THE ROYAL SOCIETY

doi 10.1098/rstb.2001.0977

On the evolution of early development in the Nematoda

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The phylum Nematoda serves as an excellent model system for exploring how development evolves, using a comparative approach to developmental genetics. More than 100 laboratories are studying developmental mechanisms in the nematode *Caenorhabditis elegans*, and many of the methods that have been developed for *C. elegans* can be applied to other nematodes. This review summarizes what is known so far about steps in early development that have evolved in the nematodes, and proposes potential experiments that could make use of these data to further our understanding of how development evolves. The promise of such a comparative approach to developmental genetics is to fill a wide gap in our understanding of evolution—a gap spanning from mutations in developmental genes through to their phenotypic results, on which natural selection may act.

Keywords: nematode; development; evolution; *Caenorhabditis elegans*

1. INTRODUCTION

Developmental genetics has much to tell us about evolution. Many of the mutations that have effects on development have ultimate effects on adult morphology, both in the laboratory and in nature. We have known for over a century of cases in which evolutionary modifications to early development have caused dramatic changes to later morphology. For example, the shell-forming embryonic cell of the bivalve *Unio* is enlarged compared with that of its close relatives, resulting in a much larger shell on its larva (Lillie 1895, 1898). Such cases are of great interest because they have the potential to tell us how mutations have caused developmental programmes to evolve, producing the diversity of organisms we find in nature. Other modifications to development affect only certain stages of development, and have little apparent effect on adult phenotype. For example, similar snails can be produced by two different types of embryonic cell lineage (Van den Biggelaar & Guerrier 1979; Freeman & Lundelius 1992). These cases are also of interest, because they tell us in what ways developmental programmes can be modified by evolution and yet produce a similar adult.

In the past decade or so, the use of nematodes for studying how development evolves has flowered (for reviews, see Fitch & Thomas 1997; Sommer 1997; Felix 1999). This subversion of *Caenorhabditis elegans* and its relatives as study organisms has, in a sense, taken a reverse path to that taken by *Drosophila* in the early 1900s. It is often unappreciated that *Drosophila* was initially adopted by Morgan and his laboratory for studying evolution, not development (Morgan 1903; Kohler 1994). Morgan chose flies primarily because they were fast and prolific breeders, traits essential to his search for mutations that subtly affected phenotype and hence that could underlie the natural variation found within a species, on which selection might be acting. Morgan's aims were derailed by his finding of much more dramatic developmental mutants than he had expected, but the traits for which *Drosophila* was initially chosen turned out to be critical in converting *Drosophila* into a model genetic system for studying development. *C. elegans*, on the other hand, was chosen by Brenner as a model genetic system for studying development (and neurobiology), again because it is a fast and prolific breeder, and because large numbers of organisms can be kept on agar plates, the laboratory habitat of choice for molecular geneticists trained on bacteria and viruses (Brenner 1974, 1988). Also, nematode embryos are translucent, allowing observation of developmental events and tracing of cell lineages throughout development (Sulston *et al.* 1983). These traits are now turning out to facilitate the conversion of *C. elegans* and its nematode relatives into a system for studying how development evolves.

Because it is possible to keep more than 30 species of free-living soil nematodes in a container the size of a breadbox on a laboratory bench, developmental events can be readily observed in a much larger number of taxa than has generally been practicable among other studies (for a review, see Raff 1996) that address how development evolves. Examining large numbers of species opens up the possibility of thoroughly mapping the evolution of early developmental traits onto a phylogeny (Voronov *et al.* 1998; Goldstein *et al.* 1998; Dolinski *et al.* 2001). This allows us to identify exactly where in evolution modifications to development have occurred (figure 1).

The greatest advantage of using nematodes to study how development evolves is the potential to employ what is known about *C. elegans* development as a basis for identifying mechanisms that may have evolved and for identifying candidate genes that may have been mutated to account for the modifications to development that we find. It should be noted that there are also disadvantages to using nematodes for studying how development evolves. First, nematodes do not show the wide range of

