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

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Abstract

All cells of a multicellular organism usually share an identical genome, faithfully transmitted through successive divisions. Yet, a number of animal species deviate from this dogma, as parts of their DNA are systematically eliminated in all their somatic nuclei, in a process called Programmed DNA Elimination (PDE). PDE leads to the unexpected reorganisation of the genome at every generation in all somatic cells but its molecular mechanism, evolutionary origins, and functional significance remain unknown. This lack of understanding partially stems from limitations in genetically tractable model species.

PDE can target an entire chromosome, or involve chromosome fragmentation followed by selective fragment retention and elimination, raising further questions on genome stability, genome integrity and mechanisms of DNA repair. PDE by chromosome fragmentation has been described in parasitic nematodes in the family Ascarididae, copepods in the genus Cyclops and unicellular ciliates. More recently, PDE has been discovered in three non-parasitic, lab-tractable nematode species from the Rhabditidae family, opening new perspectives.

In this study, we used cytological approaches to screen 25 new 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 species. This work provides a collection of lab-

tractable species that can be used to test many aspects of somatic Programmed DNA Elimination by chromosome fragmentation 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 across the phylum of ciliate protists, Ciliophora [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 excision of internal sequences, healed by non-homologous end-joining, as well as chromosome fragmentation, as found in ascarid parasitic nematodes. 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 internal eliminated sequences are remnants of transposable elements, excised by a domesticated transposase [7–9]. This, together with many internal eliminated sequences being also transposon-derived, has led a widely accepted scenario that excision of internal sequences emerged as a selfish, TE-driven process in ciliates [10]. In striking contrast, PDE through chromosome fragmentation remains poorly understood in ciliates, and its molecular dissection in animals has been largely precluded because ascarids are obligate parasites and not experimentally tractable. Moreover, the functional importance of somatic PDE is also unknown because it has not been possible to abolish PDE in any animal species so far.

Recently, PDE was identified in two nematode genera belonging to the Rhabditidae family, in a different sub-order than the one including ascarids. In *Oscheius tipulae*, telomere-to-telomere 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*. These two species eliminate approximately 30% and 20% of their genome, respectively, at all chromosome ends but also in large internal chromosomal blocks, leading to an increased somatic karyotype, as shown in Ascarids [13].

We could easily follow chromosome fragmentation in *Mesorhabditis* embryos, across the successive embryonic divisions. Indeed, in a large range of nematode species, including ascarids and Rhabditidae, the embryonic cell lineage is highly reproducible and conserved. Moreover, 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]. In *Mesorhabditis* embryos, as previously described in ascarids, we found that 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 in *Mesorhabditis*, as shown 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 [12,13,15].

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 raises the possibility that PDE, rather than having evolved independently in two distantly related clades, could be more widespread than anticipated in nematodes.

In this study, we asked whether PDE is widespread in family Rhabditidae, which originated approximately 200 million years ago [16]. 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 blocks [5,13,17]. 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 cycles and old embryos do not show cytoplasmic DNA fragments [13,15]. When fragments internal to chromosomes are also eliminated, as in ascarids or *Mesorhabditis*, the chromosome number is increased in somatic nuclei compared to germline nuclei. 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 25 new species. These species belong to 17 different genera in the Rhabditidae family, which include previously studied *Oscheius* and *Mesorhabditis* genera. We unambiguously identified cytoplasmic DNA fragments in 17 Rhabditidae new species spanning 12 genera (Fig. 1, Supp. Fig. 1).

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 then appeared rapidly (possibly during a single mitotic division) and, for any given strain, reproducibly in time. After a few cell divisions, fragments were no longer detectable suggesting they had been physically destroyed. Importantly, fragments were not detected in the germ cell primordium (Fig.1 and Supp. Fig. 1). 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 one internal chromosomal fragment has been eliminated, as shown in ascarids or *Mesorhabditis* [13,15]. We ruled out that our observed fragments corresponded to intracellular

