Somatic Programmed DNA Elimination is widespread in free-living Rhabditidae nematodes

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Abstract

All nuclei of an organism usually share an identical genome, faithfully transmitted through successive divisions. Yet, some species (e.g. in ciliates, nematodes, crustaceans, insects, vertebrates) deviate from this dogma, by systematically eliminating parts of their DNA in all their somatic nuclei, in a process called Programmed DNA Elimination (PDE). PDE leads to two coexisting genomes: a full germline genome, and a reduced somatic genome. PDE by chromosome fragmentation has been historically identified in one clade of parasitic nematodes, Ascarids, in 1887, and later also revealed in ciliate protists, in copepods and other obligate parasitic nematodes. However, and in contrast to extensive studies performed in ciliates, the molecular mechanisms, evolutionary origins, and functional significance of PDE in animals remain unclear. This lack of understanding partially stems from limitation in genetically tractable model species. Recently, PDE has been discovered in three non-parasitic, lab-tractable nematode species from the Rhabidtidae family. Identified using genomic approaches, *Oscheius tipulae* PDE targets 0.6% of its genome. We uncovered that two species in the *Mesorhabditis* genus eliminate ~30% of their genome in the soma. In this study, we used cytological approaches to screen 25 other Rhabditidae species for PDE. We found evidence of PDE in 17 species. Our work reveals that PDE is present in 12 out of 17 tested genera, demonstrating its widespread presence in Rhabditidae nematodes, with the notable exception of *C. elegans*. Genetic tools have already been established for some of them. This work provides a collection of species that can be used to test many aspects of somatic Programmed DNA Elimination in animals.

Introduction

Some species systematically eliminate portions of their genome in their soma, in a process called Programmed DNA Elimination (PDE) [1,2]. PDE can target an entire chromosome, as in songbirds or lamprey for instance [3,4]. PDE can also happen by the fragmentation and partial destruction of chromosomes, as spotted for the first time in 1887 in the parasitic nematode *Parascaris univalens* [5]. PDE by chromosome fragmentation was later identified, and extensively studied, in ciliate protists [6]. Ciliates are unicellular organisms with two nuclei: a transcriptionally inactive micronucleus (MIC) transmitted to descendants after a sexual phase, and a transcriptionally active macronucleus (MAC), copied from the MIC and amplified. During MAC development, ciliates undergo extensive PDE, often involving both small-scale and large scale PDE. PDE is pervasive in ciliates. Its study in a few species, has led to seminal discoveries on the role of small RNAs in genome rearrangement [6]. It also revealed that many eliminated short sequences are remnants of transposable elements, that are excised by a domesticated transposase [7–9]. This finding has led to a widely accepted scenario that small-scale PDE emerged as a selfish, TE-driven process in ciliates that reduces conflicts between the host and TEs [10]. In striking contrast, little is known about PDE in multicellular organisms (i.e animals), mainly because all species described until very recently were not genetically tractable. Consequently, the molecular mechanism of PDE is unknown, which makes it difficult to trace back the evolutionary history of PDE. By contrast, hypotheses on its evolution in ciliates emerged once the molecular nature of the PDE nuclease was identified. The contribution of PDE is also unknown because it has not been possible to analyse the consequence of abolished PDE in any animal species.

Recently, PDE was identified in two nematode genera belonging to the Rhabditidae family, in a different sub-order than Ascarids. In *Oscheius tipulae*, state-of-the-art genome reconstruction revealed that 0.6 % of the genome is eliminated at chromosome ends [11,12]. Almost concomitantly, and by serendipity, we discovered substantial PDE in two other Rhabditidae species - although distant from *O. tipulae* - using cytology initially: *Mesorhabditis belari* and *Mesorhabditis spiculigera*. They eliminate 30% and 20% of their genome, respectively, at all chromosome ends but also in internal chromosomal regions, leading to an increased somatic karyotype [13].

In a large range of nematode species, including Ascarids and Rhabditidae, the embryonic cell lineage is highly reproducible and conserved so that the germline precursor cell is easily identified: it is the smallest and most posterior cell; it does not divide until late in embryogenesis, or even after hatching in some species; and it harbours highly compacted chromatin [14]. Somatic cells also divide in a relatively conserved order, allowing for easy identification of cell types from one species to the other, by analogy with what has been well described in *C. elegans* [14]. We could therefore follow chromosome fragmentation in *Mesorhabditis* embryos, during embryonic divisions. As previously described in Ascarids, we found PDE starts with chromosome fragmentation in all five somatic precursor cells, successively and independently, as they enter mitosis. This is followed by fragment exclusion during mitosis and DNA destruction later in development. We also found, as show for Ascarids or *O. tipulae*, that telomeres of the germline inherited chromosomes are

systematically eliminated in the soma, and that new telomeres are assembled on the new extremities of all somatic chromosomes [13].

The finding that free-living nematode species undergo PDE following the same mechanism as in Ascarids demonstrates that PDE is not a parasitic oddity in nematodes and raise the possibility that PDE, rather than having evolved independently in two distant genera within Rhabditidae, could be more widespread than anticipated in nematodes.

