REPRODUCTIVE BIOLOGY

Males as somatic investment in a parthenogenetic nematode

Manon Grosmaire[1]*, Caroline Launay[1]*, Marion Siegwald[2], Thibault Brugière[1], Lilia Estrada-Virrueta[1], Duncan Berger[3]†, Claire Burny[1]‡§, Laurent Modolo[1], Mark Blaxter[3], Peter Meister[4], Marie-Anne Félix[5], Pierre-Henri Gouyon[2], Marie Delattre[1]¶

We report the reproductive strategy of the nematode *Mesorhabditis belari*. This species produces only 9% males, whose sperm is necessary to fertilize and activate the eggs. However, most of the fertilized eggs develop without using the sperm DNA and produce female individuals. Only in 9% of eggs is the male DNA utilized, producing sons. We found that mixing of parental genomes only gives rise to males because the Y-bearing sperm of males are much more competent than the X-bearing sperm for penetrating the eggs. In this previously unrecognized strategy, asexual females produce few sexual males whose genes never reenter the female pool. Here, production of males is of interest only if sons are more likely to mate with their sisters. Using game theory, we show that in this context, the production of 9% males by *M. belari* females is an evolutionary stable strategy.

Pseudogamy, also called sperm-dependent parthenogenesis or gynogenesis, is a special type of asexual reproduction in which females use the sperm of males, usually from another species, to activate their oocytes. However, the sperm DNA is not used, and the embryos develop only from the maternal DNA to give rise to females (1, 2). The pseudogamous terrestrial nematode *Mesorhabditis belari* was initially studied in the 1920s and 1940s as a species in which pseudogamous females produce their own males at low frequency (3, 4). We recently reisolated strains of *M. belari* and revisited these initial observations (see supplementary text).

We chose *M. belari* JU2817 as our reference strain (see supplementary text). We confirmed that JU2817 females could not reproduce in the absence of JU2817 males. In our laboratory conditions, JU2817 females produced about 9% males during their entire lifetime, with more being produced early (table S1). Crosses with males from other *Mesorhabditis* species did not yield any progeny (supplementary text and table S2). Thus, *M. belari* constitutes a special example of autopseudogamy.

We analyzed the production of males and females by following embryos after fertilization with a combination of differential interference contrast (DIC) live imaging and cytology. As previously described, we found that *M. belari* females produced two types of embryos (4, 5) (Fig. 1, A and B): gynogenetic and amphimictic. The most common class were gynogenetic embryos (n = 227 of 258 fixed embryos). After fertilization, female meiosis resumed, but a single division was observed, giving rise to a single polar body and a female pronucleus containing 20 chromosomes (fig. S1). The male DNA did not decondense, but the sperm centrosomes formed robust asters that eventually contacted the female pronucleus to establish the first mitotic spindle (movie S1). A small percentage of the embryos (n = 31 of 258 fixed embryos) were amphimictic. After fertilization, two rounds of female meiotic division were observed, leading to two polar bodies and a female pronucleus containing 10 chromosomes (fig. S1). A male pronucleus was observed associated with its centrosomes and was joined by the female pronucleus within 1 hour to form the first mitotic spindle (movie S2). Both categories of embryos were diploid, because the lack of paternal DNA was compensated for by the absence of one female meiotic division in gynogenetic embryos. Thus, the sperm of *M. belari* males is required to activate all the oocytes and to provide centrosomes to the zygote, regardless of the status of its DNA. Next, we recovered the embryos scored for their number of pronuclei by DIC time-lapse imaging and let them develop until adulthood (Fig. 1B). Out of 86 gynogenetic embryos, 85 developed as females (one embryo hatched but died as a young larva). By contrast, 30 out of 31 amphimictic embryos developed as males (one also died as a young larva) (Fig. 1C). We confirmed that only males are the result of a true fertilization using single-nucleotide polymorphism (SNP) genotyping. We

crossed JU2817 females to males of another strain of *M. belari* and analyzed the segregation of nine independent SNPs (see supplementary text). Out of 1104 genotyped F$_1$ females, 1103 carried only the maternal allele, consistent with asexual reproduction, whereas 121 out of 122 genotyped F$_1$ males harbored one allele from each parent (except for SNPs 31 and 317, see below), as expected from amphimixis (Table 1 and table S3). The remaining one animal is likely an experimental error but could also reflect the production of very rare amphimictic females (see supplementary text). We concluded that *M. belari* produce females asexually (because females arise by gynogenesis), whereas males are produced sexually (through amphimixis).

