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# Review

# (In)discrete charm of the polyembryony: Evolution of embryo cloning

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**Abstract**. Polyembryonic development, where multiple embryos are formed from a single zygote, evolved at least 15 times in six different phyla in animals. The mechanisms leading to polyembryony and the forces that shaped the evolution of the polyembryonic developmental program have remained poorly understood. Recent studies of the polyembryonic development in the endoparasitic wasp *Copidosoma floridanum* have revealed that the evolution of polyembryony is associated with the evolution of developmental novelties such as total cleavage, early specification of embryonic and extra-embryonic fates, and a specific cell proliferation phase. These changes cumulatively result in the formation of thousands of embryos from a single egg. Laser ablation studies and analysis of early cell fate specification have revealed that a single blastomere representing the progenitor of the primordial germ cell regulates the proliferation of the embryos. We propose that evolutionary changes in cell cleavage, cell interactions, and the cell-differentiation program, reminiscent of interactions between the germinal stem cell and stem cell niche in fly ovaries, underlies the evolution of polyembryony.

**Keywords.** Evolution of development, polyembryony, total cleavage, stem cells, embryo cloning, neoplastic tumors, twinning.

## Introduction

Polyembryony represents the form of development and reproduction where a single zygote gives rise to multiple embryos. The offspring produced by polyembryony represent genetic clones but are different from their mother due to meiotic recombination preceding fertilization. The embryo cloning characteristic for polyembryonic development can be initiated in the embryonic stage (Platyhelminthes: Monogenea; Arthropoda: Insecta; Bryozoa and Chordata: mammals) where a single zygote (egg) gives rise to multiple embryos, or in larval stages, by budding (Cnidaria, Platyhelminthes: Cestoidea and Trematoda; Arthropoda: Crustacea and Echinodermata), where the proliferation of the larval cells creates new individuals [1]. The obligate form of polyembryony, in contrast to the facultative polyembryony that is recorded in many species in different phyla, is rare. Nevertheless, it evolved at least 15 independent times within six animal phyla, which despite their phylogenetic distance share common life histories [1]. Polyembryonic species develop as internal parasites or placental embryos capable of taking up nutrients from the external environment. The extent of polyembryonic development ranges from the creation of four embryos from a single zygote in the Nine-banded

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armadillo to several thousand in the polyembryonic wasp, *Copidosoma floridanum,* and up to a million progeny in a single cyst of the endoparasitic flatworm, *Echinococcus granulosus* [2].

This unusual form of development raises questions: which evolutionary forces shaped the evolution of polyembryony and which mechanistic changes in development underlie the embryo cloning process? In larval and embryonic polyembryony, the cloning process appears to be based on the plasticity of the cell differentiation process. In larval polyembryony, the development of future progeny is initiated from a special cell(s). The developmental potential of this cell(s) and its origin are currently unknown [1], but it must include either de-differentiation of the somatic cells or proliferation of "set-aside" undifferentiated cells to create multiple progeny. In embryonic polyembryony, the initial creation of thousands of undifferentiated cells is a prerequisite for later formation of numerous embryos.

One of the most studied forms of polyembryony in animals is the creation of multiple embryos from the single egg in insects. Polyembryony in insects evolved five independent times, exclusively in parasitic species that develop within the body of the host. This form of development evolved in Strepsiptera [3] and four families of parasitic wasps (Hymenoptera), including Encyrtidae, Driinidae, Platygasteridae and Braconidae [4]. One of the most dramatic cases of polyembryony is polyembryonic development of the wasp *C. floridanum,* where a single egg gives rise to up to 2000 embryos created through the embryonic clonal proliferation [5].