In this study, we asked whether PDE is widespread in family Rhabditidae. Using simple DNA staining, we reveal that 17 out of 25 newly tested species show evidence of PDE. This corresponds to 12 out of 17 genera in which at least one species undergoes PDE. This work reveals that PDE is widespread in Rhabditidae, with the notable exception of *C. elegans*, and has gone unnoticed for more than a century of nematode research.

Results

Identification of Programmed DNA Elimination by DNA staining

In *Ascarids* and *Mesorhabditis* species, DNA elimination is immediately visible in early embryos because chromosomes are fragmented into large portions [5,13,15]. Prior to elimination, no fragments are visible and somatic nuclei harbour the same number of chromosomes as germ cells. During elimination, chromosome fragments can be seen excluded from the spindle during mitosis or are visible in the cytoplasm in interphasic cells. After elimination, DNA fragments are eventually destroyed after a few cell cycle and old embryos do not show cytoplasmic DNA fragments [13,16]. When fragments internal to chromosomes are also eliminated, as in Ascarids or *Mesorhabditis*, the chromosome number is smaller in the nuclei of germline precursor cell than in nuclei of somatic cells. Overall, it is possible to detect PDE using simple DNA staining on embryos at different time points during their development.

In order to detect PDE, we performed DNA staining on embryos from 28 species, including previously described *Oscheius tipulae*, *Mesorhabditis belari* and *Mesorhabditis spiculigera*. These species belong to 17 different genera in the Rhabditidae family. We unambiguously identified cytoplasmic DNA fragments in many Rhabditidae species (Fig. 1, Supp. Fig. 1).

DNA staining in the cytoplasm could reflect the presence of intracellular bacteria. If this were the case, cytoplasmic DNA should be visible at any stage in embryos. We reconstructed the dynamics of fragment production in all species (Fig.1 and Supp. Fig. 1). We found that fragments were initially absent in 1, 2 and 4-cell stage embryos. Fragments suddenly appeared at a specific time point which seems conserved for a given strain. After a few cell divisions, fragments were not detectable anymore suggesting they had been physically destroyed. Importantly, fragments were not detected in the germ cell primordium (Fig.1 and Supp. Fig. 1). This finding confirmed that DNA fragments appear and disappear specifically in somatic cells. For some species we could confirm that in cells that have generated cytoplasmic fragments, the number of chromosomes in their nuclei was higher than the number of chromosomes found in early embryos or in germ cells (Supp. Fig. 1). For instance, we counted 2n=12 chromosomes in the germline of *Pelodera teres* JU4407, and found more than 26 small fragments in somatic cells from old embryos (Supp Fig. 1). This is compatible with a scenario where for each chromosome, at least a fragment internal to chromosome has been eliminated. From these results, we concluded that DNA cytoplasmic fragments do not correspond to intracellular bacteria but reflect, as shown in Ascarids or *Mesorhabditis*, the destruction of part of chromosomes after their fragmentation [13,16].

We found large variation in the amount of eliminated DNA (Fig. 1 and Supp. Fig. 1). In some cases, we found very large and numerous fragments, as in *Mesorhabditis simplex*, or more impressively in *Cruznema tripartitum* JU4406 (Fig. 1). For other species, the amount of eliminated DNA seems more limited as fragments are small. This is the case for instance in *Rhabditeall axei* (Fig.1), *Oscheius dolichura* or *Caenorhabditis monodelphis* (Supp. Fig. 1). With this method, we did not detect any fragments in *O. tipulae*, most likely because it eliminates 0.6% of its genome only (Supp. Fig. 1) [11,12]. This suggested that we

may have underestimated the number of eliminating species using our DNA staining method.

We next asked if as found in *Ascarids*, *Mesorhabditis belari* and *Oscheius tipulae*, germline telomeres are also eliminated in somatic cells of newly identified species undergoing PDE. To this end, we performed DNAFISH against the conserved telomeric repeat TTAGGC in several species (Fig. 1). We confirmed germline telomere elimination in a few species only, because often the FISH signal was too noisy to unambiguously conclude (see Material and Method). However, germline telomere elimination could be confirmed in *Pelodera teres*, *Rhabditis rainai*, *Oscheius tipulae*, *Auanema freiburgensis* and *Caenorhabditis monodelphis*. By contrast, we showed that germline telomeres are maintained in *C. elegans*, as expected from a species that does not undergo PDE (Fig. 1). We also found maintenance of germline telomeres in *P. stronglyloides*, a species for which we did not detect cytoplasmic DNA fragments, reinforcing the idea that this species also does not undergo PDE (Supp. Fig. 1). Interstingly, after DNA elimination, the telomeric signal remained absent from the somatic nuclei, for instance in *Oscheius tipulae* (Fig. 1), and as previously shown in *M. belari* [13]. This is consistent with the detection, from sequencing reads, of very short neo-telomeres at the new somatic chromosome ends in both species [11,13]. In contrast, the telomeric signal reappears progressively in the somatic nuclei of *Pelodera teres or Rhabditis rainai*, suggesting that somatic telomeres are longer in this species than in *Mesorhabditis* species.

Altogether, this result reveals that at least 17 out of these 25 newly studied Rhabditidae species undergo PDE and that most likely they all eliminate germline telomeres, in addition to internal chromosomal fragments in some cases.

