

gingivalis (Nematoda: Cephalobina: Panagrolaimoidea)

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Summary – This paper describes the nearly complete embryonic cell lineage of the terrestrial nematode, *Halicephalobus gingivalis*, up to somatic muscle contraction, resulting in the formation of 536 cells, of which 24 undergo programmed cell death. *Halicephalobus gingivalis* has a 94% lineage homology with both *Caenorhabditis elegans* and *Pellioiditis marina*, and a fate homology of only 86% and 78%, respectively. Although *H. gingivalis* belongs to a different superfamily than *C. elegans* and *P. marina*, its cell lineage is remarkably consistent with them. Variations in the fate distribution of cells among the different species were only observed at the end of the cell lineage. The data presented here show that the polyclonal cell specification is much more widespread in clades 9 and 10 and is not a highly derived trait that is specifically linked to the fast development of the model organism *C. elegans*.

Keywords – cell lineage homology, embryology, evolution, lineage analysis.

Nematodes are a highly diverse group of organisms with a wide spectrum of morphological adaptations, from interstitial bacterivores to obligate parasites. This diversity allows them to thrive in a wide range of ecological habitats, ranging from deep-sea sediments to arid deserts (De Ley, 2006). However, it is still unclear how the morphological diversity of nematodes relates to their developmental diversity. Most of the current research on the development of nematodes is focused on the model organism *Caenorhabditis elegans*. Sulston *et al.* (1983) established the complete embryonic cell lineage of *C. elegans* and showed that a constant number of cells is formed by a fixed division pattern. These authors also argue that, despite the fixed relationship between its division pattern and cell fate, there is no obvious correlation between them. According to Sulston *et al.* (1983), the tissues of *C. elegans* have a polyclonal origin – cells that are part of the same tissue are derived from different lineages, which suggests a complex patterning mechanism. More recent studies have shown that these mechanisms constitute a complex mix of lineage-based and organ-based patterning mechanisms (reviewed by Labouesse and Mango, 1999). Bolker (1995) cautions against applying the developmental data of model organisms to other organisms. These data are often biased as a consequence of the criteria used for selecting a model organism. These crite-

ria include rapid development and short generation time, developmental canalisation and life history. The study of the embryogenesis of more nematode species will provide insights into the different patterns that form a nematode body plan and how these patterns are linked evolutionarily.

Recent research on other nematode species throughout the phylum has uncovered greater diversity in developmental mechanisms than was previously thought. Most of this research focuses on specific phases or areas of development, such as early development (reviewed in Schierenberg, 2006) or development of the gonad, vulva and male tail (reviewed in Sommer, 2005). Until recently, no detailed, complete description of the embryonic development of any nematode other than *C. elegans* has been available (Sulston *et al.*, 1983). Thus, it remains unclear if these variations in development are part of a different mode of development or fit into the highly invariant ‘*C. elegans*-like’ polyclonal developmental strategy. Is this cell lineage a highly derived feature, specific to the fast developing *C. elegans*, or is it a more general mode of nematode development? With the establishment of the embryonic cell lineage of a related nematode, *Pellioiditis marina*, which belongs to the same family as *C. elegans*, we have demonstrated that this cell lineage is conserved

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within clade 9 according to the molecular phylogeny of Holterman *et al.* (2006) (Houthoofd *et al.*, 2003).

In this study we present the nearly complete embryonic cell lineage of a nematode from the adjacent clade 10, *Halicephalobus gingivalis* (Cephalobina: Panagrolaimoidea: Panagrolaimidae). This allows us to examine the extent of conservation of this polyclonal embryonic cell lineage in another nematode clade. In most countries, *H. gingivalis* is classified as a free-living bacteriophagous nematode but in reality it is a facultative parasite of horses and occasionally humans (Blunden, 1987; Nadler *et al.*, 2003). We recorded the embryonic development of *H. gingivalis* from zygote to comma-stage embryo with a 4D microscope, followed each individual cell in time and space, established the almost complete division pattern or cell lineage by identifying most of the cell divisions, and fixed the 3D position of almost all cells. This gives us a detailed comparison between these cell lineages on the level of each individual cell.

Materials and methods

NEMATODE CULTURES

The soil nematode, *Halicephalobus gingivalis* JB128, Stefanski, 1954, was cultured on 1% agar plates with *Escherichia coli* OP50 as the food source. Culture and handling are as described by Brenner (1974).

4D MICROSCOPY AND LINEAGE ANALYSIS

Early stage embryos were obtained by cutting gravid females in distilled water. One-cell embryos were selected under a dissecting microscope, mounted on a 5% agar pad, covered with a cover slip and sealed with Vaseline® (Sulston & Horvitz, 1977).

Three single embryos were recorded at 25°C using a 4D microscope (Schnabel *et al.*, 1997; Houthoofd *et al.*, 2003). Every 30 s, 25 focal planes were recorded through the embryo and stored on a laser videodisk. The recording started at the 2-cell stage and continued until the body muscles started to contract. Once muscle contraction started, it was no longer possible to follow the cells between two time frames.

The lineage and 3D displays of each recording were reconstructed using the Simi Biocell software equipped with an automatic collision manager (version 3.5, 4.0, Simi, Unterschleissheim, Germany) (Schnabel *et al.*, 1997). This collision manager automatically detects whether

other nuclei are present close to a newly fixed nucleus and greatly reduces the error rate. Since the recording ends at somatic body muscle contraction, it is not possible always to identify accurately the cell type of a given tissue. When discussing the nervous system, no distinction can reliably be made between neurons, sockets or sheaths; this is also the case for cell types of the pharynx.