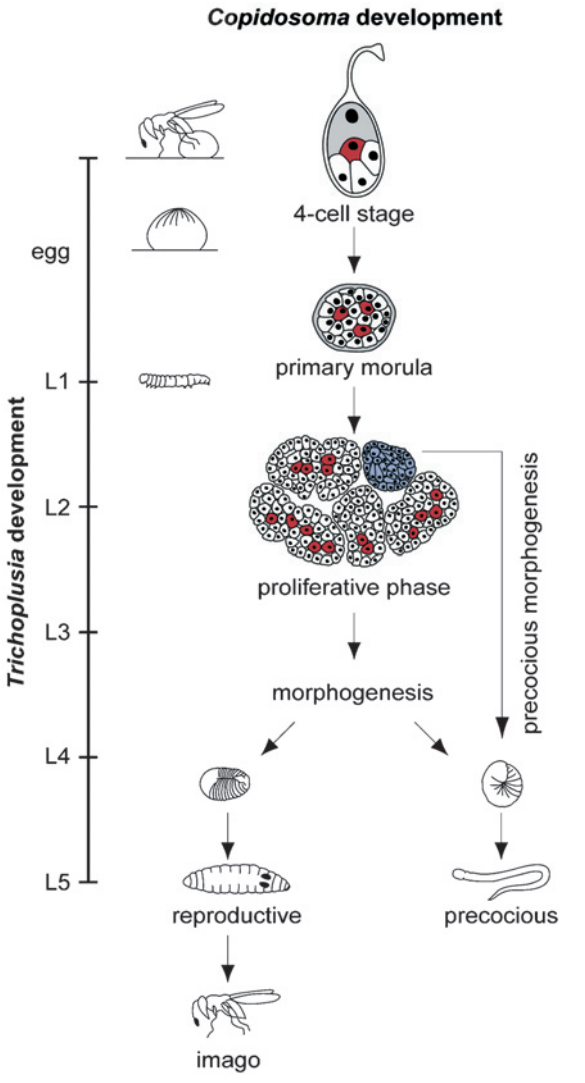


**Figure 1.** Embryonic development of *Copidosoma floridanum* and its host *Trichoplusia ni.* For *Copidosoma*, polar body and polar body-derived extra-embryonic membrane are shown in gray; germ line (primordial germ cell, PGC cells) in red; proliferating morulae without PGC (progenitors of the precocious embryos) in blue. L1–L5, larval instars of *T. ni.*

## Polyembryony in *Copidosoma*: a challenge for the *Drosophila* paradigm of development

*C. floridanum* is a parasitic wasp that parasitizes the eggs of the host, the moth *Trichoplusia ni* (Fig. 1). After parasitization, the host emerges and undergoes five host instars. During the process of host development *Copidosoma* undergoes embryonic development within the host body surrounded by the nutritive insect blood (hemolymph). As a result of embryonic proliferation, up to 2000 larvae are formed synchronously during the fifth host instar. These larvae pupate and emerge as adult wasps.

The embryonic development of this endoparasitic insect differs dramatically from the development of other insects. First, *Copidosoma* oviposits tiny yolkless eggs (50 μm in size, a size similar to mouse eggs) that are surrounded by a thin chorion. The first cleavage of the egg is total, and leads to the formation of two posterior blastomeres (which will give rise to the embryo proper) and an anteriorly localized polyploid cell (Fig. 1, gray) that results from the fusion of polar nuclei [6]. This cell will form the polyploid syncytial extra-embryonic membrane (gray). The second embryo cleavage creates one small blastomere and three equal-size blastomeres. The small cell (Fig. 1, red) is different from the other cells as it retains an injected fluorescent tracer, and is thus dye-uncoupled from other cells [7]. Embryonic blastomeres then undergo cleavages and become enveloped by the syncytial extra-embryonic membrane, and embryos emerge from the chorion into the host hemolymph and form primary morula. The primary morula implants in the host tissue and

initiates the proliferative phase of development that increases cellular mass many fold [6]. In monoembryonic animals, developmental progression from that point in embryogenesis would include a transition from morula-stage embryo to gastrulation and segmentation, leading to a completely segmented animal. In contrast, "insertion" of the proliferative phase in the canonical monoembryonic developmental program represents the developmental novelty responsible for clonal production of thousand embryos in *Copidosoma*. The proliferative phase is initiated by the split of the primary morula and creation of the polymorula, which consists of many proliferative morulae. Each proliferative morula at this stage consists of hundreds of round, apparently non-differentiated cells (Fig. 1, Fig 4b), surrounded by the extra-embryonic membrane [6]. These packages of cells become subdivided by the ingressing extra-embryonic membrane into progressively smaller clusters of cells. When the number of cells per cluster reaches about 20-30 at the fourth host instar larva, these cells undergo a change in cell shape from round to fibroblastic. The establishment of cell contacts results in cell compaction and simultaneous *de novo* formation of 2000 embryonic primordia [6]. Following compaction, each embryo undergoes gastrulation and segmentation to form larva. Thus, polyembryonic embryogenesis in *Copidosoma* shows similarities to mammalian embryonic development, including early separation of embryonic and extra-embryonic lineages, morula morphology, implantation, compaction and most importantly the net increase of the embryonic mass that is unique to mammals [8, 9]. These evolutionary changes apparently represent convergent evolution driven by the similar developmental environment: placental development in mammals and nutritive host environment in *Copidosoma*. Obligatory polyembryony evolved in mammals (armadillo) [10] and insects (parasitic wasps), but while in mammals polyembryony is conceptually compatible with the regulative development of the mouse embryo, polyembryony in insects is in sharp contrast with maternal pre-patterning of the *Drosophila* embryo.