Evolution of PDE within Rhabditidae

The phylogeny of Rhabditidae has been previously resolved [17]. We generated a new phylogeny including only 28 species examined here (27 are shown, but we also included *Cruznema*. sp. DF5015), based on rDNA18S and 28S sequences, on which we mapped PDE (Fig. 2) (see Material and Methods). Our phylogeny is consistent with previous publications, except on a couple of occasions which we discuss below.

We found that PDE occurs in two large monophyletic groups, both strongly supported (Fig. 2). Clade1 encompasses the *Mesorhabditis* genus, as well as three other genera: *Pelodera Teratorhabditis* and *Rhomborhabditis*. All five analysed *Mesorhabditis* species undergo PDE. However, we found that *Pelodera strongyloides* does not eliminate whereas *Pelodera teres* does. We note however, that *Pelodera strongyloides* has an ambigious position in the phylogenetic tree within Clade1 due to a divergent 18S and 28S sequences (see Material and Methods). Previous publications also reported a weak support of the branch for this particular species. At this stage, we don't know if *P. teres* and *P. strongyloides* belong to the same genus, with variability of PDE within that genus or whether they have been wrongly classified in the same genera [17,18]. Regarless of the position of *P. strongyloides within Clade1*, this result suggests that PDE was present in the common ancestor of Clade1, and has been lost in *P. strongyloides*.

We also found PDE in a large Clade 2, encompassing the *Oscheius genus* and seven other genera.

Here, all ten species studied undergo PDE, although the amount of eliminated DNA vary a lot across species, from very little in *O. tipulae* to massive PDE in *Cruznema tripartitum* JU4406. During our survey, we also analysed DF5015, another putative strain of *Cruznema tripartitum* (Supp. Fig. 1), for which we found very limited PDE whereas *Cruznema tripartitum* JU4406 eliminates a lot of DNA. This suggested that the amount of eliminated DNA can vary drastically within species. However, when we performed crosses between JU4406 and DF5015, we found they produce dead embryos only (see Material and Methods), demonstrating they belong to different species (though they are probably closely related as males and females can still mate). Although our phylogeny represents a single Cruznema species, we counted JU4406 and DF5015 as two studied species. This result demonstrated that the extent of PDE can vary on a small evolutionary time scale, i.e within the same genus (Fig. 2).

Outside Clade1 and Clade2, we detected PDE in *Caenorhabditis monodelphis*, suggesting PDE also occurs in a third Clade3, encompassing the *Caenorhabditis* genus. PDE is however absent in *C. elegans, C. briggsae* and *C. angaria*, demonstrating again variability at the genus level. Whether PDE was present in the common ancestor of all *Caenorhabditis* and lost in some species, notably *C. elegans*, or specifically gained in *C. monodelphis,* will require more investigation of that specific genus.

Finally, we did not detect PDE in 4 other species, each belonging to a different genus: *Diploscapter , Protorhabditis sp.*, *Choriorhabditis cristata* and *Pristionchus pacificus*. The position of *Pristionchus pacificus* and *Choriorhabditis cristata* is inconsistent with previous publication, which placed them prior to the divergence between Clade 1 and Clade 2/Caenorhabditis/Diploscapter [17]. However, regardless of the phylogenetic position of these two species, our current sampling is insufficient to distinguish between several independent origins and a single ancient origin with subsequent losses of PDE in Rhabditidae

Altogether, these results reveal that PDE is found independently in at least three clades within the Rhabditidae family.

Variation in the timing of PDE

In *Ascarids*, chromosomes start to be fragmented in ABa and ABp cells at the 4-cell stage [5,15,16]. We found the same early fragmentation starting in ABa and ABp (the two most anterior largest cells) in *Teratorhabditis palmarum* and also in all *Mesorhabditis* species, although embryos already had five blastomeres when ABa and ABp started dividing [13] (Supp. Fig. 1). However, within Clade1, DNA fragments are visible only when embryos reach ~9 cells for *Pelodera teres* and *Rhomborhabditis regina*. This delay of approximately one cell cycle for the onset of DNA fragmentation was also observed in all Clade 2 species and in *C. monodelphis*. Thus, the timing of PDE initiation varies between species, with no obvious correlations with the topology of the phylogeny (Supp. Fig. 1).

PDE occurs regardless of reproductive systems or chromosome number

Last, we analysed PDE in the context of the reproductive system or the chromosome number of the species, two other parameters that were known [14,19] or that we collected during our analysis (see Material and Methods and Supp. Fig. 1). Various reproductive systems are found in Rhabditidae nematodes: regular sexual reproduction, hermaphroditism, a combination of the two with the presence of three sexes [20], strict parthenogenesis or autopseudogamy, another form of asexuality [21]. We found no obvious link with the reproductive systems (Fig. 2). For instance, some sexual species undergo PDE, such as *Pelodera teres*, whereas others do not, such as *Pelodera strongyloides* or *Caenorhabditis angaria*. Conversely, PDE is found in species that reproduce through hermaphroditism (e. g. *Oscheius* species), species with three sexes such as *Auanema freiburgensis*, or asexually, such as *Mesorhabditis belari* and *Mesorhabditis simplex*.

