Décryptage de la méiose atypique de Mesorhabditis belari

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Deciphering the atypical meiosis of *Mesorhabditis belari*

Décryptage de la méiose atypique de *Mesorhabditis belari*

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“The gift of the great microscopist is
the ability to think with the eyes
and see with the brain.”

Daniel Mazia
U. C. Berkeley cell biologist, 1996

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En octobre 2019, j'ai l'opportunité et la chance d'intégrer la petite équipe de Marie Delattre. Je n'ai pas hésité. Petite équipe en effet, elle était alors composée de deux membres Marie, « la Cheffe », et de ma chère homonyme, Caroline Launay. Plus tard, nous ont rejoint Carine Rey, post-doc en proie aux combats de chaises, et Eva Wenger, assistance-ingénieure amatrice de convivialité, puis Brice Letcher, post-doc et seul représentant masculin de cette équipe. Cette équipe s'évertue à décortiquer les mystères d'un petit ver de composte, *Mesorhabditis belari*. Certains y trouverons quelques ressemblances avec *Caenorhabdtis elegans*. Mais ne vous y méprenez pas, ils n'ont rien à voir : ils sont beaucoup plus mignons. Pour y parvenir, l'équipe n'hésite pas à tout chambouler, à user et à développer des nouvelles techniques ainsi qu'à se remettre en question pour mieux avancer, parfois un peu trop. Et les résultats sont là. La rigueur et la passion que l'équipe consacre à la recherche scientifique n'ont d'égal que leur joie de vivre et leur bienveillance.

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Résumé

Au sein du genre de nématodes *Mesorhabditis*, plusieurs espèces se reproduisent par auto-pseudogamie : les femelles asexuées produisent peu de mâles sexuels (amphimictiques), qui sont nécessaires à la production de femelles gynogénétiques (asexuées). Dans ce système, une femelle peut produire des ovocytes haploïdes classiques qui, une fois fécondés par un spermatozoïde, produisent des œufs diploïdes. Ces œufs se développeront en mâles. Dans la plupart des cas, cependant, les femelles produisent des ovocytes diploïdes, pour lesquels le programme méiotique est modifié. Après la fécondation de ces ovocytes, l'ADN du spermatozoïde est mis de côté, et le zygote se développe uniquement à partir du génome diploïde maternel, et deviendrons ainsi des femelles (Grosmaire, et al. 2019). Mon projet de thèse s'articule en trois parties :

Je me suis demandé quelle(s) modification(s) de la méiose permet la production d'ovocytes diploïdes et comment une femelle donnée peut systématiquement produire 90 % d'ovocytes non réduits et 10 % d'ovocytes réguliers. J'ai également consacré du temps au développement d'outils techniques pour l'étude de cette espèce non-modèle.

Caractérisation du programme méiotique lors de la production d'ovocytes gynogénétiques

Ceci représente la partie principale sur laquelle j'ai travaillé pendant ma thèse. La gonade femelle de *Mesorhabditi*s belari offre une organisation spatiale idéale pour suivre la progression méiotique. J'ai constaté que lors de la production d'ovocytes non réduits, les chromosomes homologues s'apparient pendant la prophase méiotique et subissent des crossing-overs. Ensuite, bien que les bivalents ségrégent initialement en univalents au cours de la méiose I, l'anaphase ne se déroule pas et les univalents finissent par se réorganiser au centre du fuseau, en préparation de la méiose II. La méiose II se déroule alors normalement, donnant naissance à des ovocytes diploïdes avec un assortiment de chromatides non-sœurs, qui se sont recombinées pendant la prophase. Compte tenu de ce mécanisme et de l'absence de contribution du génome paternel, le génome des femelles *M. belari* devrait être homozygote sur l'ensemble du génome, sur le long terme.

En parallèle, nos collaborateurs du laboratoire de Nicolas Galtier (Montpellier, FR) a réalisé le séquençage du génome entier des descendants de 10 femelles indépendantes échantillonnées dans 10 endroits différents dans le monde, appartenant à l'espèce *M. belari* ou à l'espèce sexuelle *Mesorhabditis spiculigera*. En utilisant la quantification du déséquilibre de liaison, ils ont découvert dans les deux espèces une perte rapide du déséquilibre de liaison, confirmant l'existence d'une recombinaison homologue chez *M. belari*. De manière inattendue, nous avons également identifié un niveau élevé d'hétérozygotie dans les deux espèces, environ 1%, à l'échelle du génome.

Pour réconcilier nos résultats, nous avons émis l'hypothèse qu'après la recombinaison homologue, seules les chromatides sœurs qui ont recombinées, ou bien, celles qui n'ont pas recombinées, sont ségréguées dans les ovocytes, ce qui permettrait le maintien de l'hétérozygotie à long terme. Comme preuve ultime de cette ségrégation biaisée, j'ai développé une technique basée sur l'EdU (5-éthynyl-2'-dexosyuridine, un analogue de la thymidine) chez *M. belari*, pour démontrer premièrement que tous les 10 chromosomes se recombinent à chaque génération et deuxièmement pour montrer la ségrégation des chromatides recombinées.

II) Décrypter le mécanisme par lequel une femelle donnée est capable de suivre un programme méiotique classique (pour la production de mâles) ou incomplet (pour la production de femelles)

La production de mâles varie en fonction de l'âge de la femelle : les jeunes femelles produisent plus de mâles que les vieilles. Partant de ce constat, l'équipe a émis l'hypothèse qu'au cours de l'ovogenèse, la production de mâles pourrait être biaisée par un dépôt préférentiel d'ARNm maternels dans l'ovocyte. Nous avons généré des données transcriptomiques à partir d'ovocytes amphimictiques et gynogénétiques afin d'identifier ces types d'ARNm. J'ai ensuite validé les gènes candidats qui sont exprimés différemment selon le type d'ovocyte en utilisant le smiFISH (single molecule inexpensive FISH) et la RT-qPCR sur des embryons uniques. Par la suite, j'ai effectué une analyse fonctionnelle du gène validé en utilisant l'ARNi et CRISPR Cas9.

III) Développement de CRISPR chez *M. belari*

M. belari est une espèce non modèle pour laquelle l'équipe avait d'ores et déjà développé l'ARNi. Lorsque j'ai commencé mon doctorat, j'ai tenté de mettre en place la technique CRISPR, qui a d'abord échoué en raison de la cellularisation de la lignée germinale. J'ai trouvé un moyen de permettre à Cas9 et à l'ARN guide de se diffuser dans les cellules

germinales et j'ai établi des Knock-Out CRISPR chez *M. belari*. Par la suie, j'ai utilisé cette astuce chez *C. elegans*, et j'y ai réussi à obtenir des mutants F1 à partir d'animaux ayant reçu une injection dans la tête, facilitant ainsi la transgénèse pour les expérimentateurs non qualifiés. Parallèlement, j'ai usé de nombreuses techniques différentes pour obtenir des lignées Knock-in chez *M. belari*, sans succès jusqu'à présent.

Abstract

Within the nematode genus *Mesorhabditi*s, several species reproduce by auto-pseudogamy: asexual females produce few sexual males (amphimictic), while females are produced asseuxally and are gynogenetic. Nonetheless, the production of males is necessary to generate asexual females. In this system, a female can produce regular haploid oocytes, which once fertilized by a sperm, produce diploid eggs. These eggs develop as males. In most cases, however, females produce diploid oocytes, for which the meiotic program is modified. After fertilization of these oocytes, the sperm DNA is set aside, and the zygote develops only from the maternal diploid genome and becomes female (Grosmaire et al., 2019). My thesis project is articulated in three parts:

I asked which modification to meiosis allows the production of diploid oocytes and how a given female can systematically produce 90% unreduced oocytes and also 10% regular oocytes. I also spend time developing technical tools for studying this non-model species.

Characterization of the meiotic program during the production of gynogenetic oocytes/future female oocytes

This is the main part I worked on during my thesis. The female gonad of *Mesorhabditis belari* offers an ideal spatial organization to follow the meiotic progression. I found that during the production of unreduced oocytes, homologous chromosomes pair during meiotic prophase and undergo crossing-overs. And cytology supports that recombination is not limited to the tips of the chromosomes. Next, although bivalents initially segregate into univalent during meiosis I, anaphase fails to proceed and univalents eventually reorganize in the centre of the spindle in preparation for meiosis II. Meiosis II then proceeds normally, giving rise to diploid oocytes

with an assortment of non-sister chromatids, which have recombined during prophase. Given this mechanism, and the absence of paternal genome contribution, the genome of *M. belari* females should be homozygous genome wide, in the long term.

In parallel, our collaborators in Nicolas Galtier Lab (Montpellier, FR) performed whole genome sequencing of the descendants of 10 independent females sampled in 10 different locations in the world, belonging to the *M. belari* species or the sexual species *Mesorhabditis spiculigera*. Using quantification of linkage disequilibrium, they uncovered a rapid loss of linkage disequilibrium in both species, confirming the existence of homologous recombination in *M. belari*. Unexpectedly, we also identified a high level of heterozygosity in both species, around 1%, genome-wide.

To reconcile our results, we hypothesize that after homologous recombination, only the sister chromatids that have recombined, or those that have not recombined, are segregated in the oocytes, allowing the maintenance of heterozygosity in the long term. As a final proof of this biased segregation, I developed an EdU -based (5- ethynyl-2'-dexosyuridine, a thymidine analogue) technique in *M. belari*, to demonstrate first that all the 10 chromosomes recombine at each generation and secondly to show the co-segregation of the recombined chromatids.

II) Deciphering the mechanism by which a given female is able to follow a regular meiotic program (for the production of males) or an incomplete one (for the production of females)

The production of males varies according to the age of a female: young females produce more males than old ones. Based on this assumption, the team hypothesized that during oogenesis the production of males could be biased by a preferential deposit of maternal mRNAs in the oocyte. We generated transcriptomic data from amphimictic and gynogenetic oocytes in order to identify these types of mRNA. I then validated the candidate genes that are expressed differently depending on the type of oocyte using smiFISH (single molecule inexpensive FISH) and RT-qPCR on single embryos. Then I performed a functional analysis of the validated gene using RNAi and CRISPR Cas9.

III) Development of CRISPR in *M. belari*

M. belari is a non-model species for which the team had already developed RNAi. When I started my PhD, I established CRISPR, which was initially unsuccessful due to the

cellularization of the germline. I found a way to allow the Cas9 and guide RNA to diffuse into the germ cells and established CRISPR Knock outlines in *M. belari*. I then used this trick in *C. elegans* and managed to obtain F1 mutants from animals injected in the head; therefore, facilitating transgenesis for unskilled experimenters. In parallel, I tried many different techniques to obtain Knock-in lines in *M. belari*, without success.

Introduction

In this thesis, I attempted to elucidate the cellular mechanism that enables a species to reproduce asexually, i.e. to produce zygotes which inherit only one parental genome. Meiosis is the most ancestral among eukaryotes and sexuality is the most common reproductive system among this eukaryotes (Leonard, 2013, 2013; Maynard Smith, 1978). In metazoans, sexuality results from the mixing of two genomes to produce a progeny. To generate a new individual that shares the same ploidy as its progenitors, males and females must undergo a specific cell division that results in a two-fold division of chromosomes, called meiosis. The process of meiosis is universal, extremely conserved and ancestral (Ramesh et al., 2005; Speijer et al., 2015). However, several forms of reproduction without sex exist (reviewed in de Meeûs et al., 2007; Suomalainen, 1950; Suomalainen et al., 1987). In this manuscript, asexuality (i.e. parthenogenesis) will be defined as the production of new individuals from a single parental genome. This is made possible by modifications to meiosis, which allow the production of unreduced gametes. Asexuality has emerged multiple times in evolution from sexual ancestors, demonstrating that the meiotic program can be modified (Avise, 2008; Delmotte et al., 2001; Normark, 2014). The genomic consequences of some asexuals have been well studied, from an empirical and theoretical point of view (Birky Jr, 1996; Glemin & Galtier, 2012; Mark Welch & Meselson, 2000; Maynard Smith, 1978). However, the cellular and molecular changes at the origin of new types of meiosis in asexuals have been less explored (Cole-Clark et al., 2017; Terwagne et al., 2022; Triantaphyllou, 1981). Hence, many questions are still unresolved, in particular, which modifications are possible and which ones are more constrained, what are the genes involved in the changes, etc. To address these questions, cytological approaches are required.

