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Embryonic cell lineage of the marine nematode *Pellioditis marina*☆

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Abstract

We describe the complete embryonic cell lineage of the marine nematode *Pellioditis marina* (Rhabditidae) up to somatic muscle contraction, resulting in the formation of 638 cells, of which 67 undergo programmed cell death. In comparison with *Caenorhabditis elegans*, the overall lineage homology is 95.5%; fate homology, however, is only 76.4%. The majority of the differences in fate homology concern nervous, epidermal, and pharyngeal tissues. Gut and, remarkably, somatic muscle is highly conserved in number and position. Partial lineage data from the slower developing *Halicephalobus* sp. (Panagrolaimidae) reveal a lineage largely, but not exclusively, built up of monoclonal sublineage blocs with identical fates, unlike the polyclonal fate distribution in *C. elegans* and *P. marina.* The fate distribution pattern in a cell lineage could be a compromise between minimizing the number of specification events by monoclonal specification and minimizing the need for migrations by forming the cells close at their final position. The latter could contribute to a faster embryonic development. These results reveal that there is more than one way to build a nematode.

Keywords: 4D microscopy; *Caenorhabditis elegans*; Cell lineage; Development; Embryo; Evolution; *Halicephalobus* sp.; Nematoda; *Pellioditis marina*; Rhabditida

Introduction

Embryological changes are the first morphological and anatomical manifestations of an altered genetic composition, and therefore a logical starting point for the study of the evolutionary mechanisms that have led to nematode diversity. The limited and manageable number of cells, possible deep metazoan origins (Vanfleteren et al., 1990), and the high number of different species available (Hugot et al., 2001) make nematodes ideal multicellular organisms for comparative analysis of embryonic development and evolution.

In 1983, the complete cell lineage of the model organism *Caenorhabditis elegans* was published by Sulston et al. (1983), giving the necessary blueprint of an organism upon which future molecular diagnoses have been based. At first, the developmental processes of *C. elegans* were assumed to be representative for most species of the phylum (Wood, 1988). More recent research, however, shows that there is much more diversity regarding developmental mechanisms in other nematode species than previously appreciated (Sommer and Sternberg, 1996; Felix, 1999; Fitch, 2000; Schierenberg, 2000; Borgonie et al., 2000). Comparative embryological analyses have mainly focused on subsets of the lineage, such as early development (Schierenberg, 2000) and vulva (Felix, 1999; Sommer and Sternberg, 1996) and male tail ontogeny (Fitch, 2000). Since the embryonic development is modular in nature (Raff, 1994), it contains much more information that cannot be extracted from analysis of a single or a limited number of cell lineage subsets alone.

The embryonic cell lineage of *Pellioditis marina* and *Halicephalobus* sp. is part of a larger research effort that focuses on the comparative embryonic development of several nematode species. To that end, we obtained, isolated, cultured, and screened 250 nematode species, from different families. In a first phase, we lineaged a species which is

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phylogenetically close to *C. elegans*. As a first step, the embryonic cell lineage of the free-living nematode *P. marina* has been traced from zygote up until the initiation of muscle contraction, marking the second detailed description of the embryonic development of a nematode. As a second step, nematode species from other families in the order Rhabditida are selected for embryonic cell lineage analysis. Partial cell lineage data of *Halicephalobus* sp. (family Panagrolaimidae) are presented for comparison with *P. marina* and *C. elegans*.

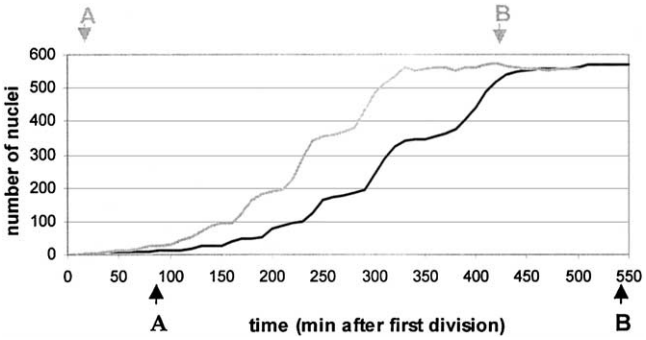

Fig. 1. Comparison of the number of nuclei during embryogenesis of *P. marina* (black) and *C. elegans* (gray). (A) Division of E. (B) Start muscle contraction.

Materials and methods

Nematode culture

Two nematode species have been selected out of a large nematode culture collection of more than 250 different nematode species, mainly extracted out of soil samples. Screening resulted in interesting species from different families (Rhabditidae, Panagrolaimidae, Cephalobidae, Plectidae, Monochidae).

The free-living, marine nematode *P. marina* (Bastian, 1865; Andrássy, 1983; Familia Rhabditidae, Ordo Rhabditida) was collected on rotting seaweed in the intertidal zone in Paje, Zanzibar by D. Verschelde. The strain TM02 is cultured on artificial sea agar plates (solution A: 23.9 g NaCl, 10.8 g $MgCl_2.6H_2O$, 1.52 g $CaCl_2.2H_2O$, 0.004 g $SrCl_2.6H_2O$, 0.68 g KCl, and 0.01 g KBr; dissolved to 856 ml; solution B: 40 g Na_2SO_4, 0.2 g $NaHCO_3$, 0.003 g NaF, and 0.027 g H_3BO_3; dissolved to 1000 ml. Mix solution A and solution B; buffer with Tris–HCl to keep a neutral pH if necessary). This solution was used to make 1% agar plates (1/3 nutrient agar/agar); 1 ml cholesterol (5 mg/ml in EtOH) was added. *Escherichia coli* OP50 was used as a food source. Stock cultures were kept at 15°C; the maximum temperature of *P. marina* in culture is 25°C (Vancoppenolle et al., 1999). *P. marina* can also be maintained on typical *C. elegans* agar plates with *E. coli* OP50 as food source, but the culture declines over time and eventually dies out. All data presented here are derived from cultures on sea agar plates.

The free-living soil nematode *Halicephalobus* sp. JB128 (Fuchs, 1930; Familia Panagrolaimidae, Ordo Rhabditida) is cultured on 1% nutrient agar plates with *E. coli* OP50 as food source. Culture and handling is as described by Brenner (1974).

Analysis of generation time

The generation time of *P. marina* was assessed in Vancoppenolle et al. (1999). To assess the generation time of *Halicephalobus* sp., young gravid females were dissected with a scalpel to release developing eggs. Thirty eggs were transferred by means of a mouth-pipette to an embryo plate with distilled water. Due to starvation, the development of the L1 larvae arrested in the same stage. The L1 larvae were transferred to sterile agar plates. Generation time was calculated as the time between the first laying of the parental generation and the first egg laying of the F_1 generation (see Table 3).

4D microscopy and lineage analysis

Early developmental stages were obtained by dissecting gravid females in seawater. One-cell embryos were selected under a stereomicroscope. *P. marina* embryos were mounted on a sea agar pad and *Halicephalobus* sp. embryos on an agar pad, covered with a coverslip and sealed with Vaseline (Sulston and Horvitz, 1977).