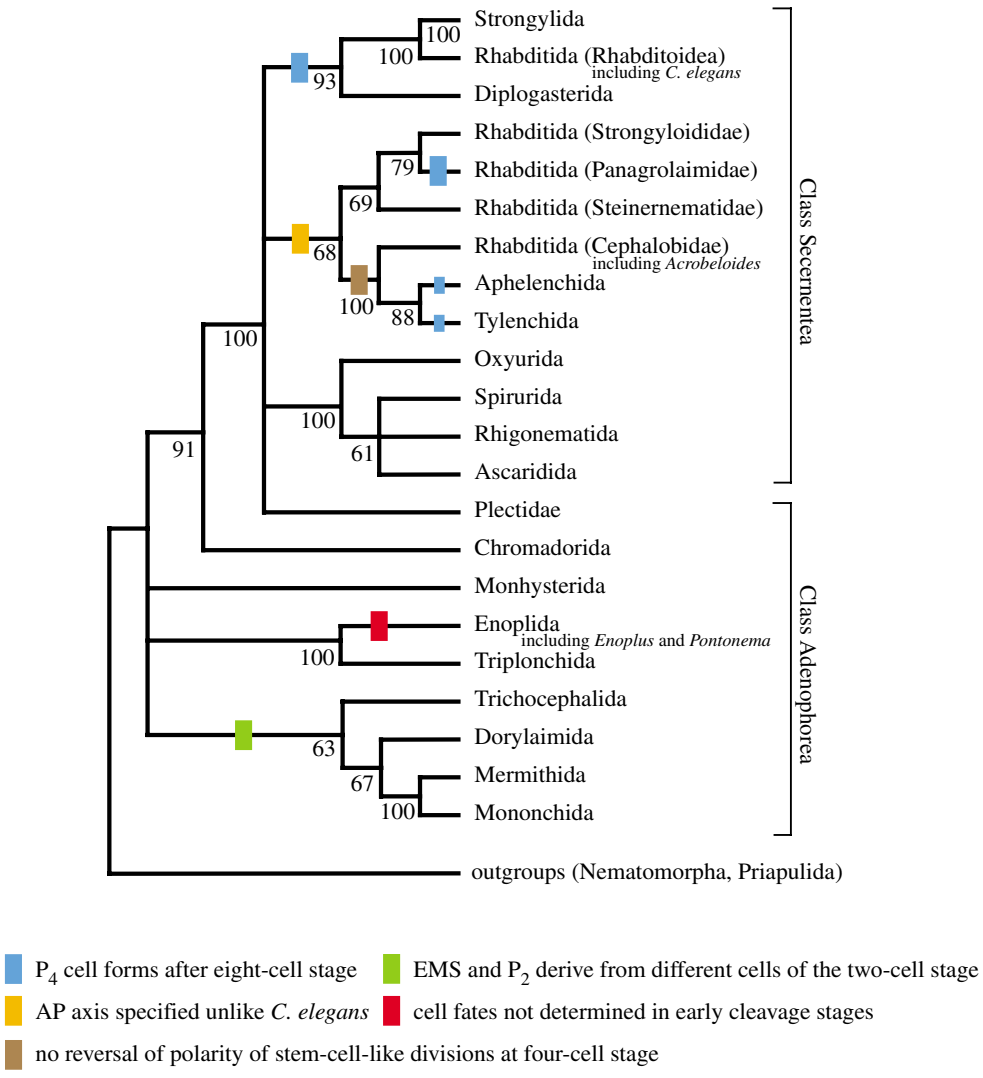

Figure 1. A mapping of evolutionary shifts in early nematode development discussed in this review onto the molecular phylogeny of the orders of nematodes generated by Blaxter *et al.* (1998). This mapping is only a preliminary, speculative version, as some of these characters have not yet been examined in many of the orders depicted: the two characters on the left side of the key have been examined primarily in the Secernentea; and reversal of polarity of stem-cell-like divisions at the four-cell stage has been examined in Rhabditoidea, Panagrolaimidae and Cephalobidae (Skiba & Schierenberg 1992) and one species of Tylenchida (*Meloidogyne incognita*) (B. Goldstein, unpublished data). Also, the polarity of change in the two characters on the right side of the key is not yet known. Nematodes have been separated traditionally into two classes (listed at right). Some aspects of the molecular phylogeny are not yet well resolved (Blaxter *et al.* 1998; for an updated phylogeny of the Secernentea, see Dolinski *et al.* 2001). Numbers reflect confidence in the monophyly of the clades indicated by indicating the maximal percentage bootstrap support in neighbour-joining analyses (Blaxter *et al.* 1998). Orders are shown, and, for the order Rhabditida, which is paraphyletic, families are indicated in parentheses. The family to which *C. elegans* belongs is indicated, and three other genus names discussed in the text are shown. Tick marks indicate each evolutionary shift, as coloured in the key. Small tick marks indicate that only some members of this taxon share the trait that is indicated as derived. Note that data from Borgonie *et al.* (2000) suggest that a species of Mononchida may develop like the Enoplida, rather than as drawn here based on Malakhov (1994). The results of Blaxter *et al.* (1998) suggest that the class Adenophorea is not a monophyletic group. The outgroups indicated were used in constructing the phylogeny (Blaxter *et al.* 1998), and not for speculating on the position and polarity of the evolutionary shifts in developmental characters.

morphological diversity found in such phyla as molluscs or arthropods. It is undeniable that most nematodes are vermiform, although many strikingly non-vermiform nematodes exist (Tarjan *et al.* 1977) and scanning electron microscopy has revealed incredible diversity in fine structure (DeLey 2000). Second, nematodes almost entirely lack a fossil record, which means that we cannot pin dates to phylogenies. This problem does not of course preclude the construction of a phylogeny; it only precludes determining when the divergences between clades occurred. There is a potential route around this; for cases in which parasitic nematodes can be established to have co-speciated with their hosts (Brooks & Glen 1982; Mitter & Brooks 1983), the divergence times of the hosts might be used to establish divergence times for the nematodes. It might be possible to use such a method to calibrate the clock of molecular evolution for at least parts of the phylogeny.

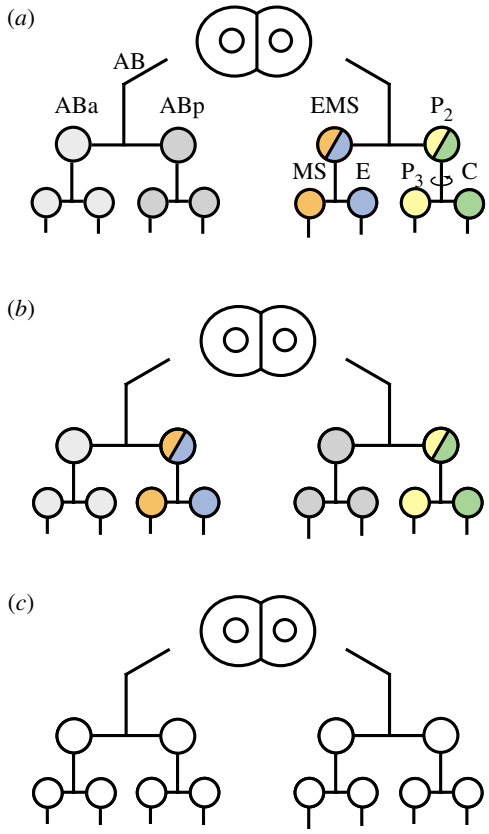

Figure 2. (*a–c*) Three patterns of cell lineage found in the nematodes (Malakhov 1994). Anterior is to the left, posterior is to the right in each lineage. Colours indicate cell types found to be produced by each cell: grey cells produce ectoderm (light grey is ABa derivatives, dark grey is ABp derivatives); orange cell produces pharynx, mesoderm and anterior ectoderm; blue cells produce endoderm (gut); green cells produce primarily posterior ectoderm, and yellow cell produces a somatic daughter and the germline precursor. White cells in (*c*) indicate that cells cannot be identified at these early stages (see text (§ 2a)). The arrow in (*a*) indicates variation in the orientation of the germline precursor division (Skiba & Schierenberg 1992), as discussed in the text (§ 2b). The patterns indicated in (*a*) occur in all Secernentean orders studied and some Adenophorean orders. Timing of cell divisions is not represented on these lineages.