RELIABILITY

The lineage presented here is based on three recordings. A consensus lineage was obtained, by which uncertainties of cell positions or cell divisions in two complete recordings were resolved by comparison with the third recording. As the recordings were made only up to body muscle contraction, information about late development is not recorded. In our recordings the consensus lineage contains 571 cells, so the majority of cells present later in the first-stage juvenile have already formed. Although there is some loss of lineage information, most cells have adopted their final fate and position. In *C. elegans*, 12 late divisions occur after the first contraction of the body muscles; 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). The mitoses of some cells could not be resolved in all three recordings and are marked as unresolved mitoses (mitosis was detected but the position of the daughter cells could not be determined). Also the position and/or fate of some terminal cells could not be resolved and are marked as unresolved cells.

NOMENCLATURE

The cells are named according to Sulston and Horvitz (1977), Deppe *et al.* (1978), and as adapted by Sulston *et al.* (1983). Here we summarise this nomenclature to help the reader follow this study. Founder cells formed in the first division rounds are given arbitrary names in capital letters according to Deppe *et al.* (1978). When a founder cell divides, each daughter is named by adding to the name of the mother cell a single lower-case letter representing its position immediately after division relative to its sister cell. For anterior-posterior divisions, the anterior and posterior daughters are indicated with an 'a' and 'p', respectively. Dorso-ventral divisions are indicated with 'd' and 'v'. Left-right divisions are indicated with 'l' and 'r'. For example, when founder cell E divides in an anterior-posterior direction, the daughters are named Ea and Ep. When Ep divides in a left-right direction its daughters are named Epl and Epr. A pair of

cells may be designated by the use of internal parentheses, e.g., Ea(l/r)aa means Ealaa and Earaa. In the cell lineage tree the 'a', 'd' and 'l' daughters are represented by the left branches, and the 'p', 'v' and 'r' daughters by right branches. Long cell names are split in groups of three letters with spaces to improve readability. For example, the cell ABalaaaaaa will be noted as ABala aaa aaa.

COMPARISON OF CELL LINEAGES

Considering the huge amount of data present in any given lineage, we have sometimes opted to include a comparison with the embryonic cell lineage of *C. elegans* (Sulston *et al.*, 1983) and *P. marina* (Houthoofd *et al.*, 2003) in the results section in order to aid clarity and facilitate discussion. When performing a pairwise comparison between the cell lineages of the three different species, we defined two types of homology. Lineage homology between two species is calculated as the percentage of cells at the time of muscle contraction of one species that have 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 of one species that have the same cell fate in the other species at the time of muscle contraction. Some figures, referred to, for example, as 'Fig. S1', are too large to be printed as part of the manuscript and are freely available as supplementary material on

the web at: <http://www.nematology.ugent.be/develop.htm> (see Appendix).

Results

FIRST DIVISIONS

The early development of *H. gingivalis* starts with a series of unequal, asynchronous cell divisions, during which a larger anterior somatic founder cell and a smaller posterior germ line precursor cell (P-cell) are formed (Figs 1, 2). The zygote P₀ divides into an anterior somatic cell AB and a posterior germline cell P₁ (Fig. 1A). This germline cell P₁ divides into an anterior somatic cell EMS and a posterior germline cell P₂ (Fig. 1B). AB divides in a perpendicular direction. After the division of the P₁ cell, one of the daughters of AB migrates to the anterior side of the embryo; this cell will be assigned as ABa. The posterior daughter ABp moves posteriorly dorsally of EMS and leads to the rhomboidal pattern (Fig. 1C). In *H. gingivalis*, the perpendicular division axes of AB and P₁ are clearly visible, resulting in a transient T-shape that immediately converts to the rhomboid configuration (Fig. 1B). The position of EMS determines the ventral side of the embryo (Fig. 1C). EMS divides into an anterior founder cell MS (mainly mesoderm) and a posterior cell E (endoderm) (Fig. 1D). The posterior daughter of the

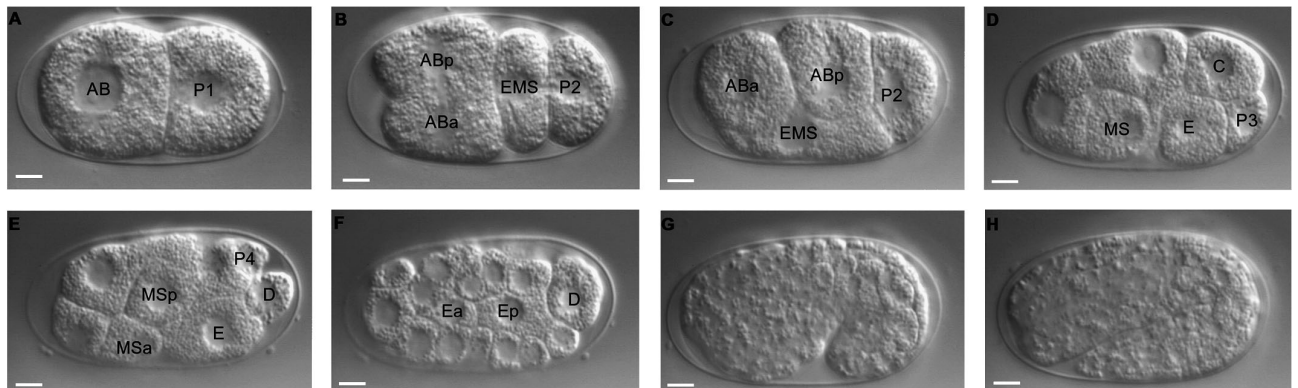


Fig. 1. Nomarski images of *Halicephalobus gingivalis* embryogenesis. All stages anterior to the left, scale bar = 5 μ m. A: 2-cell stage; B: Early 4-cell stage, left lateral view, just after division of AB and P₁. Note the perpendicular division axes of the two cells, which results in a T-shaped embryo; C: Late 4-cell stage, left lateral view. Rhomboid shape. EMS lies at the future ventral side of the embryo, ABp at the future dorsal side; D: 8-cell stage, left lateral view. EMS has divided in an anterior MS cell and a posterior E cell. P₂ has divided into a ventral P₃ cell and a dorsal C cell; E: 14-cell stage, left ventral view. P₃ has divided in a dorsal D cell and a ventral P₄ cell; F: 26-cell stage, ventral view. The two E cells lie at the ventral side just before gastrulation, when these two cells move inwards; G: Early comma stage, during morphogenesis (elongation of the body); H: Late comma (2-fold) stage at the onset of body muscle contraction.