There are however difficulties in the study of meiosis in asexuals. The first one is technical because, in many species, including sexual species, the analysis of the meiotic divisions is complicated because the germ cells are not easily accessible. Besides, the transition towards asexuality often occurred a long time ago and the mechanisms may have evolved since the first changes in meiosis. In those species, through time, new constraints have been established that potentially mask the initial changes. For these reasons, we choose to study a species which features an interesting case of transition towards asexuality, *i.e.* that presents both types of reproductive systems. In such species, the establishment of asexuality is constrained

by the necessity to also maintain sexuality. We anticipate that the proximal changes will be easier to identify. We also choose species for which meiotic divisions are easy to observe.

The lab has previously described the reproductive system of the auto-pseudogamous nematode, *Mesorhabditis belari* (Grosmaire et al., 2019; Launay et al., 2020). In *M. belari*, females are produced asexually by gynogenesis (they inherit only the maternal genomes) whereas males are produced sexually, also called amphimixis (by mixing of the parental genomes). Hence, regular/canonical meiosis leads to the production of amphimictic oocytes (the males) and alteration of meiosis generates gynogenetic oocytes (the females). In this species, fertilization by the sperm is required to resume meiosis of both types of oocytes. Moreover, the population of *M. belari* is 90% females and only 10% males. In this atypical reproductive system, males are necessary but not for their genes, which are never passed on to females (Grosmaire et al., 2019).

During my thesis, I asked which modification of meiosis allows a given female to produce both reduced and unreduced gametes, and what are the consequences on the genome of *M. belari*. I have first described the overall process and then investigated the underlying mechanisms. The characterisation was possible because many aspects of *Mesorhabditis* meiosis resemble the meiosis that has been extensively described in the model species *Caenorhabditis elegans*. I have also established tools to develop functional approaches in this non-model species. I have assembled my results in the form of articles, even when the projects were not finalised. During the 2020 locked-down, I explored the literature on the cytology of asexuals. I have summarised this work into a review which is presented in Chapter 1.

All animals perform meiosis unless proven otherwise

My work is focused on animals. The literature on asexuality in plants or fungi is vast and I have decided to concentrate on studies performed in animals (Giraud et al., 2008; Mogie, 1992). Consequently, the vocabulary and the definitions which will be used in this manuscript are not always adapted to the plant or fungi fields.

Sexuality is the most common reproduction system (Ashman et al., 2014; Leonard, 2013; Maynard Smith, 1978; Williams, 1975). In this thesis, sexuality is defined as the mixis of a male and a female genome and is also called amphimixis (Figure 1). To this aim, both the

male and the female produce haploid gametes through the highly conserved mechanism of meiosis. It is now well-established that all asexual species derived from sexual ancestors (Bell, 1982). In asexual species, the zygote inherits only the genome of one parent and is therefore not the product of genetic mixing. In those species, modifications of meiosis have conditioned the emergence of asexuality. During my thesis, I studied the emergence of asexuality from a cellular and mechanistic perspective. In this chapter, I first give background on how meiosis is executed in sexual species and what the mechanistic constraints of meiosis and fertilisation are. Next, I describe the actual and theoretical alterations of meiosis that have been described in asexual species. Finally, I discuss the different advantages of sexuality and asexuality and their consequences for the genome of the species and the current hypothesis on why asexual species are rare compared to sexual species.

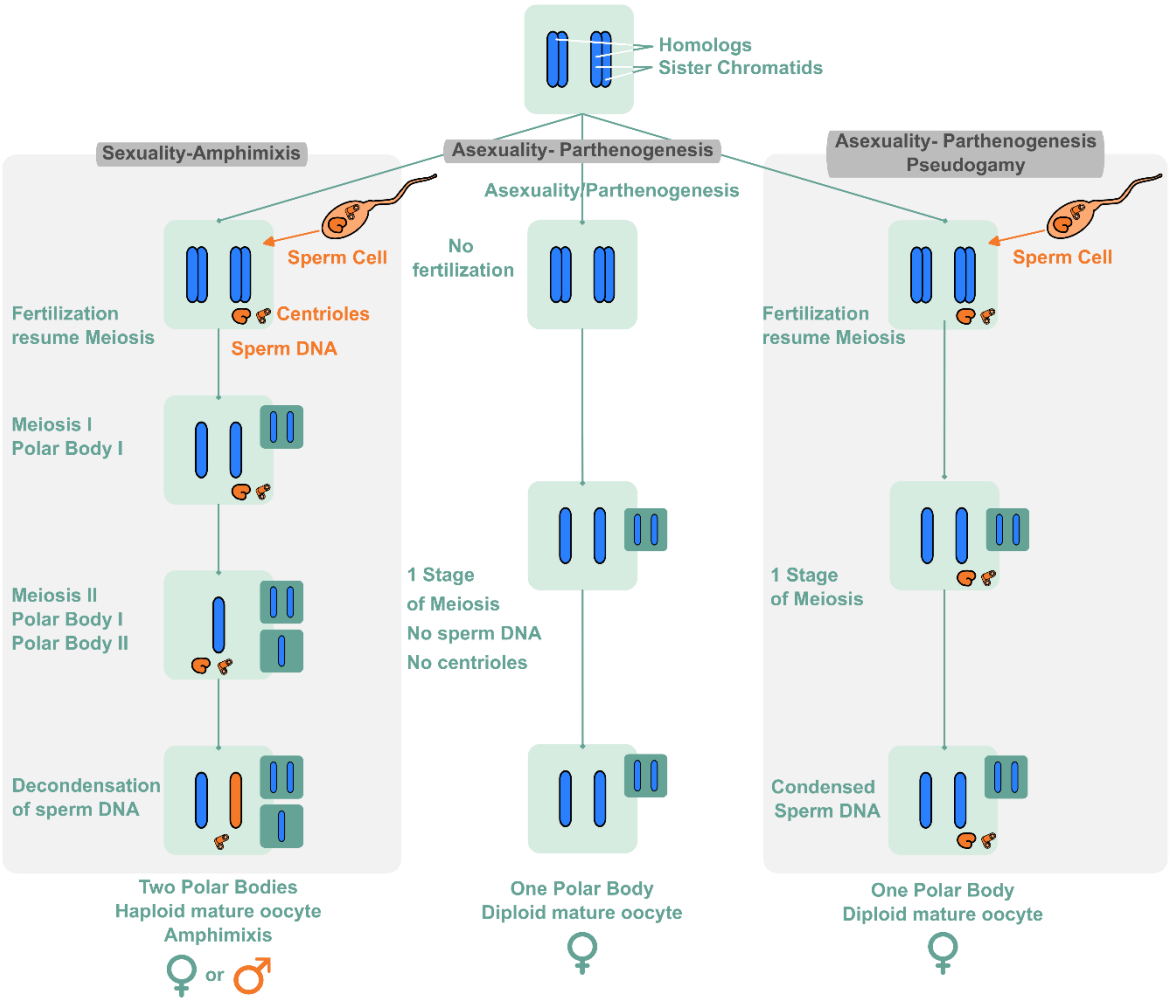

Figure 1: Three types of reproductive systems Amphimixis, Parthenogenesis and Pseudogamy. Female oocyte is in green and male components in orange

What is sexuality from a cellular point of view?

Sexuality is defined as the mixis of a male and a female gamete. To achieve amphimixis, each progenitor must produce specific reduced cells called gametes that possess half a copy of each chromosome by contrast with other somatic cells of the progenitor. For instance, if the progenitor is diploid, i.e. two copies of each chromosome, the gamete has to be haploid, i.e. one copy per chromosome. In both males and females, gametes are produced via meiosis. Therefore, sexuality is tightly linked to the meiotic process. Sexuality is also dependent on mechanisms linked to the fusion of the gametes. Importantly, the male gamete, the sperm cell, does not only provide a haploid genome to the oocyte but also other features which impose constraints on the evolution of asexuality (Engelstädter, 2008).

Female meiosis, male meiosis and mitosis are fundamentally different cell divisions

Mitosis and meiosis are two distinct types of cell division (Ohkura, 2015). They differ in the type of cell in which they occur, in their functions and the content of the chromatids. Mitosis ensures the proliferation and maintenance of the somatic cells and germ cell progenitors. Mitosis is generally a symmetrical division where a mother cell generates two daughter cells. After chromosome replication, the cell undergoes one cycle of cell division during which sister chromatids are separated. Thus, daughter cells inherit an assortment of non-sister chromatids and have, therefore, the same genetic content as the mother cell (Figure 2).

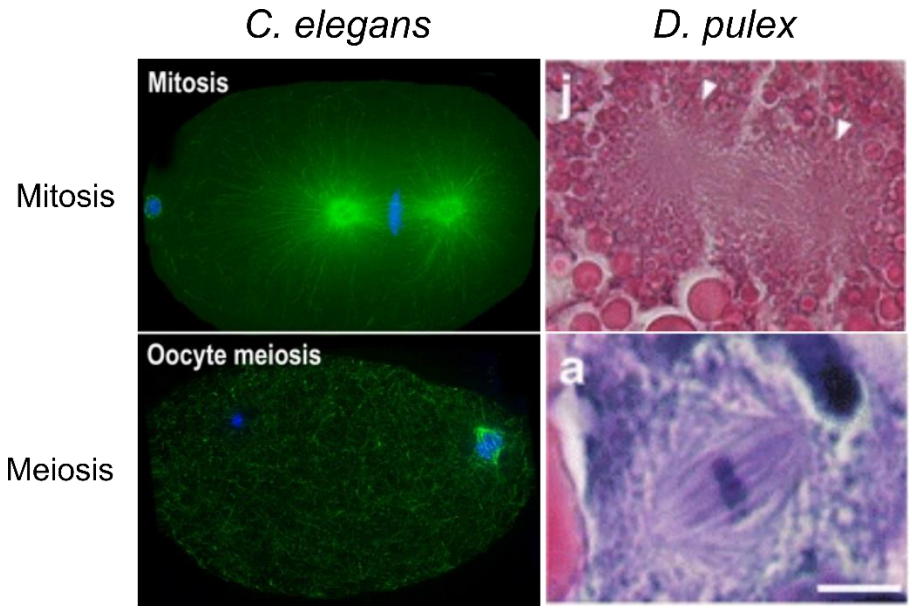

Figure 2: Mitoisis and meiosis in *Caenorhabditis elegans* and *Daphnia pulex*. Left colomn shows immunostainings of a *C. elegans* spindle of the first mitosis of the embryo (top) and a meiotic division (bottom). DNA is in blue and microtubules are in green. **https://sites.northwestern.edu/wignall-lab/research/** Right colomn shows histological observation of oogenesis in parthenogenetic *D. pulex*. i) First mitotic cytokinesis. a) metaphase 1 of meiosis. (Hiruta et al., 2010)

Canonical meiosis occurs only in germline cells and generates haploid gametes. Meiosis consists of two successive divisions, without replication in between, that lead to the production of haploid oocytes in females or sperm cells in males. In the meiotic prophase, the chromosomes replicate, form pairs between homologs and often undergo homologous recombination. During homologous recombination, there is an exchange of chromatid strands, also called crossing-over. At the end of prophase I, in the diakinesis stage, recombination is observable by the presence of chiasmata. In many species, the crossing-overs are necessary to maintain the attachment between homologous chromosomes, although some species are achiasmatic and have evolved alternative mechanisms to maintain the homologs together (Dernburg et al., 1998; Engebrecht et al., 1990; Mather, 1938). During the first meiotic division (Meiosis I), the homologous chromosomes of a given pair are separated. The second division (Meiosis II) consists of the segregation of the sister chromatids. Therefore, meiosis produces four products,

each containing a single chromatid. The mixing of the haploid gametes from the male and the female will restore the initial ploidy in the zygote (Figure 3).