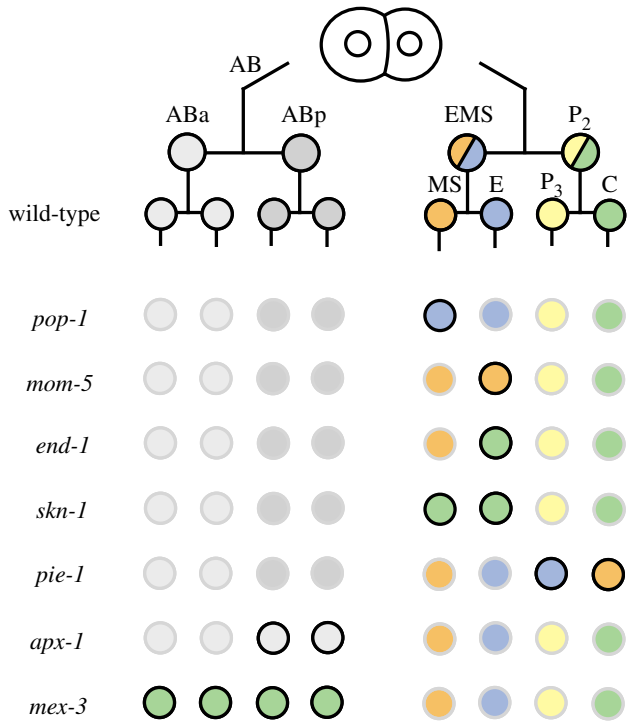

Figure 3. Some genes that specify cell fate in *C. elegans*. The colour code for cell fates is the same as that used in figure 2. Seven genes are shown, along with simplified interpretations of their loss-of-function mutant phenotypes. Cells whose fates are affected by mutations in a gene are circled in black to the right of the gene name, with the altered colour inside the black circle indicating how the cell develops in mutants; those either unaffected, or known to be affected in a way that does not suggest a simple adoption of the fate of another eight-cell stage blastomere, are outlined in grey (for a review, see Schnabel & Priess 1997).

This review discusses the evolutionary modifications to early (pregastrulation) stage nematode development that have been found so far, and proposes some experiments that may further our understanding of how development evolves. Most of the work to date has been descriptive, laying the groundwork for understanding how early development evolves by identifying what evolutionary modifications to early development have occurred during nematode evolution. Some recent studies have involved embryological perturbations that begin to address how alternative developmental mechanisms work (Wiegner & Schierenberg 1998, 1999). Genetic perturbations have not yet been performed to address how early development has evolved (although such experiments are being done for evolutionary modifications to later development; reviewed recently by Fitch & Thomas (1997), Sommer (1997) and Felix (1999)). In some cases, evolutionary modifications to early development bear some resemblance to the phenotypes of known loss-of-function mutations in *C. elegans* (Malakhov 1994; Riddle *et al.* 1997; Dolinski *et al.* 2001). It may be too much to expect single mutations like these to have caused the evolutionary alterations to development that are found, particularly since many of these mutations are lethal in *C. elegans*, but such genes at least provide a starting point towards elucidating the molecular underpinnings of such evolutionary transitions.

2. EVOLUTION OF THE EARLY EMBRYONIC CELL LINEAGE

(a) *Cell lineages of diverse nematodes*

How have cell lineage patterns diverged through the evolutionary history of nematodes? To begin to answer this question, we would like to have cell lineage data from representatives of diverse groups of nematodes. Such information would tell us whether diverse nematodes develop in a lineage pattern resembling that found in *C. elegans*, or whether cell lineage patterns have been modified through evolution and in what manners they have done so. Comparative cell lineage data for the diverse members of a phylum are available for very few phyla, but the shortlist of such phyla does in fact include the nematodes. This work, which represents the broadest stroke to date at understanding how early development

has evolved in the nematodes, was done primarily by the Soviet embryologists Drozdovskii, Malakhov and their co-workers, beginning in the late 1960s. Most of the work is published in Russian. In 1986, the results were compiled in a Russian-language book, which was updated and published in English after Soviet obstacles to publishing abroad were lifted (Malakhov 1994). Cell lineages were produced by following cell divisions until early in gastrulation. Although the lineages produced are far short of the complete lineage generated for *C. elegans* development (for review, see Sulston 1988), at least an assessment of many cells to major tissue types could be made by noting cell placements among tissues that can be recognized in early gastrulae. The results they found are striking. Instead of all nematodes having early cell lineages that resemble *C. elegans*, three broad categories of lineages were found. In one category, early cell divisions occur roughly as they do in *C. elegans* (figure 2*a*), although with some variations, as will be discussed below (§ 2b–d, 3a,b, 4). A second category differs in that cell fates are divided up by the four-cell stage in a different manner (figure 2*b*). The third category (figure 2*c*) differs more dramatically: cells cannot be distinguished from each other during early cleavage stages on the basis of size or division rate. Mapping these three categories of lineage patterns onto a molecular phylogeny (Voronov *et al.* 1998; Blaxter *et al.* 1998) suggests that each exists in a distinct part of the phylogeny (figure 1). Although some authors have made guesses as to which was the mode of development used by ancestral nematodes (e.g. Malakhov 1994), current data cannot yet resolve this issue with any degree of certainty.

Nematodes with the second category of cell lineage (figure 2*b*) have great potential to serve as a model for understanding the molecular underpinnings of how a pattern of cell-fate specification can evolve. In these nematodes, similar cell fates are produced by a different lineage pattern from that found in *C. elegans*: two cells (called P_2 and EMS) produced by the posterior cell of the two-cell stage in *C. elegans* are instead each produced by a different cell of the two-cell stage. Given that we have some understanding of how these cells are specified in *C. elegans*, of what genes are involved, and of genes involved in specifying other cell fates in the early embryo of *C. elegans* (figure 3) (for a review, see Schnabel & Priess 1997), examining the distribution and function of homologues of the relevant gene products may form a fruitful avenue for further research.

The third category of lineage (figure 2*c*), in which cells are indistinguishable in the early embryo and which occurs in nematodes of the order Enoplida (see figure 1), has been characterized further by injecting lineage tracers into embryonic cells of two nematodes, *Enoplus brevis* and *Pontonema vulgare* (Voronov *et al.* 1986, 1989; Voronov & Panchin 1998). To determine whether the indistinguishable cells of the early embryos also have variable fates, a cell of the two-cell stage was injected with lineage tracer, and the border between labelled and unlabelled cells was examined in developing embryos. For both species, the position of the border was found to vary from one injected embryo to another, indicating that these two cells can contribute to varying parts of the embryonic lineage and, hence, that fate specification is unlikely to have occurred by the two-cell stage. This is unlike many other nematodes, including *C. elegans*, in which the contributions of the cells of the two-cell stage to the developing embryo are invariant (Sulston *et al.* 1983). Despite this difference, one aspect of early cell lineage appears conserved throughout nearly all nematodes studied to date: one cell produced at the third round of division forms the entire gut. Injecting lineage tracers into cells of the eight-cell stage of *Enoplus brevis* and *Pontonema vulgare* has revealed this is true even in the nematodes with variable lineages (figure 4) (Voronov & Panchin 1998). Unlike this gut founder cell, at least some of the other seven cells of the eight-cell stage must normally make variable contributions to the developing embryo, as more than eight patterns of labelled progeny resulted from labelling single eight-cell stage blastomeres (Voronov *et al.* 1989; Voronov & Panchin 1998). How the gut precursor cell's fate is specified in *Enoplus brevis* and *Pontonema vulgare* is not yet clear, although this cell's fate is unlikely to be irreversibly determined as early as the eight-cell stage, as a preliminary report of removing single blastomeres of the four- or eight-cell stage indicates that this consistently has no effect on development (Voronov & Panchin 1998).