Finally, we found no correlation between the occurrence of PDE and the number of germline chromosomes (Supp. Fig. 1 and Fig. 2). PDE can occur in species with a large number of germline chromosomes, such as *Mesorhabditis longespiculosa* (n=17) or a small number, such as *Teratorhabditis palmarum* (n=4). Conversely, PDE is not found in species with a single chromosome such as *Diploscapter corronatus* or in *Pelodera stronglyloides* which has 11 chromosomes. Many species in this family harbour six chromosomes, some of which undergo PDE, such as *Caenorhabditis monodelphis* or *Oscheius dolichura* and others do not, such as *Caenorhabditis elegans* (Fig. 2).

Discussion

Programmed DNA elimination has been described in 1887 in a parasitic nematode [5] and we had recently found by serendipity, similar massive PDE in *Mesorhabditis* species [13]. In this study, we revealed PDE in 17 species, out of 25 newly tested species. This corresponds to 12 out of 17 genera in which at least one species undergoes PDE. Although the extant Rhabditidae species is much larger than our collection, we anticipate that PDE is widespread in Rhabditidae. Our work also reveals that this process, has been missed by pionners of nematode research, before *C. elegans*, an outsider when considering PDE, had been chosen as a model representative of nematodes.

We found three clades, in which PDE is found in at least one species. In Clade1, all nine species eliminate except *P. strongyloides*. In Clade2, all ten species undergo PDE. In Clade3, for which we analyzed four species only, we found PDE in a single species, *C. monodelphis*. Given our current sampling, it is not possible to conclude whether PDE has emerged independently three times within Rhabtidae or was present in the common ancestor with independent losses. In particular, we have found that PDE can vary a lot within a genus, demonstrating that the study should be extended to many more species per genera. Nevertheless, if we consider PDE in Ascarids, it is tempting to speculate that PDE is very ancient in nematodes and has been lost several times, including in the most notorious species *C. elegans*. Exploration of more species, in particular outside the Rhabditidae family will be required to conclude on this point. Identification of the mechanisms of the molecular effectors of PDE in all these branches will also shed light on its evolutionary trajectory.

Interestingly, elimination of the germline telomeres is a feature shared by Ascarids, *O. tipulae* and *Mesorhabditis*. Indeed *O. tipulae* which eliminates very little DNA and does not fragment its chromosomes internally, eliminates all its germline chromosome telomeres. We confirmed this feature in few other species. To date, no species has been found to eliminate exclusively internal portions while retaining chromosome ends, indicating that telomere elimination may represent a conserved ancestral feature

We found variation in the developmental timing at which chromosome fragments appear in the cytoplasm. One possibility is that fragmentation begins at the same stage in all species, but that fragments are subsequently processed or sorted at different times, preventing their cytoplasmic detection. Alternatively, this variation may reflect species-specific differences in the timing at which the PDE machinery is transcribed or becomes functional

Within our collection, *C. elegans* appears as one of the rare species that does not undergo PDE. Obviously, our understanding of PDE would have been different if the scientific community would have picked another Rhabditidae species, as a model in the middle of the XXth century [22]. Nevertheless, our work now reveals that PDE is found in free-living species, all being lab-tractable species. Genetic tools are available in some of them, including *Oscheius tipulae* [12,23] and *Auanema* [24,25] , and we also established CRISPR and RNAi in some Clade1 species (unpublished). This opens avenues- at last- for the exploration of the PDE mechanisms in animals.

We uncovered PDE by serendipity because we were analysing chromosome inheritance in *Mesorhabditis* embryos [13]. Our study calls for a systematic search for signs of PDE by chromosome fragmentation, using cytological approaches as described here, when embryos are easy to stain, or using bioinformatic approaches in any animal species because PDE could be more widespread than anticipated even outside nematodes.

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Competing interests: All the authors declare having no competing interests.

Material and methods

Species collection

Strains are maintained at 20°C on NGM plates seeded with *E. coli* OP50, following *C. elegans* protocols, as described in [23]. Strains are all listed in Supplementary Figure 1. Strains were obtained from the CGC stock center, from Marie-Anne Félix or from David Fitch.

Genetic Crosses

DF5015 and JU4406 were both matching *Cruznema tripartitum* on public databases, based on the 18S sequencing. Because they revealed very different amount of eliminated DNA, we asked whether they belong to the same species. To answer this question, we crossed 3 virgin females from one strain with one male of the other strain. In both direction of the cross, females produced dead embryos only. From this result, we concluded that DF5015 and JU4406 belong to two distinct species.

DNA staining and DNA FISH and immunno staining

Samples were fixed with methanol and the freeze-cracking method, as described in [13]

We fixed embryos to analyse DNA fragments or count chromosomes. We also fixed dissected female gonads in order to count the number of chromosomes in meiotic cells. At this stage, chromosome are compact and pair with their homologs which facilitate counting.

After washing in PBS, the slides were counter-stained with Hoechst (0.5 µg/ml).

For DNAFISH, we used the following fluorescent oligo probes at 500 nM (ordered at Sigma) to detect telomeres: 5' [TAM]TTAGGCTTAGGCTTAGGCTTAGGC 3'

Embryos and larvae were freeze-cracked and processed as described in [21]. Images were taken on an Zeiss LSM-800 confocal.

Phylogeny based on 18S and 28S sequences

Sequencing and sequence extraction

To build a phylogeny of the 28 species we studied in family Rhabditidae, we amplified the DNA sequences of 18S and 28S ribosomal RNA by PCR using universal primers, as described in [17].