## Early development of *Drosophila*: is maternal embryo pre-patterning compatible with evolution of polyembryony?

How can convergent evolution arise in two developmental systems that have so different early developmental potentials? Insect development exemplified by the paradigm of *Drosophila* development represents the prime example of maternal pre-patterning of the embryo. Localized maternal determinants such as mRNA of the anterior morphogen *bicoid* and posterior morphogen *nanos* form diffusing gradients from the opposite poles (Fig. 2a–d) specifying the positional fate of the nuclei in the *Drosophila* syncytium [11]. They trigger a downstream patterning cascade consisting of gap, pair-rule and segment-polarity genes (reviewed in [12]). This process is facilitated by a special type of early syncytial cleavages in *Drosophila* and many other insects, where nuclear cleavages are not followed by the cleavages of cytoplasm, so that proteins can diffuse in the common compartment and specify the cell fate based on the concentration of transcription factors. In this multinucleate context, removal of a small proportion of the anterior and posterior cytoplasm causes large deletions of embryonic anterior and posterior [13], emphasizing the major role of the maternal contribution in embryo patterning. In contrast to *Drosophila* development, mammalian embryos, which undergo total cleavage immediately to form individual cells, do not use pre-localized maternal determinants for embryo patterning [8]. The destruction of early blastomeres at early stages of development results in the formation of completely normal embryos in mouse [14], demonstrating a regulative type of development. If the precursor of polyembryony relied on the system where maternal determinants specify the positional cell fates (Fig. 2), as determined in basal parasitic wasps such as *Nasonia*, the insertion of the proliferative phase in such a developmental program would create two problems. First, cells with particular positional fate could have been intermixed with cells containing different positional fate during proliferation (Fig. 2e–g) resulting in scrambled developmental asymmetry. Second, the proliferative process could dilute the maternal morphogen, resulting in incorrect cell fate in the progeny of the particular cell lineage. The final challenge is *de novo* organization of 2000 embryonic axes (Fig. 2h). If maternal determinants specifying a particular positional information are used in early development, it would be necessary to sort out the proliferated cells in 2000 embryonic primordia according to the original maternally pre-determined positions. Alternatively, maternally specified fate would have to be erased or not used at all in early development and *de novo* formation of embryonic axes would have to be done by a stochastic process [15].

## *Copidosoma* developmental plasticity: larval caste formation

*Copidosoma* exhibits another developmental peculiarity: this wasp forms two different larval morpho-