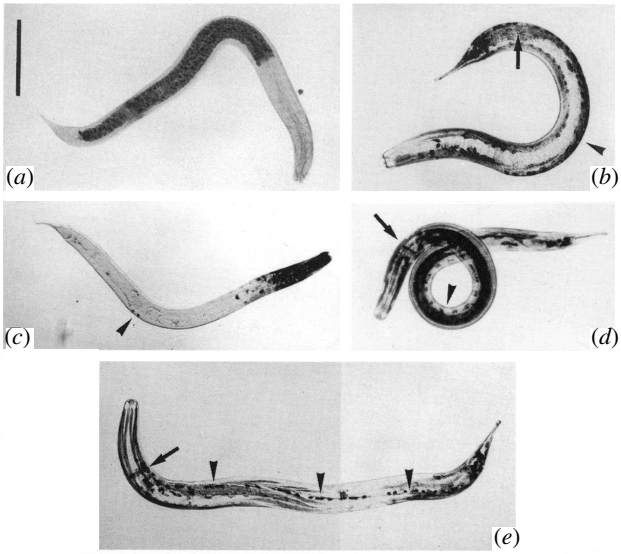

Figure 4. Patterns produced in first-stage larvae by injecting lineage tracer into one of the eight indistinguishable cells of the eight-cell stage *Enoplus* embryo. In (*a*), only the gut is labelled. In (*b–e*), multiple other tissues are labelled in each worm. Scale bar, 200 µm. (From Voronov & Panchin (1988), with permission.)

Cell lineages in some nematodes are currently being followed much further (Borgonie *et al.* 2000), up to the stage at which the embryo starts moving (this stage is about the 500-cell stage in *C. elegans*, soon after elongation of the embryo has begun). The use of four-dimensional (4D) microscopy has made such work feasible. Four-dimensional microscopy is the generation of time-lapse recordings of multiple focal planes through a subject. Software is available for then viewing the recordings forwards or backwards in time and adjusting the plane of optical section at any time (Thomas *et al.* 1996; Schnabel *et al.* 1997). Borgonie and co-workers have recently used this technique on diverse nematodes. Their preliminary

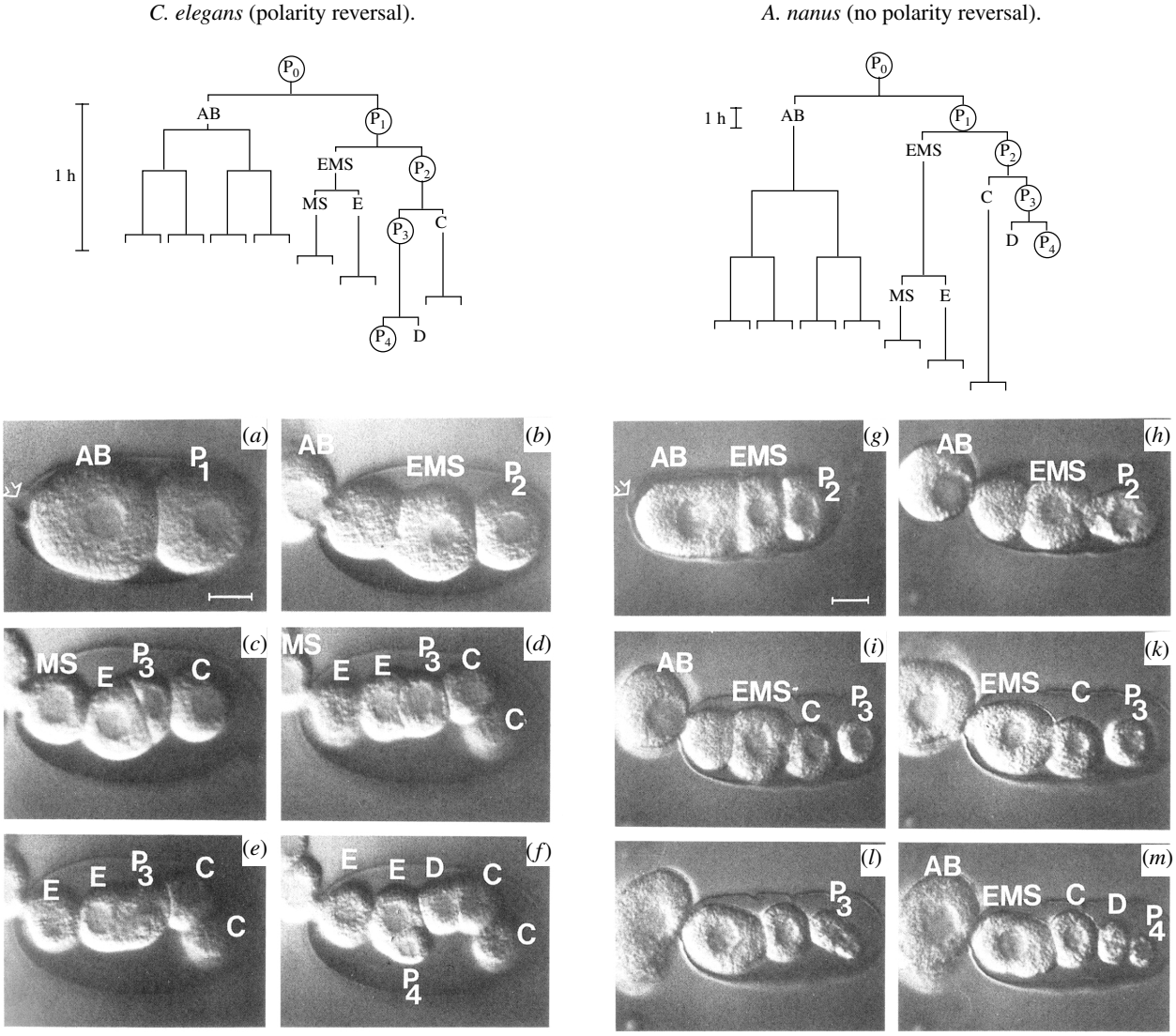

Figure 5. Germline divisions reverse polarity after two rounds of division in *C. elegans*, but not in *Acrobeloides nanus*. Cell lineages (top) and observations of germline cell division orientation upon eliminating spatial constraints by removing a cell early in development (bottom) are shown. Time-scale is shown at the left of each lineage, and germline cells are circled. Anterior is to the left and posterior to the right in both the lineages and the photographs. Scale bars, 10 μm. (Adapted with permission from Skiba & Schierenberg (1992) and Wiegner & Schierenberg (1998).)

report of both 4D microscopy and lineage tracer injection results (Borgonie *et al.* 2000) has suggested that a species in the order Mononchida also has a variable early cell lineage, contrary to a previous report in which the same species was described as developing by a cell lineage similar to that found in closely related orders (figures 1 and 2*b*) (Malakhov 1994). Future use of 4D microscopy may make it possible to resolve cell lineages in diverse nematodes in more detail than has been feasible in the past.

