaptera		Forficulidae	<i>Forficula auricularia</i>	common earwig			(2) cellular blastoderm	posterior blastoderm	LM	(Heymons, 1895)
Dermaptera		Labiduridae	<i>Labidura riparia</i>	shore earwig	OB		(1) syncytial blastoderm	posterior blastoderm	LM	(Singh, 1967)
Orthoptera	Ensifera	Gryllidae	<i>Oecanthus niveus</i>	narrow-winged tree			(3) late germ band	on either side of the dorsal vessel	LM	(Ayers, 1884)

				cricket							
Orthoptera	Ensifera	Gryllidae	<i>Acheta domesticus</i>	house cricket			(2) early germ band	posterior end of germ band	LM		(Heymons, 1895)
Orthoptera	Ensifera	Gryllidae	<i>Gryllus campestris</i>	European field cricket			(2) early germ band	posterior end of germ band	LM		(Heymons, 1895)
Orthoptera	Ensifera	Gryllidae	<i>Gryllus bimaculatus</i>	two-spotted field cricket		EX	(3) segmented germ band	abdominal mesoderm, before formation of coelomic sacs	LM, MM (Vasa and Piwi antibodies, many <i>in situ</i> ), EX (genetic manipulation)		(Donoughe et al., 2014; Ewen-Campen et al., 2013a; Nakamura and Extavour, 2016)
Orthoptera	Ensifera	Tettigoniidae	<i>Conocephalus brevipennis</i>	short-winged meadow katydid			(3) late germ band	inner walls of coelomic sacs in A1–A6 mesoderm	LM		(Wheeler, 1893)
Orthoptera	Caelifera	Acrididae	<i>Melanoplus differentialis</i>	differential grasshopper			(2) early germ band	lateral margins of germ band, A1–A9	LM		(Nelsen, 1934)
Orthoptera	Caelifera	Acrididae	<i>Locusta migratoria</i>	migratory locust			(3) late germ band	dorsal coelomic sacs of A2–A5 mesoderm	LM		(Roonwal, 1937)
Orthoptera	Caelifera	Acrididae	<i>Schistocerca gregaria</i>	desert locust			(2) early germ band	lateral margins of abdominal germ band	LM, MM (Vasa antibody)		(Chang et al., 2002)
Blattodea	Solumblattodea	Blattidae	<i>Periplaneta orientalis</i>	oriental cockroach			(2) early germ band	posterior end of germ band	LM		(Heymons, 1895)
Blattodea	Blaberoidea	Ectobiidae	<i>Blattella germanica</i>	German cockroach			(2) early germ band	posterior end of germ band; author notes that putative germ cells are indistinguishable from mesodermal cells but identifies them as germ cells based on comparison with <i>Periplaneta orientalis</i>	LM		(Heymons, 1895)
Phasmatodea	Oriophasmata	Lonchodidae	<i>Carausius morosus</i>	common stick insect			(2) early germ band or (3) late germ band	(Hammerschmidt, 1910; Wiesmann, 1926) say posterior end of germ band; (Cavallin, 1970) says abdominal mesoderm	LM		(Cavallin, 1970; Hammerschmidt, 1910; Wiesmann, 1926)
Mantodea		Mantidae	<i>Rhombodera crassa</i>				(3) late germ band	coelomic sacs in A3–A5 mesoderm	LM		(Görg, 1959)
<b>Condylognatha + Psocodea</b>											
Thysanoptera	Tubulifera	Phlaeothripidae	<i>Haplothrips verbasci</i>			OB	(1) syncytial blastoderm	posterior blastoderm	LM		(Heming, 1979)
Thysanoptera	Tubulifera	Phlaeothripidae	<i>Bactrothrips brevitubus</i>			OB	(1) syncytial blastoderm	posterior blastoderm	LM		(Haga, 1985)
Hemiptera	Heteroptera (Pentatomomorpha)	Lygaeidae	<i>Oncopeltus fasciatus</i>	large milkweed bug			(2) cellular blastoderm	posterior blastoderm	LM, MM (Vasa antibody, many <i>in situ</i> hybridization)		(Butt, 1949; Ewen-Campen et al., 2013b; Kao et al., 2025)