Three single embryos were recorded at 25°C by using a 4D microscope (Schnabel et al., 1997). Every 30 s, 25 focal planes were recorded through the embryo and stored on a laser video disk. The recording starts at the two-cell stage and continues until the body muscles start to contract; once muscle contraction starts; it is no longer possible to follow the cells between two time frames.

The lineage and 3D displays of each recording were reconstructed by using the Simi Biocell software, equipped with an automatic collision manager (Simi Gmbh, D-85705 Unterschleissheim, Germany; Schnabel et al., 1997). Since the recording ends at somatic body muscle contraction, it is not always possible to accurately identify the cell type of a given tissue. When discussing the nervous system, no distinction can always be made between neurons, sockets, or sheaths; similarly, for cell types of the pharynx.

Reliability

The lineage presented here is based on three recordings. A consensus lineage is formed, by which uncertainties in two complete recordings were resolved by comparison with the third recording. As the recordings are restricted until body muscle contraction, some information of late development is not recorded. In our recordings, the consensus lineage contains 571 cells and as such most cells later present in the L1 have already formed. Although there is

Table 1
Comparison of the number of cells at time of muscle contraction between *Halicephalobus* sp. (H), *P. marina* (P), and *C. elegans* (C).

	AB		MS			E			P4			D		C			Total	
	P	C	H	P	C	H	P	C	H	P	C	P	C	H	P	C	P	C
Nervous system	185	236	4	7	6										2	2	194	244
Body muscle	1	1	35	28	28							20	20	32	32	32	81	81
Epiderm	115	67		3										16	14	13	132	80
Pharynx	79	43	37	33	31												112	74
Intestine						18	20	20									20	20
Gonad									2	2	2						2	2
Other	20	35	2	10	14												30	47
Total survivors	400	382	78	81	79	18	20	20	2	2	2	20	20	48	48	47	571	550
Deaths	58	92	7	9	13											1	67	106
Total produced	458	474	85	90	92	18	20	20	2	2	2	20	20	48	48	48	638	656

some loss of lineage information, most cells have adopted their final fate and position. In *C. elegans*, 12 late divisions occur after muscle contraction: that means the formation of 12 extra cells over a total of 570 cells (or 2% of the number of cells) (Sulston et al., 1983).

Nomenclature

Since the first cell divisions, during which the founder cells are formed, are analogous to *C. elegans*, the same nomenclature has been adopted (Sulston and Horvitz, 1977; Deppe et al., 1978).

Comparison with cell lineages of C. elegans and Halicephalobus sp.

Considering the huge amount of data present in any given lineage, we have opted to sometimes include a comparison with the *C. elegans* embryonic cell lineage (Sulston et al., 1983) in Results for clarity and to facilitate discussion. Also cell lineages of four founder cells, MS, E, C, and P_4, of the *Halicephalobus* sp. embryo have been established.

Lineage homology between two species is calculated as the percentage of cells at the time of muscle contraction of one species that has a homologous cell with the same lineage history in the other species. Fate homology between two species is calculated as the percentage of homologous cells at the time of muscle contraction of one species that has the same cell fate in the other species.

Results

General description

In the three recorded specimens of *P. marina*, somatic muscle contraction starts at 550, 640, and 590 min after start of the recording. In *Halicephalobus* sp., somatic muscle contraction starts at 635, 650, and 688 min after start of the recording. At muscle contraction, the embryonic consensus lineage of *P. marina* consists of 571 cells; 638 are formed of which 67 undergo programmed cell death (Fig. S1; Table 1).

By reconstructing the embryonic cell lineage of three specimens of *P. marina*, we can determine variability in the lineage between individuals. However, no significant variation was detected when comparing identical lineage subsets between the recorded specimens. The lineage is very rigid; no differences in fate were ever detected when comparing identical cells between the three recorded specimens. Lineage homology shows an overall match of 95.5% with the *C. elegans* lineage (Table 2). Since reconstruction of the lineage ends at first somatic muscle contraction, we suspect that the overall match might actually be higher because of some late divisions occurring after the end of the recording; although, in *C. elegans*, 12 late divisions occur after muscle contraction. That means the formation of 12 extra cells over a total of 570 cells (2%).

Fate homology, however, shows an overall match of 76.4% between *P. marina* and *C. elegans* (Table 2). A possible increase in overall match cannot be evaluated, since no predictions can be made about the fate of any cell formed after somatic muscle contraction.

Early development

The early development of the *P. marina* embryo starts with a series of unequal, asynchronous cell divisions, during which a larger somatic founder cell and a smaller germ line precursor cell (P-cell) are formed (Fig. S1). The sequence of the first divisions is identical to in the *C. elegans* embryo, except for the cleavage of P_3 that divides before C and the 8 AB cells. This means that the germ line precursor cell P_4 is already present in the 15-cell stage instead of the 24-cell stage in *C. elegans*. In one specimen, P_3 divides earlier, before MS and E, so P_4 is already present in the 13-cell stage. The early development of *P. marina* is relatively

Table 2
Comparison of the lineage homology and fate homology between *Halicephalobus* sp. (H), *P. marina* (P), and *C. elegans* (C). n.d., not determined.

		Lineage homology (%)	Fate homology (%)
AB	*H-P*	n.d.	n.d.
	H-C	n.d.	n.d.
	P-C	94.3	69.9
MS	*H-P*	77.6	58.8
	H-C	74.1	57.6
	P-C	95.6	86.7
E	*H-P*	70.0	70.0
	H-C	70.0	70.0
	P-C	100.0	100.0
P_4	*H-P*	100.0	100.0
	H-C	100.0	100.0
	P-C	100.0	100.0
D	*H-P*	n.d.	n.d.
	H-C	n.d.	n.d.
	P-C	100.0	100.0
C	*H-P*	100.0	95.8
	H-C	100.0	93.8
	P-C	100.0	97.9
Total	*H-P*	n.d.	n.d.
	H-C	n.d.	n.d.
	P-C	95.5	76.4

slower in comparison with *C. elegans* (Fig. 1). The time until the division of E is 2.3 times longer than in the *C. elegans* embryo, while the period until muscle contraction is only 1.3 times slower than in *C. elegans.* The development quickens after 200 min at a tempo similar to in *C. elegans* (Fig. 1).

Gastrulation starts at 140 min, in the 24-cell stage with the inward migration of the intestine precursors Ea and Ep from the ventral side, near the posterior end of the embryo. Next, at 200–240 min, P_4 and the MS cells migrate inwards. Between 250 and 300 min, D and the myogenous descendants of C sink inwards in the ventral posterior part of the embryo.

After birth, each founder cell divides with characteristic periods (Fig. 2). The division rate correlates with the size and the time of origin of the founder cell: AB divides the fastest, D the slowest. E is an exception: it arises earlier than C but divides slower, so that the fourth generation of C divides earlier than the third generation of E. The length of the division rounds, that is the time between the first and the last division of each division round, also increases later in development (Fig. 2). But the stages of the AB-lineage divide in a much shorter period than the corresponding stages of the other founder cells. For example, the 16-AB cell stage divides in the 6th division round in a shorter period than the 16- MS stage, which divides in the 8th division round (Fig. 2). The length of the division rounds of each founder cell is much more correlated with the division round in the embryo than those of the founder cell. For example, in the 6th division round, the 16-cell stage of AB and the 4-cell stage of MS divide in a comparative period of time.