114 bacteria, as cytoplasmic DNA fragments appeared and disappeared specifically in somatic cells, and not in
115 the germline primordium.
116
117 We found large variation in the amount of eliminated DNA (Fig. 1 and Supp. Fig. 1). In some cases,
118 we found very large and numerous fragments, as in *Mesorhabditis simplex*, or more dramatically even in
119 *Cruznema sp.* JU4406 (Fig. 1). For other species, the amount of eliminated DNA seems more limited as
120 fragments are small. This is notably the case in *Rhabditella axei* (Fig.1), *Oscheius dolichura* and
121 *Caenorhabditis monodelphis* (Supp. Fig. 1). With our DNA staining method, we did not detect any
122 fragments in *O. tipulae*, most likely because it eliminates 0.6% of its genome only (Supp. Fig. 1) [11,12].
123 This suggested that we may have underestimated the number of eliminating species using this method.
124
125 We next asked if as found in ascarids, *Mesorhabditis belari* and *Oscheius tipulae*, germline
126 telomeres are also eliminated from somatic cells in our newly identified species. To this end, we performed
127 DNA-FISH against the conserved telomeric repeat TTAGGC in several species (Fig. 1). We confirmed
128 germline telomere elimination in a few species only, as the FISH signal was often too noisy to
129 unambiguously conclude (see Material and Method). However, germline telomere elimination could be
130 confirmed in *Pelodera teres*, *Rhabditis rainai*, *Oscheius tipulae*, *Auanema freiburgensis* and *Caenorhabditis*
131 *monodelphis*. As a control, we showed that germline telomeres are maintained in *C. elegans* somatic nuclei,
132 as expected from a species that does not undergo PDE (Fig. 1). We also found maintenance of germline
133 telomeres in *Pelodera strongyloides*, a species for which we did not detect cytoplasmic DNA fragments,
134 strongly suggesting that this species also does not undergo PDE (Supp. Fig. 1). In some species, following
135 DNA elimination the telomere signal did not reappear in somatic nuclei, including in *Oscheius tipulae* (Fig.
136 1), and in *Mesorhabditis belari*, confirming our previous finding [13]. This is consistent with the detection,
137 from sequencing reads, of shorter neo-telomeres at the new somatic chromosome ends than in the germline
138 in *Oscheius tipulae* [11]. In contrast, the telomeric signal progressively reappears in the somatic nuclei of
139 late embryos in *Pelodera teres* and *Rhabditis rainai*, suggesting that somatic telomeres are longer in these
140 species than in *Mesorhabditis* species. This will need to be confirmed using genomics.
141

142 Altogether, our results reveal that at least 17 out of these 25 newly studied Rhabditidae species
143 undergo PDE, that they most likely all eliminate germline telomeres, and in some cases additionally
144 eliminate internal parts of chromosomes as well.
145

146 *Evolution of PDE within Rhabditidae*

147 To identify phylogenetic patterns of PDE presence/absence, we generated a new phylogeny containing the 28
148 species we studied here (*M. belari*, *M. spiculigera*, *O. tipulae* and 25 new species), using 18S and 28S
149 ribosomal DNA (rDNA) sequencing data (Fig. 2). Our phylogeny is consistent with previous publications
150 [18], except on one occasion that we discuss below.
151 We found that PDE occurs in two large monophyletic groups, both with strong bootstrap support
152 (Fig. 2 and Supp. Fig. 2). The first clade encompasses the *Mesorhabditis* genus plus three other genera:
153 *Pelodera, Teratorhabditis* and *Rhomborhabditis*. All five analysed *Mesorhabditis* species undergo PDE.
154 However, we found that *Pelodera strongyloides* does not eliminate whereas *Pelodera teres* does. We note
155 that the phylogenetic placement of *Pelodera strongyloides* is ambiguous in our tree, due to highly divergent
156 18S and 28S sequences (see Material and Methods). Previous publications also reported weak branch support
157 for this specific species [18,19]. It thus remains to be determined whether *P. teres* and *P. strongyloides* truly
158 belong to the same genus, and where exactly *P. strongyloides* branches. At this stage, we do not know if
159 PDE was present in the common ancestor of this clade and subsequently lost in the lineage leading to *P.*
160 *strongyloides* (Fig. 2), or whether it emerged after the divergence from *P. strongyloides*.
161 The second large monophyletic clade with PDE encompasses the *Oscheius* genus plus seven other
162 genera (Fig. 2). All ten studied species from this clade undergo PDE, with striking variability in the amount
163 of eliminated DNA, ranging from very little in *O. tipulae* to massively in *Cruznema sp.* JU4406 (Fig. 1).
164 During our survey, we also found that DF5015, another putative strain of *Cruznema tripartitum* displayed
165 much less eliminated DNA than JU4406 (Supp. Fig. 1). While this suggested strong intra-species variability,
166 crosses between JU4406 and DF5015 produced dead embryos only, likely suggesting they are in fact
167 different species (see Materials and Methods). Overall, our results suggest that the extent of PDE can vary
168 over a relatively short evolutionary time scale, i.e. at least within the same genus (Fig. 2).
169

170 Outside of these two clades with many tested eliminating species, we also detected PDE in a third
171 clade with one eliminating species, *Caenorhabditis monodelphis*. In this clade, we found PDE is absent in *C.*
172 *elegans, C. briggsae* and *C. angaria*, demonstrating again variability at the genus level. In a recent study,
173 deeper investigation within this genus confirmed using genomics that PDE occurs in *C. monodelphis*, as
174 found here, and also identified PDE in two other basal *Caenorhabditis* species, *C. auriculariae* and *C.*
175 *parvicauda*, with cytological confirmation in *C. auriculariae* [20]. That study found an absence of PDE in all
176 other *Caenorhabditis* species - including the three we tested here - leading to the conclusion that PDE was
177 subsequently lost in the genus *Caenorhabditis* [20].
178
179 Finally, we did not detect PDE in four other species, each belonging to a different genus:
180 *Diploscapter sp., Protorhabditis sp.*, *Choriorhabditis cristata* and *Pristionchus pacificus*. The position of
181 *Pristionchus pacificus* is consistent with previous publications placing it basal to all other species studied
182 here [16,18]. However, the position of *Choriorhabditis cristata* is inconsistent with one previously published
183 phylogeny [18],that also placed it basally, while here *C. cristata* falls between the *Caenorhabditis* genus and
184 the clade containing *Oscheius*. Future whole genome-based phylogenies will enable definitively resolving
185 this inconsistency. .
186 Irrespective of the placement of *C. cristata*, our results suggest that PDE has been lost and/or gained
187 at least three times independently within the family Rhabditidae (Fig. 2).
188