Hemiptera	Heteroptera (Pentatomomorpha)	Blissidae	<i>Blissus leucopterus hirtus</i>	hairy chinch bug			(2) cellular blastoderm	posterior blastoderm	LM		(Choban and Gupta, 1972)
Hemiptera	Heteroptera (Pentatomomorpha)	Pyrrhocoridae	<i>Pyrrhocoris apterus</i>	European firebug			(3) segmented germ band	from the mesoderm in segments A1–A8	LM		(Seidel, 1924)
Hemiptera	Heteroptera (Cimicomorpha)	Reduviidae	<i>Rhodnius prolixus</i>	kissing bug			(2) cellular blastoderm	posterior blastoderm	LM, SEM		(Heming and Huebner, 1994; Kelly and Huebner, 1989; Mellanby, 1935)
Hemiptera	Sternorrhynca	Monophlebidae	<i>Icerya purchasi</i>	cottony cushion scale			(2) cellular blastoderm	posterior blastoderm	LM		(Shinji, 1919)
Hemiptera	Sternorrhynca	Pseudococcidae	<i>Pseudococcus citri</i>	citrus mealybug			(2) cellular blastoderm	posterior blastoderm	LM		(Schrader, 1922)
Hemiptera	Sternorrhynca	Aphididae	<i>Acyrtosiphon pisum</i>	pea aphid		OB	(2) cellular blastoderm	posterior blastoderm	LM, MM (Vasa, Nanos antibody, <i>vasa in situ</i> )		(Chang et al., 2006; Chang et al., 2007; Lin et al., 2014; Miura et al., 2003)
Psocodea	Psocomorpha	Archipsocidae	<i>Archipsocus fernandi</i>				(2) cellular blastoderm	posterior blastoderm	LM		(Fernando, 1934)
Psocodea	Troctomorpha	Liposcelididae	<i>Liposcelis divergens</i>	book louse			(2) cellular blastoderm	posterior blastoderm	LM		(Goss, 1952; Goss, 1953)
<b>Holometabola</b>											
Hymenoptera	Apocrita (Aculeata)	Formicidae	<i>Lasius niger</i>	black garden ant		OB	(1) cellular blastoderm	posterior pole of blastoderm	LM, MM (Vasa antibody, <i>nanos</i> , <i>oskar in situ</i> )		(Rafiqi et al., 2020)
Hymenoptera	Apocrita (Aculeata)	Formicidae	<i>Camponotus floridanus</i>	Florida carpenter ant		OB	(1) cellular blastoderm	posterior pole of blastoderm	LM, MM (Vasa antibody, <i>nanos</i> , <i>oskar in situ</i> )		(Rafiqi et al., 2020)
Hymenoptera	Apocrita (Aculeata)	Formicidae	<i>Messor pergandei</i>	black harvester ant		OB	(1) cellular blastoderm	posterior pole of blastoderm	LM, MM ( <i>oskar</i> , <i>vasa</i> , <i>nanos in situ</i> )		(Khila and Abouheif, 2010; Lynch et al., 2011)
Hymenoptera	Apocrita (Aculeata)	Apidae	<i>Apis mellifera</i>	western honeybee			(3) segmented germ band	genital ridges in A3–A6 mesoderm	LM, SEM, MM ( <i>vasa</i> , <i>nanos in situ</i> )		(Dearden, 2006; Fleig and Sander, 1986; Nelson, 1915)
Hymenoptera	Apocrita (Parasitoida)	Pteromalidae	<i>Nasonia vitripennis</i>	jewel wasp		EX	(1) syncytial blastoderm (nuclear cycle 8)	pole cells at posterior pole of blastoderm	LM, MM ( <i>nanos</i> , <i>oskar in situ</i> ), EX (genetic manipulation), TS		(Bull, 1982; Lynch and Desplan, 2010; Lynch et al., 2011; Quan et al., 2019)
Hymenoptera	Apocrita (Parasitoida)	Trichogrammatidae	<i>Trichogramma evanescens</i>			OB	(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM		(Gatenby, 1917; Gatenby, 1918)
Hymenoptera	Apocrita (Parasitoida)	Encyrtidae	<i>Copidosoma floridanum</i>			EX	(1) 4-cell stage	B4 blastomere	LM, MM (Vasa <i>in situ</i> and antibody), EX (cell ablation)		(Donnell et al., 2004; Grbic, 2003; Zhurov et al., 2004)