We next explored why amphimictic eggs of *M. belari* only give rise to males. To this end, we first asked whether *M. belari* relies on sex chromosomes for sex determination. The genomes of males and females were sequenced separately and the reads mapped onto a de novo assembled genome that had been obtained from a mixed sexes population [see supplementary text and (6)]. This allowed the identification of genes and contigs present in males only, suggesting the presence of a Y chromosome (fig. S2). By contrast, some contigs were twofold overrepresented in females compared with males, as expected from an X chromosome (fig. S2). By genotyping on SNPs 31 and 317 located on these female-biased contigs, we found that F$_1$ males inherited only the maternal allele, in agreement with the expected segregation of an X chromosome (Table 1 and table S3). We therefore concluded that *M. belari* relies on a XX/XY sex determination system.

Because none of the amphimictic eggs give rise to viable females, we hypothesized that either only Y-bearing sperm was produced by *M. belari* males or X-bearing sperm was not able to fertilize these eggs. To determine at which step the Y bias occurs, we analyzed the chromosomal content of sperm cells using DNA fluorescence in situ hybridization (FISH) with a Y-specific probe (6). In the male gonad and the female spermatheca, 50% of sperm nuclei were positive for Y (see Fig. 2A, fig. S3, and supplementary text). This demonstrated that both X- and Y-bearing spermatozoa were produced by males and transferred to females. After fertilization, the condensed sperm DNA in gynogenetic embryos was positive for the Y-probe in 66 out of 76 embryos (Fig. 2B), indicating that in *M. belari*, embryos are fertilized by Y-bearing spermatozoa in 90% of the cases, thus in both gynogenetic and amphimictic embryos. If 10% of eggs are fertilized by an X-bearing sperm, they will give rise to mostly gynogenetic embryos, and a few (10% of 9% = 0.9%) XX amphimictic eggs may also be produced and die before giving rise to a female. Such a low proportion of unviable amphimictic XX animals is compatible with the observed lethality of JU2817 (after embryo recovery or in the whole population; see supplementary text). We thus propose that X-bearing spermatozoa are mostly unable to penetrate the