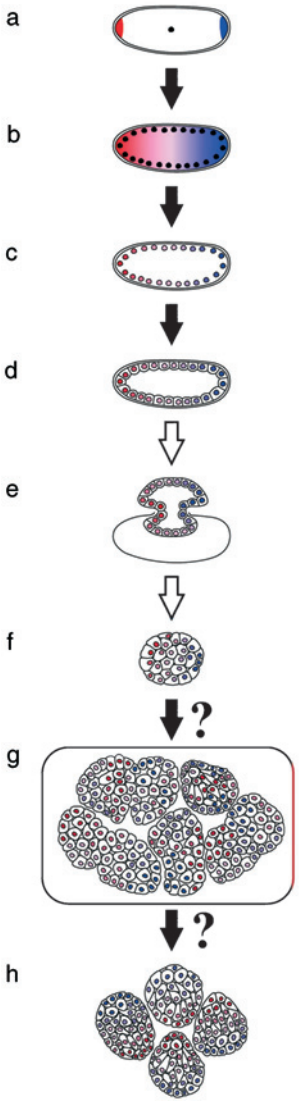


**Figure 2.** Hypothetical ancestor with maternal pre-patterning system and polyembryonic development. (*a*) Polar localization of maternal determinants. (*b*) Diffusion of maternal determinants in syncytium. (*c*) Establishment of the nuclear fate. (*d*) Cellularized embryo with established positional cell fate. (*e*) Emergence of maternally pre-patterned embryo from chorion. (*f*) Formation of primary morula. (*g*) Proliferative phase: scattering of positionally determined cells during the proliferation leading to the dilution of the maternal determinants. (*h*) *De novo* formation of 2000 embryonic axes: how to re-establish the original polarity? Red: anterior determinant; blue: posterior determinant; shades of red and blue: positional fate of cells based on the concentration of determinants; ?= hypothetical mechanism of proliferation and *de novo* formation of embryonic axes.

types from a single zygote. During the early host instars, while the majority of embryos undergo proliferation, some embryos initiate morphogenesis, forming the special larval caste termed 'precocious larvae' (Fig. 3a). These larvae have elongated and slender bodies with prominent mandibles (Fig. 3a, inset). They circulate through host hemolymph and perform functions analogous to those of worker and soldier castes in social insects such as ants and bees. They defend their siblings from the interspecific competitors [16] and are involved in the sex ratio adjustment of the broods [17]. They never molt and are ultimately consumed by their reproductive siblings. In contrast to the precocious larvae that initiate morphogenesis early and asynchronously, the reproductive embryos simultaneously form 2000 embryonic primordial at the fourth host instar. They undergo morphogenesis forming embryonic axes at random relative to each other [6], form compact larvae that consume the host (Fig. 3b), pupate and form adult wasps. The reproductive to precocious larval ratio under normal development is 19 to 1, but the number of precocious larvae can increase in the presence of an interspecific competitor [18].

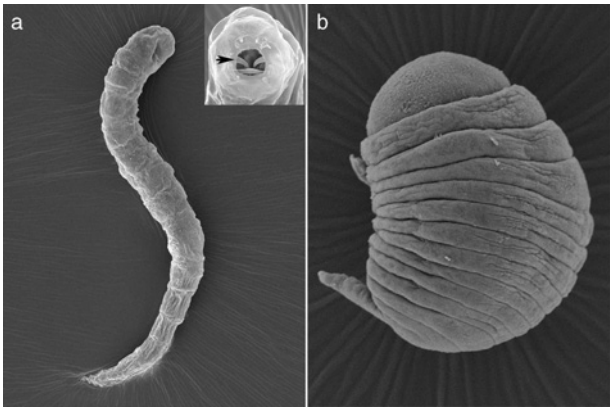


**Figure 3.** SEM of the precocious and reproductive larvae. (*a*) Precocious larva, inset: close-up of the head, arrow points to the mandible. (*b*) Reproductive larva. Anterior is up.

In contrast to caste polyphenism in ants and bees, where castes are formed from the same genome but under different environmental conditions (exposure of worker and queen larvae to different food and hormones) [19], *Copidosoma* larval castes are clones created from the same zygote that develop in the same environment of the host. Thus, the question remains: how is the larval polyphenism specified in clonal embryos?

## Maternal pre-patterning in *Copidosoma*: specification of the germ line

The germ line is one of the first developmental fates specified in many organisms [20]. The RNA helicase *vasa* is the ubiquitous germ-line marker in metazoans involved in the specification of the primordial germ cell (PGC) lineage. PGCs represent the first cells that