Hymenoptera	Apocrita (Parasitoida)	Braconidae	<i>Macrocentrus cingulum</i>		OB		(2) cellular blastoderm	inside the posterior blastocoel	LM, MM (Vasa antibody)	(Grbic', 2003; Sucena et al., 2014)
Hymenoptera	Apocrita (Parasitoida)	Braconidae	<i>Coeloides vancouverensis</i>		OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Ryan, 1963)
Hymenoptera	Apocrita (Parasitoida)	Braconidae	<i>Habrobracon juglandis</i>		OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Amy, 1961)
Hymenoptera	Apocrita (Parasitoida)	Ichneumonidae	<i>Cosmoconus meridianator</i>		OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	TEM	(Klag and Bilinski, 1993)
Hymenoptera	Apocrita (Parasitoida)	Ichneumonidae	<i>Lissonota catenator</i>		OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	TEM	(Klag and Bilinski, 1993)
Hymenoptera	Apocrita (Parasitoida)	Ichneumonidae	<i>Pimpla turionellae</i>		EX	EX	(1) syncytial blastoderm	pole cells at posterior pole of blastoderm (note: evidence that germ line can also regenerate)	LM, EX (damage cytoplasm)	(Achtelig and Krause, 1971; Bronskill, 1959; Meng, 1968)
Hymenoptera	Eusymphyta	Tenthredinidae	<i>Nematus ribesii</i>	gooseberry sawfly			(3) segmented germ band	posterior midgut rudiment	LM	(Shafiq, 1954)
Hymenoptera	Eusymphyta	Tenthredinidae	<i>Athalia rosae</i>	turnip sawfly			(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM, MM (Vasa antibody)	(Nakao et al., 2006)
Raphidioptera		Inocelliidae	<i>Inocellia japonica</i>				(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM, SEM	(Tsutsumi and Machida, 2006)
Megaloptera		Sialidae	<i>Sialis mitsuhashii okamoto</i>	alderfly			(2) late blastoderm	posterior end of ventral plate	TEM	(Suzuki et al., 1981)
Strepsiptera		Stylopidae	<i>Stylops spp.</i>				(3) extended germ band	posterior end of the germ band	LM	(Noskiewicz and Poluszyński, 1927)
Coleoptera	Adephaga	Carabidae	<i>Carabus insulicola</i>				??	no pole cells observed	LM, SEM	(Kobayashi et al., 2013)
Coleoptera	Adephaga	Gyrinidae	<i>Dineutus mellyi</i>	whirligig beetle			??	no pole cells observed	LM, SEM	(Komatsu and Kobayashi, 2012)
Coleoptera	Adephaga	Dytiscidae	<i>Hydaticus pacificus</i>	diving beetle			??	no pole cells observed	LM, SEM	(Niikura et al., 2017)
Coleoptera	Polyphaga (Elateroidea)	Lampyridae	<i>Nipponoluciola cruciata</i>				??	no pole cells observed	LM	(Kobayashi and Ando, 1985)
Coleoptera	Polyphaga (Elateroidea)	Lampyridae	<i>Pyrocoelia rufa</i>				??	no pole cells observed	LM	(Kobayashi et al., 2006)
Coleoptera	Polyphaga (Hydrophiloidea)	Hydrophilidae	<i>Hydrophilus piceus</i>	great silver water beetle			(3) segmented germ band	from the walls of the coelomic sacs of abdominal mesoderm	LM	(Heider, 1889)
Coleoptera	Polyphaga (Scarabaeoidea)	Passalidae	<i>Odontotaenius disjunctus</i>	patent-leather beetle, horned passalus			(3) late germ band	dorsal side of abdominal germ band, near A6–A7	LM	(Krause, 1947)
Coleoptera	Polyphaga (Scarabaeoidea)	Scarabaeidae	<i>Phyllophaga fervida</i>				(2) cellular blastoderm	posterior blastoderm	LM	(Luginbill, 1953)
Coleoptera	Polyphaga (Bostrichoidea)	Dermestidae	<i>Dermestes frischii</i>		OB		(2) cellular blastoderm	posterior blastoderm	LM	(Küthe, 1966)
Coleoptera	Polyphaga (Tenebrionoidea)	Meloidae	<i>Lytta viridana</i>	blister beetle			(2) cellular blastoderm	posterior blastoderm	LM	(Church and Rempel, 1971; Rempel and Church, 1969)
Coleoptera	Polyphaga (Tenebrionoidea)	Tenebrionidae	<i>Tenebrio molitor</i>	yellow mealworm beetle			(2) cellular blastoderm	posterior blastoderm	LM	(Ullmann, 1964)
Coleoptera	Polyphaga (Tenebrionoidea)	Tenebrionidae	<i>Tribolium confusum</i>	confused flour beetle			(2) cellular blastoderm	posterior blastoderm	LM	(Hodson, 1934; Stanley and Grundmann, 1970)
Coleoptera	Polyphaga (Tenebrionoidea)	Tenebrionidae	<i>Tribolium castaneum</i>	red flour beetle		EX	(2) cellular blastoderm	posterior blastoderm	LM, MM ( <i>vasa in situ</i> ), EX (genetic manipulation)	(Ansari et al., 2018; Schröder, 2006)
Coleoptera	Polyphaga (Curculionidea)	Curculionidae	<i>Sphenophorus callosus</i>	southern corn billbug	EX		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM, EX (cytoplasmic cauterization)	(Hegner, 1911; Hegner, 1914; Wray, 1937)
Coleoptera	Polyphaga (Curculionidea)	Curculionidae	<i>Otiorhynchus ligustici</i>	alfalfa snout beetle	OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Butt, 1936)
Coleoptera	Polyphaga (Curculionidea)	Curculionidae	<i>Sitophilus oryzae</i>	rice weevil			(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Tiegs and Murray, 1938)
Coleoptera	Polyphaga (Chrysomeloidea)	Chrysomelidae	<i>Calligrapha multipunctata</i>	common willow calligrapha	EX		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM, EX (cytoplasmic removal)	(Hegner, 1908; Hegner, 1909a; Hegner, 1909b)
Coleoptera	Polyphaga (Chrysomeloidea)	Chrysomelidae	<i>Leptinotarsa decemlineata</i>	Colorado potato beetle	EX		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM, EX (cytoplasmic cauterization/removal)	(Hegner, 1908; Hegner, 1911; Hegner, 1914)
Coleoptera	Polyphaga (Chrysomeloidea)	Chrysomelidae	<i>Callosobruchus maculatus</i>	cowpea weevil	EX		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM, MM ( <i>oskar</i> ; <i>vasa</i> ; <i>tudor in situ</i> ), EX (UV irradiation)	(Brauer, 1925; Brauer, 1949; Quan, 2018)
Coleoptera	Polyphaga (Chrysomeloidea)	Chrysomelidae	<i>Acanthoscelides obtectus</i>	bean weevil	OB		(1) syncytial blastoderm (nuclear cycle 8)	pole cells at posterior pole of blastoderm	LM, MM ( <i>vasa in situ</i> ), TEM	(Jung, 1966; Lynch et al., 2011)