In some cases, PCR amplification or sequencing did not work or gave truncated sequences. In these cases, we turned to public databases to obtain sequences from the same species that we studied by cytology. In these cases, we used the SILVA database of ribosomal gene sequences [27], downloading all SSU (18S) and LSU (28S) sequences for taxon Chromadorea (taxon ID: 119089), a superset of Rhabditidae. For any given species, when multiple hits were available in SILVA, we used the hit referenced in [17] in which the authors built a phylogeny of many of the same species of Rhabditidae as studied here, also using 18S and 28S sequences (plus the large subunit of RNA Polymerase II where available). These hits can be found in

Table S1 of Kiontke *et al.* (2007)[17].

We summarise the origin of each sequence for each species we studied in table 1 below.

Species	**18S origin**	**18S strain**	**28S origin**	**28S strain**
M. belari	This study	JU2817	This study	JU2817
M. simplex	This study	JU2864	-	-
M. monhystera	This study	JU2855	-	-
M. longespiculosa	This study	DF5017	This study	DF5017
M. spiculigera	This study	AF72	-This study	AF72
T. palmarum	U13937.1	DF5019	This study	DF5019
P. strongyloides	U13932.1	DF5022	-	-
P. teres	AF083002.1	EM437	This study	JU4407
Rh. regina	This study	DF5012	This study	DF5012
O. tipulae	This study	CEW1	EU195969.1	CEW1
O. dolichura	This study	JU1182	EU195971.1	JU460
O. myriophilus	This study	DF5020	AY602176.1	DF5020
R. rainai	This study	JU1368	EU195966.1	DF5091
A. freiburgensis	This study	VSL2220	KY680646.1	SB372
R. axei	U13934.1	DF5006	AY602177.1	DF5006
R. terricola	This study	JU4408	EF417152.1	NK3
P. typica	U13933.1	DF5026	EU195962.1	DF5025
P. neopapillosa	This study	JU1104	-	-
Cruznema tripartitum	This study	JU4406	EU195974.1	SB202
Choriorhabditis cristata	EU196013.1	SB300	EU195976.1	SB300
C. elegans	This study	N2	This study	N2
C. briggsae	U13929.1	PB102	AY604481.1	PB800
C. angaria	JN636068.1	PS1010	AY604482.1	PS1010
C. monodelphis	JN636070.1	SB341	AY602170.1	SB341
D. coronatus	This study	JU4418	BBTA01000078.4838	PDL0010
Protorhabditis sp.	This study	JB122	EU195958.1	JB122
P. pacificus	AF083010.1	PS312	This study	PS312
Bunonema reticulatum	EU196017.1	PDL1002	-	-

Table 1. **Origin and strain of the 18S and 28S rDNA sequences used to build our Rhabditidae phylogeny**. For each of our 28 species (rows), the origin columns show whether we used our sequencing, where successful ('This study'), or if publicly-available data was used, in which case we provide the NCBI Nucleotide accession. The sequenced strains, of our study, or of the publicly-available data, are also provided. '-' indicates we did not sequence and no public data was available - this was only the case for 28S sequences.

We note from Table 1 that, in a number of cases, the publicly-available sequences we used did not come from the same strain that we studied by cytology (see Supp. Figure 1), and/or the public sequences we used were not from the same strains between 18S and 28S. However, we checked our resulting phylogeny against previously-built phylogenies of Rhabditidae, and found our final phylogeny to be consistent with previous knowledge, except on few instances that are discussed above, validating the use of different strains when the strain we imaged was not available. In addition, when we used our own sequencing, we consistently found that our sequencing matched almost identically with publicly-available sequences of the same species (where available).

Phylogeny building

For each set of gene sequences (18S and 28S), we next produced multiple sequence alignments using mafft v7.490 [28] (`--adjustdirection --reorder`), and trimmed the alignment with clipkit v2.3.0 [29] to remove aligned positions with more than 80% gaps (-m gappy -g 0.8). We then built a phylogeny using IQ-TREE v2.3.6 [30] with auto-detection of the best-fitting substitution model (here, GTR+F+I+R3 for both the 18S tree and the 28S tree) and 1000 bootstrap replicates (-B 1000). We then used weighted ASTRAL v1.23.3.7 [31](--treeweights) to combine the two gene trees into a species tree, using a hybrid weighting scheme (--mode 1) that accounts for both bootstrap support and branch lengths to resolve gene tree discordance, and using 16 rounds of placement and subsampling (-R). We also gave twice as much weight to the 28S tree compared to the 18S tree, as the 18S tree placed the *Diploscapter*/*Protorhabditis* group incorrectly relative to the *Caenorhabditis* group.

Phylogenetic trees were visualised and rendered with FigTree (https://github.com/rambaut/figtree/).

Figure legend

Figure 1: Somatic chromosome fragmentation and telomere elimination is found in several Rhabditidae species.