(b) *The polarity of an early cell division*

Schierenberg (1987) and Skiba & Schierenberg (1992) found that stem-cell-like divisions occur with alternative polarities among nematodes of the category shown in figure 2*a*, defining two further subcategories of developmental strategies. Early divisions in *C. elegans* produce a germ cell by successive stem-cell-like divisions: at each of the first four rounds of division, there is one cell whose division produces one precursor of only somatic cells and one precursor of both somatic and germ cells. The primordial germ cell (called P_4) is produced at the fourth stem-cell-like division. After two divisions in *C. elegans*, the polarity of stem-cell-like divisions reverses; this is not obvious in intact embryos, as cell division orientations are constrained by other cells and by the eggshell, but it can be revealed by examining unconstrained divisions after extruding other cells through a hole made in the eggshell (Schierenberg 1987). When Skiba & Schierenberg (1992) carried out this manipulation in *Acrobeloides nanus* (formerly referred to by them as *Cephalobus* sp.), they found that germ-cell-producing divisions did not reverse after two divisions (figure 5). Examining several species for this character suggests that the *Acrobeloides* pattern was derived in the evolution of a clade that includes the family Cephalobidae and order Tylenchida (figure 1).

Exactly what sort of cellular mechanism has evolved here remains an open and interesting question. We do not yet understand how this polarity reversal occurs in *C. elegans*, although one set of findings regarding how P granules are segregated might be relevant. (P granules are ribonucleoprotein particles that are segregated to one side of a cell before each of the four stem-cell-like divisions leading to the formation of the primordial germ cell. P granule components are required for the germline to develop (Seydoux & Strome 1999).) Hird *et al.* (1996) have imaged P granules in living *C. elegans* embryos, and found that they are segregated in one pattern before each of the first two divisions and in a different pattern before each of the following two divisions, in which polarity is reversed. The pre-reversal pattern involves P granules moving in the cytoplasm unattached to any apparent larger structure and coincident with bulk movements of cytoplasm. The post-reversal pattern involves the P granules temporarily associating with the nuclear envelope as the nucleus migrates to one side of the cell. Both forms of P granule segregation occur during interphase. One might expect that in the absence of reversal at the four-cell stage in *Acrobeloides nanus*, the mechanism used during the first two division cycles in *C. elegans* would simply be used in all four divisions leading to the formation of the germ cell progenitor. Instead, a third pattern was found to occur in all four early divisions, by examining P granules in fixed embryos (figure 6) (Goldstein *et al.* 1998). P granules stay symmetrically distributed in *Acrobeloides* embryos throughout interphase, then surround the metaphase plate of chromosomes, and do not become asymmetrically localized until anaphase; no bulk movements of cytoplasm can be detected at the time the P granules are segregated. Whether the change in polarity of stem cell divisions and the change in the mode of P granule localization evolved together or separately is an open question; although these characters have been examined in some other species (Skiba & Schierenberg 1992; Goldstein *et al.* 1998), they have not yet been surveyed together in a large number of species.

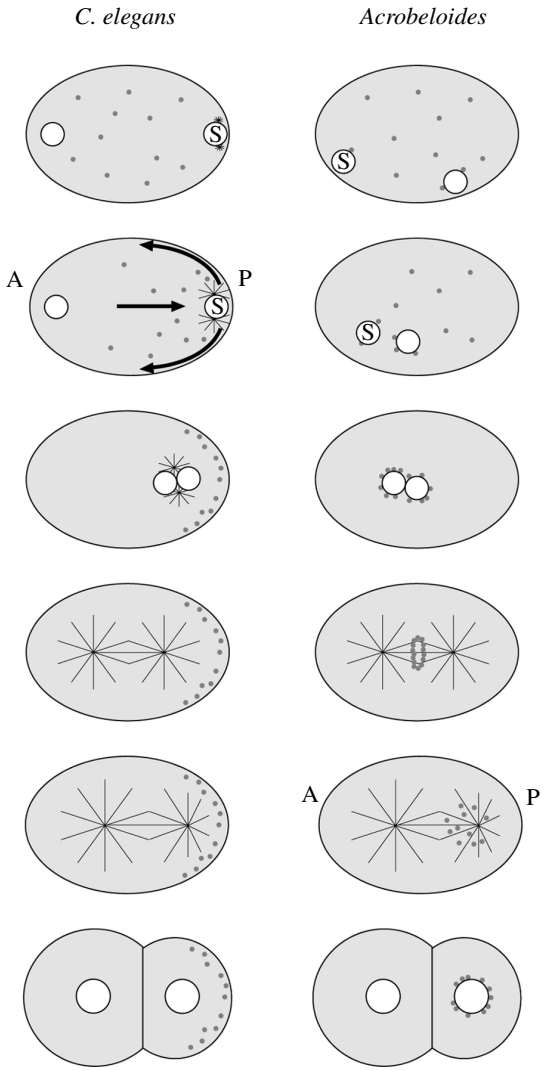

Figure 6. Mechanisms for AP axis specification differ between *C. elegans* and *Acrobeloides*. Embryos are drawn for each species at successive stages from top to bottom: during pronuclear appearance, pronuclear migration, pronuclear meeting, metaphase, anaphase and the two-cell stage. P granules are drawn in dark grey; straight lines indicate astral microtubules; arrows indicate flows of cytoplasm. The stage at which P granules first become asymmetrical in each species is labelled with A for anterior, P for posterior (Goldstein *et al.* 1998).