Coleoptera	Polyphaga (Chrysomeloidea)	Chrysomelidae	<i>Euryope terminalis</i>	milkweed leaf beetle	OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Paterson, 1931)
Coleoptera	Polyphaga (Chrysomeloidea)	Chrysomelidae	<i>Platycorynus compressicornis</i>		OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Paterson, 1935)
Trichoptera	Annulipalpia	Stenopsychidae	<i>Stenopsyche griseipennis</i>				(3) segmented germ band	genital ridges in A2–A7 mesoderm	LM	(Miyakawa, 1974)
Lepidoptera	Micropterigoidea	Micropterigidae	<i>Neomicropteryx nipponensis</i>				(3) segmented germ band	mediodorsal coelomic sacs in A5 mesoderm	LM	(Kobayashi and Ando, 1984)
Lepidoptera	Glossata (Hepialoidea)	Hepialidae	<i>Endoclita signifer</i>				(2) early germ band	posterior germ band	LM	(Ando and Tanaka, 1980)
Lepidoptera	Apoditrysia (Tortrichoidea)	Tortricidae	<i>Epiphyas postvittana</i>	light brown apple moth			(2) early germ band	between germ band and yolk	LM	(Anderson and Wood, 1968)
Lepidoptera	Obtectomera (Papilionoidea)	Nymphalidae	<i>Euvanessa (Nymphalis) antiopa</i>	mourning cloak			(2) early germ band	midline of ventral plate, about one-third length from the posterior	LM	(Woodworth, 1889)
Lepidoptera	Obtectomera (Papilionoidea)	Nymphalidae	<i>Pararge aegeria</i>	speckled wood butterfly	OB		(2) cellular blastoderm	ventral blastoderm	LM, MM ( <i>nanos in situ</i> ), TS	(Carter et al., 2013; Carter et al., 2015)
Lepidoptera	Obtectomera (Papilionoidea)	Pieridae	<i>Pieris rapae</i>	cabbage white, cabbage butterfly			(3) segmented germ band	coelomic sacs of A4–A5 mesoderm	LM	(Eastham, 1931)
Lepidoptera	Obtectomera (Papilionoidea)	Papilionidae	<i>Parnassius glacialis</i>	glacial Apollo, Japanese clouded Apollo			(2) late cellular blastoderm/early germ band	midline of ventral plate, about one-third length from the posterior	LM	(Tanaka, 1987)
Lepidoptera	Obtectomera (Papilionoidea)	Papilionidae	<i>Luehdorfia japonica</i>	Gifu butterfly			(2) early germ band	midline of germ band, about one-third length from the posterior	LM	(Tanaka, 1987)
Lepidoptera	Obtectomera (Papilionoidea)	Papilionidae	<i>Byasa alcinous alcinous</i>	Chinese windmill			(2) early germ band	midline of germ band, about one-third length from the posterior	LM	(Tanaka, 1987)
Lepidoptera	Obtectomera (Gelechioidea)	Gelechiidae	<i>Pectinophora gossypiella</i>	pink bollworm	OB		(2) cellular blastoderm	posterior, ventral blastoderm	TEM	(Berg and Gassner, 1978)
Lepidoptera	Macroheterocera (Noctuoidea)	Amatidae	<i>Amata fortunei</i>	white-spotted moth			(2) early germ band	midline of germ band, near the center	LM	(Tanaka, 1985)
Lepidoptera	Macroheterocera (Noctuoidea)	Noctuidae	<i>Helicoverpa zea</i>	corn earworm			(2) early germ band	midline of germ band, near the center	LM	(Presser and Rutschky, 1957)
Lepidoptera	Macroheterocera (Noctuoidea)	Arctiinae	<i>Spilosoma virginica</i>	Virginian tiger moth			(3) late germ band	coelomic sacs in abdominal mesoderm	LM	(Johannsen, 1929)
Lepidoptera	Macroheterocera (Bombycoidea)	Bombycidae	<i>Bombyx mori</i>	domestic silk moth	EX	EX	(2) cellular blastoderm	midline of ventral plate, about one-third length from the posterior		(Miya, 1958; Nakao, 1999; Nakao and Takasu, 2019)
Lepidoptera	Macroheterocera (Bombycoidea)	Saturniidae	<i>Antheraea pernyi</i>	Chinese (oak) tussar moth			(3) segmented germ band	mesodermal coelomic sacs of all abdominal segments	LM	(Saito, 1937)
Mecoptera		Panorpidae	<i>Panorpa pryeri</i>		OB		(2) early germ band	posterior end of germ band	LM	(Suzuki, 1990)
Mecoptera		Panorpididae	<i>Panorpodes paradoxa</i>				(2) early germ band	posterior end of germ band	LM	(Suzuki, 1990)
Mecoptera		Bittacidae	<i>Bittacus laevipes</i>				(2) early germ band	posterior end of germ band	LM	(Suzuki, 1990)
Mecoptera		Boreidae	<i>Boreus westwoodi</i>				(2) early germ band	posterior end of germ band	LM	(Suzuki, 1990)
Siphonaptera		Pulicidae	<i>Ctenocephalides felis</i>	cat flea			(1) syncytial blastoderm (nuclear cycle 6)	pole cells at posterior pole of blastoderm	LM	(Kessel, 1939)
Diptera	Psychodomorpha	Psychodidae	<i>Phlebotomus papatasi</i>	sand fly	OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Abbassy et al., 1995a; Abbassy et al., 1995b; Abbassy et al., 1995c)
Diptera	Psychodomorpha	Psychodidae	<i>Clogmia albipunctata</i>	moth fly		EX	(2) cellular blastoderm	posterior blastoderm; no morphologically distinct pole cells	SEM, LM, MM (Vasa antibody, <i>nanos in situ</i> ), EX (genetic manipulation)	(Jiménez-Guri et al., 2014; Yoon et al., 2019)
Diptera	Culicomorpha (Chironomoidea)	Simuliidae	<i>Simulium pictipes</i>	black fly	OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Gambrell, 1933)
Diptera	Culicomorpha (Chironomoidea)	Chironomidae	<i>Chironomus sp.</i>				(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Klomp et al., 2015; Weissmann, 1863)
Diptera	Culicomorpha (Chironomoidea)	Chironomidae	<i>Chironomus confinis</i>	nonbiting midge	OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Hegner, 1914)
Diptera	Culicomorpha (Chironomoidea)	Chironomidae	<i>Smittia sp.</i>		OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM, TEM	(Zissler and Sander, 1973)
Diptera	Culicomorpha (Culicoidea)	Culicidae	<i>Aedes aegypti</i>	yellow fever mosquito	OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM, MM ( <i>oskar in situ</i> )	(Juhn and James, 2006; Raminani and Cupp, 1975)
Diptera	Culicomorpha (Culicoidea)	Culicidae	<i>Culex quinquefasciatus</i>	southern house mosquito	OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM, MM ( <i>nanos, oskar in situ</i> )	(Davis, 1967; Juhn et al., 2008)