189 *Variation in the timing of PDE*

190 In ascarids, chromosomes start to be fragmented in ABa and ABp cells at the 4-cell stage [5,15,17]. We
191 found the same early fragmentation starting in ABa and ABp (the two largest and most anterior cells) in
192 *Teratorhabditis palmarum* and also in all *Mesorhabditis* species, although embryos already had five
193 blastomeres when ABa and ABp started dividing in *Mesorhabditis* [13] (Supp. Fig. 1). However, DNA
194 fragments are visible only when embryos reach ~9 cells for *Pelodera teres* and *Rhomborhabditis regina*.
195 This delay of approximately one cell cycle for the onset of DNA fragmentation was also observed in all
196 species of the second large clade and in *C. monodelphis*. *Mesorhabditis*, *Pelodera* and *Rhomborhabditis* are
197 closer phylogenetically than either is to *C. monodelphis* (Fig. 2). Thus, the timing of PDE initiation varies

198 between species, but with no obvious ties to the topology of the phylogeny.

199

200 *PDE occurs regardless of reproductive systems or chromosome number*

201 Last, we analysed PDE in the context of the reproductive system or the chromosome number of the species,
202 two other parameters that were known [14,21] or that we collected during our analysis (see Materials and
203 Methods and Supp. Fig. 1). Various reproductive systems are found in Rhabditidae nematodes: regular
204 sexual reproduction, hermaphroditism, a combination of the two with the presence of three sexes [22], strict
205 parthenogenesis (full asexuality) [23] or autopseudogamy (pseudo-sexuality) [24]. We found no obvious link
206 between PDE presence/absence and reproductive systems (Fig. 2). For instance, some sexual species
207 undergo PDE(e.g. *Pelodera teres)*, while others do not (e.g. *Pelodera strongyloides* and *Caenorhabditis*
208 *angaria)*. Similarly, PDE is also found in species that reproduce through all reproductive modes:
209 hermaphroditism (e. g. *Oscheius* species), species with three sexes such as *Auanema freiburgensis*,
210 or through autopseudogamy such as *Mesorhabditis belari* and *Mesorhabditis simplex,* but is not found in
211 other hermaphrodites (e.g. *C. elegans*) or asexuals (e.g. *Diploscapter*).
212 Finally, we found no correlation between the occurrence of PDE and the number of germline
213 chromosomes (Supp. Fig. 1 and Fig. 2). PDE can occur in species with a large number of germline
214 chromosomes, such as *Mesorhabditis longespiculosa* (n=17) or a small number, such as *Teratorhabditis*
215 *palmarum* (n=4). Conversely, PDE is found neither in *Diploscapter coronatus*, a species with a single pair of
216 germline chromosomes, nor *Pelodera strongyloides* that has 11. Many species in Rhabditidae have six
217 chromosomes, some of which undergo PDE, such as *Caenorhabditis monodelphis* or *Oscheius dolichura* and
218 others that do not, such as *Caenorhabditis elegans* (Fig. 2).

219

220

221 Discussion

222 Programmed DNA Elimination (PDE) was first described in 1887 in a parasitic nematode [5] and we had
223 recently uncovered the same process in free-living *Mesorhabditis* species that eliminate up to 30% of their
224 genome in the soma [13]. In this study, we revealed PDE in 17 other free-living species (out of 25 newly
225 tested species). This corresponds to 12 out of 17 genera in which at least one species undergoes PDE. We