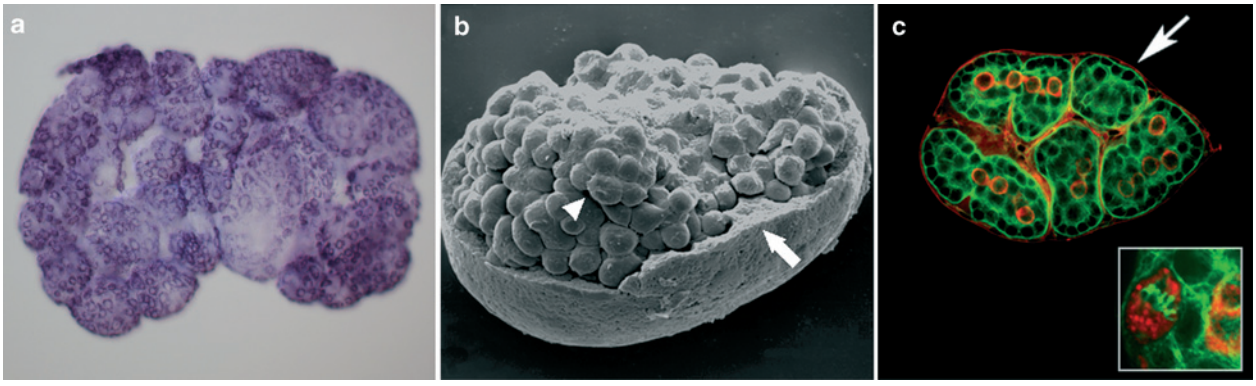


**Figure 4.** The proliferative phase of development. (*a*) Expression of alkaline phosphatase mRNA (blue) in the extra-embryonic membrane. (*b*) The round morphology of the proliferating cell in the individual proliferative morulae; arrow marks extra-embryonic membrane (removed from the top), arrowhead marks individual cell. (*c*) Expression of the Vasa protein in the proliferating morulae. Red: Vasa protein, green cell outlines visualized with anti-tubulin staining, white arrows points to the morulae without Vasa-positive cells. Inset: Division of Vasa-positive cells; both cells inherit Vasa antigen.

give rise exclusively to germ cells by clonal mitotic divisions [21]. PGCs are progenitors of germ-line stem cells (GSCs) that undergo self renewal, differentiate into gametes, and ultimately produce all of the cell types in future offspring.

Isolation of the *Copidosoma vasa* mRNA (*Cfvas*) homologue and examination of its pattern of expression showed that *Cfvas* is transcribed in nurse cells in *Copidosoma* ovaries [22]. Vasa protein localizes in the structure called oosome [22], which was proposed to be homologous to the *Drosophila* germ-line pole plasma (nouage). Thus, in *Copidosoma* at least one asymmetrically localized maternal determinant is deposited in the forming egg. After oviposition Vasa protein is invariably localized into the dye-uncoupled small cell, showing that this early cellular asymmetry is also paralleled by a molecular asymmetry. In the primary morula this asymmetry is perpetuated in several cells that express Vasa protein (Fig. 1, red). Following the initiation of the proliferative phase Vasa-positive cells are scattered in individual proliferating morulae (Fig. 1, Fig. 4c). During the process of division, the daughter cells all inherit Vasa protein, suggesting that they represent cell lineage. Following the entrance into the morphogenetic phase each reproductive embryonic primordium receives two Vasa-positive cells. These cells remain localized at the posterior and give rise to the embryonic gonads (Fig. 1). Thus, maternal cellular asymmetry marked by the expression of Vasa protein perpetuates throughout the proliferative phase and becomes continuous with the germ line, suggesting that *Copidosoma* specifies the PGC maternally.

## Development of the precocious embryos: differential distribution of PGC specifies the reproductive potential

While it was known that the reproductive larvae give rise to adults and have a reproductive function, the reproductive potential of the precocious larvae in *Copidosoma* was uncertain. They do not molt and become consumed by their reproductive sibling. This poses the question of whether they have a reproductive potential that is simply not realized due to their premature death or they entirely lack potential for the reproduction. In social hymenoptera, workers are sterile in contrast to fertile queens. However, this sterility is often conditional. Both queens and workers have germ-line progenitor cells, but in queens the reproductive apparatus become hypertrophic, while in workers ovarioles degenerate [23]. However, in some cases workers can restore their reproductive potential and become reproductives [24]. During the proliferative phase in *Copidosoma* it was noticed that some proliferative morulae do not contain Vasa-positive cells (Fig. 1, blue, Fig. 4c). These morulae undergo differentiation and give rise into the precocious embryos that do not inherit PGCs. Thus, the mechanism based on segregation of PGC lineage in reproductives, and the failure of the precocious embryos to inherit PGCs represents novel cell-sorting mechanism that specifies the caste fate. This mechanism specifies in an all-or-none fashion a different reproductive capacity in genetically identical embryos.