Diptera	Culicomorpha (Culicoidea)	Culicidae	<i>Culiseta inornata</i>	winter marsh mosquito	OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Harber and Mutchmor, 1970)
Diptera	Culicomorpha (Culicoidea)	Culicidae	<i>Anopheles gambiae</i>	African malaria mosquito	OB		(1) syncytial blastoderm (nuclear cycle 9)	pole cells at posterior pole of blastoderm	LM, MM ( <i>oskar in situ</i> )	(Juhn and James, 2006)
Diptera	Bibionomorpha	Cecidomyiidae	<i>Miastor metraloas</i>	gall midge	OB		(1) syncytial blastoderm (nuclear cycle 3)	pole cells at posterior pole of blastoderm	LM, TEM	(Hegner, 1914; Mahowald, 1975)
Diptera	Bibionomorpha	Cecidomyiidae	<i>Mayetiola destructor</i>	Hessian fly			(1) syncytial blastoderm (nuclear cycle 5)	pole cells at posterior pole of blastoderm	LM	(Metcalf, 1935)
Diptera	Bibionomorpha	Sciaridae	<i>Bradysia coprophila</i>	black fungus gnat	OB		(1) syncytial blastoderm (nuclear cycle 5)	pole cells at posterior pole of blastoderm	LM	(DuBois, 1932; de Saint Phalle and Sullivan, 1996)
Diptera	Bibionomorpha	Sciaridae	<i>Sciara sp.</i>		OB		(1) syncytial blastoderm (nuclear cycle 5)	pole cells at posterior pole of blastoderm	LM	(Butt, 1934)
Diptera	Cyclorhapha (Platypezoidea)	Phoridae	<i>Megaselia abdita</i>	scuttle fly	OB		(1) syncytial blastoderm (nuclear cycle 9)	pole cells at posterior pole of blastoderm	SEM, LM, MM (Vasa antibody)	(Wotton et al., 2014)
Diptera	Schizophora (Tephritoidea)	Tephritidae	<i>Bactrocera tryoni</i>	Queensland fruit fly	OB		(1) syncytial blastoderm (nuclear cycle 10)	pole cells at posterior pole of blastoderm	LM	(Anderson, 1962)
Diptera	Schizophora (Ephydroidea)	Drosophilidae	<i>Drosophila melanogaster</i>	common fruit fly, vinegar fly	EX		(1) syncytial blastoderm (nuclear cycle 9)	pole cells at posterior pole of blastoderm	LM, EM, MM (many), EX (genetic manipulation, cytoplasmic transplant)	(Counce, 1963; Ephrussi and Lehmann, 1992; Huettner, 1923; Illmensee and Mahowald, 1974; Mahowald, 1968)
Diptera	Schizophora (Ephydroidea)	Drosophilidae	<i>Drosophila hydei</i>		OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM, TEM	(Counce, 1963; Mahowald, 1968)
Diptera	Schizophora (Ephydroidea)	Drosophilidae	<i>Drosophila willistoni</i>		OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM, TEM	(Counce, 1963; Mahowald, 1968)
Diptera	Schizophora (Ephydroidea)	Drosophilidae	<i>Drosophila immigrans</i>		EX		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	TEM, EX (cytoplasmic transplant)	(Mahowald, 1968; Mahowald et al., 1976)
Diptera	Schizophora (Ephydroidea)	Drosophilidae	<i>Drosophila busckii</i>		OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Counce, 1963)
Diptera	Schizophora (Ephydroidea)	Drosophilidae	<i>Drosophila virilis</i>		OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM, MM (Vasa, Oskar antibody, many <i>in situ</i> )	(Counce, 1963; Rivard et al., 2025)
Diptera	Schizophora (Ephydroidea)	Drosophilidae	<i>Drosophila americana</i>		OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Counce, 1963)
Diptera	Schizophora (Ephydroidea)	Drosophilidae	<i>Drosophila repleta</i>		OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Counce, 1963)
Diptera	Schizophora (Ephydroidea)	Drosophilidae	<i>Drosophila gibberosa</i>		OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Counce, 1963)
Diptera	Schizophora (Ephydroidea)	Drosophilidae	<i>Drosophila funebris</i>		OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Counce, 1963)
Diptera	Schizophora	Hippoboscidae	<i>Melophagus ovinus</i>	sheep ked, sheep tick	OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Lassmann, 1936)
Diptera	Schizophora	Muscidae	<i>Stomoxys calcitrans</i>	stable fly			(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM, TEM, SEM	(Ajidagba et al., 1983)
Diptera	Schizophora	Muscidae	<i>Musca domestica</i>	house fly	OB		(1) syncytial blastoderm (nuclear cycle 6)	pole cells at posterior pole of blastoderm	LM	(West et al., 1968)
Diptera	Schizophora (Oestroidea)	Calliphoridae	<i>Calliphora vicina</i>	blowfly	OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Hegner, 1914)
Diptera	Schizophora (Oestroidea)	Calliphoridae	<i>Phormia regina</i>	black blowfly	OB		(1) syncytial blastoderm	pole cells at posterior pole of blastoderm	LM	(Auten, 1934)
Diptera	Schizophora (Oestroidea)	Calliphoridae	<i>Lucilia sericata</i>	sheep blowfly	OB		(1) syncytial blastoderm (nuclear cycle 10)	pole cells at posterior pole of blastoderm	LM	(Davis, 1967; Fish, 1947a; Fish, 1947b; Fish, 1949; Fish, 1952)