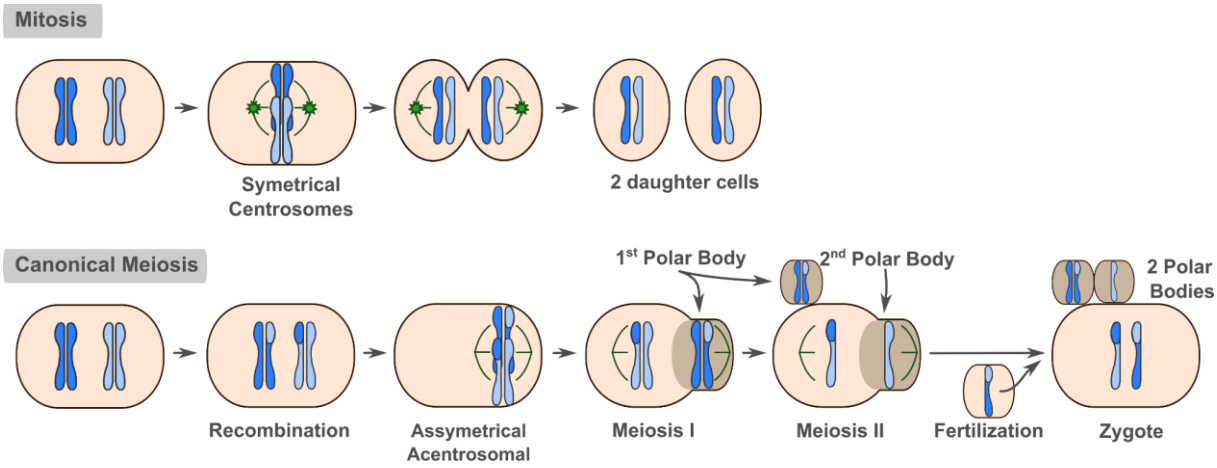

Figure 3: Schematic representation of mitosis and canonical meiosis of females. Mitosis is a symmetrical division that produces two daughter cells. Centrosomes (green stars) are present in mitosis. Meiosis consists of two successive divisions (meiosis I and II). Meiosis is asymmetrical and acentrosomal. Recombination occurs in the meiotic prophase. In meiosis I, homologous chromosomes segregate and, in meiosis II, sister chromatids are separated. This is an example of meiosis in females where polar bodies are extruded. The mature oocyte is haploid and the diploidy is restored by fertilization. The sister chromatids are in dark and light blue, corresponding to homologous chromosomes. Microtubules are in dark green.

Although the segregation of chromosomes is identical in both sexes, the cell divisions during meiosis are very different in metazoan males and females. In males, the divisions that produce sperm cells are symmetrical: from one cell, four haploid cells are formed. They will differentiate into small sperm cells, optimised for their movements towards the oocyte. This is achieved by the purging of unnecessary compounds and drastic and specific modifications of cell shape. In contrast, the meiosis that generates oocytes in metazoan females is asymmetric: out of the four products of meiosis, only one will become the haploid oocyte. This will allow the production of a single large oocyte, full of cellular components necessary for the early development of the zygote (such as proteins, RNAs and yolk). In some species, this is achieved by a very asymmetric positioning of the meiotic spindle. Hence, after cytokinesis and asymmetric cell divisions, tiny polar bodies and one large oocyte are formed. In other species, the segregation of chromatids during the meiotic divisions is not accompanied by cell cleavages, leaving the large size of the oocyte intact. The four products of meiosis align and only one of those will become the oocyte nucleus while other are degraded during embryogenesis.

Here, we saw that meiosis and mitosis differ in their function, the type of cell where the division occurs, the number of cell divisions, the possibility of recombination and the quality of chromatids present in the resulting cell. As we will see later, this is an important distinction in the context of asexuality.

Interestingly, although meiosis is an ancient mechanism, the mechanisms of meiosis have evolved even in sexual species. For instance, homologous recombination is absent in some species, the sequence of events during chromosome pairing changes depending on the species, or the essential proteins for chromosome and chromatid dissociation have evolved (see Chapter 1). These are examples demonstrating that changes in the meiotic process are possible and such variations have probably conditioned the emergence of asexuality.

Sperm cells do not only provide a haploid genome to the oocyte in sexual species

During fertilization, sperm cells carry the genetic material of the male to the oocyte. Once in the oocyte, the sperm DNA fuses with the oocyte DNA and the initial ploidy is restored. In the absence of sperm cells, zygotic development is impaired but not only because of a defect in ploidy. Indeed, besides their DNA content, sperm cells provide other essential features at fertilization (Engelstädter, 2008).

In most species described so far, during meiosis I the oocyte undergoes an arrest of development. For instance, in mammalian female as in jellyfish or nematodes, meiosis arrests in prophase I (Dorée & Hunt, 2002; Huelgas-Morales & Greenstein, 2018; Jessus et al., 2020; Mehlmann, 2005; Von Stetina & Orr-Weaver, 2011). Several hypotheses have been proposed to explain the existence of a meiotic arrest (Mira, 1998). Meiosis can only resume when the sperm cell fuses with the oocyte plasma membrane (Nishiyama et al., 2010). Furthermore, in many sexual species, the sperm entry point determines either the anterior-posterior axis or the dorsoventral axis of the future zygote (Albertson, 1984; Goldstein & Hird, 1996; Wangler & Bellen, 2017). Hence, sperm entry is essential for the organisation of cell divisions and the development of the zygote.

Moreover, in many sexual species, the sperm cell provides the first centrioles to the zygote (Figure 4A). Centrioles are the structures at the core of centrosomes which once

assembled, control the nucleation of microtubules and orchestrate them during mitotic divisions (Figure 4).

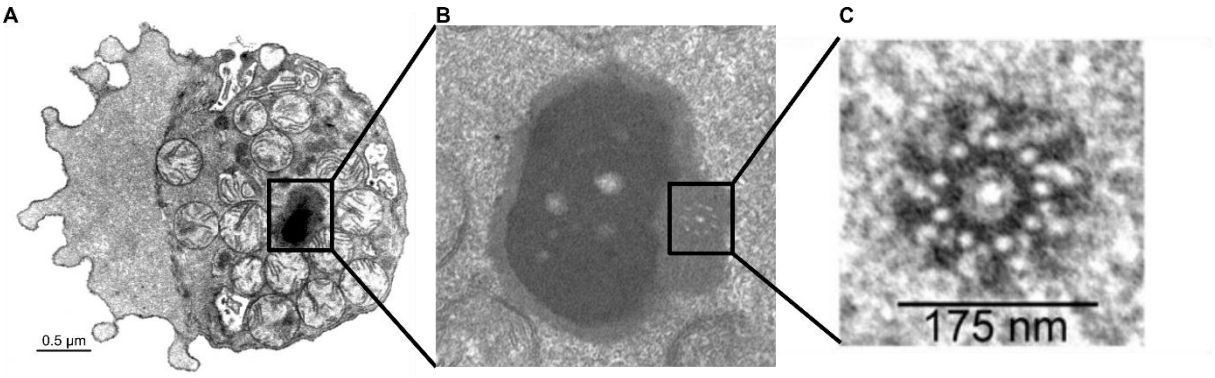

Figure 4: Sperm cell carrying the centrosome and centrioles in *C. elegans* mitosis. A) Transmission electron micrograph of the sperm cell structure. https://www.wormatlas.org/Wardsperm/spermatozoontemleg.htm B) Nucleus of sperm cell and its centrosome. One centriole is on top and the other on the side. C) Transmission electron micrograph of the centriole structure. **https://www.hfsp.org/hfsp-news/molecular-license-converts-daughter-centriole-mother**

In mitotic divisions, two centrosomes are located on each side of the metaphase plate. Via microtubules, centrosomes bind the chromosomes. The spindle microtubules are necessary for the alignment of chromosomes on the metaphase plate and, in anaphase, for the displacement of the sister chromatids towards each future daughter cell. Each centrosome is composed of two centrioles that duplicate at every cell cycle (Delattre & Gönczy, 2004). In most species, female meiosis is acentriolar, with centrioles being eliminated during oogenesis, before meiosis II (Dumont & Desai, 2012; Manandhar et al., 2005; Szollosi et al., 1972). The stage of centriole elimination differs depending on the species. In mice, it occurs during the breakdown of the oocyte nuclear envelope (Szollosi et al., 1972). In *C. elegans*, it occurs during pachytene (Mikeladze-Dvali et al., 2012). In contrast, in starfish, the centrioles of oocytes are still present during meiosis I (Manandhar et al., 2005). During male gametogenesis, however, the centrioles are maintained in most species (Delattre & Gönczy, 2004). Therefore, at fertilization in most species, the centrioles are provided paternally, by the sperm cell. When the sperm is also devoid of centrioles, like in rodents, centrioles are formed de novo in the oocyte cytoplasm, after fertilisation (Delattre & Gönczy, 2004). How asexual species trigger their

meiotic division, generate centrioles and polarize the zygote without fertilization by sperm cells is an essential question.

What is asexuality from a cellular point of view?

The term asexuality in animals is usually called parthenogenesis. Parthenogenesis originates from the ancient Greek *παρθένος* (parthénos, "virgin") and *γένεσις* (génesis, "origin, creation, generation") and is often referred to as "virgin birth". Parthenogenesis is a reproductive system in which a zygote develops spontaneously from a single gamete. As the zygote possesses the genetic heritage of its sole progenitor, parthenogenesis is often described as clonal. As we will see below, clonality is not systematic in asexuals. The spontaneous development of an unfertilised oocyte in females is called thelytoky. Whereas, parthenogenetic females producing a male progeny is known as arrhenotoky.

During the two-step division of meiosis in sexual species, the genetic complexity is reduced and only one chromatid per pair of homologous chromosomes is present in the gametes. In parthenogenetic species, the process of meiosis must be modified to produce unreduced gametes. This way, the progeny inherits two chromatids per pair of homologous chromosomes from its mother only. Descriptions of meiosis performed in asexuals have shown that a great diversity of mechanisms can lead to the association of two chromatids. The referencing of these mechanisms was first initiated at the end of the 20th century by Suomalainen, who described a large number of asexual species among metazoans (Suomalainen, 1950; Suomalainen et al., 1987). In two rich opuses, Suomalainen classifies for the first time asexual animals according to their mechanisms of meiosis. In the end, 18 types of meiosis alterations have been proposed (the count includes each type of meiosis with or without recombination, Figure 5). In each case, the underlying cellular mechanisms can vary between species. For instance, an abortive meiosis I can result from incorrect segregation of homologs (Dumont et al., 2010; Hollis et al., 2020; Siomos et al., 2001), from tripolar spindle poles (Yamashita et al., 1993), from incomplete spindle rotation (McNally et al., 2016) or from a defect in polar body extrusion (Carmena et al., 1998; K.-T. Yang et al., 2010).s

We can also try to further group these modifications according to the extent of changes compared to the regular two-step division program in canonical meiosis: i) regular two-step meiosis with a post-meiotic fusion of two meiotic products (Figure 5 - B and C), ii) regular two-

step meiosis with a pre or post-meiotic chromosomes endoreplication (Figure 5 - E and F) and iii) meiosis with one abortive division (Figure 5 - D, G, H, I and J).