226 have discovered that PDE is widespread in Rhabditidae. To confirm this finding, more species in this family
227 can be tested, beyond our collection. Our work also reveals that this process was missed by early pioneers of
228 nematode research, even before *C. elegans*- an outsider when considering PDE- was chosen as a model
229 system [25].
230
231 Within Rhabditidae, we found three distinct clades in which PDE is found in at least one species.
232 Given our current sampling, it is not possible to conclude whether PDE has emerged independently three
233 times within this family, was present in the common ancestor with independent losses, or a combination of
234 both. In particular, we have found that the amount of eliminated DNA can vary substantially within a genus,
235 calling for extending observations to more species per genus. Nevertheless, if we consider PDE in ascarids, it
236 is tempting to speculate that PDE is an ancient process in nematodes and has been lost several times,
237 including in the well-studied species *C. elegans* [20]. An interesting parallel is the unicellular ciliates, an
238 extremely broad phylum in which most studied representatives so far have been found to undergo PDE,
239 except in genus *Loxodes* [26]; this includes species in genera *Blepharisma*, *Euplotes*, *Oxytricha,*
240 *Tetrahymena* and *Paramecium* [6,27]. Here too, the timing and amount of eliminated DNA vary widely. To
241 what extent PDE is ancestral in ciliates, and/or evolved repeatedly and convergently, remains to be fully
242 elucidated.
243
244 In the case of nematodes, exploration of more species - in particular, outside the Rhabditidae family -
245 will be required to further explore the history of PDE. Identification of the mechanisms of the molecular
246 effectors of PDE, none of which are known so far in nematodes, in all these branches will also shed light on
247 its evolutionary trajectory.
248
249 Interestingly, elimination of the germline telomeres is a feature shared by ascarids, *O. tipulae*,
250 *Mesorhabditis*. Indeed *O. tipulae* which eliminates very little DNA and does not fragment its chromosomes
251 internally, eliminates all its germline chromosome telomeres. We confirmed this feature in four other species
252 and it has also been confirmed for *Caenorhabditis parvicauda* and *Caenorhabditis auriculariae* [20] To date,
253 no species has been found to eliminate exclusively internal portions while retaining chromosome ends,

254 indicating that telomere elimination may represent a conserved, ancestral feature.

255

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

261

262 Within our collection, *C. elegans* appears as one of a minority of species that does not undergo PDE.
263 Perhaps our understanding of PDE would have been different if the scientific community had picked another
264 Rhabditidae species as a model species, in the mid-20th century [28]. Nevertheless, our work now reveals
265 that PDE is found in many free-living species, all of which are potentially lab-tractable. Genetic tools are
266 already available in some of them, including *Oscheius tipulae* [12,29] and *Auanema* [30,31] , and we have
267 also established CRISPR and RNAi in some *Mesorhabditis and Pelodera* species (unpublished). This at last
268 opens avenues for the mechanistic exploration of the PDE in animals.

269

270 We originally discovered PDE in *Mesorhabditis* by serendipity when analysing chromosome
271 inheritance in young embryos [13]. Our study calls for a systematic search for signs of PDE by chromosome
272 fragmentation, using cytological approaches as described here, when embryos are easy to stain, or using
273 bioinformatic approaches in any animal species (for example using the tool delfies [32]), because PDE could
274 be more widespread than anticipated even outside nematodes.

275

276

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285
286
287 **Material and methods**

288 *Species collection*

289 *Strains* are maintained at 20°C on NGM plates seeded with *E. coli* OP50, following *C. elegans* protocols, as
290 described in [33]. Strains are all listed in Supplementary Figure 1. Strains were obtained from the CGC stock
291 center, or were kind gifts from Marie-Anne Félix or from David Fitch.
292

293 *Genetic Crosses*

294 DF5015 and JU4406 were both matching *Cruznema tripartitum* on public databases, based on the 18S
295 sequencing. Because they revealed contrasting amounts of eliminated DNA, we wondered whether they
296 belong to the same species. To test this, we crossed 3 virgin females from one strain with one male of the
297 other strain. In both cross directions, females produced dead embryos only. From this result, we concluded
298 that DF5015 and JU4406 belong to two distinct species, though they are probably closely related as males
299 and females can still mate.
300

301 *DNA staining and DNA FISH and immunno-staining*

302 Samples were fixed with methanol and the freeze-cracking method, as described in [13]. We fixed
303 embryos to analyse DNA fragments or count chromosomes. We also fixed dissected female gonads
304 in order to count the number of chromosomes in meiotic cells. At this stage, chromosomes are
305 compact and paired as homologs, facilitating counting. After washing in PBS, the slides were
306 counter-stained with Hoechst (0.5 µg/ml). For DNA-FISH, we used the following fluorescent oligo
307 probes at 500 nM (ordered at Sigma) to detect telomeres: 5'
308 [TAM]TTAGGCTTAGGCTTAGGCTTAGGC 3'. In many species, the telomeric signal

309 was found inside nuclei, as expected, but also outside nuclei, which we interpreted as noise. This prevented
310 us from concluding on the presence/absence of telomeres. Images were taken on a Zeiss LSM-800 confocal.
311

312 *Phylogeny based on 18S and 28S sequences*

313 *Sequencing and sequence extraction*

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

316 In some cases, PCR amplification or sequencing did not work or gave truncated sequences. In these
317 cases, we turned to public databases to obtain sequences from the same species that we studied by cytology.
318 In these cases, we used the SILVA database of ribosomal gene sequences, release 138.2, [34], downloading
319 all SSU (18S) and LSU (28S) sequences for taxon Chromadorea (taxon ID: 119089), a superset of
320 Rhabditidae. For any given species, when multiple hits were available in SILVA, we used the hit referenced
321 in [18] in which the authors built a phylogeny of many of the same species of Rhabditidae as studied here,
322 also using 18S and 28S sequences (plus the large subunit of RNA Polymerase II where available). These hits
323 can be found in Table S1 of Kiontke *et al.* (2007)[18].
324