**Table S2. Studies used for phylogram and constraint tree construction.** Clade: clade extracted from the cited study; Resolved rank: lowest taxonomic rank resolved in our phylogram from the study.

Reference	Clade	Resolved rank	Note
(Howard et al., 2022)	Panarthropoda	Phylum	
(Baker et al., 2021)	Onychophora	Family	
(Giribet, 2018)	Chelicerata	Order	Euchelicerate relationships outside of Arachnopulmonata were left as a polytomy to reflect the continued uncertainty in chelicerate phylogeny (Giribet, 2018; Sharma and Gavish-Regev, 2025).
(Sabroux et al., 2023)	Pantopoda	Family	
(Fernández et al., 2018)	Myriapoda	Class	
(Bernot et al., 2023)	Pancrustacea	Family, Order, Subphylum	Lower-rank constraints for Hexapoda, Decapoda, Isopoda, Amphipoda, Diplostraca, and Copepoda were substituted from other studies.
(Wolfe et al., 2019)	Decapoda	Family	
(Thorpe, 2024)	Isopoda	Family	
(Copilaş-Ciocianu et al., 2020)	Amphipoda	Family	
(Sun and Cheng, 2023)	Diplostraca	Family	
(Eyun, 2017)	Copepoda	Family	
(Yu et al., 2024)	Collembola	Order	
(Misof et al., 2014)	Insecta	Order	All order-level constraints were followed except for Mecoptera, which was constrained as a polytomy with Siphonaptera to reflect recent evidence supporting the paraphyly of Mecoptera (Meusemann et al., 2020; Tihelka et al., 2020).
(Chang et al., 2020)	Orthoptera	Family	
(Song et al., 2024)	Hemiptera	Family	
(Blaimer et al., 2023)	Hymenoptera	Family	(Pteromalidae + Encytridae + Trichogrammatidae) and (Tenthredinidae + Athaliidae) were left as polytomies in the constraint tree due to non-monophyly.
(Zhang et al., 2018)	Coleoptera	Family	