[1]Laboratoire de Biologie et Modélisation de la Cellule, Université de Lyon, ENS, UCBL, CNRS, INSERM, UMR 5239, U 1210, F-69364 Lyon, France. [2]Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, SU, EPHE, UA, CP 39, 57 rue Cuvier, 75005 Paris, France. [3]The Ashworth Laboratories, Institute of Evolutionary Biology, The University of Edinburgh, Edinburgh EH9 3FL, UK. [4]Cell Fate and Nuclear Organization, Institute of Cell Biology, University of Bern, 3012 Bern, Switzerland. [5]Département de Biologie, Ecole Normale Supérieure, IBENS, CNRS, Inserm, PSL Research University, 75005 Paris, France.
*These authors contributed equally to this work. †Present address: Wellcome Sanger Institute, Hinxton, Cambridgeshire CB10 1SA, UK. ‡Present address: Institut für Populationsgenetik, Vetmeduni Vienna, Vienna A-1210, Austria. §Vienna Graduate School of Population Genetics, Vetmeduni Vienna, Vienna A-1210, Austria.
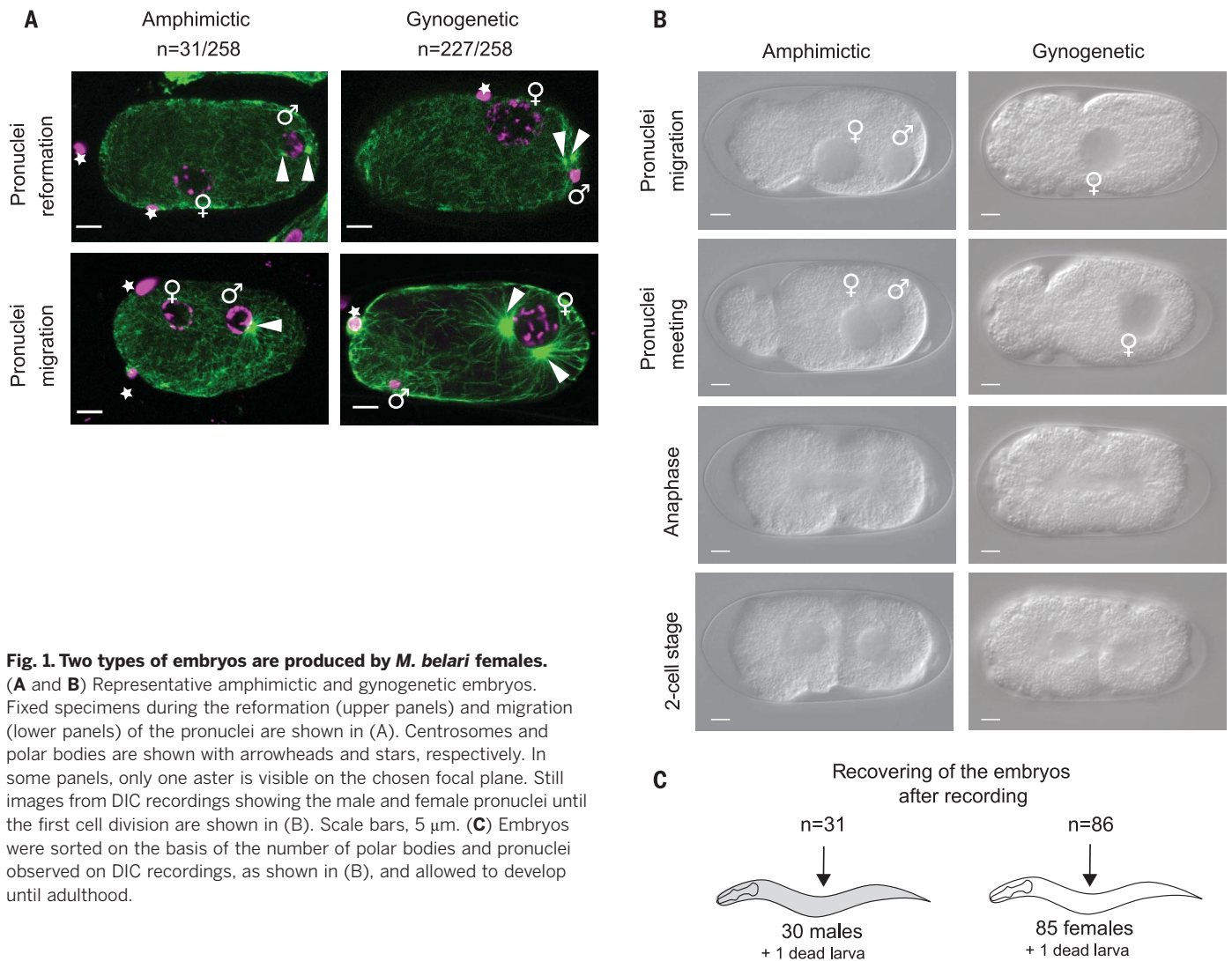¶Corresponding author. Email: marie.delattre@ens-lyon.fr

Fig. 1. Two types of embryos are produced by *M. belari* females.
(**A** and **B**) Representative amphimictic and gynogenetic embryos. Fixed specimens during the reformation (upper panels) and migration (lower panels) of the pronuclei are shown in (A). Centrosomes and polar bodies are shown with arrowheads and stars, respectively. In some panels, only one aster is visible on the chosen focal plane. Still images from DIC recordings showing the male and female pronuclei until the first cell division are shown in (B). Scale bars, 5 μm. (**C**) Embryos were sorted on the basis of the number of polar bodies and pronuclei observed on DIC recordings, as shown in (B), and allowed to develop until adulthood.

oocytes. In the few cases of successful oocyte penetration, they cannot sustain proper development of amphimictic eggs. Importantly, despite a strong bias in sperm efficacy toward Y-bearing sperm, *M. belari* JU2817 is mainly composed of females because most eggs develop by gynogenesis.