## Function of PGCs in *Copidosoma*: caste fate and proliferation

In contrast to *Drosophila*, where PGCs undergo migration through the embryo to reach their position in future gonads [25], *Copidosoma* PGCs undergo a

complex journey, which includes cell parceling during the proliferative stage, differential segregation to two castes and final localization at the embryonic gonads [22]. Clearly PGCs must be involved in the formation of the germ line, but their complex ontogeny poses the question of whether they have other functions in polyembryonic development? Besides Vasa, these cells likely contain many other determinants that may have a role in embryo germ cell specification, proliferation or caste fate. One possibility is that these cells have a cell-autonomous function in specifying the germ line as in *Drosophila*. This scenario predicts that the removal of PGC progenitor cell will result in formation of the reproductive larvae without gonads. Alternatively, this cell could have non-cell autonomous function(s) so that the germ-line specification is coupled with other developmental processes. Laser ablation of Vasa-positive cell at the four cell stage (Fig. 1, red) has revealed that it has multiple functions [22, 26]. As a consequence of ablation, *Copidosoma* reproductive embryos did not proliferate as detected by lack of formation of the reproductive embryos [22]. However, the precocious larvae development was not affected, resulting in normal numbers. Laser ablation of the Vasa-positive cell reduced 95% of polyembryonic proliferation. In contrast, ablation of any of the large blastomeres at the same stage (Fig. 1, white cells) restores the development and proliferation of reproductive embryos [22]. This suggests that the PGC progenitor has a dual function: it regulates proliferation and the reproductive caste fate.

## Cellularization and uncoupling of the germ line and morphogenetic program are prerequisites for evolution of polyembryony

Development of *Copidosoma* emphasizes early compartmentalization and modularity of the developmental program as key elements underlying the evolution of polyembryony. First, evolution of new, cellular environments allow specification of individual cell fates in contrast to the specification of nuclear fate *en masse* in syncytium of *Drosophila*. This is reflected in early separation of embryonic and extra-embryonic lineages in the wasp *via* formation of a novel structure: the polar body-derived extra-embryonic membrane. In other insects polar bodies degenerate and do not have a role in development. However, in *Copidosoma* the polar body-derived extra-embryonic membrane has an active role in embryogenenesis, enveloping and subdividing clusters of proliferative cells. This novel structure expresses alkaline phosphatase (Fig. 4a), an insect enzyme associated with cell membrane of the gut and implicated in uptake of nutrients [27]. This reinforces the trophic role of polar body-derived membrane, supporting the proposal that both mouse and *Copidosoma* embryos defer they embryo patterning to build first the trophic apparatus [28], resulting in convergent evolution of development.

In addition, the new cellular environment allows spatial and temporal uncoupling of processes that occur simultaneously in syncytially cleaving insects. In *Drosophila* and basal ectoparasitic wasps, including *Nasonia,* the posterior patterning is tightly associated with specification of the germ line. In these species the posterior patterning and germ line specifying gene products are localized together in the posteriorly localized pole plasma [29, 30]. *Nanos* represents an example where a single gene plays roles both in posterior body morphogenesis and maintenance of PGCs [29, 31]. Thus, the existence of dual, patterning and germ-line roles of *nanos* appear to be ancestral in syncytially cleaving hymenoptera.

In *Copidosoma,* Vasa protein becomes localized to the oosome and consequently to the PGC progenitor at the four-cell stage embryo. *Copidosoma nanos* is also localized to the small blastomere (Zhurov and Grbić, unpublished), which is consistent with the scenario in *Drosophila*, where Vasa protein is involved in localization of *nanos* mRNA [29]. Thus, it is likely that *Copidosoma nanos* is used for the germ-line maintenance, its ancestral function determined in other metazoans [32]. We currently do not know the complete pattern of *nanos* expression, but its localization to the PGC progenitor would mean that *nanos* would segregate to the reproductive but not to the precocious morphs, and thus would not be active in body patterning. Together with the expression of gap and pair-rule genes in morphogenesis of both morphs (Terzin and Grbić, unpublished), it appears that the specification of the germ line and embryo axial polarity is uncoupled in *Copidosoma.* Ultimately, these evolutionary changes lead to the developmental program where the germ line is specified maternally, while embryo body patterning is initiated apparently by the zygotic genes.