(Nie et al., 2020)	Chrysomelidae	Subfamily	
(Kawahara et al., 2019)	Lepidoptera	Family	
(Tihelka et al., 2020)	Siphonaptera + Mecoptera	Family	
(Wiegmann et al., 2011)	Diptera	Family	
(Suvorov et al., 2022)	Drosophilidae	Species	
(daSilva et al., 2020)	Culicidae	Genus	

**Table S3. Hypothesized transitions in germ cell specification in Panarthropoda.** Clade name = monophyletic group in whose ancestral lineage the transition is specified to have occurred; Node\_number = node labels in Figures S1 and S2; Transition = gain of germ plasm (GP) or reversion to induction (IND); Parsimony = support from maximum parsimony (Figure S1); Likelihood = support from maximum likelihood (Figure S2).

See Table S3 at [https://github.com/rishabhraj Kapoor/panarthropoda\\_gc\\_specification\\_evolution](https://github.com/rishabhraj Kapoor/panarthropoda_gc_specification_evolution), under supplementary\_tables, commit ID ff9945c.

**Table S4. Genomic and transcriptomic datasets used for phylogenetic inference.**

We were unable to find sequence data for species in the “sub\_species” columns, and instead substituted the genomes or transcriptomes for species in the same taxonomic family. Species in the “sub\_species” columns are replaced by “iq\_tree\_label” in trees.

See Table S4 at [https://github.com/rishabhraj Kapoor/panarthropoda\\_gc\\_specification\\_evolution](https://github.com/rishabhraj Kapoor/panarthropoda_gc_specification_evolution), under supplementary\_tables, commit ID ff9945c.

**Table S5. Literature-derived fossil calibrations.** Node = clade name calibrated by fossil bounds; Spanning set = list of two taxa spanning the full clade in our tree; Min = minimum node age (millions of years) used for soft fossil constraints in MCMCTree; Max = maximum node age used for soft fossil constraints in MCMCTree. In the Node column, “+” indicates a node representing the common ancestor of two monophyletic lineages. Note that Mecoptera was treated as paraphyletic in this study (see Table S2), such that the “Siphonaptera and Mecoptera” node represents the common ancestor of all Mecoptera and Siphonaptera.

Node	Spanning set	Min	Max	Reference
Panarthropoda	Parachela, <i>Drosophila_virilis</i>	528.8	636.1	(Howard et al., 2022)
Chelicerata	Nymphonidae, <i>Parasteatoda_tepidariorum</i>	509.0	636.1	(Howard et al., 2022)
Euchelicerata	Pseudoscorpiones, <i>Parasteatoda_tepidariorum</i>	500.5	636.1	(Howard et al., 2022)
Arachnoplumonata	Euscorpidae, <i>Parasteatoda_tepidariorum</i>	435.2	514.0	(Howard et al., 2022)
Holometabola	Liposcelididae, <i>Camponotus_floridanus</i>	306.9	450.0	(Misof et al., 2014)
Pterygota	<i>Epiophlebia_superstes</i> , <i>Drosophila_virilis</i>	324.0	450.0	(Misof et al., 2014)
Dicondylia	<i>Thermobia_domestica</i> , <i>Drosophila_virilis</i>	411.5	450.0	(Misof et al., 2014)
Thysanoptera + Hemiptera	Phlaeothripidae, <i>Rhodnius_prolixus</i>	207.0	450.0	(Misof et al., 2014)
Hymenoptera	Tenthredinidae, <i>Camponotus_floridanus</i>	221	450.0	(Misof et al., 2014)
Formicidae + Apidae	<i>Camponotus_floridanus</i> , <i>Apis_mellifera</i>	89.8	221.0	(Misof et al., 2014)
Gyrinidae + Carabidae	<i>Gyrinus_marinus</i> , <i>Elaphrus_aureus</i>	205.6	306.0	(Misof et al., 2014)
Tenebrionidae + Curculionidae	<i>Sitophilus_oryzae</i> , <i>Tribolium_castaneum</i>	207.0	306.0	(Misof et al., 2014)
Lepidoptera	Hepialidae, <i>Bombyx_mori</i>	112.0	306.9	(Misof et al., 2014)
Papilionoidea	Papilionidae, <i>Pararge_aegeria</i>	34.1	112.0	(Misof et al., 2014)
Siphonaptera and Mecoptera	<i>Ctenocephalides_felis</i> , <i>Boreidae</i>	160.5	306.9	(Misof et al., 2014)
Diptera	<i>Phlebotomus_papatasi</i> , <i>Drosophila_virilis</i>	230.0	306.9	(Wiegmann et al., 2011)
Schizophora	<i>Bactrocera_tryoni</i> , <i>Drosophila_virilis</i>	64.0	230.0	(Wiegmann et al., 2011)
Sciaroidea	<i>Bradysia_coprophila</i> , <i>Mayetiola_destructor</i>	180.0	230.0	(Wiegmann et al., 2011)
Arthropoda	<i>Limulus_polyphemus</i> , <i>Drosophila_virilis</i>	514.0	636.1	(Bernot et al., 2023)
Branchiopoda	Triopsidae, <i>Daphnia_magna</i>	405.0	521.0	(Bernot et al., 2023)
Collembola	Tomoceridae, <i>Tetodontophora_bielanensis</i>	405.0	521.0	(Bernot et al., 2023)
Peracarida	Gammaridae, <i>Armadillidium_nasatum</i>	358.2	521.0	(Bernot et al., 2023)