Our results imply that *M. belari* females produce males whose DNA is not transmitted to females because females are produced asexually. We asked whether, conversely, males tend to exclude the genes of the females by preferentially transmitting their alleles from fathers to sons. To test this, we followed the transmission of four independent SNPs on autosomes. We found that heterozygote males transmitted the autosomal alleles of their mother or father at the same frequency to their sons (Table 1 and table S3). Thus, over time, the male genome most likely converges with the female genome, with the exception of the Y chromosome, because in each generation, the paternal genome is diluted with a mother genome in its sons.

From the viewpoint of the genes of *M. belari* females, production of sons is of interest for a female only if her sons mate with her daughters (to ensure that a maximum of eggs is fertilized) and do not mate unrelated lineages of females (to which they do not transmit any DNA). In this context, we asked in what proportion should *M. belari* gynogenetic females produce their own males. To this end, we adopted a game theory model to explore the evolutionary stable strategy (ESS) of sex ratio (7–9). We postulated that the progeny of a female constitutes a patch of size k. Males and females can migrate in and out of the patches to find a mate, with a similar rate m. Here, m can also be interpreted as a rate of mating with unrelated females if the isolation is behavioral rather than geographical. The fitness of a female playing a sex ratio x in a population where the others play a sex ratio x^* is computed as the number of fertilized eggs that her daughters produce. We first developed an analytical model, considering that one male is sufficient to make all eggs fertile within a patch

(see supplementary text). We found that the evolutionary stable proportion of females is

$$(1 - x^*) = \sqrt[k]{1/[1 + k \ (1 - m)^2]}$$

Consequently, in a panmictic population with complete dispersal (m = 1), the stable sex ratio is equal to 0, because a female has no advantage to produce sons if they mate mostly with the daughters of unrelated females. This would secondarily lead to population extinction. However, with decreased dispersal between patches, the stable sex ratio increases (Fig. 3). Second, we explored the ESS sex ratio, considering that the sperm could be a limiting factor, using computer simulation (see supplementary text). We found that, at a given dispersal rate, limiting sperm slightly increases the ESS proportion of males (Fig. 3 and fig. S4). The curves being relatively flat, dispersal rates between 0 (no migration) and 0.5 are compatible with the observed sex ratio of JU2817, around 0.09. We also showed that mating preference between siblings clearly

Table 1. Females of *M. belari* come from gynogenesis and males from amphimixis. Females and males from two different strains of *M. belari* were crossed, and the progenies were genotyped (first column, *n* individuals) at different independent SNP loci (second column, selected randomly on different contigs of the assembled genome). The number of individuals showing the genotype of the mother or a heterozygous genotype are shown in the third and fourth columns, respectively. Among the *n* individuals, some were sequenced at different SNPs; see table S3 for a detailed structure of the genotyping. In bold are contigs of the X chromosome. A * indicates that the same individual is a heterozygote on three SNPs.

Progeny	Contig number	Homozygous-like mother	Heterozygous
Female F$_1$	11	539	0
(*n* = 1104)	13	149	0
	29	56	0
	52	65	0
	103	275	*1
	117	108	*1
	541	187	*1
	31	**73**	**0**
	317	**77**	**0**
Male F$_1$	11	0	49
(*n* = 122)	13	0	19
	52	0	28
	103	1	29
	117	0	4
	31	**20**	**0**
	317	**23**	**0**
Male F$_2$	11	24	24
(*n* = 185)	13	33	28
	103	30	41
	117	6	2
	541	14	11

Fig. 2. *M. belari* embryos are mostly fertilized by Y-bearing sperm. (**A** and **B**) One representative spermatheca of a *M. belari* female showing individual sperm cells (A) and one representative gynogenetic embryo (B) labeled by DNA FISH with a probe specific for the Y chromosome (in red). DNA is shown in blue. Insets in (B) show higher magnification and Z projection of 10 images of the red signal in the female pronucleus (left) and the sperm DNA (right). The polar body is shown with a white star. Scale bars, 5 μm.