Beside dual function in proliferation and specification of the caste fate, the Vasa-positive cell appears to maintain other cells in the proliferative morulae in undifferentiated state. Formation of morulae without PGCs results in morphogenesis of precocious larvae, indicating that escape from the PGC influence results in initiation of differentiation.

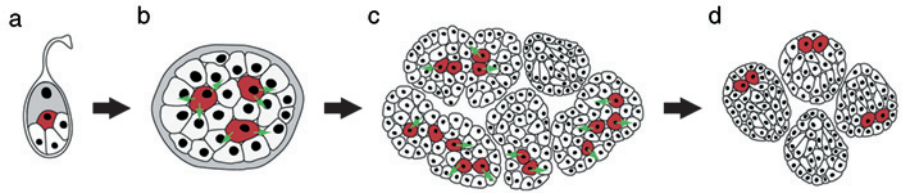


**Figure 5.** Model of polyembryonic development. (*a*) Specification of the PGC progenitor. (*b*) Formation of the primary morula and initiation of signaling from PGC lineage leading to proliferation. (*c*) Proliferation and prevention of the differentiation by PGC. (*d*) Termination of the proliferation and initiation of the germ-line formation and morphogenesis of the embryonic primordium. PGC red; green arrows depict putative signal(s) inducing proliferation and preventing differentiation.

## Model of *Copidosoma* development: evolutionary changes in the cell differentiation mechanism underlie polyembryonic development

Our understanding of polyembryonic development is limited compared to other major developmental systems. However, laser ablation data coupled with description of PGC development serve as a starting point for building the testable model of *Copidosoma* development (Fig. 5).

In *Copidosoma* maternal determinants clustered in the oosome are segregated into the small blastomere initiating PGC lineage. However, beside germ-line determinants this cell apparently inherits other determinants for embryo proliferation. This is consistent with our laser ablation studies where elimination of PGC progenitor cell at four-cell stage terminates both proliferation and reproductive caste formation. In *Copidosoma* it is important to create a mass of undifferentiated cells in sufficient numbers for the formation of numerous embryos. Thus, the progeny of Vasa-positive cells could be a source of signals that would maintain cells in proliferating morulae in an undifferentiated state and stimulate their proliferation.

Striking differences in the early developmental program between *Drosophila* and *Copidosoma* make it difficult to extend the paradigm of the fly early developmental program to that of polyembryonic development. On the other hand, the stem cell-based mechanism for the formation of germ cells in *Drosophila* is remarkably analogous to the scenario of *Copidosoma* early development. In *Drosophila* ovaries, cap cells represent a special cellular environment that constitutes the stem cell niche populated by GSCs [33]. The stem cell niche emits short-range signals to GSCs, inducing them to divide asymmetrically. This creates one GSC that stays in contact with the niche and another one that is formed away from the stem cell niche. The cell without contact with the stem cell niche undergoes differentiation to form the cystoblasts that will ultimately form the oocyte. Overexpression of the bone morphogenenetic protein homolog Decapentaplegic (Dpp), the major component of niche signaling, causes overproliferation of GSC and de-differentiation of cystoblasts [34]. In contrast, GSC mutants in components of Dpp signaling pathway undergo precocious differentiation [35]. By analogy, in *Copidosoma* elimination of PGCs causes termination of proliferation. In addition, lack of PGCs in proliferating morulae appears to initiate differentiation of the precocious embryos. In this system, PGCs would be equivalents of the stem cell niche by promoting proliferation and preventing differentiation.

The cells in the proliferative morulae have a round morphology (Fig. 4b), and form three-dimensional structures resembling *Drosophila* neoplastic tumor mutants. Neoplastic tumors are formed in mutant imaginal discs where cells loose their epithelial architecture forming spherical masses of cells as they overproliferate [36]. Current models for the formation of neoplastic tumors range from cell mispolarization causing disruption of signaling pathways to mislocalization of cell fate determinants that alter fate-regulating signaling pathways, leading to expansion of a proliferative "stem-cell"-like population [36]. In both cases, it is clear that alteration of cell signaling plays a major role in cell proliferation and that it affects the ability of cells to differentiate. Thus, one appealing hypothesis, consistent with laser ablation data and features of *Copidosoma* development, is that evolutionary changes in the cell differentiation mechanism, reminiscent of that of the stem cell regulation program, underlie polyembryonic development.