**Table S6. phytools model fits for maximum likelihood ancestral state reconstruction.**

SYM = symmetric substitution rates; ARD = asymmetric substitution rates; SYM.G = symmetric substitution rates with gamma heterogeneity; ARD.G = asymmetric substitution rates with gamma heterogeneity; HRM2 = hidden rates model with symmetric substitution rates and two hidden rate categories. log(L) = log likelihood score; d.f. = degrees of freedom (number of model parameters); AIC = Akaike information criterion; Weight = model importance via ANOVA.

Model	log(L)	d.f.	AIC	Weight
SYM	-68.10199	1	138.204	0.0065576866
ARD	-67.93943	2	139.879	0.0028382863
SYM.G	-62.41206	2	128.824	0.7137752031
ARD.G	-62.36246	3	130.725	0.2759361780
HRM2	-67.09620	4	142.192	0.0008926461

**Table S7. Marginal probabilities at select nodes.** Probabilities of germ plasm nodes at select ancestors derived via maximum likelihood. Node\_number = labels at internal nodes of Figures S1 and S2; IND = induction; GP = germ plasm.

Clade	Node_number	P_IND	P_GP
Panarthropoda	1	0.994	0.006
Arthropoda	2	1.000	0.000
Chelicerata	3	1.000	0.000
Myriapoda	4	0.994	0.006
Pancrustacea	5	1.000	0.000
Decapoda	6	0.119	0.881
Hyalidae + Talitridae	7	0.084	0.916
Poduromorpha + Symphypleona	8	0.668	0.332
Collembola	9	0.987	0.013
Insecta	10	0.999	0.001
Holometabola	11	0.806	0.194
Hymenoptera	12	0.283	0.717
Coleoptera	13	0.986	0.014
Chrysomelidae + Curculionidae	14	0.120	0.880
Lepidoptera	15	0.996	0.004
Mecoptera + Siphonaptera	16	0.518	0.482
Antliophora (Diptera + Mecoptera + Siphonaptera)	17	0.532	0.468
Diptera	18	0.385	0.615

**Table S8. Results of Pagel's test of correlated rates in germ band type and germ cell specification mode.**

Model	log(L)	AIC	p-value
Independent	-79.52302	167.0460	n/a
Bidirectional	-65.03560	146.0712	7.90998e-06
GC dependent	-69.61696	151.2339	4.98718e-05
GB dependent	-68.75534	149.5107	2.10696e-05

**Table S9. Results of search for *oskar* orthologs.** BUSCO scores may differ from Table S4 for the seven unannotated genomes used in our analysis (*Clogmia albipunctata*, *Mayetiola destructor*, *Macrocentus cingulum*, *Epiphyas postvittana*, *Nymphalis antiopa*, *Pyrrhocoris apterus*, *Tribolium confusum*), as BUSCO scores and *oskar* presence are determined from AUGUSTUS genome annotations. “Blondel\_*oskar*\_count” column indicates the total number of *oskar* orthologs previously identified across all datasets from this species (Blondel et al., 2021). Empty cells indicate species that were not analyzed in our previous study on *oskar* ortholog evolution (Blondel et al., 2021). “*oskar*\_present” column indicates that *oskar* was detected in the current analysis and/or in our previous work (Blondel et al., 2021). “*data\_type*” indicates whether the protein predictions were obtained directly from protein fasta files on NCBI (“NCBI-annotated genome”), from TransDecoder run on transcriptome shotgun assembly nucleotide sequences (“TSA”), or from *ab initio* annotations generated with AUGUSTUS (“AUGUSTUS-annotated genome”). We were unable to find sequence data for species in the “*sub\_species*” column, and instead substituted the genomes/transcriptomes for species in the same taxonomic family. The “*oskar*\_accession” columns provide either NCBI protein accessions for NCBI-annotated genomes (“<https://www.ncbi.nlm.nih.gov/protein/>”), TSA nucleotide accessions for TSAs (“<https://www.ncbi.nlm.nih.gov/genbank/tsa/>”), or AUGUSTUS-generated accessions for AUGUSTUS-annotated genomes. All Oskar protein sequences are available in *oskar\_proteins.fa*.

See Table S9 at [https://github.com/rishabhraj Kapoor/panarthropoda\\_gc\\_specification\\_evolution](https://github.com/rishabhraj Kapoor/panarthropoda_gc_specification_evolution), under supplementary tables, commit ID ff9945c.