325 We summarise the origin of each sequence for each species we studied in table 1 below. All sequences are
326 available on Zenodo: https://doi.org/10.5281/zenodo.18890442
327

Species	**18S origin**	**18S strain**	**28S origin**	**28S strain**
Mesorhabditis belari	This study	JU2817	This study	JU2817
Mesorhabditis simplex	This study	JU2864	-	-
Mesorhabditis monhystera	This study	JU2855	-	-
Mesorhabditis longespiculosa	This study	DF5017	This study	DF5017
Mesorhabditis spiculigera	This study	AF72		
Teratorhabditis palmarum	U13937.1	DF5019	This study	DF5019
Pelodera strongyloides	U13932.1	DF5022	-	-

Pelodera teres	AF083002.1	EM437	This study	JU4407
Rhomborhabditis regina	This study	DF5012	This study	DF5012
Oscheius tipulae	KP756939.1	CEW1	EU195969.1	CEW1
Oscheius dolichura	EU196010	JU460	EU195971.1	JU460
Oscheius myriophilus	U81588.1	DF5020	AY602176.1	DF5020
Rhabditis rainai	AF083008.1	PS1191	EU195966.1	DF5091
Auanema freiburgensis	KY680647.1	SB372	KY680646.1	SB372
Rhabditella axei	U13934.1	DF5006	AY602177.1	DF5006
Rhabditis terricola	This study	JU4408	EF417152.1	NK3
Pellioditis typica	U13933.1	DF5026	EU195962.1	DF5025
Phasmarhabditis neopapillosa	This study	JU1104	-	-
Cruznema tripartitum	U73449.1	DF5015	EU195974.1	SB202
Cruznema sp.	This study	JU4406		
Choriorhabditis cristata	EU196013.1	SB300	EU195976.1	SB300
Caenorhabditis elegans	This study	N2	This study	N2
Caenorhabditis briggsae	U13929.1	PB102	AY604481.1	PB800
Caenorhabditis angaria	JN636068.1	PS1010	AY604482.1	PS1010
Caenorhabditis monodelphis	JN636070.1	SB341	AY602170.1	SB341
Diploscapter coronatus	This study	JU4418	BBTA01000078.4838	PDL0010
Protorhabditis sp.	This study	JB122	EU195958.1	JB122
Pristionchus pacificus	AF083010.1	PS312	EU195982.1	PS312
Bunonema reticulatum	EU196017.1	PDL1002	This study	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 in one instance that is 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 [35] (`--adjustdirection --reorder`), and trimmed the alignment with clipkit v2.3.0 [36] 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 [37] 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 [38](--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 (Supp. Fig. 2).

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

The raw Newick-formatted tree files, and code to generate the trees from the input sequences, are available on Zenodo: https://doi.org/10.5281/zenodo.18890442

360 **Figure legend**

361

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

364 **A.** Embryos from different species and at various stages of development (from the youngest on the left to the
365 oldest on the right) were fixed and stained. DNA is shown in blue. The contours of the cells are shown in
366 dotted white lines. The DNA of the germline precursor cell is shown in red. Polar bodies, when visible, are
367 circled with a solid white line. For the pseudogamous species *Mesorhabditis simplex*, the paternal DNA stay
368 highly condensed after fertilization [33] and is shown circled with a solid yellow line. White arrows point
369 towards cytoplasmic fragments that become visible from the 5-cell stage in *Mesorhabditis simplex* and later
370 in *Cruznema sp.* and *Rhabditella axei*. The name of the strain for each species is shown in parentheses. A
371 zoomed-in view of the image of each embryo in the third column is shown in the inset on the right. In the
372 inset, the nuclei are encircled in white and the DNA fragments excluded from the nuclei are indicated by an
373 arrow. **B.** Embryos from different species are shown and at various stages of development, after DNA FISH
374 treatment against telomeric repeats. On the left, embryos are undergoing chromosome fragmentation in a
375 least one of their somatic cells. Enlargement of one cell is shown in the inset. DNA is in blue and telomeres
376 are in red. In the inset, the nuclei are encircled in white and the telomeres excluded from the nuclei are
377 indicated by an arrow. Older embryos, after chromosome fragmentation are shown on the right. In
378 *Caenorhabditis elegans*, all cells in young and old embryos maintain their telomeres, whereas in species
379 undergoing PDE, somatic telomeres do not reappear (*Oscheius tipulae, Caenorhabditis monodelphis*) or
380 reappear progressively (*Rhabditis rainai* and *Pelodera teres*).