Bilateral symmetry

The founder cells form bilateral symmetric groups in their first or second division round (Fig. 3). The anterior daughters of MS, C, D, and P_4 lie in the left part of the embryo, while the posterior daughters lie in the right part. In AB and E, the left–right symmetry is established in the second division round.

In MS, E, D, and P_4, the bilateral symmetry is preserved for the next division rounds: bilateral symmetric cells are formed by equivalent lineages. In the E- and D-lineage, the bilateral symmetry is preserved until after their last division round and is visible in the tissues they form. The intestine is

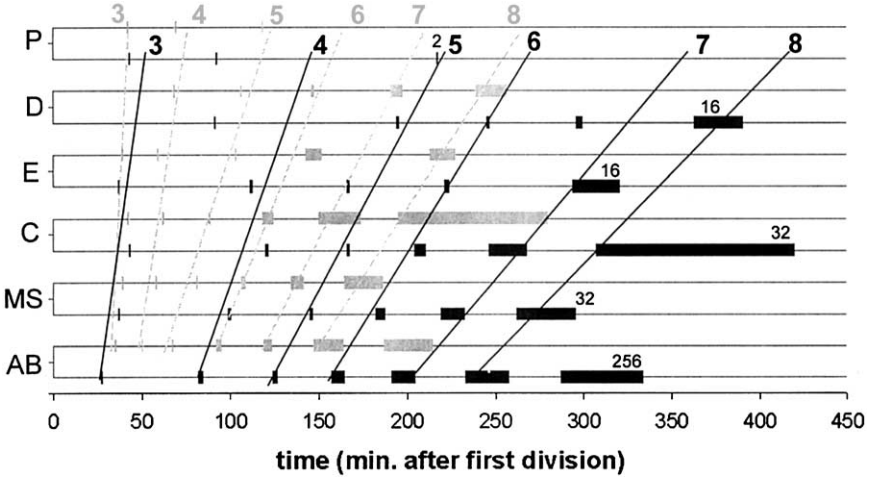

Fig. 2. Comparison of times and rounds of cell divisions between *P. marina* (black) and *C. elegans* (gray). On each horizontal line, the cell division events of one founder cell are given. The order from top to bottom is by decreasing length of cell cycle. Each box indicates the time from the division of the first cell to the division of the last cell of a lineage. The number above the last box of each lineage indicates the number of cells of that lineage present after that division. Black full (*P. marina*) and gray dashed (*C. elegans*) lines indicate rounds of cell division.

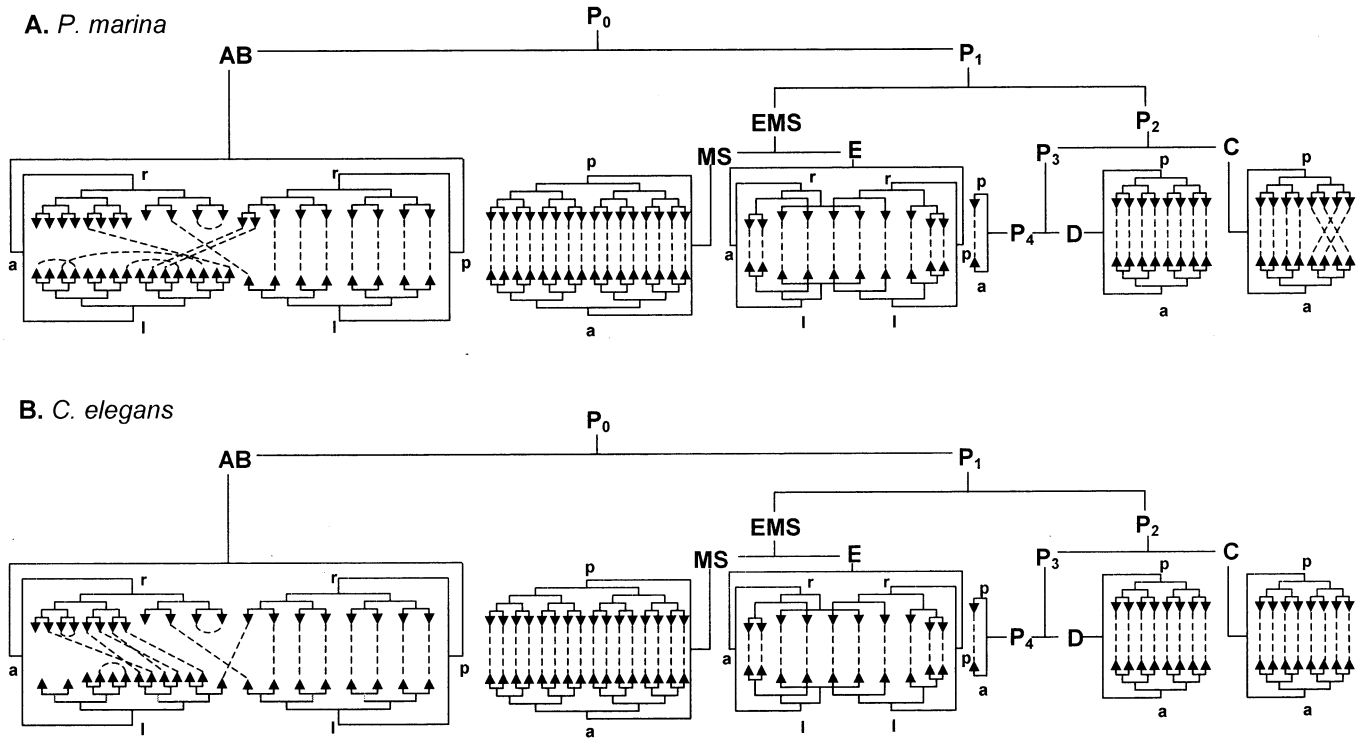

Fig. 3. The bilateral symmetry in the *P. marina* (A) and *C. elegans* (B) embryo. Dorsal view, anterior left. Left branches are the anterior daughters of divisions; right branches are the posterior daughters. Differences occur in Caa and Cpa and anteriorly in ABar and ABal. a, anterior; p. posterior, I, left; r, right.

completely formed by the bilateral symmetrical E-lineage and D forms bilateral symmetric muscle cells. In the MS-lineage, the symmetry is retained until the 32-cell MS stage. The anterior daughters of MSa and MSp, MSaa and MSpa, form a double inner row of eight cells. The posterior daughters, MSap and MSpp, which lie more lateral, form an double outer row. After the 7th division round of MS, the symmetry is lost.

In the third division round of C, a symmetry breakage in the body muscle precursors Cap, and Cpp lineage is corrected to maintain the bilateral symmetry (Fig. 4). Cap and Cpp divide in a left–right direction. The outer daughters Capa and Cppp migrate anteriorly and lie before their sisters. Consequently, a secondary symmetry arises, where the descendants of Capa and Capp are symmetric with, respectively, Cppp and Cppa, hence the crossed lines in Fig. 3A.