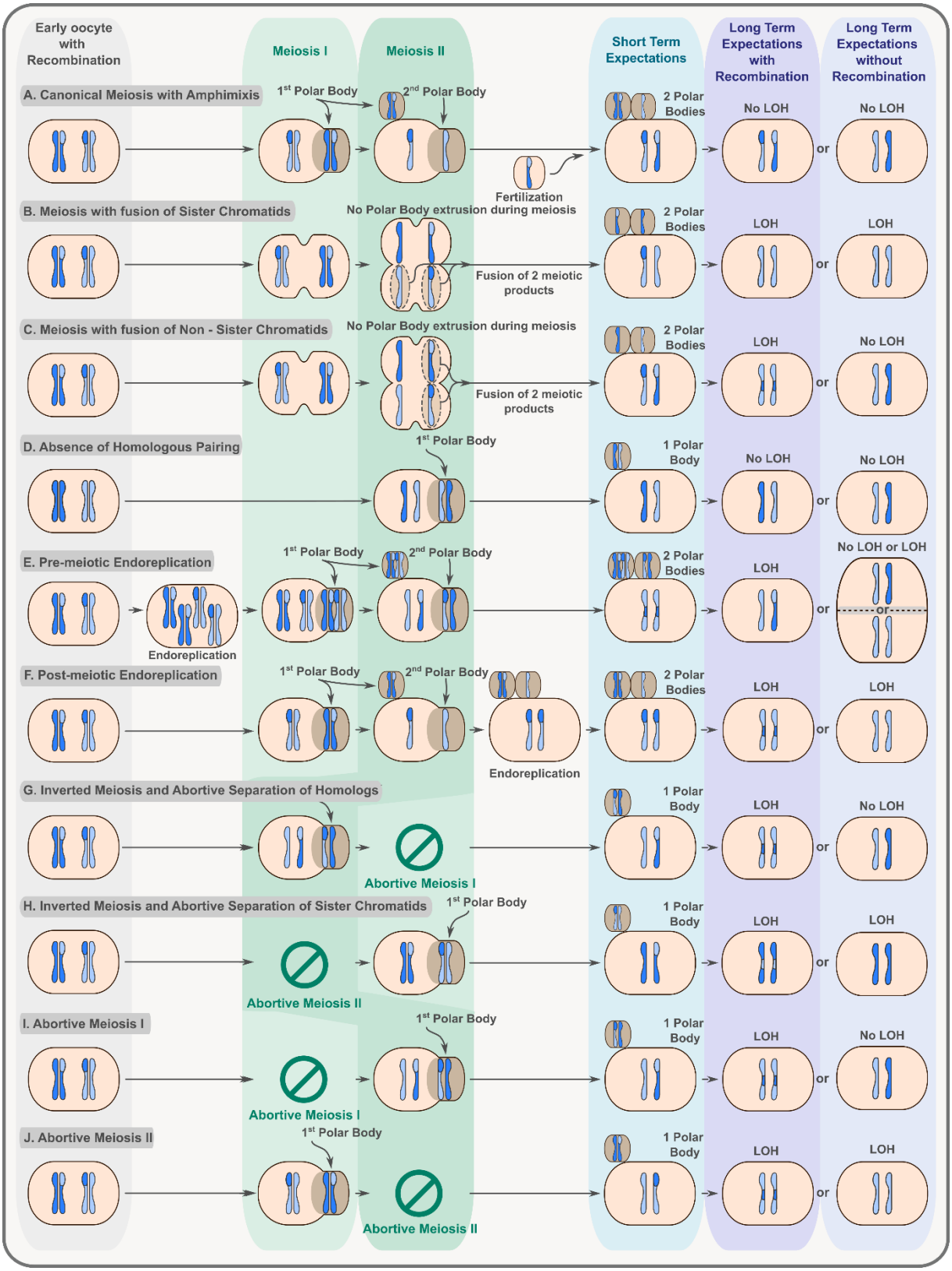

Figure 5: List of 18 possible alterations of meiosis and their theoretical consequences on the genome. A single pair of chromosomes is shown for simplicity. The count includes each type of meiosis with or without recombination**.** The first line refers to canonical meiosis. The two columns on the right show the genomic expectations in the long term with and without recombination. In the case of pre-meitotic endoreplication (E), the assortment of two sister chromatids with recombination would also result in LOH. The sister chromatids are in dark and light blue, corresponding to homologous chromosomes. Recombination is represented by an exchange of colors chromatids. LOH means Loss of Heterozygosity (LOH)

These studies on asexual species have revealed that asexuality has emerged multiple times in the course of evolution, and many different modifications of meiosis have been observed. We have seen that besides the modification of meiosis for the maintenance of ploidy across generations, the production of a zygote in an asexual species must be associated with a mechanism to release the meiotic cell cycle arrest independently of the sperm, with the determination of the poles and axes of the zygote without the sperm, and with de novo assembly of centrioles or maintenance of the maternal centrioles during female meiosis (Galis & van Alphen, 2020).

Very few studies have studied how parthenogenetic animals circumvent the necessity of sperm cells. For instance, in the parthenogenetic hymenopteran *Muscidifurax uniraptor*, centrosomes are formed de novo after egg activation (Riparbelli et al., 1998). Other species as the stick insects of the *Bacillus* genus, generate centrioles de novo in both sexual and parthenogenetic species (Marescalchi et al., 2002). This suggests that the ability to generate centrioles de novo in sexual species has favoured the emergence of new parthenogenetic species in this genus. Concerning embryo polarisation, parthenogenetic species also successfully bypass the absence of sperm cells. In the nematode sexual species *C.elegans*, the entry of the sperm defines the posterior pole of the embryo (Goldstein & Hird, 1996). In some closely related parthenogenetic nematodes such as *Diploscapter coronatus* the selection of the posterior pole is random, suggesting an auto-organisation of the egg axis (Eweis et al., 2022; Lahl et al., 2006). In contrast, in *Diploscapter pachys*, the posterior pole correlates with the position of the meiotic spindle, suggesting the meiotic spindle serves as a polarity cue instead of the sperm (Eweis et al., 2022).

To conclude on this part, as explained in detail in Chapter 1, when homologous chromosomes have lost their ability to form pairs, the only possible meiotic division consists in the segregation of sister chromatids, as it would happen during mitosis (sister chromatid

segregation without recombination). Although this type of meiosis has been often referred to as "ameiotic" or "mitotic-like" in the literature, we do not want to use this term. We believe it has led to many misinterpretations and misunderstandings because it suggests that the meiotic program has been lost. Despite being highly modified (with loss of pairing and recombination and one abortive division), the meiotic program is still present; and still generates a gamete. We prefer the term "non-recombining abortive meiosis I" or "non-recombining assortment of non-sister chromatids".

The evolutionary paradox of sexuality

Advantages and disadvantages of sexual and asexual species

In this paragraph, I expose the different theoretical advantages and disadvantages of being sexual or asexual and the impact of these differences on the genome and the life history traits. We have seen that a key component of sexuality is mixis. Indeed, during meiosis, genetic shuffling through recombination modifies the allelic arrangement within the chromosomes and at fertilisation, genetic mixing combines the alleles from different individuals. Mixis generates new genetic combinations that potentially favour the adaptability of a sexual species (Bell, 1982; Ghiselin, 1974; Roughgarden, 1991; J. M. Smith, 1980; Van Valen, 1973). Nonetheless, genetic shuffling can also break beneficial allelic arrangements, which is a drawback.

Asexuality is often associated in the literature with a loss of recombination. In the absence of recombination (genetic shuffling), combinations of beneficial alleles will be preserved, which confers an advantage for asexual species. On the other hand, the absence of recombination results in the irreversible accumulation of slightly deleterious mutations known as Muller's ratchet (Muller, 1932, 1964). This accumulation of mutations is potentially detrimental to the species, especially if the type of meiosis does not expose the mutations at the homozygous state: the deleterious mutations cannot be counter-selected and purged. As we will see below, many asexual species still undergo homologous recombination, and therefore, the advantages and disadvantages of recombination cannot be systematically invoked when comparing these two modes of reproduction.

On the other hand, there are theoretically clear costs imposed by sexuality. Sexuality is first associated with a "two-fold cost of sex" (Maynard Smith, 1971, 1978; Williams, 1971, 1975). If a sexual female produces 50% of sons, she invests 50% of her resources into them. Thus, producing sons reduces the per capita birth rate of sexual females. In contrast, asexual females invest 100% of their resources only into their daughters. Therefore, in the short term, the per capita birth rate of asexual species should be twice as high as that of sexual species. For this reason, in the short term, asexuality is considered largely advantageous in comparison to sexuality. On the same line, a sexual individual gives only half of its genome to its progeny whereas a zygote generated asexually inherits the entire genome of its single progenitor. The genetic investment is therefore better in asexual than in sexual species.

Furthermore, there are costs associated with the production of males, the search for a mating partner, by the sexual competitiveness in mating, but also by an unequal investment over provisioning the progeny. Mating itself is dangerous because it exposes the individuals to predators, or to infectious diseases. Despite all these disadvantages, sexuality is the most abundant reproductive system across phylogenies (Ashman et al., 2014). The parthenogenetic species are scarce in comparison to the sexual species. For instance, there are less than 0.1% obligate parthenogenetic vertebrates (Freitas et al., 2019) and 0.1% asexual Hexapoda (Normark, 2014; Rabeling & Kronauer, 2013). But given the costs of sexuality, it is not obvious that genetic mixis is sufficient to overcome all these costs. Other non-exclusive mechanisms compensating for the costs of sexuality have been proposed (Red Queen hypothesis (Van Valen, 1973) Tangle bank hypothesis (Bell, 1982; Ghiselin, 1974) or Fluctuating Selection (Roughgarden, 1991; J. M. Smith, 1980). Nevertheless, the prevalence of sexuality remains a paradox in today's evolutionary biology.

Cellular constraints for the emergence of new asexual species

We have seen that the emergence of asexuality requires the implementation of various cellular changes, concerning meiosis, egg activation, possibly egg polarisation and centriole inheritance. For this reason, it is possible that the scarcity of asexuality in the Tree of Life is not only due to the genomic consequences of reproduction without mixis but also to the sophistication of the mechanisms to be established for the successful transition (Engelstädter, 2008). In other words, asexuals are maybe rare because they arise rarely. Indeed, from a mechanical perspective, many cellular innovations are required to transition to asexuality,

which may not occur very often in nature (Lenormand et al., 2016). In some sexual species, including many insects, unfertilized eggs can develop: the sperm is not required to activate the eggs, centrosomes can be formed *de novo* in the egg cytoplasm and the haploid cell becomes a viable adult. In large hyper-diverse orders, such as Hymenoptera, males are produced through such unfertilized egg development (haplo-diploid sex determination system). Not surprisingly, many asexual species are found in Hymenoptera, most likely because relaxation on these cellular constraints facilitates the emergence of asexuality in this specific taxon (Neiman et al., 2014). On the other side of the spectrum, pseudogamous species, also called sperm-dependent parthenogens (see below), are all-female species that use the sperm of males from other species to get their eggs activated but do not use the sperm DNA (Lehtonen et al., 2013). In this case, there is still a dependency on the sperm for egg activation, and maybe for centrosomes, but not for ploidy restoration. Why modifications of the meiotic divisions were facilitated while autoactivation of eggs was not possible? Which mutations are required for these processes to be modified and how many of them? Are these changes progressive like suggested by the case of pseudogamous species, or do they arise synchronously? Generally, while different mechanisms of unreduced gamete formation have been described (see above), the molecular basis of these changes are largely unknown. The mechanisms that could enable egg autoactivation or *de novo* centrosome formation are almost entirely mysterious. The reason is most likely because asexual species are often not amenable to lab culture and experimental approaches, thus data are missing.