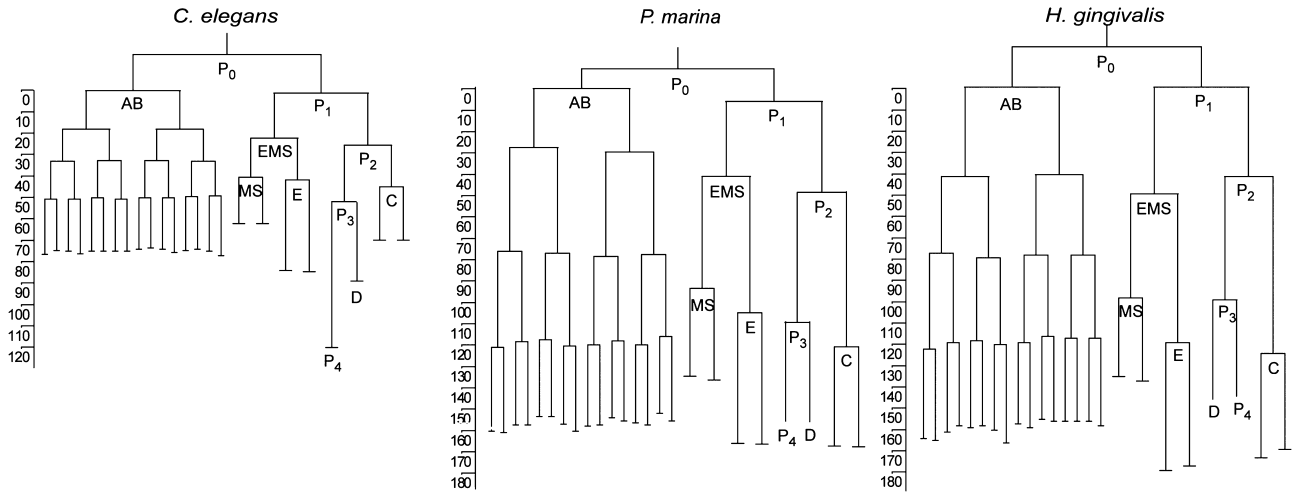


Fig. 2. The early cell lineages and the formation of the founder cells (somatic cells AB, MS, E, C, D and germline precursor P₄). A: *Caenorhabditis elegans*; B: *Pellioditis marina*; C: *Halicephalobus gingivalis*. Vertical axis represents time. Timeline in min after first division of AB. The scale of the timeline is equal in all four cell lineages. Note that the developmental speed and the division sequence are different in the three species. Left branch is anterior daughter, right branch posterior daughter.

germline cell P₁ is called P₂ (Fig. 1C) and is positioned more dorsally from its sister EMS.

In *H. gingivalis* there is a double reversal of cleavage polarity in the germline. At the division of P₂, polarity of the germline reverses so that P₃ is positioned ventrally from its sister C (Fig. 1D), i.e., similar to *P. marina* and *C. elegans*. However, at the division of P₃, the polarity reverses again so that P₄ is positioned dorsally from its ventral sister D. This is in contrast to *P. marina* and *C. elegans* in which P₄ lies ventrally from its sister D after the division of their mother cell P₃. As a result, the cells are orientated from ventral to dorsal: E-D-P₄-C (Fig. 1E). In *H. gingivalis*, the two primordial germ cells migrate in between the two daughters of D, after which they migrate inwards together.

DEVELOPMENTAL TEMPO

Halicephalobus gingivalis has the slowest early developmental tempo and is 2.9 times slower than *C. elegans* (Table 1). Subsequently, the developmental rate speeds up so that the period until muscle contraction is only 1.8 times slower than *C. elegans* (Fig. 3).

In all species, the median cell cycle length of each AB generation increases during the course of embryonic development (Fig. 4). Comparing the three species, *C. elegans* has an overall shorter median cell cycle length in AB than *H. gingivalis* and *P. marina* (Fig. 4). Two different patterns can be distinguished. In *C. elegans* and

Table 1. Division sequence of the early divisions of the founder cells. *Caenorhabditis elegans* (Ce), *Pellioditis marina* (Pm) and *Halicephalobus gingivalis* (Hg). The germline cells are marked in bold. Divisions that occur simultaneously or that vary within the same species are separated with a slash. The relative early developmental tempo was measured from the first division of AB and the division of E and normalised with the tempo of *C. elegans*. The *C. elegans* data are from Skiba and Schierenberg (1992).

Sequence of cell divisions	Ce	Pm	Hg
1	P₀	P₀	P₀
2	AB	AB	AB/P ₁
3	P₁	P₁	
4	2AB	2AB	P₂
5	EMS	EMS	2AB
6	P₂	P₂	EMS
7	4AB	4AB	4AB
8	MS	MS	MS
9	E	E	P₃
10	C	P₃	8AB
11	P₃/8AB	C	E
P ₄ present	24	15	14
Relative early developmental tempo	1.0	2.5	2.9
Relative tempo until muscle contraction	1.0	1.7	1.8
Number of specimens	10	3	3