At the division of the 4-AB cell stage, a difference between the ABa and the ABp lineage occurs. In the ABa lineage, the bilateral symmetry is completely lost, while in the ABp lineage, it is largely retained, except for ABplaaa and ABpraaaa/p, which form bilateral symmetric fields with, respectively, ABarpap and ABalpaap/pa (Fig. 3).

Tissue description

Intestine

The 20 intestinal cells are exclusively derived from the founder cell E, which does not give rise to any other tissue. The daughter cells Ea and Ep enter the body ventrally during gastrulation (140 min). The next left–right division establishes the bilateral symmetry: Eal and Epl form the left row, Ear and Epr the right row. After the next 2 division rounds (510 min), the intestinal tube is elongated and consists of 2 rows of 8 cells. Because of the more ventral position of Ealp, its anterior daughter, Ealpa, migrates between Ealaa and Ealap and its posterior daughter, Ealpp, between Eplaa and Eplap. The first pair of cells divides dorsoventrally to form a ring of four cells, connecting the intestine with the pharynx. The last pair of cells divides once more to form a 9th row of intestinal cells that make contact with the rectum. The intestine is built identically as in the *C. elegans* embryo, except for the orientation of the 2nd to the 4th ring. In *C. elegans*, those rings rotate 90° counterclockwise immediately after the division of the first pair of cells and before attachment to the pharyngointestinal valves. This twist is not observed in the analogous elongation period until muscle contraction in the *P. marina* embryo.

Gonad

At the time of body muscle contraction, the primordial gonad consists of two germ line cells and two somatic cells. Both have separate origins. The germ line cells are the two daughter cells of the founder cell P_4. They are closely associated with the fifth intestinal ring, P_4a with Ealpp and P_4p with Earpp. The somatic cells originate from identical

lineages from MSa and MSp ($MS^{a}/_{p}$ppaap) in the anterior part of the embryo and migrate posteriorly, where they attach to the germ line cells.

Pharynx

There are 112 pharynx cells identified at the time of muscle contraction; 79 are generated by the founder cell AB and 33 by the founder cell MS (Fig. S1; Table 1). The MS cells form the posterior part of the pharynx. The initial bilateral symmetry of MS is still visible in the MS-derived part of the pharynx. Particularly, the anterior daughters of MSa and MSp form pharynx cells, together 29 of the 33 cells. In the AB-lineage, pharynx cells are mainly formed by ABalp and ABara, together 63 of the 79 cells (80%). ABalp descendants lie largely in the left part of the pharynx, ABara in the right part, although the bilateral symmetry is not strict.

At the time of muscle contraction, the pharyngeal primordium is visible as a distinct mass of cells in the anterior of the embryo. It is not possible to make a detailed 3D reconstruction of the pharynx and the different cell types, since the morphogenesis of the pharynx has not yet been completed. The pharynx elongates anteriorly and so reaches the buccal cavity.

Body muscle

The 81 body muscle cells have a mixed origin: 20 are generated by the blastomere D, 32 by C, 28 by MS, and 1 by AB (Fig. S1, Table 1). These cells are arranged into 4 anteroposterior strips, 2 dorsolaterally and 2 ventrolaterally.

The D blastomere forms body muscle cells exclusively. Bilateral symmetry is established after the first division: Da in the left part, Dp in the right part of the embryo. Two granddaughters of the founder cell C each form 16 body muscle cells: Cap at the left side, Cpp at the right side. The MS-derived muscle cells are bilaterally evenly distributed: 13 MSa cells and 1 MSp cell in the left muscle strips and 14 MSp cells in the right muscle strips. The founder cell AB forms 1 body muscle cell posteriorly in the right–ventral muscle strip.

Epidermis

The epidermis has a mixed origin and comprises 132 cells: 115 AB cells, 3 MS cells, and 14 C cells (Fig. S1; Table 1). In the AB-lineage, epidermis cells are particularly formed by the ABarp, ABpla, and ABpra blastomeres (79 of the 115 cells). ABpla and ABpra form bilaterally symmetric lateral fields, while the epidermal ABarp descendants lie dorsally with bifurcated endings. Two granddaughters of C form epidermal cells: 6 Caa-derived cells behind each other in the left dorsal field and 8 Cpa-derived cells behind each other in the right dorsal field.

At midembryogenesis, between 330 and 450 min after the first division, the dorsal epidermal nuclei undergo a contralateral migration, where they cross each other in the midline.

Some basic patterns in the organogenesis are identical to *C. elegans*, where ABpla- and ABpra-descendants form, respectively, left lateral and right lateral epidermis cells, while Caa-, Cpa-, and ABarp-descendants form dorsal epidermis cells, the latter with the typical bifurcated endings. More variation is found in the epidermis cells that are specified later in the last division rounds.

Nervous system

There are 194 neurons identified at the start of muscle contraction. Ninety-five percent are formed by the AB-lineage (185 neurons), while MS forms 7 neurons and C only 2 (Fig. S1; Table 1). Eighty-five percent of the nerve cells formed in *P. marina* have an identical fate in *C. elegans.* Twenty-nine neurons are specific for *P. marina.* These cells in *C. elegans* undergo cell death (10), become pharynx (11), epidermis (2), divide once more (5), and 1 forms a pharyngointestinal valve cell. The 65 neuron cells, specific for *C. elegans,* form in *P. marina* pharynx cells (29), epidermis (33), and 3 undergo programmed cell death. Since reconstruction of the lineage ends at first body movement, we estimate that the number of nerve cells in *P. marina* is an underestimation of the actual number occurring in its embryogenesis.

Programmed cell death

Until muscle contraction, 11% (67/638; *C. elegans* 16%, 106/656) of the cells undergo apoptosis (Fig. S1; Table 1). All cell deaths occur in the AB- (58) and the MS-lineage (9) in the anterior half of the embryo and none in the C-lineage, unlike in *C. elegans* (Fig. S1). Ninety-five percent of the cell deaths in *P. marina* occur also in *C. elegans,* including the 14 prominent cell deaths in the 9th generation of AB and the 1 early cell death in the 4th generation of MS. There are 3 cell deaths in *P. marina,* which become neurons in *C. elegans.* The difference between *P. marina* and *C. elegans* lies in the cell deaths after the last division round. Since reconstruction of the lineage ends at first body movement, we estimate that the number of cell deaths in *P. marina* is an underestimation of the actual number occurring in its embryogenesis.

The partial embryonic lineage of Halicephalobus sp.

Four founder cells of the *Halicephalobus* sp. embryo have been lineaged: MS, E, C, and P_4.

The MS founder cell has formed 85 cells at the time of somatic muscle contraction: 37 pharynx cells, 35 body muscle cells, 4 nerve cells, and 2 cells with another cell fate; 7 cells undergo programmed death. *P. marina* and *C. elegans* form, respectively, 90 and 92 cells (Table 1). The lineage homology between the MS- lineage of *Halicephalobus* sp. and those of *P. marina* and *C. elegans* is, respectively, 77.6% and 74.1% (Table 2). The fate homology, however, is substantially lower: 58.8% and 57.6%, respectively (Table 2).