(c) *Cell lineage timing*

The speed with which steps in embryogenesis occur is perhaps the only character in early development known to evolve that has a readily apparent adaptive significance: organisms that can develop faster may be represented by more individuals over time because they can produce more of themselves in a shorter time. Skiba & Schierenberg (1992) found variation in the speed and order in which cells divide among nematodes of the category shown in figure 2*a*. The order of somatic cell divisions in the species examined is nearly identical to that found in *C. elegans*, but the pace of somatic cell divisions versus the pace of germline divisions varies considerably. This variation roughly correlates with developmental tempo; the somatic cell divisions occur up to about 20 times more slowly in the slowly developing species, whereas the germline divisions occur only up to about four times more slowly. As a result, in the fast-developing *C. elegans*, the primordial germ cell (called P_4) is present at a stage when there are 23 somatic cells, whereas in *Acrobeloides nanus*, in which embryonic development occurs more slowly at the same temperature, the primordial germ cell is present at a stage when there are only five somatic cells (figure 5). The timing of divisions in *Acrobeloides* means that certain inductive cell interactions discovered in *C. elegans* (for a review, see Schnabel & Priess 1997) must involve different cells in *Acrobeloides*, or the relevant cell fates in *Acrobeloides* must be specified without a need for cell interactions. Cell-fate specification does in fact appear to work very differently in *Acrobeloides*, as will be discussed below (§ 3a,b).

The evolutionary history of germline division rates in the class Secernentea was reconstructed by Dolinski *et al.* (2001), using original observations in 37 species and literature reports of 33 other species. Two characters

relevant to cell lineage timing were scored—whether the cells of the two-cell stage divide near the same time as opposed to one dividing more than once before the other divided, and whether the P_4 cell formed before or after there were seven other cells in the embryo. Mapping the data onto a phylogeny and comparing the data with the character states of an outgroup suggested that alterations in each character have occurred multiple times in the evolution of nematodes (figure 1).

(d) *A single species with two cell lineages*

Some parasitic nematodes alternate between free-living and infective generations. Such species offer unusual opportunities to examine how selection may produce differing modes of embryonic development not by creating two different genomes, as occurs in speciation events, but by modifying a single genome to produce two alternating developmental programmes (similar cases may be found between the two sexes of a species, although in *C. elegans*, differences in the embryonic lineages between the sexes are slight (Sulston 1988)). Spieler & Schierenberg (1995) have asked whether the early embryonic cell lineages of alternating generations differ from each other, in the toad-infecting nematode *Rhabdias bufonis*. In this species, parasitic individuals living in the lungs of toads produce 130 µm long embryos that are released from the toad in its faeces; these embryos grow up to become free-living adults, which produce 90 µm long embryos that as larvae can infect toads again. Despite the difference in size of embryos, the two forms have very similar cell lineages. There are, however, some differences in their cell lineages: the free-living embryos (the embryos that will develop into free-living worms) develop *ca.* 20% more slowly, and the germline precursor cell P_3 divides before its sister cell C, whereas it divides after C in the parasitic embryos. Also, the adult free-living hermaphrodite has about 870 non-gonadal nuclei, whereas the adult parasite has about 4700 non-gonadal nuclei, in large part due to extra post-embryonic cell divisions in the gut (Spieler & Schierenberg 1995). Understanding how these two developmental programmes are produced will be an interesting topic for future study.

3. EVOLUTION OF SPECIFICATION MECHANISMS

(a) *Anteroposterior (AP) axis specification*

Even AP axis specification, the initial step in embryonic pattern formation in nematodes, can evolve significantly. In *C. elegans*, the AP axis is specified by the position of the fertilizing sperm: when the position of sperm entry is altered, the orientation of the AP axis is altered as well. The mechanism by which the sperm generates asymmetries is not completely clear, but it involves in part generating both polarized flows of cytoplasmic material and polarized movement of P granules to one side of the fertilized egg. It has been proposed that the cytoplasmic flows and P granule movements are driven by an interaction of astral microtubules, associated with the sperm pronucleus, with the actin-rich cortex of the fertilized egg (Goldstein & Hird 1996), and there is evidence that the generation of other molecular asymmetries is dependent on the position of astral microtubules at this stage as well (Wallenfang & Seydoux 2000).

As some nematodes lack sperm altogether and develop from parthenogenetically activated eggs, it is clear that the sperm must not be used as a cue for AP axis specification in all nematodes. If nematodes could be identified in which the sperm is not used as a cue, it would be possible then to examine to what extent this mechanism has evolved during the evolutionary divergence of the taxa involved. To find such nematodes, the events of the first cell division cycle were examined in 30 species (Goldstein *et al.* 1998). Several species were found in which isolated individuals were capable of reproducing, and no sperm pronucleus appeared during pronuclear migration, providing evidence that these species were in fact parthenogens. Other species were found in which a sperm pronucleus appeared, but the position of the sperm pronucleus was not a predictor of the posterior pole of the developing embryo. By studying one such species in detail, it was found that the mechanism for axis specification differs dramatically: as well as the sperm not being used to specify the axis, astral microtubules are not associated with the sperm pronucleus, polarized flows of cytoplasm do not occur, and the distribution of P granules becomes asymmetrical at a different time (figure 6). Although this mechanism for axis specification clearly differs from that used in *C. elegans*, exactly how or when the AP axis is specified in this species is not yet clear.

To reconstruct the evolutionary history of mechanisms for AP axis specification, the presence or absence of polarized flows of cytoplasm in each species was mapped onto a molecular phylogeny of the nematodes. The results suggest that the mechanism used by *C. elegans* is the ancestral mechanism for the class Secernentea, and another mechanism evolved, most probably either once or twice, in ancestors of some relatives of *C. elegans* (figure 1).

(b) *Evolution of cell-fate specification*

Wiegner & Schierenberg (1998, 1999) have taken the study of how early nematode development evolves a step beyond description, by conducting experiments that perturb normal development to address how cell fates are specified. They have found remarkable differences in mechanisms of cell-fate specification between *C. elegans* and *Acrobeloides nanus*: cell-fate specification appears to occur by about as different a method as possible, given the similarity of their cell lineages (see figures 2*a* and 5). In both species, the gut is derived from a single eight-cell stage blastomere, and ablating this cell prevents gut differentiation (Wiegner & Schierenberg 1998). Specification of this gut founder cell in *C. elegans* depends on a cell–cell interaction at the four-cell stage (for a review, see Thorpe *et al.* 2000). Wiegner & Schierenberg found that while removing the inducing cell in *C. elegans* could prevent gut development, removing the same cell in *Acrobeloides* could not, suggesting that this inductive interaction does not occur in *Acrobeloides*. These experiments turned out to be the tip of the iceberg of how differently cell-fate specification works in *Acrobeloides*. Gut development could occur when either one or the other cell of the two-cell stage was removed, suggesting that repressive interactions must normally occur between blastomeres to ensure that only one cell normally becomes the gut founder cell (Wiegner & Schierenberg 1998). Perhaps most surprisingly, when the mother cell (called EMS) of