Interestingly, in some orders such as mammals, no parthenogenesis is referenced, whether obligatory, cyclic or facultative. The few cases reported (hamsters, rats, mice, monkeys, and humans) concern parthenogenesis induced by environmental factors, chemicals, and viruses (Kharche & Jha, 2016). The absence of non-natural parthenogenesis in mammals could be explained by the existence of genomic imprinting. In contrast to other metazoans, the zygote experiences genomic imprinting during embryogenesis. In eutherian mammals and marsupials, a small proportion of genes are specifically silenced during gametogenesis in one sex (Barlow & Bartolomei, 2014). A diploid zygote has a pool of haploid chromosomes inherited from the mother and a second haploid pool inherited from the father. Some genes are therefore present in two copies in the zygote but only one copy is expressed while the other copy is genomically imprinted. Among those genes, some are specifically maternally silenced and other paternally silenced. Of the 25,000 genes in the mouse genome, over 100 are genomically imprinted (Li & Sasaki, 2011). Genomic imprinting is required for these genes to

be properly expressed for normal development, fetal growth, nutrient metabolism and adult behaviour. The sophistication of the genomic imprinting process, and the mechanical constraints it generates, could explain why parthenogenesis does not emerge spontaneously in mammals: because a gene inherited only from the mother, for instance, could be totally silenced. Again, the absence of parthenogenesis in this class of metazoans may be explained by the difficulties in bypassing the strong constraints applied to sexual reproduction.

Whether the emergence of asexuality is prevented in some taxa because of cellular constraints, and/or whether the consequences of asexuality are too negative on the long term for these species to be maintained remains an open question.

The paradox of heterozygosity maintenance in asexual species

Heterozygosity reflects the diversity of the genome. A gene is considered heterozygous when, in a diploid organism, it is represented by two different alleles on the two homologous chromosomes. Conversely, homozygous means that only one allele is present on the two homologous chromosomes. The majority of sexual species has heterozygous genomes. It is also well established that a loss of heterozygosity (LOH) in sexual species, for example via genetic inbreeding, can be detrimental to the population, as it reveals deleterious recessive alleles at the homozygous state. As expected, the majority of asexual species analysed so far display heterozygous genomes (Birky Jr, 1996; Jaron et al., 2021). Nevertheless, some exceptions are to be mentioned such as the parthenogenetic stick insects of the genus *Timema*. Interestingly, there is no fitness decline in those *Timema* species (Bast et al., 2018) and they even show signs of positive selection (Jaron et al., 2021). Importantly, that in the absence of recombination can sustain heterozygosity and is even theoretically expected to amplify it (Dedukh, Altmanová, et al., 2022; Fradin et al., 2017; Marais, 2012; Triantaphyllou, 1963, 1981). This phenomenon is called the "Meselson effect", and claims that the homologous chromosomes that are not subjected to recombination accumulate independent mutations that increase their divergence over time (Birky Jr, 1996; Mark Welch & Meselson, 2000).

As discussed in detail in Chapter 1, there are theoretically different genetic expectations for all the meiosis modifications described above (Lampert et al., 2007). If we classify these modifications according to the genetic content of the resulting gametes, we see that there are

only four classes: association of sister chromatids (Figure 5 - B, F, H and J) or of non-sister chromatids (Figure 5 - C, D, E, G and I), and with or without the maintenance of recombination. Importantly, most of these modifications should theoretically lead to a total or partial loss of heterozygosity in the genome, called LOH. Indeed, homogenisation of the genome should happen in one generation in the case of post- or pre-meiotic chromosome duplication (Figure 5 - E and F). In the case of fusion of the two first products of meiosis (Figure 5 - B and C), or abortion of meiosis I (Figure 5 - D, G and I), non-sister chromatids will be assorted. If recombination occurs and if chromatids are randomly assorted in the oocyte, some chromatids will share the same regions, distally to the crossing-over, and therefore heterozygosity will be lost in those regions. In the next generation, other portions with LOH will be generated. In the long term, the genome will have large homozygous regions. Similarly, in the case of fusion of the last products of meiosis (Figure 5 – B) or after abortion of meiosis II (Figure 5- H and J), sister chromatids will be assorted and LOH is expected proximally to the crossing-over in the long term. Of note, when sister chromatids are associated in unreduced gametes, the rate of LOH should be higher than when non-sister chromatids are assorted. The rate of LOH also differs according to the localisation of the recombination. For intanse, in sister chromatids assortment (Figure 5-H and J) with proximal centromere recombination, LOH occurs in one generation. Wherease subtelemoric recombination results in a slower LOH rate. Furthermore, the rate of LOH increases with the rate of recombination (Lynch, 1984). Only the absence of recombination joined with an abortive step or a fusion, or the absence of chromosome pairing, which necessarily prevents recombination, can theoretically maintain a heterozygous genome (Figure 5 – C, D, E, G and I).

Consequently, in theory, the transition to parthenogenesis should result in a genome-wide LOH, with the rate of LOH depending on the mechanism of parthenogenesis. But, as discussed in Chapter 1, during the evolution of asexuals, it is therefore expected that mechanisms that prevent or slow down LOH should have been selected in the majority of cases. This is theoretically possible by preventing homologous pairing (and consequently recombination) (Dedukh, Altmanová, et al., 2022), by suppressing recombination, reducing the rate of recombination which only delays LOH (Rey et al., 2011), or by limiting recombination to the tips of the chromosomes (Oldroyd et al., 2021) causing only local stretches of LOH. Importantly, few studies have systematically confronted the cytological evidence for changes in the recombination landscape, and the genomic data. Therefore, other mechanisms may also exist. As discussed in Chapter 1 and below, many asexual species have been categorised as non-

recombining asexuals only because their genomes were highly heterozygous. Recent studies have revealed homologous recombination for some of these iconic species (Simion et al., 2021).

To conclude here, more work combining cytology and genomics is required to understand which cellular and molecular changes allow the transition towards asexuality while preserving healthy genomes. Ultimately, this should help to understand the adaptive value of sex.

Coexistence of sexual and asexual reproductive systems

Studying the mechanisms of transition from a sexual to an asexual mode of reproduction is a complicated task. In particular, the mechanisms that were initially responsible for cellular changes have continued to evolve in today's asexual species. As we will see, some asexual species are extremely ancient, which makes the analysis even more complicated. Obligate parthenogenesis is probably the system that has required the most modification. In addition to the modification of meiosis, the system has evolved to cope with the absence of spermatozoa. As I describe in this paragraph, pseudogamy or gynogenesis is an interesting case because asexual reproduction still depends on fertilization by a sperm cell. In this sperm-dependent type of parthenogenesis, only modifications of female meiosis are required to transition from sexuality to asexuality. I have studied more particularly a case of auto-pseudogamy in the species *Mesorhabditis belari*, where females produce females by sperm-dependent parthenogenesis and males by sexual reproduction. Here, a given female is able to switch meiosis to produce unreduced gametes (for the production of females) and regular haploid oocytes (for the production of males). For this reason, *M. belari* constitutes an ideal model to study which cellular and molecular modifications of meiosis can happen during the transition towards asexuality.

Before describing the *M. belari* system, I present in this section examples of animals belonging to the different categories of parthenogenesis, namely obligatory parthenogenesis, cyclic parthenogenesis, facultative parthenogenesis and finally the main focus of my work, pseudogamy. The lists of metazoan species presented in these sections are of course not exhaustive. For each category, I illustrate that there is a diversity of possible modifications of meiosis, each with its own genetic implication. More details and other examples are also presented in Chapter 1.

Some species reproduce by obligate parthenogenesis where only one individual is required to form a zygote. This reproductive system is strictly asexual. Nonetheless, other forms of parthenogenesis exist and combine both sexual and asexual modes of reproduction: facultative parthenogenesis, cyclic parthenogenesis and pseudogamy also called gynogenesis or sperm-dependent parthenogenesis. In these species, although both females and males are present, some individuals are produced asexually.

In most studies, parthenogenesis is demonstrated by isolating a virgin female (thelytoky), and assessing whether the female produces viable and fertile offspring on its own. This sort of approach missed the other forms of parthenogenesis such as gynogenesis. Some species have been categorised as asexual by studying the segregation of microsatellite markers, or heterozygous specific loci, over generations. If the same genotype was observed from parents to offspring over generations, the species was considered clonal, i.e. asexual (de Meeûs et al., 2007; Sköld et al., 2009; Suomalainen, 1950). In general, cytological evidence of asexuality remains rare. Moreover, most cytological studies were conducted before 1990 by researchers such as Esko Suomalainen (Suomalainen, 1950; Suomalainen et al., 1987) or, in nematodes, Anastasios C. Triantaphyllou (Triantaphyllou, 1966, 1969, 1987) using their contemporary techniques. There is for instance only one description of the meiosis of oribatid mites from 1958 (Taberly, 1958). Before the work of the Van Doninck group in 2021, the last cytological description of meiosis in bdelloid rotifers, the iconic ancient asexuals, dated back to 1956 (W. S. Hsu, 1956a, 1956b). Given the evolution of cytological techniques, there will be an interest in re-examining the parthenogenic species to better apprehend how this system might emerge.

Obligate parthenogenesis

Obligate parthenogenesis describes strict asexuality where eggs always develop in the total absence of sperm and fertilization. Among metazoan, obligate parthenogenetic species have been estimated at 1 in 1000 species (Beukeboom & Vrijenhoek, 1998). This estimation is based on vertebrates. Nonetheless, obligate parthenogenesis has been described so far in Rotifera, Crustacea, Arachnida, Hexapoda, Nematoda, and Reptilia. True obligate parthenogenesis is extremely rare and represents, for instance, less than 0.1% of vertebrates (Avise, 2008). Bdelloid rotifers, darwinulid ostracods and oribatid mites have long been considered as "evolutionary scandals", despite the total absence of sex and genetic mixing, these

species have persisted over millions of years (Maynard Smith, 1978). In the following sec tions, I briefly describe the obligatory parthenogenetic system in these species as well as in Hexapoda, Nematoda or Reptilia.

The bdelloid rotifers are iconic obligate parthenogenetic species. This bilaterian constitutes the freshwater zooplankton. Bdelloid rotifers have long been a paradox known as "evolutionary scandals" (Maynard Smith, 1986). Indeed, analyses of their fossils revealed that bdelloid species survived for over 50 million years without sex (Poinar & Ricci, 1992; Tang et al., 2014). Moreover, 460 morphologically distinct species have been described, suggesting that diversification is largely possible without sexuality (Segers, 2007). In these rotifers, females are exclusively produced via parthenogenesis. The species *Adineta vaga* has long been incorrectly described as apomictic, i.e. ameiotic with mitotic-like divisions (see Chapter 1). More recent cytological studies have revealed that *A. vaga* is actually a recombining asexual, undergoing an abortive meiosis I, resulting in the formation of an unreduced diploid gamete containing non-sister chromatids (Simion et al., 2021; Terwagne et al., 2022).