The E founder cell has formed 20 intestinal cells of which 2, Eaap and Epap, undergo apoptosis. The lineage homology with *P. marina* and *C. elegans* is 70% (14 of the 20 cells) (Table 2). The cells are arranged in 9 rows of 2 cells, 3 rows are formed by the anterior daughter of E; 6 rows are formed by the posterior daughter.

The C founder cell has formed 48 cells at the time of somatic muscle contraction. The fate topology is symmetric: the two anterior granddaughters of C, Caa and Cpa, each form 8 epidermis cells; the two posterior granddaughters, Cap and Cpp, each form 16 body muscle cells (Table 1). The lineage topology is identical as in *P. marina* and *C. elegans;* an identical number of cells is formed in an identical cell lineage. There is only a small difference in fate topology: Caapaa and Caappp form nerve cells in *P. marina* and *C. elegans,* and Caapap undergoes programmed cell death in *C. elegans.*

The founder cell P_4 divides once more to form two primordial germ cells, which are closely associated with the fifth intestinal row.

Discussion

The lineage origin of the tissues varies from highly conserved to highly variable

The embryonic development of *P. marina* is as invariant as in *C. elegans.* Comparison of the tissue configuration in the *P. marina* embryo with this in *C. elegans* shows a variable degree of conservation. The intestine, the primordial gonad, and the body muscles are highly conserved in the two species, while the pharynx, the epidermis and the nervous system have a more variable configuration.

All intestinal cells arise from identical cell lineages of the E blastomere in both species. In both embryos, the intestine is built in an identical way, except for the orientation of the second to the fourth ring. In *C. elegans,* those rings rotate 90° counterclockwise immediately after the division of the first pair of cells and before attachment to the pharyngointestinal valve cells (Sulston et al., 1983; Leung et al., 1999). This twist is not observed in the analogous period in the *P. marina* embryo. It is not known whether this rotation occurs beyond our recording, although we think that such a rotation would be improbable since after somatic muscle contraction the intestine is firmly attached to the valves of the pharynx and the rectum. The asymmetric expression of LIN-12 in the left and the right intestinal cells in the *C. elegans* embryo is probably the basis for asymmetry in later cell–cell interactions within the intestinal primordium that lead directly to the intestinal twist (Hermann et al., 2000). It is thought that the intestinal twist in *C. elegans* contributes to the gonad morphogenesis since *lin-12* mutants have aberrant positioning of the gonad relative to the intestine (Hermann et al., 2000). Moreover, LIN-12 is involved in the orientation of the migration of the lateral blast cells P(11/12)L/R, which is highly biased in *C. elegans* and is linked to the whole body handedness of the adult (Delattre and Felix, 2001). In another *Pellioditis* species, *P. typica,* this migration is apparently fully random (Delattre and Felix, 2001). No such asymmetry in the adult intestine and gonad is observed in *P. marina,* suggesting that there is no intestinal twist in *P. marina.*

In all investigated nematodes so far, the intestine originates from a single founder cell E (Malakhov, 1994). In the Rhabditida, there is a small variation in the number of intestinal cells in the hatchling: from 18 in *Turbatrix aceti, Panagrellus redivivus* (Sulston et al., 1983), and *Halicephalobus* sp., 20 in *C. elegans* (Sulston et al., 1983) and *P. marina* to 22 in *Acrobeloides nanus* (Wiegner and Schierenberg, 1998). Most of the *T. aceti* embryonic cell lineage from Sulston et al. (1983) cannot be used for lineage comparison because no terminal fate assignments have been made. Only the E-lineage is complete and is identical with the one of *Halicephalobus* sp. Although based on limited cell lineage data, this similarity may reflect their close relationship; they are both members of the family Panagrolaimidae. Although another gut differentiation mechanism has been identified in *A. nanus* in comparison with *C. elegans,* the result is the same: a gut lineage originating from a single blastomere (Wiegner and Schierenberg, 1998). In the 8-cell embryo of the primitive nematode *Enoplus brevis,* an endoderm precursor cell can be identified, while the other blastomeres give rise to a variable set of cell types (Voronov and Panchin, 1998). So it seems that the origin of the midgut from the E cell is a very conservative feature among nematodes.

Also very conserved is the behavior of the primordial germ cells (PGC). In the three species, the germ line cells P_4a and P_4p are closely associated with the cells of the fifth intestinal ring. Electron micrographs of a *C. elegans* embryo in the second half of the embryogenesis show that the germ cells protrude large lobes into the two intestinal cells (Sulston et al., 1983). Probably the germ cells are also nursed by the intestine until their attachment to the somatic gonadal cells, since the latter are essential for the survival and the further development of the gonad (Kimble and White, 1981). Also in the enoplid *Enopus demani,* two large primordial germ cells are found closely associated with the intestine, but it is not clear from which blastomere they come (Malakhov, 1994). Also, in many other phyla, the PGCs are closely associated with the early endoderm at the vegetal pole of the egg (e.g., in Amphibia) and the endoderm is necessary for the correct migration of the PGCs to the somatic gonads (*Drosophila,* Warrior, 1994; Jaglarz and Howard, 1995; Amphibia, Bounoure, 1934; Ressom and Dixon, 1988; Kloc et al., 1993; Mammalia, Heath, 1978). The fact that these two tissues perform the two most primitive Metazoan functions—feeding and reproduction—could explain the strong conservation and association of them in *P. marina, Halicephalobus sp.,* and *C. elegans* and throughout the animal kingdom.

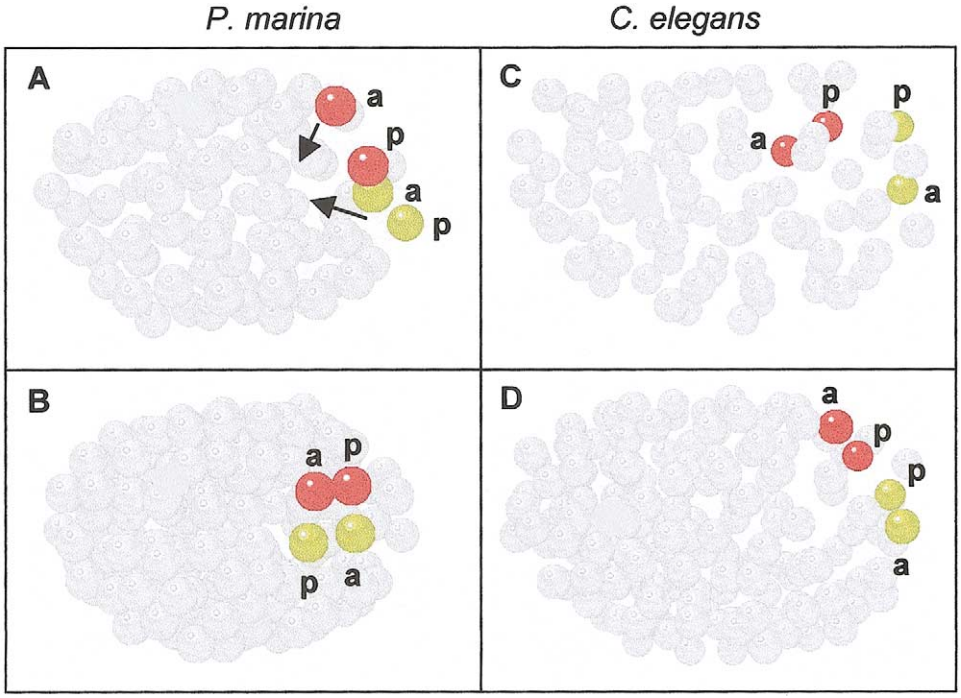

Fig. 4. Correction of bilateral symmetry breakage in the body muscle precursors Cap (red) and Cpp (yellow) in *P. marina* (A, B) compared with *C. elegans* (C, D). Ventral view, anterior to the left. (A) 240 min (90-cell stage). (B) 300 min (171-cell stage). (C) 123 min (87-cell stage). (D) 160 min (167-cell stage).