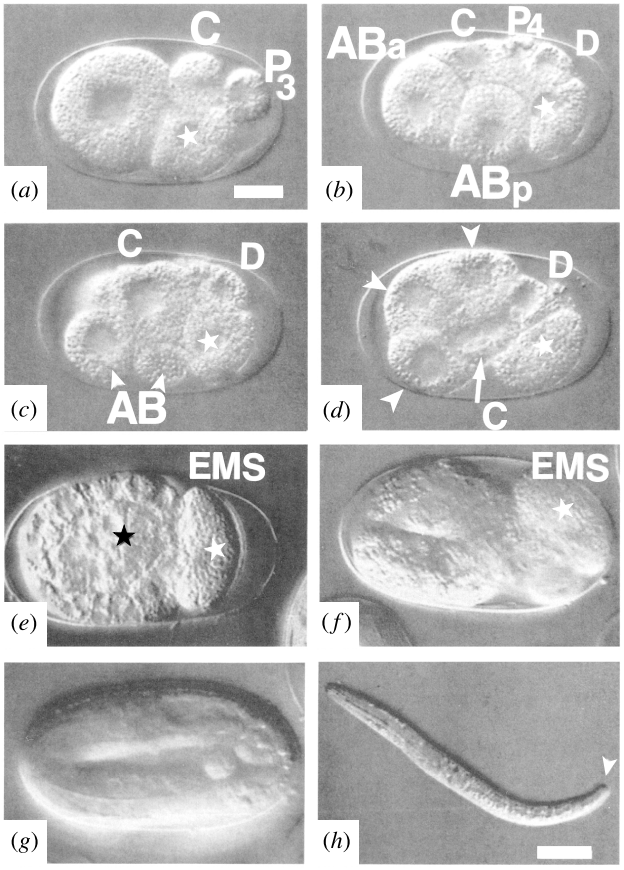

Figure 7. A normal larva can develop after laser ablation of the EMS cell in *Acrobeloides*. (*a–h*) The ablated EMS cell is marked in successive stages by white stars. The larva shown in (*h*) moulted and reproduced normally. Scale bar in (*a*), 10 µm; in (*h*), 25 µm. (From Wiegner & Schierenberg (1999) with permission.)

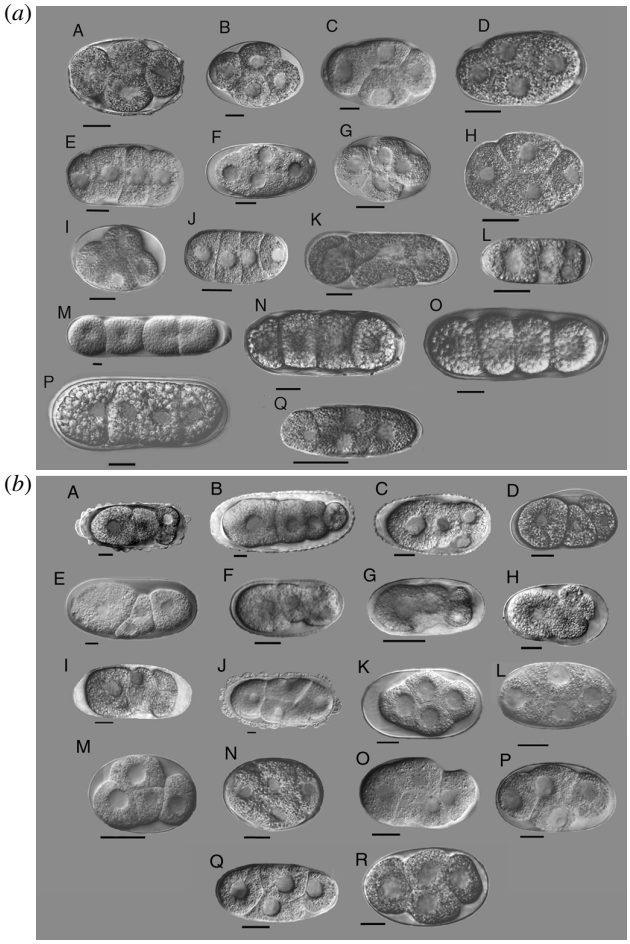

Figure 8. Orientation of cells at the four-cell stage in 35 species. Families and genera are, for (*a*) A–J, Rhabditina: A, *Teratorhabditis*; B, *Bunonema*; C, *Cruznema*; D, *Mesorhabditis*; E, *Diploscapter*; F, *Rhabditella*; G, *Rhabditis*; H, *Caenorhabditis*; I, PS1010; J, *Protorhabditis*. K, Aphelenchidae (*Aphelenchus*). L, Aphelenchoididae (*Aphelenchoides*). M–P, Tylenchina: M, Belonolaimidae (*Belonolaimus*); N, Heteroderidae (*Meloidogyne*); O–P, Pratylenchidae (*Pratylenchus* and *Nacobbus*). Q, Teratocephalidae (*Teratocephalus*). (*b*) A–I, Cephalobidae: A, *Chiloplacus*; B, *Zeldia*; C, *Cephalobus*; D, *Acrobeloides*; E. *Acrobeles*; F, *Eucephalobus*; G, *Pseudoacrobeles*; H, *Nothacrobeles*; I, *Cervidellus*. J–L, Diplogastrina: J, *Diplenteron*; K, *Aduncospiculum*; L, *Pristionchus*; M, *Goodeyus*. N–Q, Panagrolaimidae: N, *Halicephalobus*; O, *Panagrellus*; P, *Panagrobelus*; Q, *Panagrolaimus*. R, Brevibuccidae (*Plectonchus*). All scale bars are 20 µm. (From Dolinski *et al.* (2001) with permission.)

the gut founder cell is ablated in *Acrobeloides*, another cell (called C) takes its place, in what appears to be a cell lineage transformation, and in some cases a worm develops that appears fairly normal, has roughly the normal number of nuclei, and can even reproduce (figure 7). Ablating the somatic founder cell of the two-cell stage can give a similar result, by way of at least two successive cell-fate transformations. Germline cells, however, are not replaced upon ablation. The results suggest that several repressive cell interactions ensure that normal cell fates are executed in *Acrobeloides* development and, in the absence of these interactions, somatic founder cells can be replaced by other somatic founder cells (Wiegner & Schierenberg 1999). Wiegner & Schierenberg (1998) have also found different requirements for early zygotic gene expression between *C. elegans* and *Acrobeloides*. Bossinger & Schierenberg (1996) have found differences in gap junctional communication and the pattern of cytoplasmic bridges between cells, although the developmental significance of this finding is not yet clear. The differences between *C. elegans* and *Acrobeloides nanus* development are formidable, and it will be interesting to see how genetic pathways have evolved to produce such differences. Genes involved in specification of the gut, such as WNT pathway genes (Thorpe *et al.* 2000) and transcription factors required for gut development (Schnabel & Priess 1997) must be regulated very differently in *Acrobeloides*.