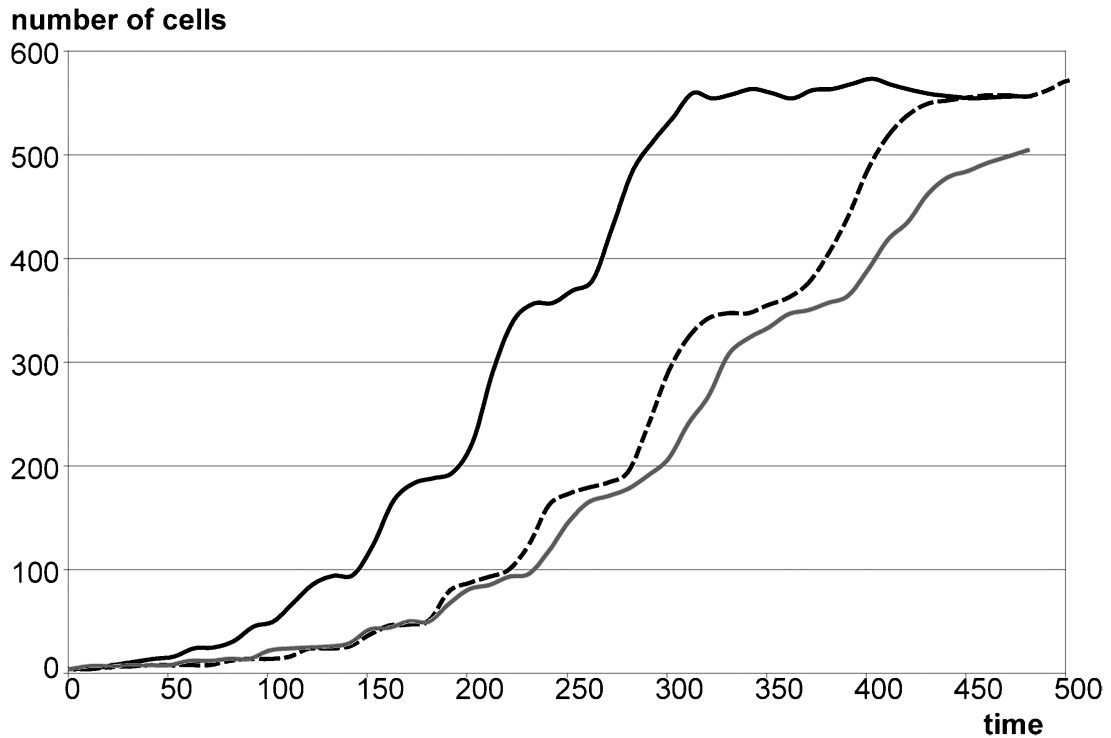


Fig. 3. Number of cells during embryonic development. *Caenorhabditis elegans* (black), *Halicephalobus gingivalis* (grey) and *Pellioditis marina* (dashed). Time in the X-axis is normalised from the first division of AB. The cell count of *H. gingivalis* stops prematurely since the precise cell number could not be determined due to the unresolved cells and mitoses at the end of the recordings.

H. gingivalis, the cell cycle length increases slowly at an equal rate. In *P. marina*, the median cell cycle length of AB shows a peak in cell cycle length at the 4AB generation after which the tempo briefly speeds up again for two cell cycles.

GASTRULATION

In *H. gingivalis* gastrulation starts at the 29-cell stage, with the two intestinal precursors, Ea and Ep, which lie ventrally, ingressing to the interior of the body (see Appendix – Supplementary material: Movie S1). Ea gastrulates first at 180 min in between the mesodermal precursors MSap and MSpp just before the second division round of E. These MS cells gastrulate little after the two daughters of Ea. After the division of E, the two Ep daughters start to gastrulate. Immediately behind these cells, the big D founder cell divides in a left-right orientation and its daughters Da and Dp gastrulate after the Ep daughters. Also, the germinal precursor P₄ divides and the two daughters migrate anteriorly between the gastrulating D daughters. The MSaa and MSpa descendants divide

anterior-posteriorly and migrate posteriorly over the gastrulating intestinal precursors. Next, these cells migrate into the interior of the embryo. Meanwhile, the mesodermal descendants of C, Cap, and Cpp migrate from the dorsal side to the ventral side and lie on both sides of the two P₄ cells. In a final phase, these cells migrate together to the inside as the AB descendants start to grow over the gastrulating cells.

LINEAGE AND FATE SIMILARITY

At muscle contraction, the consensus lineage of *H. gingivalis* contains 597 terminal cells (Fig. S1; Tables 2, S1; Appendix). The fate and position of 536 (90%) of these has been determined (the position and fate of 48 cells and the division of 13 cells could not be resolved). The lineage similarity with *C. elegans* and *P. marina* is 94%, and 504 of the 536 determined cells have an equivalent terminal cell in *C. elegans* and *P. marina* (Fig. 5). Fate similarity with *C. elegans* is 86%, i.e., 434 of these cells also have an equivalent cell fate. Fate similarity with *P. marina* is lower at 78% (only