A spermatheca - 79/161 positive sperm cells

DNA/Y chromosome

B gynogenetic embryo - 66/76 Y-bearing sperm

DNA/Y chromosome
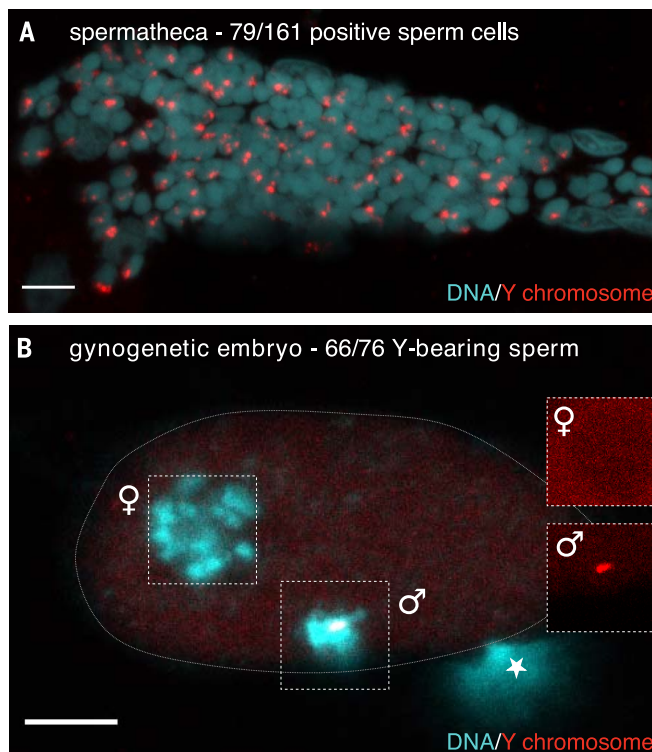
increased the ESS sex ratio (fig. S4). Hence, the asexual females of *M. belari* can afford the production of 9% males if these males are more likely to mate with their sisters, either because of limited dispersal or by an active choice of males or females toward their siblings.

The reproductive system of *M. belari* represents a distinctive state, where asexual females systematically produce few sexual males, and male genes never reenter the female pool. Although other examples of species with mixed sexual-asexual systems exist (haplo-diploid, cyclical parthenogens, and so on), none of them feature exclusively asexual females and progressive loss of males and sexuality (*10*). Most likely, if a mutation allowed *M. belari* females to activate their eggs independently of the sperm, such strict parthenogens would invade the population. Thus, activation of the egg by the sperm, as well as paternal contribution of centrioles, most likely constitutes strong cellular constraints that allow the maintenance of pseudogamy. Whereas other pseudogamous species find the source of sperm and centrioles in other species (*1*), the strategy of *M. belari* consists in producing just enough of its own males. Such a strategy may avoid competition for males with females of another species. Males of *M. belari* can therefore be seen as a nongenetic, or somatic, investment in centrioles for clonally produced daughters. Self-fertilizing pseudogamous species of nematodes have been also described, which may constitute one more step toward a minimal investment for a source of centrioles (*3*).