Both *P. marina* and *C. elegans* have the same 81 body muscle cells in a similar configuration. Also, in the *Halicephalobus* embryo, 83 body muscle cells are present. Apparently, the program for muscle formation is strongly conserved. Probably not much variation in the course of evolution was possible for the proper functioning of the muscles. There is no obvious explanation for this conservative pattern. Nevertheless, *mes-1* mutants of *C. elegans* in which P_4 forms extra body muscles cells seem to function well (Strome et al., 1995). Also, *C. elegans* embryos, lacking 20 of the 81 embryonic body wall muscle cells by ablation of the D blastomere, become viable adults, indistinguishable from wild type animals (Moerman et al., 1996).

In *P. marina*, ABp contributes pharyngeal cells. In *C. elegans*, a P_2 to ABp interaction prevents the ABp lineage from responding to an MS-derived pharynx- inducing signal to produce pharyngeal cells (Hutter and Schnabel, 1994; Mango et al., 1994; Mello et al., 1994; Moskowitz et al., 1994). This could mean two things. First, the P_2 signal is absent in *P. marina*, or second, later signals induce secondary production of pharyngeal cells. If this signal in *C. elegans* is absent, then ABp adopts an ABa-like fate (Mello et al., 1994), while in *P. marina* only a few ABp cells adopt a pharyngeal fate. So, the pharyngeal cells in ABp are most likely induced secondarily, later in embryonic development.

The epidermis is a more variable tissue, although some basic patterning in both species is similar. More variation between both species is found in the epidermis cells that are specified later in the last division rounds. So, it seems that there is a conserved pattern of epidermis formation that can be adapted to a specific epidermis tissue by adding or deleting epidermis cells in the last division rounds of the cell lineage.

The configuration of the nervous system is adjusted to specific functional needs, by transforming cells to neurons that originate close to their final position, rather than by moving the existing neurons to a new position. One could expect that cell deaths exhibit the same plasticity. The high degree of homology of the cell deaths between *P. marina* and *C. elegans* contrasts somewhat with the lower degree of similarity in the nervous system. Although rewiring of the pharynx could be a possibility, there is a high morphological similarity between the pharynxes of both species, making this rather unlikely. More lineage data of other nematode species is needed to assess the importance of the differences observed.

Monoclonal vs polyclonal fate specification and possible influence on speed of development

High lineage homology but low fate homology

The embryonic lineage of *P. marina* closely resembles that of *C. elegans* but differs considerably from that of *Halicephalobus* sp. (Panagrolaimidae). However, even between *P. marina* and *C. elegans* there are subtle differences demonstrating possible evolutionary adaptations. When comparing these two embryonic cell lineages, they show a high lineage homology (95.5%), but a lower fate homology (76.4%). Cells have an identical lineage history but get a

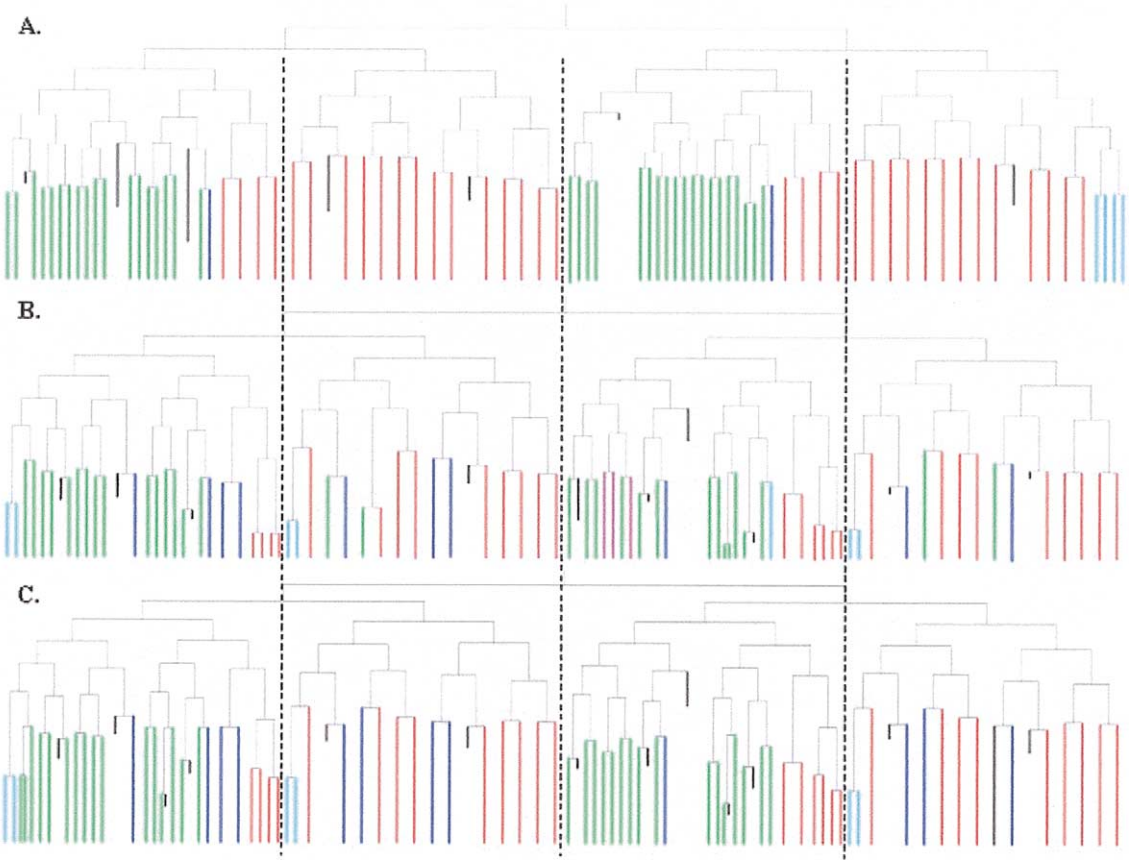

Fig. 5. Comparison of the fate topology in the MS-lineage between (A) *Halicephalobus* sp., (B) *P. marina*, and (C) *C. elegans*. The MS-lineage is subdivided in four equivalent blocks by dashed lines. blue: nervous system; red: body muscle; purple: epidermis; green: pharynx; black: programmed cell death; dark blue: other.

different fate assignment. Differences in fate of homologous cells are called fate transformations.