A.Embryos from different species and at various stages of development (from the the youngest on the left to the oldest on the right) were fixed and stained. DNA is shown in blue. The contours of the cells are shown in dotted white lines. The DNA of the germline precursor cell is shown in red. White arrows point towards cytoplasmic fragments, which become visible from the 5-cell stage in *Mesorhabditis simplex* and later in *Cruznema sp.* and *Rhabditella axei*. The name of the strain for each species is shown in parentheses. An enlargement of the image of each embryo in the third column is shown in the inset on the right. In the inset, the nuclei are encircled in white and the DNA fragments excluded from the nuclei are indicated by an arrow.
B. Embryos from different species are shown and at various stages of development. On the left, embryos are undergoing chromosome fragmentation in a least one of their somatic cells. Enlargement of one cell is shown in the inset. DNA is in blue and telomeres are in red. In the inset, the nuclei are encircled in white and the telomeres excluded from the nuclei are indicated by an arrow. Older embryos, after chromosome fragmentation are shown on the right. In *C. elegans*, all cells in young and old embryos maintain their telomeres, whereas in species undergoing PDE, somatic telomeres do not reappear (*O. tipulae, C. monodelphis*) or reappear progressively (*Rh. rainai* and *P. teres*).

Figure 2: Repartition of species undergoing Programmed DNA Elimination within the Rhabditidae family

Cladogram of species analysed in this study, based on the 18S and 28S rDNA sequencing. Species in which we detected PDE are shown in green and others in red. PDE has not been scored in Bunonema reticulatum, which serves here as an outgroup for the phylogeny only. Species undergoing PDE are grouped in two main monophyletic groups we called Clade1 and Clade2. We also found Caenorhabditis monodelphis undergo PDE within the Caenorhabditis. The reproductive modes are shown on the right (asexual, male/female, hermaphrodite and three sexes), as well as chromosome numbers (also visible on Supp. Fig. 1). *Pelodera strongyloides* is not monophyletic with *Pelodera teres*, most likely due to a divergent ribosomal DNA sequence, as discussed in the text.The study also includes Cruznema sp. DF5015 which is not shown in this tree.

Supplementary Figure 1:Cytological analysis of all species from this study

Embryos from all species analysed in this study are shown. DNA is in blue. The contours of the cells are shown in dotted white lines. White arrows point towards cytoplasmic fragments, On the left, are show embryos at the 4 or 5-cell stage. On the right, embryos at the time of chromosome fragmentation for species undergoing PDE (shown with a star) or at equivalent stage for species in which we never detected cytoplasmic fragments. Images used to quantify chromosome number are shown as well. In most cases, we analysed meiotic cells during diakinesis of prometaphase. At this stage, chromosomes are paired and we

therefore count a haploid number of chromosomes. For some species, it was possible to count chromosome number in embryos during prometaphase. In this case, we counted a diploid number. Of note, for *Diploscapter coronatus* the 2 DNA bodies observed in diakinesis correspond to the two unpaired chromosome. This species as a single chromosome which does not pair during meiosis. For *Oscheius tipulae*, *Auanema sp. VSL2220* and *Caenorhabditis monodelphis*, are show DNAFISH images revealing the telomeres in red. This allows validating elimination in these species which eliminate very little DNA. In Teratorhabditis palmarum, chromosome fragmentation starts at the 4-cell stage, as the number of chromosome, some being very small, is more than 2n=8 in mitotic cells (embryos on the left).

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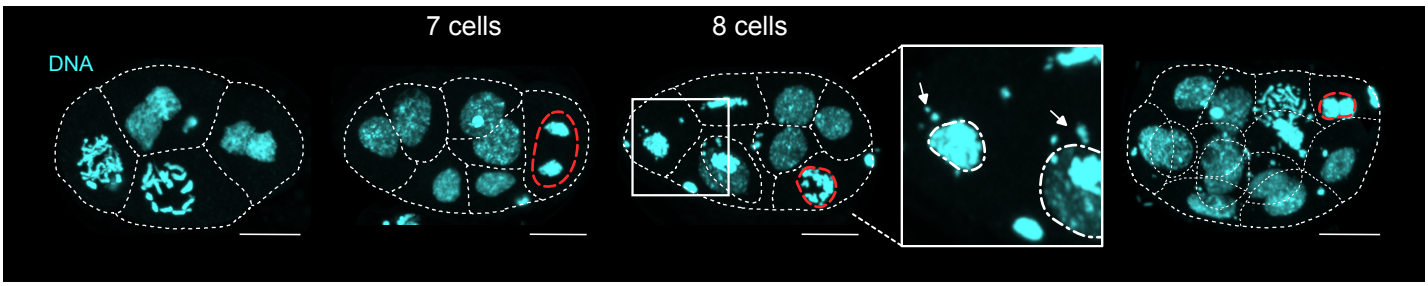
Fig.1

A. DNA staining

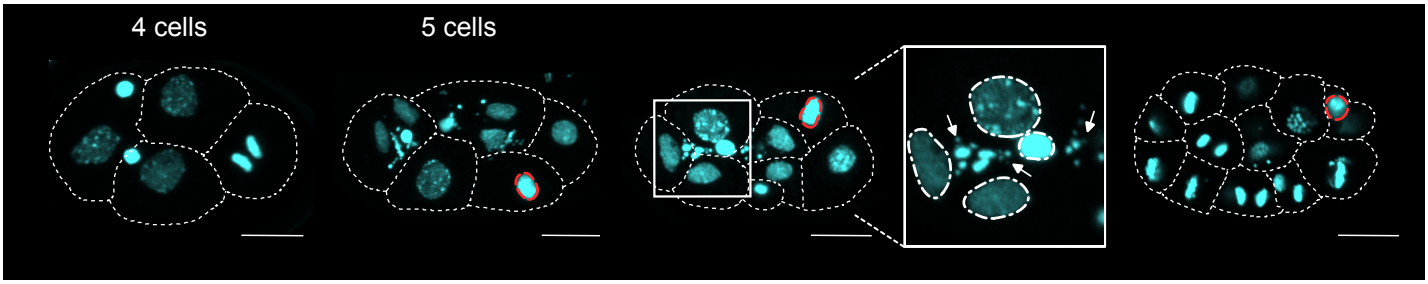
Limited elimination

Rhabditella axei (DF5006)