381

382 **Figure 2: Phylogenetic distribution of species undergoing Programmed DNA Elimination in family**
383 **Rhabditidae**

384 Cladogram of species analysed in this study, based on the 18S and 28S rDNA sequencing. Species in which
385 we detected PDE are shown in blue and others in orange. PDE has not been scored in *Bunonema reticulatum*,
386 which serves here as an outgroup for the phylogeny only. Species undergoing PDE are grouped in two main
387 monophyletic groups. We also found that *Caenorhabditis monodelphis* undergoes PDE whereas other

388 *Caenorhabditis* species do not. The reproductive modes are shown on the right (asexual, male/female,
389 hermaphrodite and three sexes), as well as chromosome numbers (also visible on Supp. Fig. 1). *Pelodera*
390 *strongyloides* is not monophyletic with *Pelodera teres*, (see discussion in the main text).
391
392 **Supplementary Figure 1: Cytological results for all 28 analysed species**
393 Embryos from all species analysed in this study are shown. DNA is in blue. The contours of the cells are
394 shown in dotted white lines. Polar bodies, when visible, are circled with a solid white line. For the
395 pseudogamous species *M. belari, M. simplex, M. monhystera* [33] *and Protorhabditis sp. (*unpublished*)*, the
396 paternal DNA stay highly condensed after fertilization [33] and is shown circled with a solid yellow line.
397 White arrows point towards cytoplasmic fragments. On the left, embryos at the 4 or 5-cell stage are shown.
398 On the right, are shown embryos at the time of chromosome fragmentation for species undergoing PDE
399 (shown with a star) or at equivalent stage for species in which we never detected cytoplasmic fragments.
400 Images used to quantify chromosome number are shown as well. In most cases, we analysed meiotic cells
401 during diakinesis of prometaphase. At this stage, chromosomes are paired and we therefore count a haploid
402 number of chromosomes. For some species, it was possible to count chromosome number in 1 or 2 cell
403 embryos during prometaphase. In this case, we counted a diploid number. Of note, for *Diploscapter*
404 *coronatus* the 2 DNA bodies observed in diakinesis correspond to the two unpaired chromosome. This
405 species as a single chromosome which does not pair during meiosis. For *Oscheius tipulae*, *Auanema sp.*
406 *VSL2220* and *Caenorhabditis monodelphis*, the images come from DNAFISH stainings, revealing the
407 telomeres in red. This allows validating elimination in these species which eliminate very little DNA. In
408 *Teratorhabditis palmarum*, the number of chromosomes, some being very small, is more than 2n=8 in
409 mitotic cells, revealing that chromosome fragmentation starts at the 4-cell stage (embryos on the left).
410
411 **Supplementary Figure 2: Phylogeny of studied species using 18S and 28S sequences**
412 Phylogenies of species analysed in this study, based on the 18S rDNA sequencing or 28S rDNA sequencing
413
414

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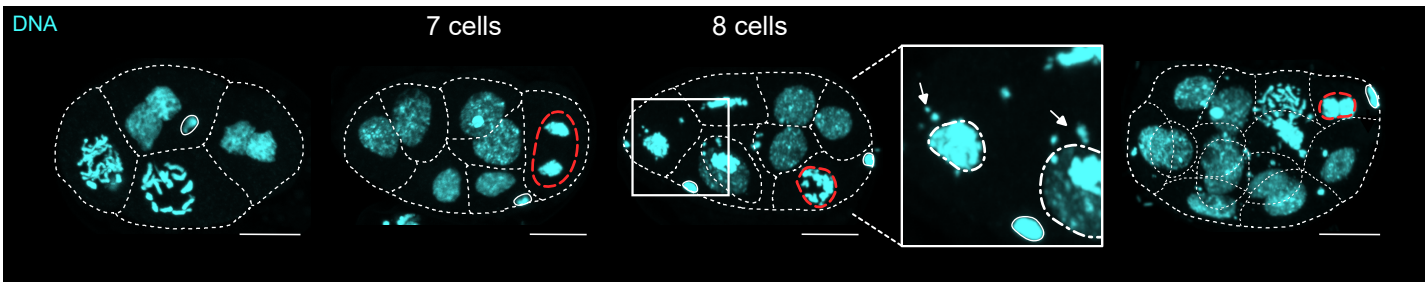
Fig.1