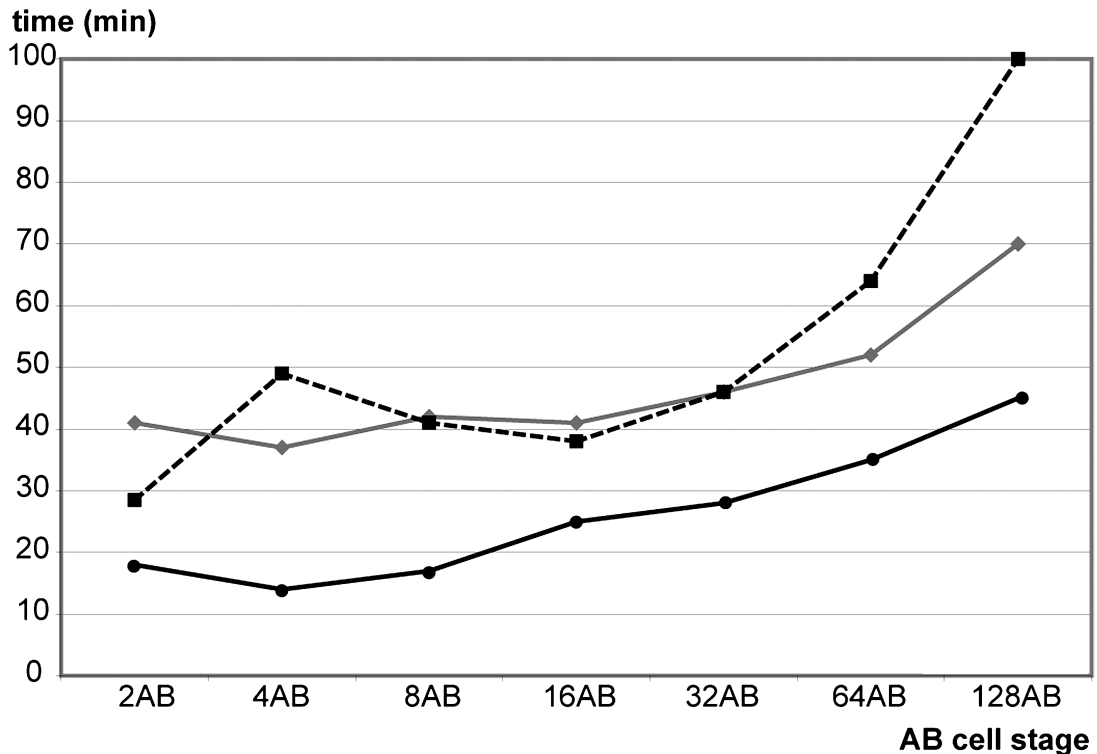


Fig. 4. The median cell cycle length of AB cells in each generation is plotted in time. *Caenorhabditis elegans* (black), *Halicephalobus gingivalis* (grey) and *Pellioiditis marina* (dashed).

Table 2. Number of cells per tissue type (row) and founder cell (column). *Halicephalobus gingivalis* (Hg), *Caenorhabditis elegans* (Ce) and *Pellioiditis marina* (Pm).

	AB			MS			E			C			D			P4			Total		
	Hg	Pm	Ce	Hg	Pm	Ce	Hg	Pm	Ce	Hg	Pm	Ce	Hg	Pm	Ce	Hg	Pm	Ce	Hg	Pm	Ce
Pharynx	60	79	56	31	33	30													91	112	86
Neuron	199	186	211	1	7	6				2	2								200	195	219
Muscle	1	1	1	33	28	28				32	32	32	20	20	20				86	81	81
Epidermis	74	114	66		3					16	14	13							90	131	79
Intestine							18	20	20										18	20	20
Other	18	20	20	7	10	11													25	30	31
Gonad																2	2	2	2	2	2
Mitosis			26			3															29
Total survivors	352	400	380	72	81	78	18	20	20	48	48	47	20	20	20	2	2	2	512	571	547
Cell death	18	58	78	4	9	13	2												24	67	91
Unresolved mitosis	13																		13		
Unresolved cell	38			10															48		
Total cells produced	421	458	458	86	90	91	20	20	20	48	48	47	20	20	20	2	2	2	597	638	638

395 of the equivalent cells in *H. gingivalis* have an equivalent fate in *P. marina*). Lineage and fate similarity

with the other species is probably higher since the fate of 48 cells in the *H. gingivalis* cell lineage could not be

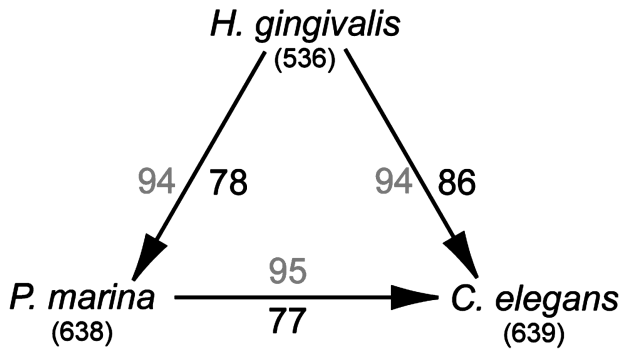


Fig. 5. Lineage and fate similarities between the three species, *Caenorhabditis elegans*, *Halicephalobus gingivalis* and *Pellioditis marina*. Lineage similarity is expressed as the percentage of resolved terminal cells in the first species that have the same lineage history in the second species. Fate similarity is expressed as the percentage of cells of the first species that has the same cell fate as the equivalent cell in the second species. Lineage similarities are marked in grey, fate similarities in black. The direction of the arrow indicates how the comparison was performed. For example, 94% of the 536 terminal resolved cells in *H. gingivalis* have an equivalent terminal cell in *P. marina*. The number of resolved terminal cells in the lineage of each species is indicated between brackets.

determined and the cell divisions of 13 cells could not be resolved.

Despite the marked similarity in development of the three species, individual differences among the different tissues were observed. The primordial gonad, intestine and the body muscles are highly conserved in the three species, whilst the pharynx, the epidermis, and nervous system have a more variable configuration. The following sections will focus on the differences amongst these three species.

INTESTINE

The 18 embryonic intestinal cells are formed exclusively by the E blastomere. Two E descendants, Eaap and Eapp, undergo programmed cell death. This division pattern differs from the *P. marina* and *C. elegans* pattern although a similar embryonic intestine is formed that consists of nine rings, each built from two bilateral symmetric cells. The morphogenesis of the intestine of *H. gingivalis* and some other close relatives is described extensively in Houthoofd *et al.* (2006).