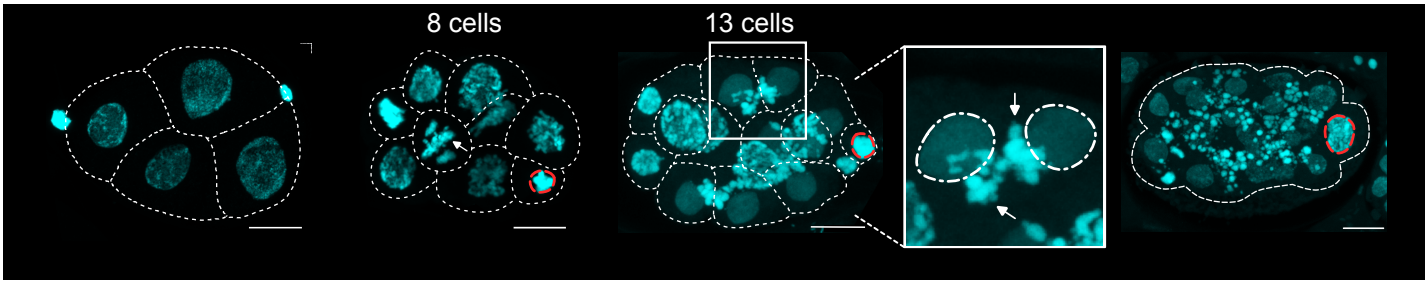
Moderate elimination

Mesorhabditis simplex (JU2864)



Massive elimination

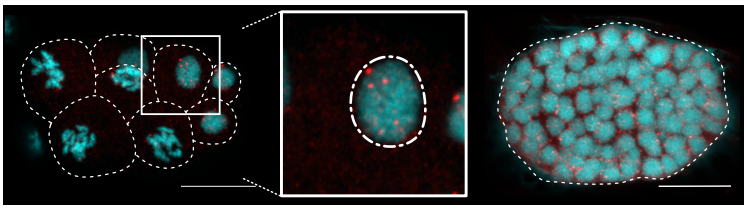
Cruznema sp. (JU4406)



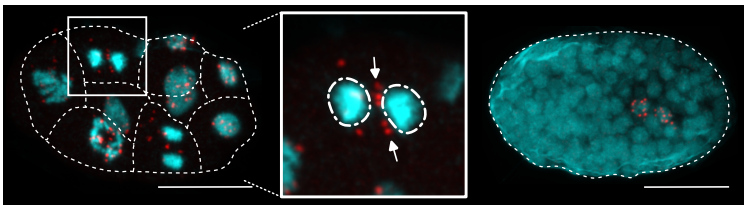
B. Telomere DNA FISH

Telomeres
DNA

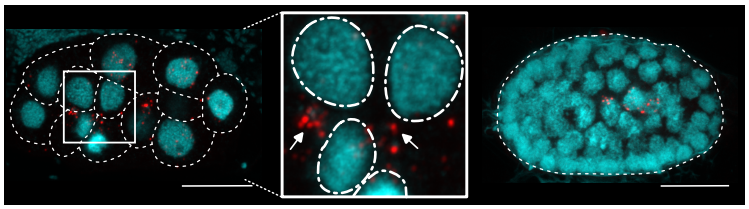
Caenorhabditis elegans (N2)



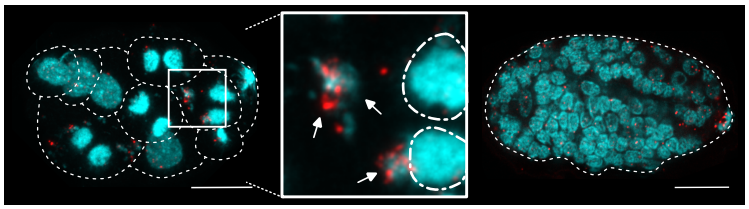
Oschieus tipulae (CEW1)



Caenorhabditis monodelphis (JU1667)



Pelodera teres (JU4407)



Rhabditis rainai (PS1191)