A. DNA staining

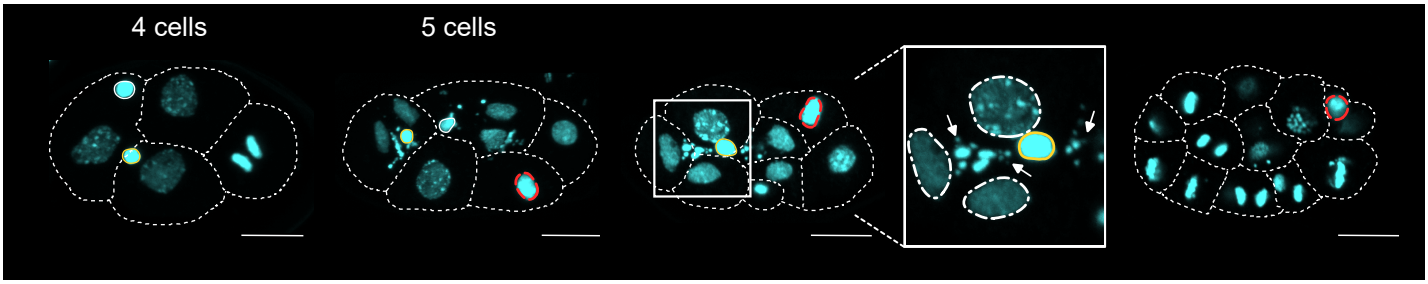
Low elimination

Rhabditella axei DF5006



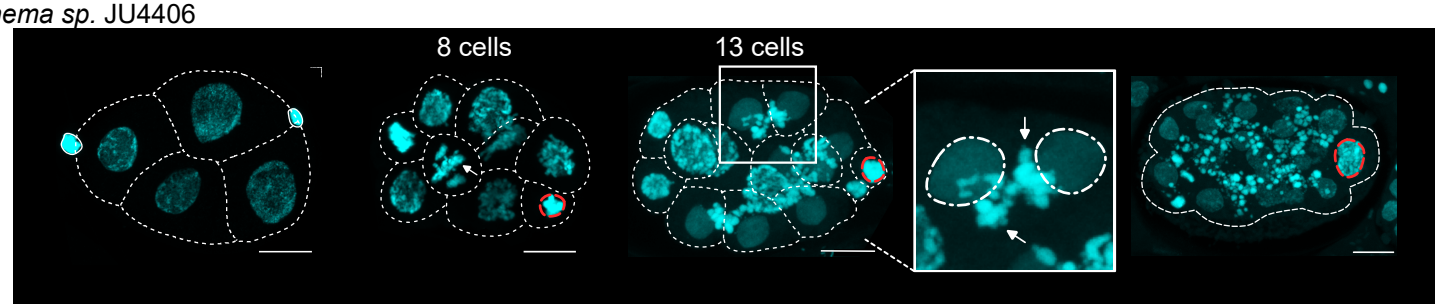
Moderate elimination

Mesorhabditis simplex JU2864



Massive elimination

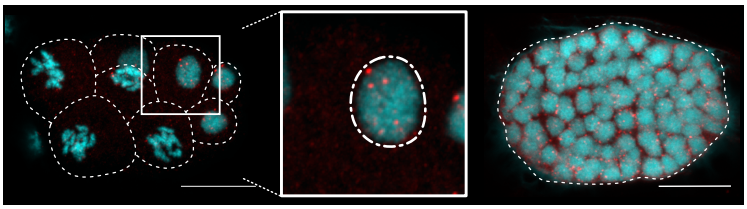
Cruznema sp. JU4406



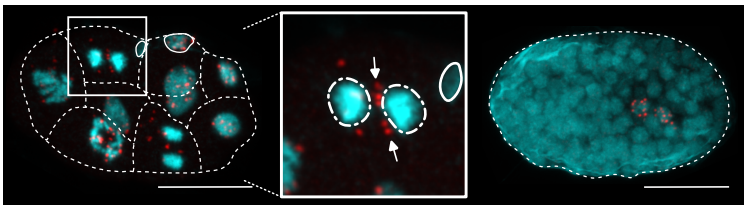
B. Telomere DNA FISH

Telomeres
DNA

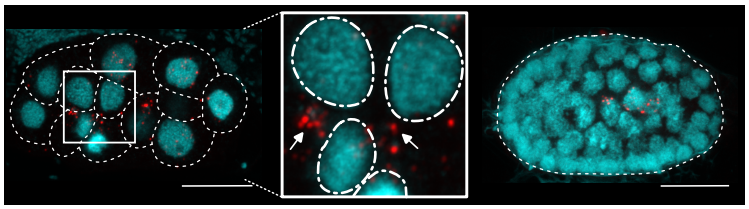
Caenorhabditis elegans N2



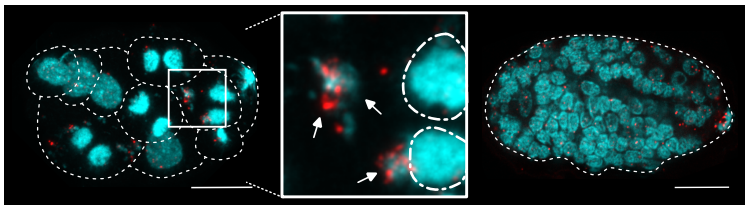
Oschieus tipulae CEW1



Caenorhabditis monodelphis JU1667



Pelodera teres JU4407



Rhabditis rainai PS1191

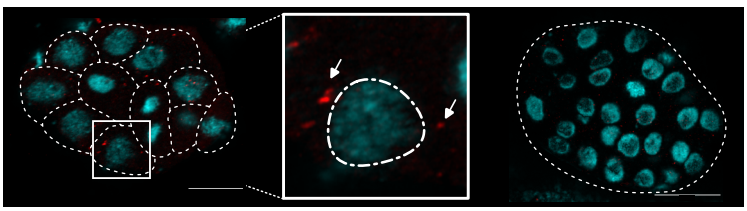
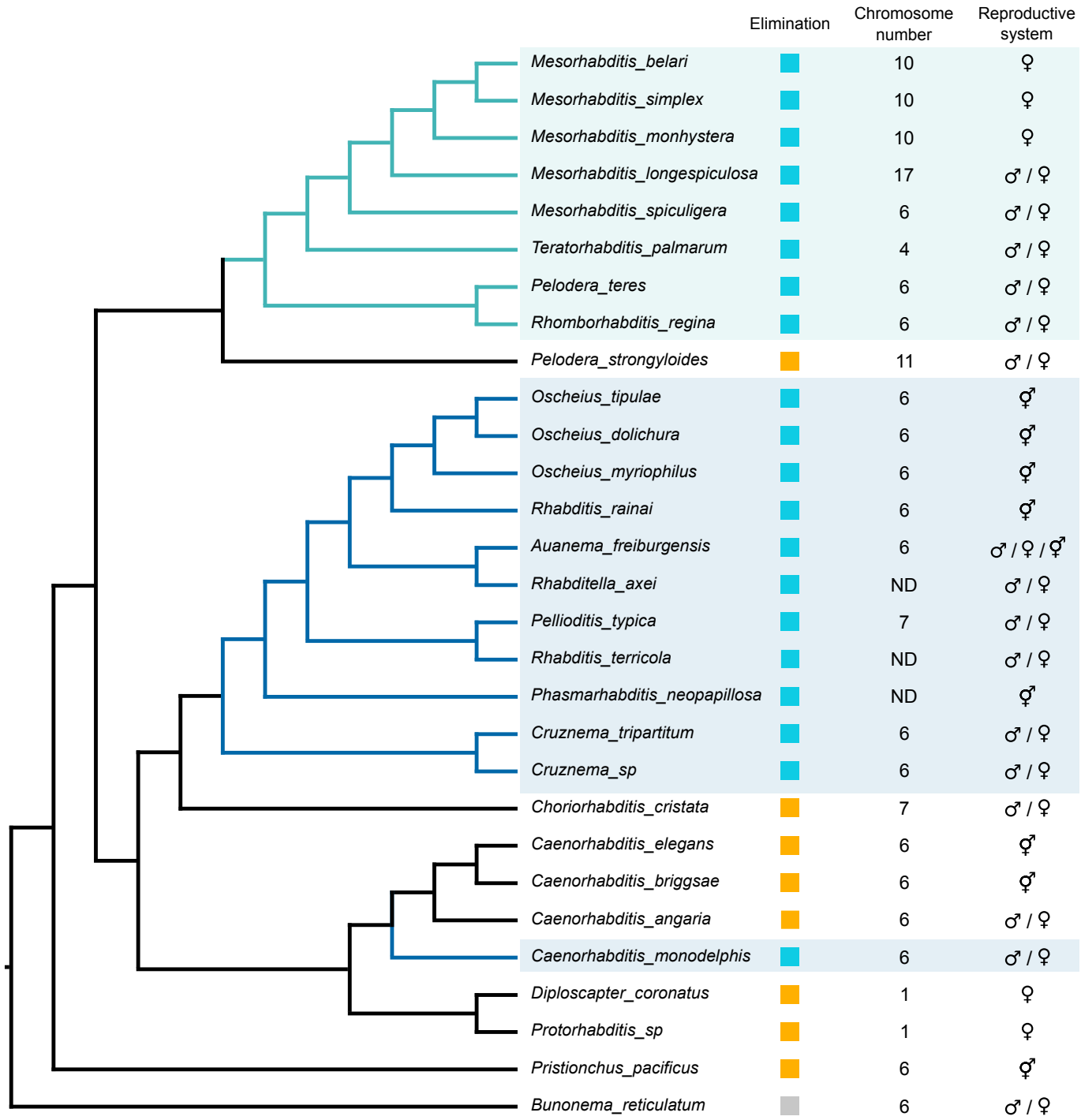


Fig.2


Elimination
Chromosome number
Reproductive system
Mesorhabditis_belari 10 ♀
Mesorhabditis_simplex 10 ♀
Mesorhabditis_monhystera 10 ♀
Mesorhabditis_longespiculosa 17 ♂ / ♀
Mesorhabditis_spiculigera 6 ♂ / ♀
Teratorhabditis_palmarum 4 ♂ / ♀
Pelodera_teres 6 ♂ / ♀
Rhomborhabditis_regina 6 ♂ / ♀
Pelodera_strongyloides 11 ♂ / ♀
Oscheius_tipulae 6 ⚥
Oscheius_dolichura 6 ⚥
Oscheius_myriophilus 6 ⚥
Rhabditis_rainai 6 ⚥
Auanema_freiburgensis 6 ♂ / ♀ / ⚥
Rhabditella_axei ND ♂ / ♀
Pellioditis_typica 7 ♂ / ♀
Rhabditis_terricola ND ♂ / ♀
Phasmarhabditis_neopapillosa ND ⚥
Cruznema_tripartitum 6 ♂ / ♀
Cruznema_sp 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 ♂ / ♀