The program regulating the proliferation and maintenance of undifferentiated cells must have a specific counting mechanism because at a certain point proliferating cells terminate proliferation and enter morphogenesis. Presumably, at this point PGCs segregated to individual embryonic primordia (marked by Vasa expression, Fig. 4c) switch off the signal(s) that keep surrounding cells undifferentiated, and assume a role in germ-line formation. This is paralleled with changes in cell shape and polarity, where

establishment of cell adhesion marks initiation of morphogenesis. The regulation of the counting mechanism can be complex, as illustrated by studies of neuroblast proliferation in *Drosophila*. In this case, neural division is regulated by the interplay of neuroblast intrinsic inputs, nutrition-dependent humoral cues from the fat body, and local signals from glial-dependent niches [37]. Thus, in the context of the complex development of *Copidosoma,* in its tight developmental synchrony with the host it is likely that both intrinsic and host factors could provide signaling cues regulating exit from proliferation.

Proposed model accounts for the sequential proliferation and morphogenesis and initiation of early morphogenesis of the precocious larvae but still does not explain the mechanism of the axial polarity specification in *Copidosoma* embryos. Posteriorly localized PGCs in reproductive embryos could represent symmetry-breaking points to initiate posterior patterning once they become segregated into individual embryonic primordia. However, precocious larvae establish their axial polarity without PGCs. Assuming that the axial patterning mechanism is the same in both morphs, the lack of these cells excludes the possibility that PGCs are involved in establishment of the axial polarity. This model suggests that the germline specification process represents a conserved module that can be co-opted for diverse functions ranging from posterior patterning in *Drosophila* to specification of the caste fate and proliferation in *Copidosoma*.

## Utility of *Copidosoma*: from evolution of development to the twinning model

Is *Copidosoma* development just an unusual fluke of evolution or does this dramatic change in evolution have broader biological significance? First, changes in *Copidosoma* early developmental program are of importance for the evolution of developmental mechanisms. They suggest that there are fewer developmental constraints in early embryogenesis compared with later stages of development. Second, it highlights the importance of cellular context and cell interactions in broadening the repertoire of evolutionary developmental changes. Third, it implicates the influence of the embryonic environment in shaping the evolution of developmental program. The independent cases of evolution of convergent developmental programs in nutritive embryonic environment [28] provide the evidence that embryonic environment contributes to shaping developmental processes in groups as divergent as mammals and insects.

Besides providing clues to understand evolutionary changes in development, *Copidosoma* can also be a useful satellite system for understanding mechanic changes in cell proliferation, differentiation and twinning. *Copidosoma* enter cell proliferation resembling the neoplastic tumor formation in *Drosophila* but *Copidosoma* proliferative morulae exit the proliferation program at the defined point and enter differentiation, forming synchronously 2000 monozygotic twins (MZT). In a broader context, MZT represents a form of polyembryonic development where multiple embryos are generated from a single zygote. The accidental form of polyembryonic development, where an individual egg occasionally forms MZT, has been described in almost all animal groups, including humans [38–42]. This accidental form of polyembryony suggests that eggs of otherwise monoembryonic species have the regulative capacity to generate multiple embryos. The causes of MZT in humans and other animals are still largely speculative [43], and a major obstacle for progress in understanding MZT is a lack of animal models of monozygotic twining [44].

The only two mammals known to undergo obligate polyembryony are the armadillos *Dasyphus novemcinctus* and *Dasyphus hybridis* [10] but difficulties associated with animal husbandry, seasonal breeding, and a month-long implantation process impairs their utility as a mammalian MZT model. From an experimental perspective, two major difficulties hinder the study of MZT. First, since accidental monozygotic twinning is unpredictable, and only a few mammals are obligatory polyembryonic (but produce very few embryos), the sheer limitation and difficulty of analyzing their embryogenesis makes certain lines of experimentation untenable. The second impediment, related to the first, is that the limited number of monozygotic twins produced by most organisms makes identification of genes operative in MZT difficult to analyze. The unique biology of *Copidosoma* overcomes the constraints on abundance of embryo twinning. Coupled with moderate genome size, 534 Mbp [45], and new approaches in molecular biology, like single cell gene profiling and RNAi, *Copidosoma* offers new opportunities for identifying genes and cellular events regulating MZT.

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