Ostracods also belong to the "ancient asexuals". They are small bivalved crustaceans. Whereas Cypridoidea and Cytheroidea exhibit cyclic parthenogenesis (see Cyclic parthenogenesis), Darwinuloidea only reproduce parthenogenetically. Within this superfamily, females give rise to females (thelytoky). *Darwinula stevensoni* is the most investigated darwinulid ostracods. This species is totally asexual, and fossils have revealed their maintenance for over 200 million years (Martens et al., 2003, 2008). Darwinulids have lower fecundity levels and longer life cycles than other ostracods, especially in higher latitudes (Geiger, 1998; Van Doninck et al., 2003). Cytogenetic analyses were conducted between the 1960s and 1970s, mainly by Tétart (Tétart, 1977), but they did not identify the mechanism of meiosis in *D. stevensoni*.

Oribatid mites are the last emblematic 'ancient asexuals'. Indeed, studies suggest that this group of arachnids diverged about 400 to 440 million years ago (Lindquist, 1984). Cytogenetic studies conducted in the 50’s revealed that one step of meiosis is aborted (Figure 6) (Taberly, 1958, 1958, 1960). Because the genome of this species is largely heterozygote (Bergmann et al., 2018; Laumann et al., 2008), Archetti proposed a complicated modification of meiosis to reconcile the cytology and the genomics (Archetti, 2022). It involves i) an inverted meiosis combined with abortion of meiosis II, ii) together with a biaised segregation of the recombinant chromatids during the first division (called Z-segregation) (Archetti, 2022).

However, further cytological investigations are required to prove the existence of inverted meiosis in this species. Besides, some studies suggested that recombination is absent in these species, in which case the maintenance of heterozygosity would not be mysterious, as shown above (Bergmann et al., 2018; Brandt et al., 2021; Palmer & Norton, 1992; Schaefer et al., 2006).s Oribatid mites constitute a typical example where cytological data should have been revisited since 1956 (Figure 6).

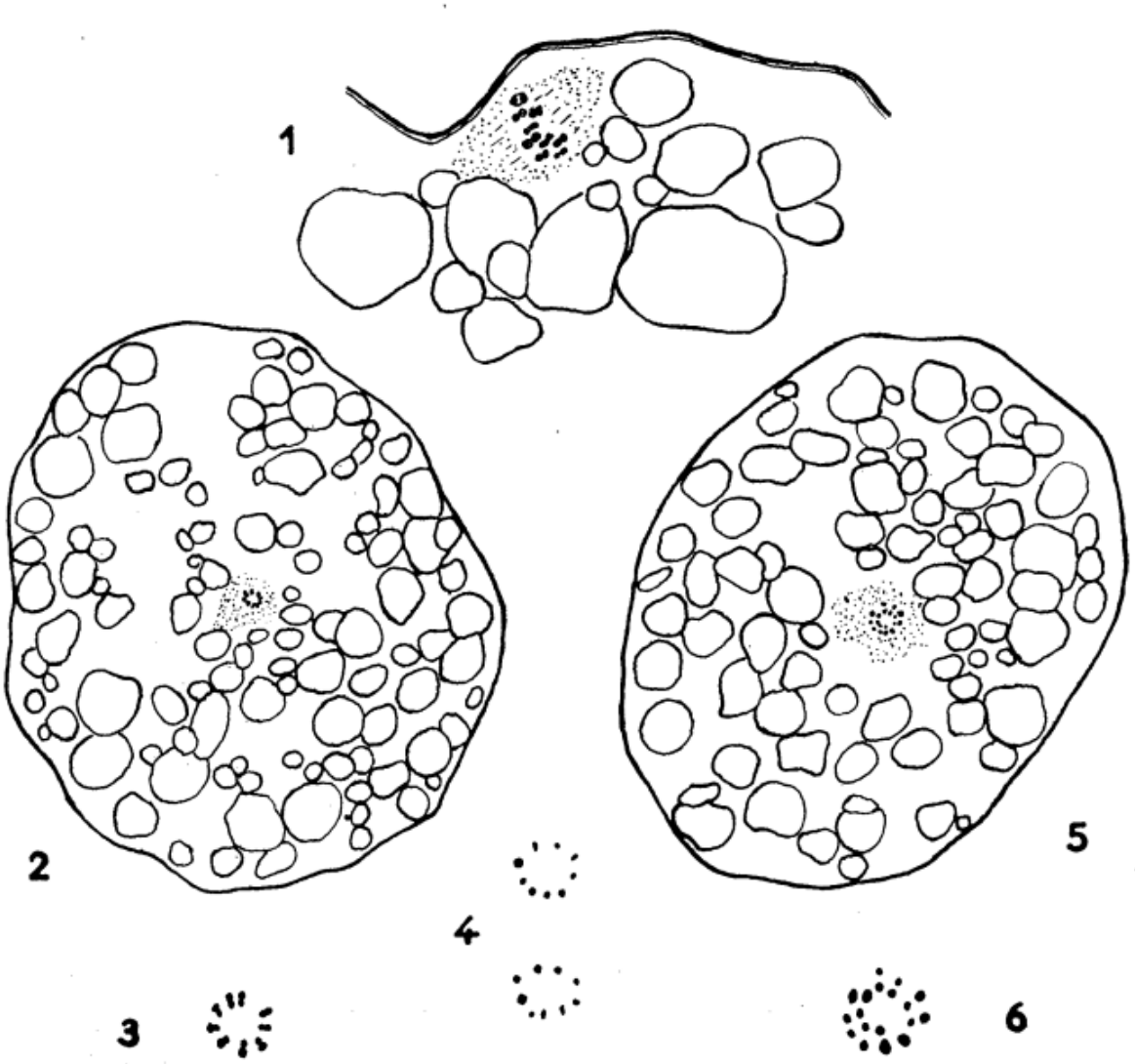

Fig. 1, 3, 4, 6 (× 2 220); fig. 2, 5 (× 730).

Figure 6: Sketches of abortive first stage of meiosis in *Platynothrus peltifer*, an oribatid mite. 1-3) Side and top view of the 9 chromosomes in metaphase I. At this stage, the chromosomes are organised in rosette and no polar bodies are present. 4) The two sets (rosettes) of 9 chromosomes of anaphase I. Taberly underlines a distance between the rosettes which are separating from each other. 5-6) At metaphase II, 18 chromosomes are visible on the metaphase plate and no polar bodies are found. Bouin-Duboscq-Brasil fixation and Feulgen staining. (Taberly, 1958)

In nematodes, obligate parthenogenesis has been described in many free-living species as well as in plant parasites (reviewed in Fradin et al., 2017; Hiraki et al., 2017; Hung & Jenkins, 1969; Merlin et al., 2003; Nigon & Félix, 2018; Triantaphyllou, 1970). Several types of modification of meiosis have been described in this phylum. In the free-living species *D. pachys*, cytogenetic evidence revealed that chromosomes do not pair. Hence, the only division observed consists of the separation of sister chromatids in meiosis II (Fradin et al., 2017). In this species, recombination is abrogated. The same meiotic process is found in the closely related species, *D. coronatus* (Hiraki et al., 2017). In the plant parasites *Meloidogyne* spp, the parthenogenesis systems differ rapidly between species. In *M. hapla*, *M. fallax* and *M. chitwoodi*, the chromosomes pair in meiosis I but meiosis II is abortive (Liu et al., 2007; Triantaphyllou, 1966; Van Der Beek et al., 1998). Hence sister chromatids are found in the unreduced gametes. While, in *M. incognita* and *M.*arenaria, there is no pairing as in *D. pachys* (Marais, 2012; Triantaphyllou, 1963, 1981). In 1963, Triantaphyllou argued that parthenogenetic nematodes such as *Heterodera betulae* could be produced by post-meiotic endoreplication after a regular meiosis, but the cytology is unclear (Triantaphyllou, 1963).

In Hexapoda, obligate parthenogenesis is also rare and represents 0.1% of the species (Normark, 2014). In Holometabola, it is mentioned in six orders: Lepidoptera, Coleoptera, Trichoptera, Hymenoptera, Strepsiptera, and Diptera (Gokhman & Kuznetsova, 2018). Whereas in non-holometabolous, parthenogens have been reported in four orders: Phasmatodea, Psocodea, Thysanoptera and Hemiptera (Vershinina & Kuznetsova, 2016). Obligate parthenogenesis is mainly identified in hymenopterans. Hymenoptera species are known to be haplodiploid. Females are diploid (XX) while males are haploid (X0). The males are only derived from arrhenotokous parthenogenesis of unfertilized eggs, whereas the females are derived from the sexual pathway. In this case, meiosis is not modified and remains canonical: a haploid gamete is produced from previous diploid oocytes and a haploid male arises from this egg. In haplodiploid animals, arrhenotokous parthenogenesis does not influence the species' genome. Maybe because of the ability to form unfertilized egg, parthenogenesis is frequent in Hymenoptera. In most cases, parthenogenesis is induced by the parasite *Wolbachia* (Braig et al., 2002; Stouthamre, 1997; Van Wilgenburg et al., 2006). However, some species like *Venturia canescens* present both thelytokous and arrhenotokous parthenogenesis with any presence of *Wolbachia* (Mateo Leach unpublished data, Beukeboom & Pijnacker, 2000; Schneider et al., 2002). *V. canescens* is a solitary endoparasitoid wasp of lepidopteran larvae (Berling, 1932; Salt, 1976). In this species meiosis I is abortive, leading to the assortment of

non-sister chromatids in the unreduced gametes (Figure 7) (Beukeboom & Pijnacker, 2000; B. Speicher et al., 1965; B. R. Speicher, 1937).