PHARYNX

In the primordial pharynx of *H. gingivalis*, 91 pharynx cells could be identified. Sixty cells are formed by AB

Table 3. The number of cells per tissue type in *Halicephalobus gingivalis* (in bold in the top row) and the number of equivalent cells in *Pellioditis marina* (Pm) and *Caenorhabditis elegans* (Ce), with the same fate (in grey box) or another fate. The grey boxes indicate the number of cells of a certain tissue in *H. gingivalis* that have the same cell type in the other species. For example, 91 cells have the pharynx fate in *H. gingivalis* (top row), 64 of the equivalent cells in *P. marina* also have pharynx fate (row 3), while the other 27 pharyngeal cells have other cell fates in *P. marina* (rows 4-9): epidermis (6), neuron (14) and cell death (7).

<i>H. gingivalis</i> (no of cells)	Pharynx (91)		Epidermis (90)		Neurons (200)		Cell death (24)	
	Pm	Ce	Pm	Ce	Pm	Ce	Pm	Ce
Pharynx	64	76	0	0	29	1	3	1
Epidermis	6	1	78	72	25	3	0	0
Neuron	14	1	5	6	132	164	0	1
Cell death	7	11	3	6	12	16	19	20
Intestine	0	0	0	0	0	0	2	2
Other	0	0	4	1	1	2	0	0
Mitosis	0	2	0	5	1	14	0	0

and 31 cells by MS. In the AB-lineage, pharynx cells are exclusively formed by ABalp and ABara (60/60 cells). Also in the MS-lineage, the pharynx cells are exclusively formed by the anterior daughters of MSa and MSp, MSaa and MSpa (31/31 cells). Since the MS cells gastrulate before the AB descendants, the MS-derived pharynx cells primarily form dorsal pharynx tissue, while the AB descendants form the ventral part of the pharynx. The initial bilateral symmetry of the early cell lineages is still visible in the primordial pharynx. MSaa forms pharynx cells on the left side of the pharynx; MSpa forms cells on the right side. In the AB-lineage the bilateral symmetry is not strict. ABara mainly forms cells on the right side; except for the AB ara apa lineage that forms cells on the left side along with the ABalp cells.

Comparing the 91 cells that form the pharynx in *H. gingivalis* with the equivalent cell in *P. marina* and *C. elegans*, 64 (70%) and 76 (84%) of those cells, respectively, also form a pharynx cell (Table 3). In *P. marina*, 27 of those cells have another cell fate: neuron (14), epidermis (6) and programmed cell death (7). In *C. elegans*, 15 of those cells have another cell fate: cell death (11), epidermis (1), and neuron (1). For two cells the division of the mother cell could not be resolved in *C. elegans* (Table 3).

BODY MUSCLE

In *H. gingivalis*, 86 body muscle cells were identified, *i.e.*, five more cells than in the *C. elegans* and *P. marina* embryo. They arise from AB (1), MS (33), C (32) and D (20). However, it was not possible to determine the spatial configuration in detail since not all the muscle cells could be followed until body muscle contraction occurred.

EPIDERMIS

In the *H. gingivalis* embryo, 90 epidermal cells of a polyclonal origin were identified. They arise from the AB-(74) and C-lineage (16). In the AB-lineage, epidermis cells were mainly formed in the posterior daughters of the ABa lineage by ABalp and ABarp (28/28 cells), and in the anterior daughters of the ABp lineage by ABpla and ABpra (41/46 cells).

Comparing the 90 cells that form the epidermis in *H. gingivalis* with the fate of the equivalent cells in *P. marina* and *C. elegans*, 78 (87%) and 72 (80%) cells, respectively, have an equivalent epidermal cell in those species (Table 3). In *P. marina*, 12 of those cells have another fate: neuron (5), cell death (3), or other cell type (4). In *C. elegans*, 18 of those cells have another fate: neuron (6), cell death (6) or other cell type (1); for five cells the division of the mother cell could not be resolved.

NERVOUS SYSTEM

In the *H. gingivalis* embryo, 200 terminal neurons were identified (Table 2). In AB, 73% of the neurons are formed by ABala (37), ABalp (28), ABplp (40) and ABprp (41). One MS cell, MSa aaa aal, forms one extra neuron. 66-82% of those 200 neuronal cells in *H. gingivalis* have an equivalent neuron in one of the other species.

Comparing the 200 cells that form neurons in *H. gingivalis* with the fate of the equivalent cells in *P. marina* and *C. elegans*, 132 (66%) and 164 (82%), respectively, of those cells have an equivalent neuronal cell in those species (Table 3). In *P. marina*, 68 of those cells have another fate: pharynx (29), epidermis (25), cell death (12) or other cell type (1). For one cell the division of the mother cell could not be resolved. In *C. elegans*, 36 of those cells have another fate: pharynx (1), epidermis (3), cell death (16) or other cell type (1); for 14 cells the division of the mother cell could not be resolved.

PROGRAMMED CELL DEATHS

In the *H. gingivalis* embryo, only 24 programmed cell deaths could be identified (Tables 2, 3). Twenty of those cells have an equivalent cell death in *C. elegans* and 19 in *P. marina*. Four cell deaths are specific for *H. gingivalis*, one in AB (ABala ppp aaa), one in MS (MSa apa pa) and two in the E-lineage (Ea(a/p)p). In *C. elegans*, the equivalent AB cell has a neuronal fate; the MS cell divides and its posterior daughter undergoes cell death, and the two E-cells divide to form two intestinal cells each. The 72 cells that undergo cell death in *C. elegans* but not in *H. gingivalis*, become neurons (24), pharynx (11), epidermis (6) and body muscle (2). The position and fate of 23 cells in the *H. gingivalis* recordings, which have a cell death in *C. elegans*, could not be resolved, nor could we determine the division of their mother cell (six cells). It is possible that these cells also undergo cell death but this could not be confirmed in the recordings.