Massive fate transformations can be found when comparing the MS-lineage of the three species (Fig. 5). A total of 77.6% of the MS cells of *Halicephalobus* sp. have an identical lineage history as the MS cells of *P. marina*. But only 58.8% of these cells have the same cell fate. These differences are less between the MS lineages of *P. marina* and *C. elegans*. Two different patterns of cell fate distribution can be distinguished. In *Halicephalobus* sp., pharynx and body muscle cells are formed in large identical blocks of sublineages (Fig. 5A). Cell fates have a monoclonal distribution in the embryonic cell lineage. Equivalent lineage blocks in *P. marina* and *C. elegans* exhibit numerous individual fate transformations in comparison with the uniform MS-lineage of *Halicephalobus* sp. (Fig. 5B and C). Here, the cell fates have a polyclonal distribution in the embryonic cell lineage.

Lineage pattern is linked with positioning of cells

These two different clonal patterns have implications on the positioning of the cells. A clear example can be found in the Caa-lineage of three species (Fig. 6A–C). In *Halicephalobus* sp., the Caa-lineage forms only epidermal cells (Fig. 6A). Those cells migrate to their final position. In the Caa-lineage of *P. marina* and *C. elegans*, two cells are specified into tail neurons (Fig. 6B and C). In *Halicephalobus* sp., those neurons are formed in the AB-lineage in the anterior part of the body and later migrate to the tail region (Fig. 6D). This migration is not necessary in *P. marina* and *C. elegans* by forming those two nerve cells in the Caa-lineage in the posterior part of the embryo close to their final position in the embryo (Fig. 6E and F). In *Halicephalobus* sp., cells are specified in clusters of monoclones which arrive in different places of the embryo and as such have to migrate to their final positions. Thus, in *Halicephalobus* sp., lineally related cells have the same fate, but arrive at a different position. While in the polyclonal pattern of *P. marina* and *C. elegans*, the cell lineage is arranged so that cells originate close to their final positions. Here, lineally related cells have a similar position but a different cell fate.

Is there a compromise between minimizing the number of specification events and minimizing the need for migration?

A cell lineage with monoclonal cell fate distribution suggests a simple specification mechanism. Possibly, less

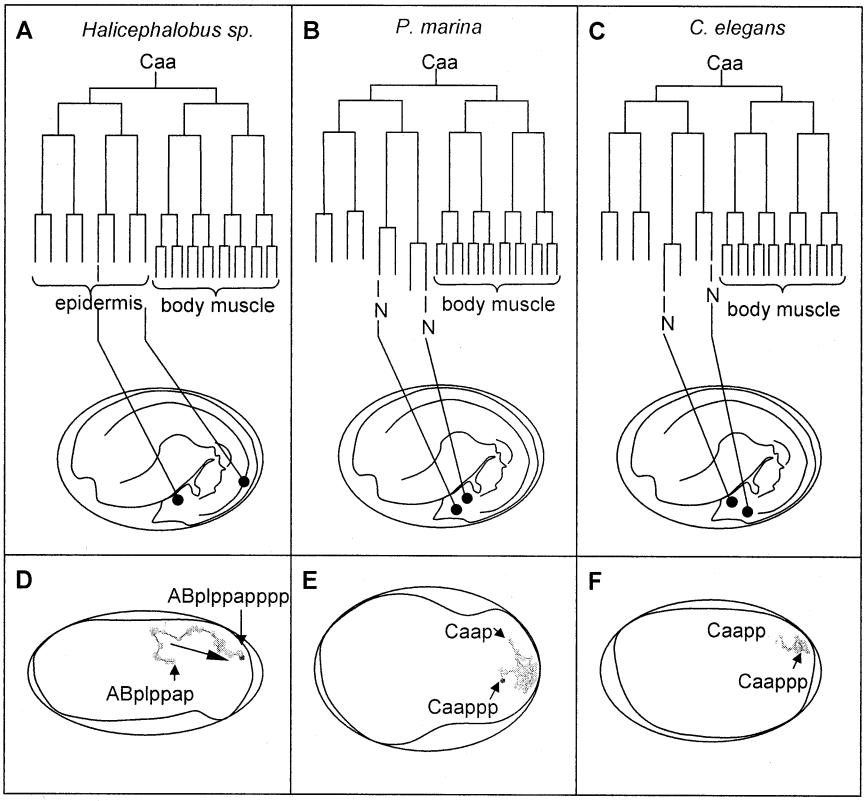

Fig. 6. (A-C) Evolutionary transformation of the Caa-lineage. Individual cells in uniform lineage blocks undergo fate transformation or apoptosis. (A) *Halicephalobus sp*.; (B) *P. marina*; (C) *C. elegans*. Embryos in left lateral view at initiation of muscle contraction. (D–F) Analysis of migration of the tail neuron precursor in the three species indicates fate transformation allows cells to originate close to the final destination, eliminating unnecessary migrations and allowing faster embryonic development. Dorsal view, anterior to the left. (D) *Halicephalobus* sp. 220–455 min; (E) *P. marina* 200–419 min; (F) *C. elegans* 159–245 min.

specification events are needed, no matter which mechanism is used (distribution of determinants, differential genetic expression, or induction signals between cells). As such, the decision can occur once at the precursor cell of the clone.

A disadvantage of this strategy is that those cells have to migrate to their final position. For example, the tail neurons in *Halicephalobus* sp. originate anteriorly in the AB cell, from which all neurons originate, and migrate posteriorly to arrive in the tail (Fig. 6D). This would suggest a cross-traffic of terminal cells and an accompanying complex signaling mechanism to get these cells to the correct position on time and thereby slows down the embryonic development. Furthermore, this poses a risk in an embryo with a limited number of cells. There is no flexibility; for example, if the leading epidermal cells do not arrive at their correct position on time, the ventral enclosure of the epidermis could fail which causes a preliminary arrest of the embryonic development (Williams-Masson et al., 1997). On the contrary, in vertebrates where clones of millions of cells are made, loss of one or more cells could easily be compensated for by others (LeDouarin, 1980; LaBonne and Bronner-Fraser, 1999).

However, a cell lineage with polyclonal cell fate distribution could allow a faster embryonic development by reducing the need for cell migrations. Indeed, the embryonic development until muscle contraction of *P. marina* and *C. elegans* is faster then in *Halicephalobus* sp., respectively, 430 and 550 min after first division against 650 min after first division. It is unlikely that this strategy alone would suffice to explain the fast embryonic development in *P. marina* and *C. elegans.* This strategy is only one of the many possible strategies for fast embryonic development. Supply of maternal gene products enhances the speed of the early development (Skiba and Schierenberg, 1992; Wiegner and Schierenberg, 1998), and the *clk-1* gene in *C. elegans* regulates physiological growth rate in embryos (Wong et al., 1995). The strategy of polyclonal specification pattern only influences the length of the period up to organogenesis, in the period where cells are formed, specified, and migrate to their final position. The strategy is correlated only with this period and not with the whole generation time. It is possible that there exists a nematode with a slower first part of embryogenesis but with a faster overall development. There are other genetic mechanisms that regulate the length of postembryonic development. For example, in *C. elegans*, heterochronic *lin* genes regulate the division timing of certain blastomeres (Slack and Ruvkun, 1997).