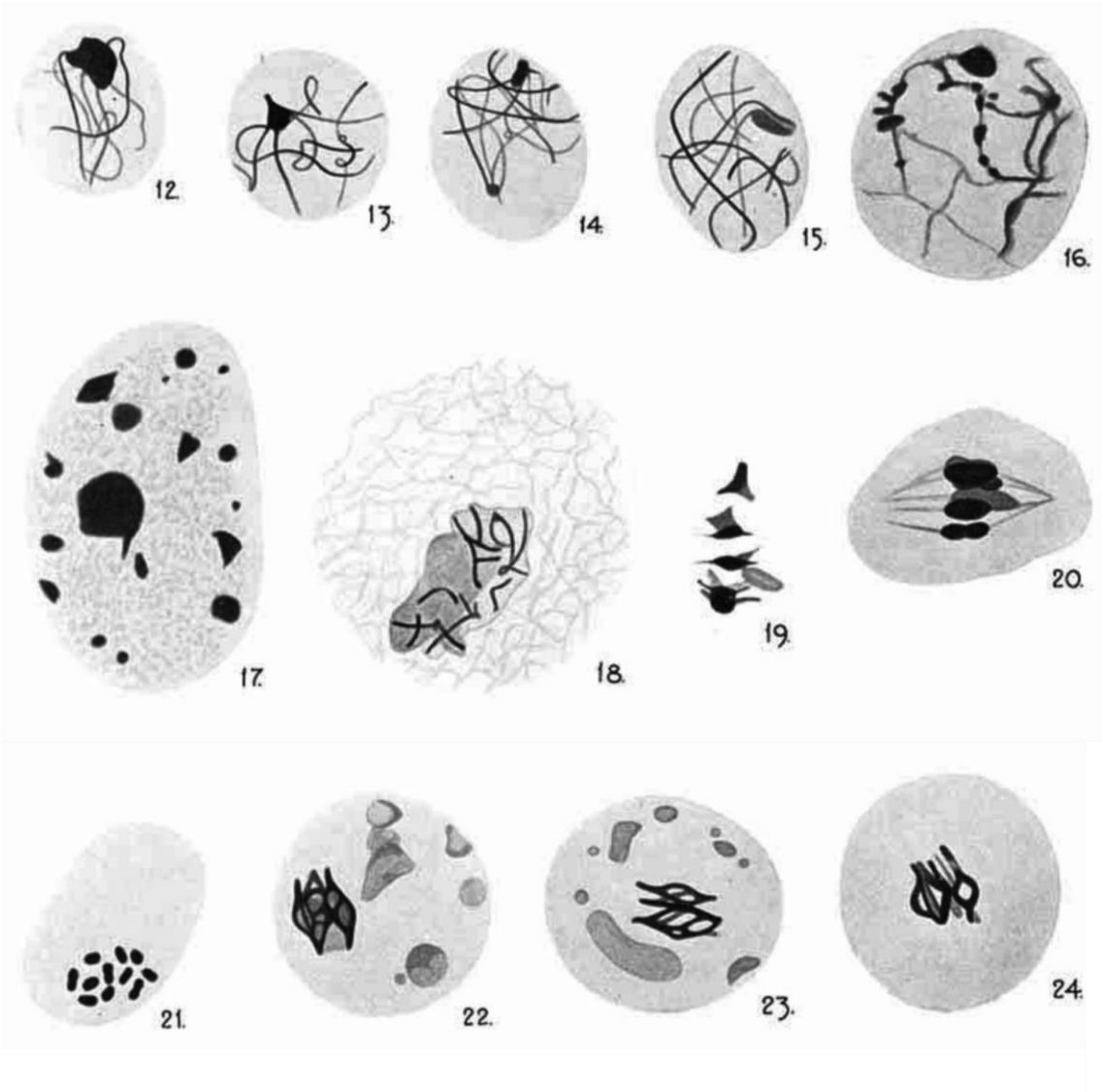

Figure 7: Drawings of oogenesis in thelytokous wasp. 12-14) First oocyte nucleus after synizesis. 15) First oocyte nucleus after synizesis. 17) Section of first oocyte nucleus in diffuse stage, showing large plasmosome and smaller granules. Staining with Strong Flemming. 18) Section of first oocyte nucleus following the diffuse stage, with chromosomes appearing within an amphinucleolus. 19) Late first diakenesis. Staining with Hot Kahle. 20) First metaphasic nucleus. A spindle is formed within a complete nuclear. Staining with Warm Bouin. 21) First metaphase nucleus, polar view, with eleven chromosomes. Staining with Warm Bouin 22-23) Nucleus from uterine egg in what is probably early anaphase. No spindle fiber visible. Staining with Bouin 24) Nucleus from newly laid egg, haying the same appearance as figures 22 and 23. Staining with Feulgen technique and Carnoy All drawings are magnified about x 3000. (B. R. Speicher, 1937)

In Reptilia, obligate parthenogenesis has been reported in various species such as a snake, an iguana and in lizards and geckos (Abdala et al., 2016; M. Adams et al., 2003; Barley et al., 2021; Dedukh, Altmanová, et al., 2022; Grismer et al., 2014; Lutes et al., 2010; Pellegrino et al., 2003; Reeder et al., 2002; Sinclair et al., 2010; Wynn et al., 1987). The vast majority of analyses of parthenogenesis in reptiles are based on genetic and genomic findings. To my knowledge, few studies, such as those on the whiptail lizards of the genus *Aspidoscelis* or of geckos, have relied on cytology to identify the underlying mechanism of parthenogenesis (Dedukh, Altmanová, et al., 2022; Lutes et al., 2010). In lizards and geckos, parthenogenesis relies on premeiotic endoreplication. This means that before meiosis, the chromosomes undergo a second cycle of replication. In these species, the meiotic process is not altered: recombination is still present and chromosome segregation is Mendelian. Nonetheless, pre-meiotic endoreplication enables the maintenance of ploidy in the zygote by doubling the number of chromatids that enter meiosis. Most obligate parthenogens described in reptiles have a hybrid origin ("Hybrid Origin Hypothesis") with the exception of xantusiids (Sinclair et al., 2010) where the authors hypothesised that parthenogenesis emerged via the accumulation of spontaneous mutations ("Mutational Origin Hypothesis", (Bullini, 1994; Suomalainen et al., 1987).

Cyclic parthenogenesis

Cyclic parthenogenesis, or cycling parthenogenesis, refers to a reproductive system in which females produce offspring sexually and periodically switch to asexual production in a reversible manner. Cyclic parthenogenesis has been described in eight lineages of metazoans and represents at least 15,000 species distributed among one order of nematodes, one of rotifers, one of the trematodes and one of the crustaceans and in four orders of Hexapoda, Hemiptera, Hymenoptera, Diptera, and Coleoptera (Hebert, 1978; Riparbelli et al., 2017). The shift between the two reproductive modes is usually induced by environmental stimuli (Hebert et al., 1988; Simon et al., 2003). Theoretically, both modes of reproduction provide benefits. Asexual animals have a higher reproductive output than sexual animals. Asexuality, therefore, avoids the double costs associated with sexual reproduction and is known to be beneficial in the short term. Whereas occasional sexuality is associated with long-term benefits such as genetic mixing and thus the ability to purge deleterious mutations (Hebert et al., 1988; Simon et al., 2003). Furthermore, these species do not suffer from the deleterious effects of parthenogenesis. Indeed, they are temporarily parthenogenetic and the transient accumulation of LOH may be

compensated by sexual events, leaving the species with a reasonable level of heterozygosity (Omilian et al., 2006). Some obligate parthenogenetic species are considered to have arisen from cyclic parthenogens via frequency-specific allelic modulations (Dedryver et al., 2013; Stelzer et al., 2010) or via “contagious” parthenogenesis in *Daphnia pulex* and pea Aphids (Jaquiéry et al., 2014; Tucker et al., 2013; Xu et al., 2015).

The most popular example is the crustacean of fresh water, *D. pulex*. In water fleas, seasonal changes, food deprivation or crowding can induce a switch to parthenogenesis (Hebert, 1978; Hiruta & Tochinai, 2012). The *D. pulex* female can produce both asexual (thelytoky) and sexual female offspring, while males are exclusively produced parthenogenetically (arrhenotoky). During the parthenogenetic stage, meiosis I is aborted and homologs are not separated. The unreduced gamete, therefore, is diploid and contains an assortment of non-sister chromatids. Although no clear chiasmata have been observed, the homologs pair at the first stage of asexual meiosis, which provides an opportunity for the chromosomes to recombine (Hiruta et al., 2010; Omilian et al., 2006). Genomic analyses have revealed stretches of LOH confirming the existence of recombination events (Lynch et al., 2022; Omilian et al., 2006). While in cyclic parthenogenetic *Daphnia* LOH can be counterbalanced by the sexual stage, what about asexual the lineage?

Concerning Aphids (Hemiptera), a majority of species described so far are subject to cyclic parthenogenesis. Yet, some cyclic species can evolve as obligate parthenogens such as *Sitobion avena* (Dedryver et al., 2013). Aphids are haplodiploids. In summer, when the photoperiod is longer and the environmental conditions are clement, the females reproduce asexually. Conversely, in winter, the females switch to a sexual mode of reproduction. In the most studied family, the Aphididae, the mode of reproduction is controlled by an asexuality-promoting signal generated by the mother's brain. This signal is influenced by photoperiod and is transmitted to the embryos during embryogenesis (Davis, 2012; Galis & van Alphen, 2020). Also, in Aphididae, using exclusively genomics data, meiosis is qualified as inverted: sister chromatids segregated first and homologs in the second round of meiotic cell division. During the asexual stage of the species, only the separation of homologs occurs (i.e. meiosis I). The zygote therefore inherits two non-sister chromatids and, in the absence of recombination, is a clone of its mother (BLACKMAN, 1987; Hales et al., 2002; Srinivasan et al., 2010).

Strongyloides (Rhabditida) are both free-living and parasitic nematodes of the intestines of vertebrates. Interestingly, during the parasitic phase in the host, the authors suggested that

females are produced asexually via auto-pseudogamy, (see Gynogenesis) (Streit, 2008; Triantaphyllou & Moncol, 1977). In *Strongyloides ransomi*, *S. ratti* and *S. papillosus*, no homologous pairing occurs (Streit, 2008). Females can also give rise to a free-living generation. In this case, females reproduce sexually and meiosis in canonical.

Facultative parthenogenesis

Facultative parthenogenesis is defined as accidental parthenogenesis that arises in a sexual species. In contrast to obligatory parthenogenesis, facultative parthenogenesis does not generally originate from a hybridisation event but occurs spontaneously in an unfertilised egg. Events of parthenogenesis occur at low frequency in those species. Facultative parthenogenesis is usually associated with a low proportion of hatching eggs and an even lower proportion that develop until adulthood (Booth et al., 2012). In metazoan, facultative parthenogenesis has been observed in both invertebrates and vertebrates. In invertebrates, facultative parthenogenesis is described in Hymenoptera. In vertebrates, it is mainly found in fish, reptiles and very rarely in birds. As mentioned in Cellular constraints, no emergence of parthenogenesis species is reported in mammals. Given the number of existing facultative parthenogenesis systems, in the following paragraphs, I only describe the most detailed ones in Hexapoda, Nematoda, Reptilia, Aves and Fishes.

Facultative parthenogenesis is found in various orders of Hexapoda: Odonata Orthoptera, Blattodea, Phasmatodea, Mantodea, Psocoptera, Lepidoptera, and Diptera (Normark & Kirkendall, 2009). As for obligate parthenogenesis, facultative parthenogenesis has been mainly investigated in eusocial insects as in Hymenoptera and Isoptera.

Isopteran clade is composed of the orders of cockroaches and termites. In both orders, species have demonstrated the capacity to undergo facultative parthenogenesis (Khan et al., 2022; Tanaka & Daimon, 2019). In this clade parthenogenetic differs depending on the species. Some mechanisms promote the association of non-sister chromatids while others present an association of sister chromatids as is the case in cockroaches (Fournier et al., 2016; Komatsu et al., 2015; Tanaka & Daimon, 2019).

In Hymenoptera, the system has been described in bees *Apis mellifera capensis* (Cole-Clark et al., 2017), the little fire ant *Mycocepurus smithii* (Rabeling et al., 2009) or in clonal raider ants *Ooceraea biroi* (Oxley et al., 2014).These species are haplodiploid. In *A. capensis*, under stress conditions, when a colony has lost a queen without a future queen being raised, the workers can produce a new queen and workers via thelytoky (Cole-Clark et al., 2017). In these species, both genetic and cytological analyses demonstrate that the ploidy is maintained by the fusion of the first products of meiosis, leading to an assortment of non-sister chromatids (Figure 8). The same mechanism occurs in the raider ants *O. biroi*.