4. EVOLUTION OF THE ORIENTATION OF CELL DIVISIONS

Evolutionary shifts in early nematode development that bear resemblance to known *C. elegans* mutants suggest tractable ways forward towards a molecular understanding of evolutionary changes to development. The case for which an evolutionary shift in early nematode development bears the most resemblance to known *C. elegans* mutants involves the orientation of cell divisions in the early embryo. Dolinski *et al.* (2001) have examined cell division orientation at the two-cell stage in the class Secernentea, observing 37 species (figure 8) and using literature reports for 33 other species, as they did for

scoring characters of cell lineage timing (see § 2c). In many species, the anterior cell positions its mitotic spindle parallel to the short axis of the embryo, and the other cell positions its mitotic spindle parallel to the long axis of the embryo, as occurs in *C. elegans*. In several other species, both cells position their mitotic spindles along the long axis of the embryo, producing a linear four-cell stage. This occurs not only in embryos with eggshells that are elongate, but also in embryos with eggshells of similar proportions to the eggshell of *C. elegans* embryos. Dolinski *et al.* (2001) reconstructed the evolutionary history of these states by mapping the results onto a phylogeny and comparing these with an outgroup; their results suggest that the *C. elegans* pattern is ancestral for the class Secernentea, and evolutionary transformations to the linear pattern have occurred at least three times. Whether such transformations had further developmental consequences is not yet clear. Progeny of the anterior cell in *C. elegans* appear to be equivalent to each other, suggesting that the division orientation of this cell may not be critical—although in *C. elegans*, cell interactions that generate differences between the progeny of the anterior cell depend on cell contacts that are altered by the linear pattern of division orientation (for a review of the *C. elegans* data, see Schnabel & Priess 1997). It will be interesting to see if evolutionary transformations of division orientation are associated with transitions in other characters, such as characters relevant to how cell diversity is generated in the progeny of the anterior cell. The multiple evolutionary transformations of division orientation implicated by Dolinski *et al.* (2001) could additionally make it possible to test for the association between evolutionary transformations of this character and other characters yet to be examined (Maddison 1990; Wray & Bely 1994).

We have some understanding of how division plane is regulated at the two-cell stage in *C. elegans*: positioning the mitotic spindle along the long axis in one cell results from an interaction of astral microtubules with a site in the cortex of that cell. A microtubule tether between a centrosome and the site in the cortex shortens, causing a 90° rotation of the future cell division machinery, which realigns the plane of cell division (Hyman & White 1987; Hyman 1989). Several genes are known to regulate this rotation, including genes required for several differences to arise between the cells of the two-cell stage (such as the *par* genes (Kemphues *et al.* 1988; Cheng *et al.* 1995)). Loss of function of certain genes, such as *par-3*, mimicks the linear pattern found in some species (Cheng *et al.* 1995). Several other genes may play more direct roles in aligning the cell division plane (for a review, see Goldstein 2000). Loss of function of these genes results in embryonic lethality in *C. elegans*; whether this is true in other nematodes as well is not yet clear. It is generally a difficult task to pinpoint the mutations that have caused evolutionary transitions to occur; it is therefore an exciting prospect that the mutations that caused the evolutionary transitions in division plane orientation might have occurred in homologues of genes that have already been identified in *C. elegans*. Identifying which genes these were, how their functions were modified, and whether the same genes were affected each time the linear pattern evolved remain interesting questions.

5. THE FUTURE OF COMPARATIVE NEMATODE DEVELOPMENTAL GENETICS

In the coming years, additional modifications to early nematode development may be identified, and the molecular underpinnings of such changes may begin to be explored. Future work can be complemented by the intense study of *C. elegans*' developmental genetics (Riddle *et al.* 1997), which provides a rich resource for asking how mutations in known developmental genes might generate evolutionary modifications to development. Several tools developed in *C. elegans* hold particular promise for the comparative developmental geneticist of the future: the genome sequence of *C. elegans* (*C. elegans* Sequencing Consortium 1998) and ongoing genome sequencing projects in other nematodes (Blaxter *et al.* 1999) should facilitate finding homologues of the most relevant genes in various species. Once such homologues are found, examining protein localization may be informative. Ultimately, understanding how these genes function will require interfering with their function. The lack of genetic maps in the vast majority of other nematodes studied means that traditional genetic methods generally cannot be used to identify genes affected in mutants of interest (for a way around this for selected genes, see Sommer 2000). Instead, it may be possible to use reverse genetic methods, for example by identifying mutations in pools of mutagenized worms by polymerase chain reaction, as can be done in *C. elegans* (Barstead 1999). It may also be possible to extend the technique of RNA interference (Fire *et al.* 1998) to other nematodes. Although success with RNA interference in other nematodes has been reported only in other species of the genus *Caenorhabditis* (Streit *et al.* 1999; Haag & Kimble 2000), success with the technique in *Drosophila* and other organisms (Sharp & Zamore 2000) suggests that it should be possible to apply the technique to other nematodes.

Currently, we have essentially no understanding of whether the modifications to early development that have been found have any adaptive consequences. It will be interesting to examine whether variations in early development described here have immediate adaptive consequences and, if not, whether they nevertheless affect the shapes of fitness landscapes by either facilitating or impeding additional evolutionary alterations to development.

How do the mutations that have occurred in nature affect development to produce the phenotypic results on which selection may act? Finding the general answers to this question, using nematodes and other organisms, will fill a large gap in our understanding of evolution.

I thank E. Schierenberg, J. Baldwin and M.-A. Felix for helpful comments on the manuscript, E. Schierenberg, J. Baldwin, and D. Voronov for permission to use figures, and J. Baldwin for sharing unpublished results. B.G. is supported by the Pew Scholars Program in the Biomedical Sciences, sponsored by the Pew Charitable Trusts, and research grant no. 5-FY99-730 from the March of Dimes Birth Defects Foundation.

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