Table 3
Generation time in days (d) or months (m) of 31 nematode species

Taxon	Species	Generation time	Reference
Rhabditoidea	Caenorhabditis elegans	2.5-4d	Hirsch et al. (1976)
	Caenorhabditis briggsae	2.6-10d	T
	Pellioditis marina	4-5d	V
	Diploscapter coronatus	4-7d	V
	Cruznema sp.	3-5d	V
	Oscheius sp.	6-7d	V
	Pelodera strongyloides dermatitica	4-7d	V
	Teratorhabditis sp.	3-6d	V
	Operculorhabditis sp.	5-7d	V
	Rhabditidae sp.	3-5d	V
	Rhabditidae sp.	3-5d	V
	Rhabditidae sp.	3-5d	V
	Rhabditidae sp.	3-4d	V
Diplogasterida	Paroigolaimella bernensis	2-3d	T
	Adunctospiculum halicti	5-10d	V
	Goodeyus ulmi	4-11d	V
	Pseudodiplogasteroides sp.	4-7d	V
	Pristionchus pacificus	3-5d	V
Panagrolaimoidea	Panagrolaimus rigidus	6-14d	V
	Halicephalobus gingivalis	3-7d	V
	Halicephalobus sp.	6d	
Cephaloboidea	Acrobeloides maximus	4-14d	V
Tylenchida	Aglenchus costatus	27-35d	T
Plectidae	Plectus palustris	12.5-18.5d	T
Chromadorida	Chromadora macrolaimoides	22d	T
	Ethmolaimus pratensis	3-12m	T
Monohysterida	Monohystera denticulata	10-197d	T
	Monohystera paludicola	4-12m	T
	Monohystera cf. stagnalis	6-7m	T
Enoplida	Enoplus communis	12m	T
	Ironus tenuicaudatus	12m	T
Mononchida	Mononchus aquaticus	14-90d	T

T. Traunspurger (2000); V. Vancoppenolle et al. (1999)

Disadvantage of the polyclonal specification strategy is the likely complex specification mechanism, where almost every cell has to be specified individually. In an embryo with limited number of cells, if a cell is wrongly specified, a wrong cell arrives at the wrong place, without the possibility for correction.

These strategies are but extremes. The embryonic development of the three species probably represents a evolutionary compromise between minimizing the number of specification events and minimizing the need for migration. Each organism uses a combination of the two strategies. In *Halicephalobus* sp., the balance is more in direction of clonal specification and minimizing the specification events. The embryonic development of *P. marina* and *C. elegans* balances more toward a polyclonal strategy and thereby minimizing the cell migrations.

Stent (1985) already recognized the monoclonal and polyclonal patterns and called these, respectively, typologically and topographically hierarchic schemes. In invertebrate species as nematodes and leeches, where the embryos have a limited number of cells and cell migration plays a minor role, two lineally related cells have a similar position rather than a similar cell fate (Weisblat et al., 1984; Shankland and Weisblat, 1984). In vertebrates, however, where cell numbers range in the millions, cell migration plays a prominent role (e.g., neuron precursor cells are formed in the neural crest and later migrate to their ultimate position; LeDouarin, 1980).

Which of the patterns is the primitive state?

Did the polyclonal fate pattern evolve from the monoclonal pattern or vice versa? If the polyclonal fate pattern is the primitive state, then fate transformations have led to a simpler pattern, by which whole sublineages form one type of tissue. This slows down embryonic development. If the monoclonal pattern is the primitive state, then the fate transformations have led to a polyclonal cell lineage. These transformations could be adaptations to avoid migrations and fasten embryonic development. Faster development shortens the vulnerable period during which embryos reside in the eggshell and in which disturbances of the developmental processes can lead to embryonic deformation and/or arrest. Furthermore, it allows explosive colonization of sudden appearing and possibly short-lived niches. The latter applies well to *P. marina*, which can rapidly colonize rotting breakaway seaweed and has a tremendous potential to

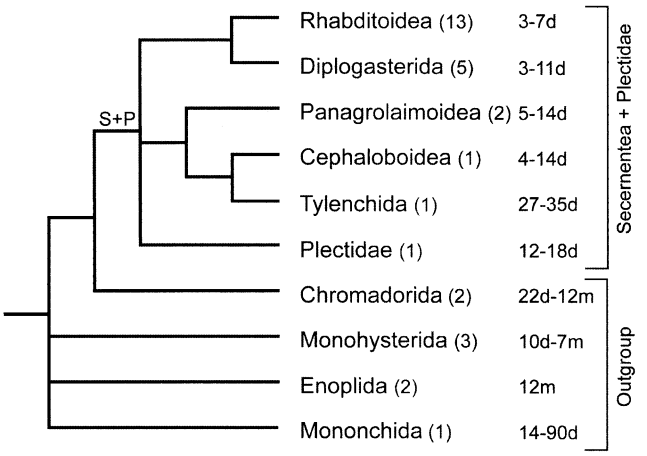

Fig. 7. Generation times of 31 nematode species per order, family, or superfamily mapped on a molecular phylogeny adapted from Blaxter et al. 1998 in days (d) or months (m). S+P indicates the clade containing Secernentea and Plectidae.

react to increased food supply in part because of its extremely short generation time (Moens et al., 1996).

The question of the ancestry of the specification mechanism is coupled to speed of development. Is increasing or decreasing the speed of development an important criterion in the evolution of development in the Phylum Nematoda or has the speed of development only changed in some taxa? Therefore, the generation time of 31 nematode species (Vancoppenolle et al., 1999; Traunspurger, 2000) was summarized per family, superfamily, or order and mapped on the molecular phylogeny of Blaxter et al. (1998) (Table 3; Fig. 7). The speed of development in the Phylum is very variable, from 3 days in the Rhabditoidea to 12 months in the Chromadorida and the Enoplida. But has the speed of development increased from a slow developing ancestor or vice versa, decreased from a fast developing ancestor. In other words, is the fast development of *C. elegans* and *P. marina* a primitive or a derived character within the phylum? First, if one compares the generation time of the taxon Secernentea + Plectidae with the outgroup, then one can conclude that the fast generation time in the taxon Secernentea + Plectidae is a derived character. Secondly, the superfamily Rhabditoidea, to which *P. marina* and *C. elegans* belong, is the fastest developing taxon within the Secernentea. So based on this limited dataset, the speed of development seems to have increased during the course of evolution in the phylum.

Based on the limited data of the embryonic cell lineage of three species and the limited data on the speed of development in the Phylum, we propose the process of fate transformations to be directly responsible for the extremely complex and chaotic lineage in *C. elegans*, as indicated by Sulston et al. (1983). If gradual fate transformations allows cells to originate closer to their final position, a highly irregular and seemingly illogic fate topology ensues. These fate transformations could greatly reduce the need for cell migrations and hence would be a potential contributing factor to faster embryonic development.

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