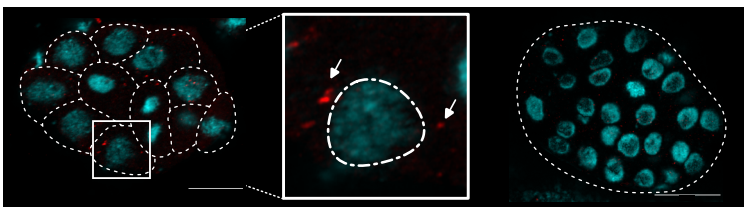
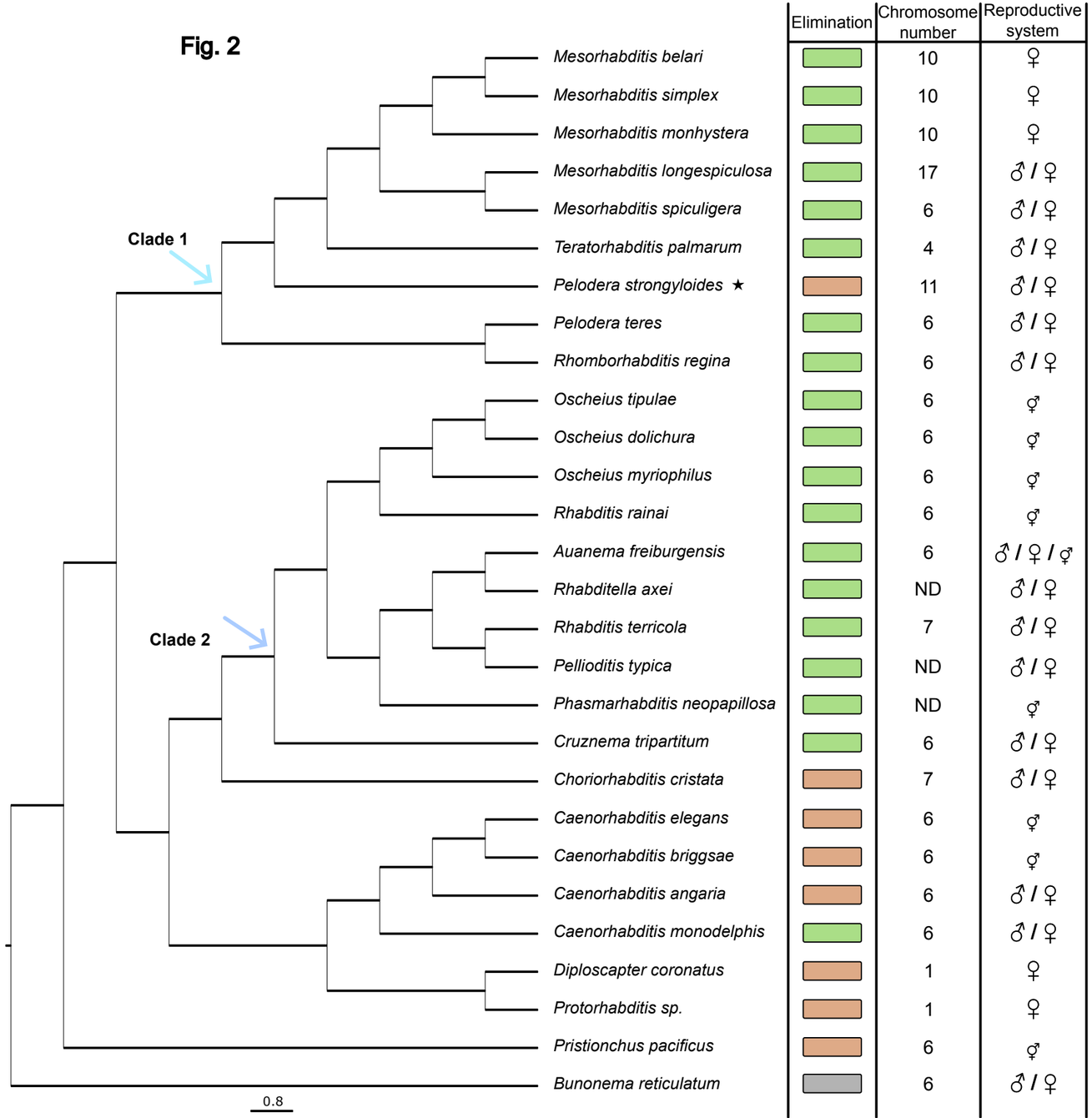


Fig. 2


Elimination
Chromosome number
Reproductive system
Clade 1
Clade 2
Mesorhabditis belari 10 ♀
Mesorhabditis simplex 10 ♀
Mesorhabditis monhystera 10 ♀
Mesorhabditis longespiculosa 17 ♂ / ♀
Mesorhabditis spiculigera 6 ♂ / ♀
Teratorhabditis palmarum 4 ♂ / ♀
Pelodera strongyloides ★ 11 ♂ / ♀
Pelodera teres 6 ♂ / ♀
Rhomborhabditis regina 6 ♂ / ♀
Oscheius tipulae 6 ⚥
Oscheius dolichura 6 ⚥
Oscheius myriophilus 6 ⚥
Rhabditis rainai 6 ⚥
Auanema freiburgensis 6 ♂ / ♀ / ⚥
Rhabditella axei ND ♂ / ♀
Rhabditis terricola 7 ♂ / ♀
Pellioditis typica ND ♂ / ♀
Phasmarhabditis neopapillosa ND ⚥
Cruznema tripartitum 6 ♂ / ♀
Choriorhabditis cristata 7 ♂ / ♀
Caenorhabditis elegans 6 ⚥
Caenorhabditis briggsae 6 ⚥
Caenorhabditis angaria 6 ♂ / ♀
Caenorhabditis monodelphis 6 ♂ / ♀
Diploscapter coronatus 1 ♀
Protorhabditis sp. 1 ♀
Pristionchus pacificus 6 ⚥
Bunonema reticulatum 6 ♂ / ♀
0.8