We found that the males of *M. belari* produce Y-bearing sperm that are much more competent than the X-bearing sperm for fertilizing the oocytes. It is tempting to speculate that if such a male drive existed in an ancestral sexual *Mesorhabditis* species, it may have favored the emergence of gynogenesis in females, as an anti-drive system.
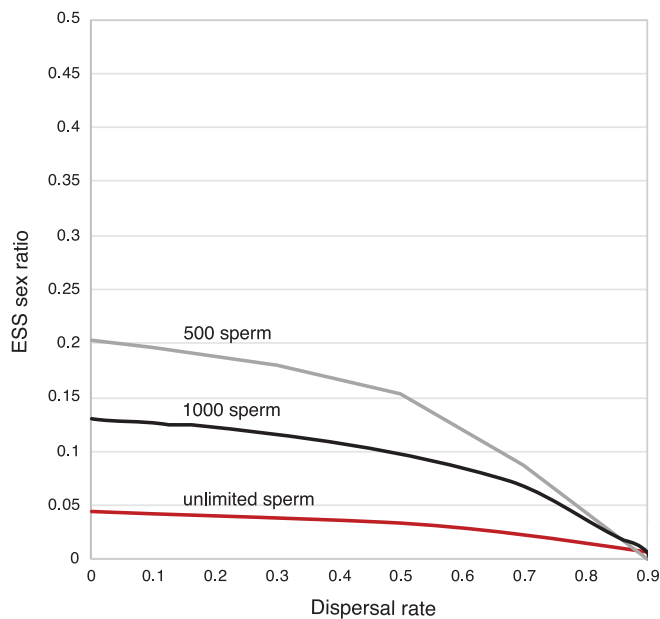
REFERENCES AND NOTES

1. L. W. Beukeboom, R. C. Vrijenhoek, *J. Evol. Biol.* **11**, 755–782 (1998).
2. J. Lehtonen, D. J. Schmidt, K. Heubel, H. Kokko, *Trends Ecol. Evol.* **28**, 297–306 (2013).
3. K. Belar, *Zeitung für Zellen und Gewebelehre* **1**, 1–21 (1924).
4. V. Nigon, *Les modalités de la reproduction et le déterminisme du sexe chez quelques nématodes libres* (Annales des sciences naturelles: Zoologie et biologie animale, vol. 11, Masson, Paris, 1949).
5. V. M. Nigon, M. A. Félix, *WormBook* **2017**, 1–84 (2017).
6. Materials and methods are available as supplementary materials.
7. W. D. Hamilton, *Science* **156**, 477–488 (1967).
8. P. D. Taylor, M. G. Bulmer, *J. Theor. Biol.* **86**, 409–419 (1980).
9. D. Haig, *J. Evol. Biol.* **6**, 69–77 (1993).
10. M. Neiman, T. F. Sharbel, T. Schwander, *J. Evol. Biol.* **27**, 1346–1359 (2014).

Fig. 3. The evolutionary stable sex ratio of _M. belari_ increases as the dispersal rate decreases. The ESS sex ratio (proportion of males) is shown as a function of the dispersal rate between patches when the sperm is not limiting (red curve) or limiting [500 (gray curve) or 1000 (black curve) sperm cells produced per male], with 100 individuals per deme. Dispersal rate of 1 corresponds to a panmictic population.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/363/6432/1210/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S4
Tables S1 to S6
References (11–37)
Movies S1 and S2
Data S1

Science

Males as somatic investment in a parthenogenetic nematode

Manon Grosmaire, Caroline Launay, Marion Siegwald, Thibault Brugière, Lilia Estrada-Virrueta, Duncan Berger, Claire Burny, Laurent Modolo, Mark Blaxter, Peter Meister, Marie-Anne Félix, Pierre-Henri Gouyon and Marie Delattre

Mysterious males

In parthenogenetic species, females produce female offspring, generally without the input of males. Given this, the production of males would seem to be a waste of resources. Grosmaire *et al.* report that in a particular soil nematode, males are regularly produced at a rate of about 9%. They found that the male sperm was required for egg activation, yet the sperm DNA never transmitted on to the subsequent female generation. Male DNA was only passed on through sibling mating, which allows for male production to be evolutionarily stable.
Science, this issue p. 1210

ARTICLE TOOLS	http://science.sciencemag.org/content/363/6432/1210
SUPPLEMENTARY MATERIALS	http://science.sciencemag.org/content/suppl/2019/03/13/363.6432.1210.DC1
REFERENCES	This article cites 35 articles, 7 of which you can access for free http://science.sciencemag.org/content/363/6432/1210#BIBL
PERMISSIONS	http://www.sciencemag.org/help/reprints-and-permissions