Discussion

REVERSAL OF POLARITY IN THE GERMLINE

In *H. gingivalis*, the polarity in the germline divisions reverses twice (Fig. 6A-E). The polarity of the division P₂ reverses, resulting in a ventral germ cell P₃ and a dorsal cell C, as in *P. marina* and *C. elegans* (Fig. 6D). In contrast to these species, the polarity of the subsequent germline division of P₃ reverses again, resulting in a primordial germ cell (PGC) P₄ that is orientated dorsally from its sister D (Fig. 6E). The resulting configuration of the posterior cells is: E-D-P₄-C. After the last division, contact between E and the germline is lost. This phenomenon of polarity reversal in the germline has been described and extensively studied in *C. elegans*. Experiments in *C. elegans*, in which the constraining factor of the egg shell is avoided, indicate that during the subsequent germline divisions, P₁ and P₂ are actually posterior daughters and P₃ and P₄ are anterior daughters (Schierenberg, 1987). Other variations in the polarity of the germline divisions are found in other species. The cephalobid *Acrobeloides nanus* (clade 11) lacks a reversal of polarity – all the P cells represent the posterior daughters, resulting in the configuration E-C-D-P₄ (Skiba & Schierenberg, 1992) (Fig. 6K-O). Laugsch and Schierenberg (2004) found intraspecific variation in two *Rhabditis* species, *R. dolichura* and *R. belari*; in some cases, the reversal of polarity is only expressed in the germline division of P₃, resulting in the transient configuration E-C-P₄-D. Despite the loss

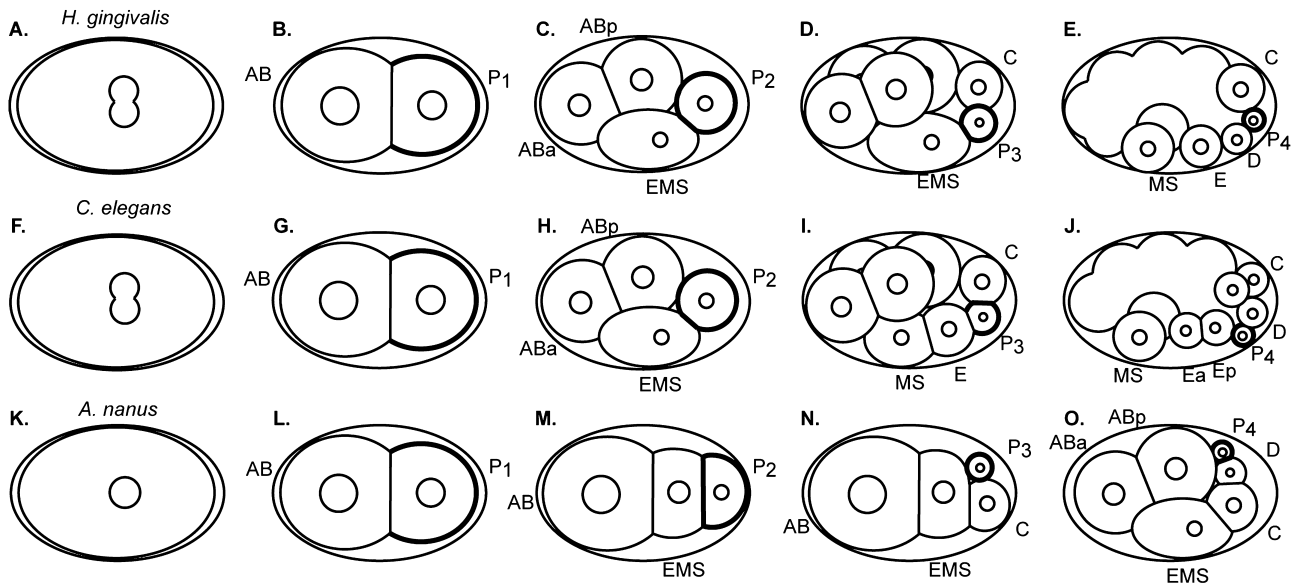


Fig. 6. Drawings of first divisions illustrating the polarity of the germline divisions. Left lateral view, anterior to the left. Big circles in cells indicate the nuclear envelope. A-E: *Halicephalobus gingivalis* double reversal of polarity in germline divisions. A: 1-cell stage; B: 2-cell stage; C: 4-cell stage; D: 7-cell stage; E: 14-cell stage, the AB progeny is indicated by outline. F-J: *Caenorhabditis elegans* single reversal of polarity. F: 1-cell stage; G: 2-cell stage; H: 4-cell stage; I: 8-cell stage; J: 24-cell stage, the AB progeny is indicated by outline. K-O: *Acrobeloides nanus*. No reversal of polarity in germline divisions. K: 1-cell stage, only one nucleus due to parthenogenetic development, in contrast with A and F, where male and female pronuclei fuse to form zygote; L: 2-cell stage; M: 3-cell stage; N: 4-cell stage; O: 6-cell stage, variations in configuration are possible (Wiegner & Schierenberg, 1998).

of contact between germline and endodermal line in all these species with partial or no reversal of polarity, this contact is restored before the onset of gastrulation through cell migrations. In *H. gingivalis*, the two primordial germ cells migrate between the two daughters of D, after which they migrate inwards together. In the six-cell stage of *A. nanus*, C and P₄ migrate over the D cell, and thus they switch places before the onset of gastrulation (Skiba & Schierenberg, 1992).

Skiba & Schierenberg (1992) postulate that the configuration in *A. nanus* is the ancestral state and that the polarity reversal observed in the rhabditids is an evolutionary modification on the 'A. nanus-like' configuration, in order to avoid compensatory migrations and to speed up the cell cycle periods and thereby developmental rate. However, recently Lahl *et al.* (2003) observed a reversal of polarity in P₂ and P₃ in several species of the Plectidae, an outgroup of the Rhabditida (Blaxter *et al.*, 1998; De Ley & Blaxter, 2002; Meldal *et al.*, 2007). Furthermore, descriptions of the early development of members of other families from clades I, II and III suggest the presence of small primordial germ precursor cells at the ventral side that contact the intestinal precursor (Malakhov,

1994). Based on the observations of the Plectidae and the other families, one can conclude that this reversal of polarity in the germline, and the maintained contact between the germline and the endodermal line found in *C. elegans* and *P. marina*, is the ancestral state in the phylum Nematoda. Consequently, the variations in reversal of cleavage polarity in the germline found within clade 9 (*R. dolichura*, *R. belari*), and clade 10 (*H. gingivalis*), and the lack of reversal of polarity in *A. nanus* (clade 11) are derived states that must have evolved independently from the ancestral state in the different branches.

EMBRYONIC CELL LINEAGE CONSERVED WITHIN CLADES 9 AND 10

A first striking conclusion is that in *H. gingivalis*, cells are built according to a cell lineage that is very similar to *P. marina* and *C. elegans*. The lineage similarities between *H. gingivalis* on the one hand, and *P. marina* and *C. elegans* on the other, are 94%, almost equal to the similarity between *P. marina* and *C. elegans* (95%). However, fate homology is greatest between *H. gingivalis* and *C. elegans* (86%), *i.e.*, 9% higher than

between the closely related *P. marina* and *C. elegans*. This indicates that variations in fate similarity do not reflect the evolutionary distance amongst the species. The lower fate homology between *P. marina* and the other two species is mainly due to the different composition of the nervous system and the pharynx, which can reflect specific morphological adaptations to the brackish environment. We only observed variations in the fate distribution of cells at the end of the cell lineage between individual species. With the establishment of the nearly complete embryonic cell lineage of *H. gingivalis* it is clear that the polyclonal cell specification is much more widespread in clades 9 and 10 and is not a highly derived trait specifically linked to the fast development of the model organism *C. elegans* (Bolker, 1995; Houthoofd *et al.*, 2003). This developmental strategy is very robust, despite the great evolutionary differences and the great genomic diversity in these clades (Parkinson *et al.*, 2004; Mitreva *et al.*, 2005).

However, this robust developmental strategy does not reflect the large morphological and ecological diversity within clades 9 and 10 (Order Rhabditida). Throughout this order, the adult body plan has undergone a wide range of evolutionary modifications that allows them to flourish in terrestrial habitats, *e.g.*, those subject to frequent episodes of rapid de- and rehydration, such as mosses and lichens or extremely xeric and/or cryogenic soils (De Ley, 2006). For example, adaptations in buccal and pharynx morphologies and different food strategies for different adult species must occur late in development after all cells are formed in the first part of embryonic development. During embryonic development, a robust basic body plan, with a pharynx primordium, intestine and four body muscle strings, is built through a stereotyped cell division pattern on which specific adaptations are superimposed during post-embryonic development. For example, a key adaptation within the Rhabditida was the development of a chemically impermeable cuticle that clearly contributed to their success as parasites, colonisers and extremophiles (De Ley, 2006). Cuticle secretion starts after the elongation of the embryo is complete. Another subject of modification in the Rhabditida is the morphology of the pharynx. This diversity mainly involves the evolution of one or more rounded muscular bulbs, which has apparently allowed for more compact body designs. The similar number of pharynx cells among the three species does not reflect the differences in the adult morphology of the pharynx in the three species compared here. *Halicephalobus gingivalis* has only one

bulb in the pharynx, whilst the other species have two. The definitive shape of the pharynx is formed during the second part of embryonic development after most of the cell divisions and after body muscle contractions. On the other hand, the differences in cellular composition of the nervous system among the three species could indicate a possible rewiring of the different pharynx morphologies.

The strong conservation of this polyclonal pattern between the species of clade 9 and 10 suggests that this strategy evolved before the diversification of these clades and played only a minor role in the evolution of faster developing species in these clades. Also, earlier descriptions of the embryonic development of *Ascaris megalocephala* (clade 3) indicated a fixed polyclonal division pattern (Müller, 1903). This strategy has probably evolved from a slower, more regulative, non-determined development in which cells are determined later in development, as can be found in the basal clades 1 and 2 (Voronov & Panchin, 1998; Borgonie *et al.*, 2000; Schierenberg, 2005).

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Appendix: Supplementary material

The following files are too large to be printed as part of the manuscript and are freely available as supplementary material on 'The embryonic cell lineage of *Halicephalobus gingivalis*' website at: <http://www.nematology.ugent.be/develop.htm>

Table S1. Comparative cell list of terminal cells with their cell fate between *Halicephalobus gingivalis* (Hg), *Pellioditis marina* (Pm) and *Caenorhabditis elegans* (Ce). AB cells are grouped per great-granddaughter of AB and in the names of the cells spaces are left between groups of three letters to improve readability. The fate of each cell is marked with a letter and colour code in accordance with the colour code of the cell lineage figures. blue: neuron (N); green: pharynx (P); purple: epidermis (E); dark blue: other fate (O); grey: programmed cell death

(D); orange: body muscle (U); brown: intestine (I); gonad (G); unresolved cell (F); unresolved mitosis (M?); mitosis (M).

Figure S1: Embryonic cell lineage of *Halicephalobus gingivalis* until body muscle contraction (650 min after first division). Vertical axis represents time. Timeline in min after first division. The AB cell lineage is cut off after 490 min, after the last division round, since not every cell could be followed until body muscle contraction. Left branch is anterior daughter, right branch posterior daughter. Terminal cells are coloured according to their fate assignment. light blue: neuron; green: pharynx; purple: epidermis; dark blue: other cell type; orange: body muscle; brown intestine. Programmed cell deaths are indicated by a cross at the end of the line.

Movie S1. Time lapse movie of the inward migration of the intestinal precursors during gastrulation in *Halicephalobus gingivalis*. Based on subsequent 3D reconstructions; balls represent the positions of the nucleus of each cell. Right lateral view, ventral side at the top. MSa, dark grey; MSp, light grey; E, brown; P₄, dark blue, D, light blue. Time is marked at right top corner in min after division of AB.

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