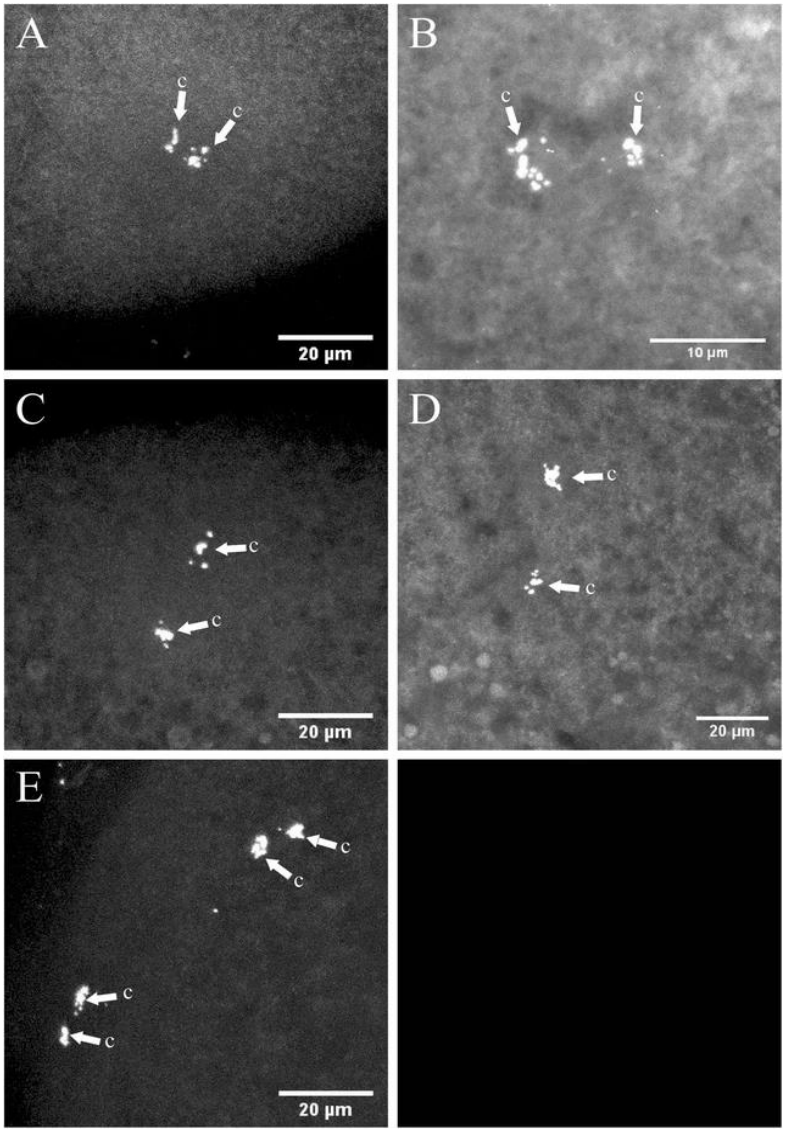

Figure 8: Meiotic events observed in worker-laid eggs of Apis mellifera capensis 0–4.5h post oviposition. a Anaphase I (0–30 min); two groups of chromosomes during their separation. b Telophase I (30–60 min); complete separation of chromosomes with clustering at the polar ends of the now-separated pronuclei. c Prophase II (1–2 h); chromosomes are condensed. d Transitional stage between

prophase II and metaphase II (2–3 h); chromosomes are condensed and lined up along the equator of each pronucleus. e Telophase II (4–4 ½ h); complete separation of the chromosomes resulting in the formation of four haploid pronuclei. c chromosomes, cr chromatin thread. All images are two-dimensional projections of three-dimensional confocal z-stacks. (Cole-Clark et al., 2017).

Reptilia belongs to one of the classes of metazoans with the highest incidence of parthenogenesis. Facultative parthenogenic species are found in lizards, Komodo dragons and snakes (Figure 9) (reviewed in Booth et al., 2012; Cubides-Cubillos et al., 2020; Stöck et al., 2021). In this class, facultative parthenogenesis is also associated with low survivorship within litters, but few individuals develop into fertile adults. Here again, evidence of the underlying parthenogenic mechanisms has so far been supported by genetic and genomic data only.

Reptiles possess a ZW sex-determination system where females are heterozygous (ZW) and males are homozygous (ZZ). Theoretically, a parthenogenic female can produce three types of zygotes: (ZW) females, (ZZ) males or (WW) females. For a long time, researchers considered the (WW) association in female progeny to be lethal. But studies performed on the Boa Constrictor, *Boa constrictor imperators*, and the Brazilian Rainbow Boa, *Epicrates cenchria cenchria*, suggested that (WW) females can be viable (Booth et al., 2011; Kinney et al., 2013). However, the great majority of these parthenogenetic species undergo arrhenotokous parthenogenesis and produce (ZZ) males, suggesting an assortment of sister-chromatids (Card et al., 2021; Jordan et al., 2015; Kinney et al., 2013; Miller et al., 2019). However, cytological analyses are required to validate this point. In whiptail lizards of the genus *Aspidoscelis*, using cytology authors demonstrated that the association of sister chromatids in the mature associate is ensured by premeiotic endoreplication of chromosomes (Lutes et al., 2010).

As expected in this sister-chromatids assortment scenario, genomic analyses demonstrated that the offspring of asexual individuals were widely homozygous, with the exception of the Burmese python, *Python molurus bivittatus* (Groot et al., 2003; Jordan et al., 2015). In *P. molurus bivittatus*, all offspring produced are females (ZW). The analysis of microsatellites and 700 Amplified Fragment Length Polymorphism (AFLP), revealed that the genome of the zygote was identical to the mother's genome. Thus, the unreduced gamete must contain an association of non-sister chromatids. In the absence of cytological data, the authors could not determine the underlying mechanisms and assess whether parthenogenesis is due to

premeiotic chromosome doubling, aborted meiosis I or a final fusion of two meiotic products that contain the non-sister chromatid.

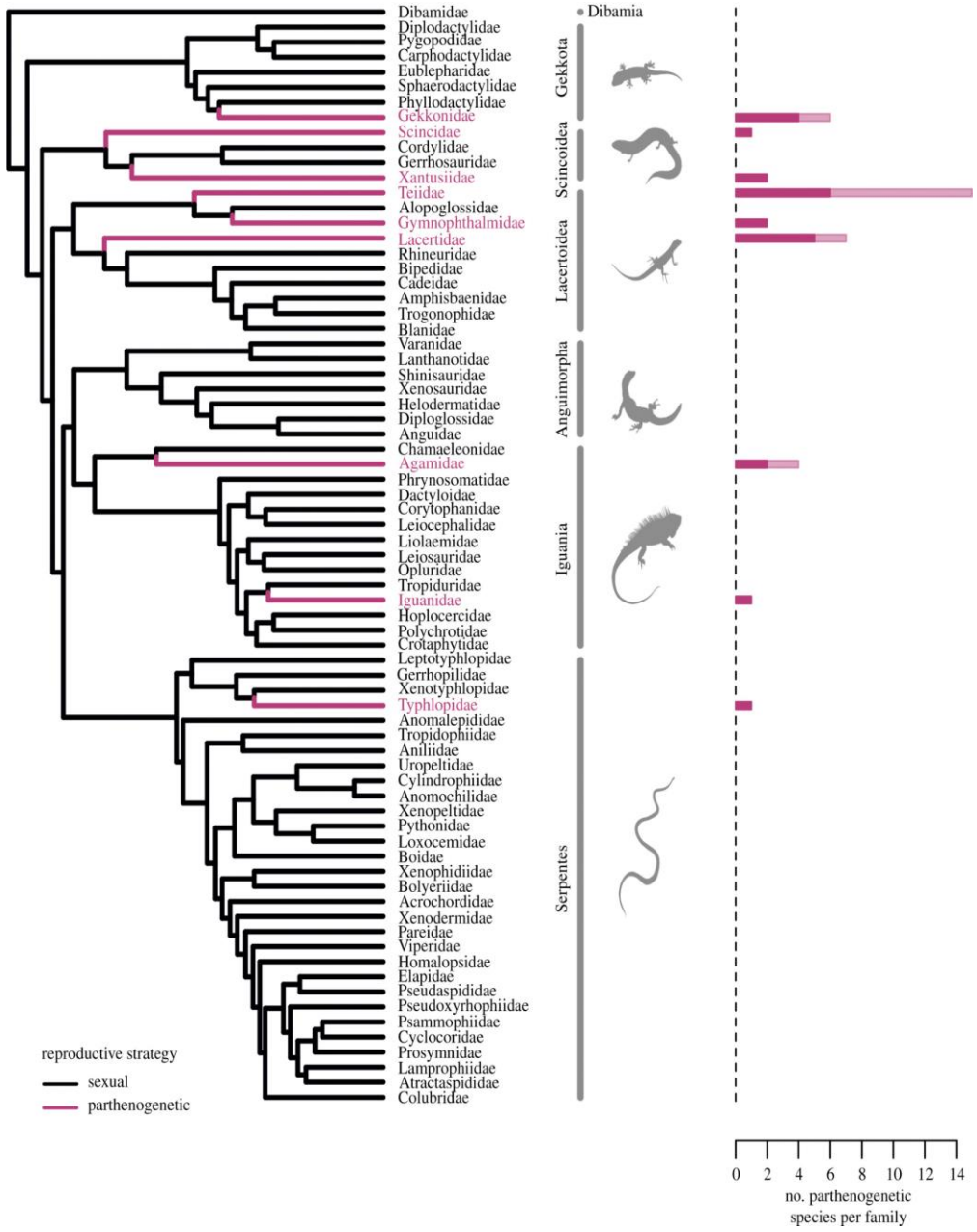

Figure 9: Incidence of true/constitutive parthenogenesis in Squamata. The tree is drawn at the family level, but all analyses were performed at the species level (n = 5388). Coloured edges/tip labels (solid purple) represent families that include parthenogenetic species**.** The bar plot represents the number of parthenogenetic species per family included (solid purple) and the number of described parthenogenetic species per family (transparent purple). Vertical grey lines and silhouettes indicate the seven major clades. (Moreira et al., 2021)

In birds, facultative parthenogenesis is extremely rare and evidences have been found almost only in animals in captivity or in intensive farming: in captive female finches *Taeniopygia guttata* (Schut et al., 2008), in the domestic pigeon Columba livia (Bartelmez & Riddle, 1924), the Chinese painted quail *Coturnix chinensis* (Parker & McDaniel, 2009), in the domestic turkey *Meleagris gallopavo* (M. W. Olsen, 1965; M. W. Olsen et al., 1968; W. W. Olsen & Marsden, 1954) and more recently in the California condors *Gymnogyps californianus* (Ryder et al., 2021). As in Reptilia, birds have a ZW chromosome system where females are heterozygous (ZW), the males are homozygous (ZZ) and (ZWW) is lethal (Bonaminio & Fechheimer, 1993). Only arrhenotokous parthenogenesis has been documented in birds where virgin females give birth to males (ZZ). Therefore, the meiosis that produces parthenogenetic males must result in the association of sister chromatids. To this aim, either meiosis II must be aborted or the two meiotic products containing the two sister chromatids Z must fuse. This hypothesis is supported by the work on Californian condors. Using 21 polymorphic loci, the authors demonstrated that the two parthenogenetic individuals born in captivity were completely homozygous whereas the mother had heterozygous loci (Ryder et al., 2021).

Interestingly, in parthenogenic birds, embryogenesis is rarely initiated or stops during embryological development (Schut et al., 2008). Chicks resulting from parthenogenesis are therefore rare and fertile adults are even scarcer. Nonetheless, studies on Beltsville Small White turkeys demonstrated that eggs produced by parthenogenesis can develop. Moreover, the incidence of arrhenotokous parthenogenesis in poultry turkeys has increased dramatically from 16.7% to 41.5% in five generations (M. W. Olsen, 1965). In the asexual Chinese painted quail, the females produced smaller eggs but increased the size of the clutch (Parker & McDaniel, 2009; F. E. Robinson et al., 1990, 1991). Here, the authors assisted in a genetic selection for parthenogenesis. Nevertheless, it should be noted that this experiment took place in a battery and not in the wild. In a battery, the animals are protected from environmental variations and the immigration of genes from other populations. In this environment, the detrimental effect of certain mutations could be counterbalanced by the presence of shelters or antibiotics.

Facultative parthenogenesis in fish has been documented mainly in chondrichthyans and almost exclusively in elasmobranchs (Chapman et al., 2007, 2008; Feldheim et al., 2010, 2017, 2022; Harmon et al., 2016; Portnoy et al., 2014; D. P. Robinson et al., 2011). The Elasmobranch

subclass consists of 96% rays and sharks. To date, no facultative parthenogenesis has been detected in teleost fish. However, only gynogenesis has been often documented in teleost fish (see Gynogenesis). In chondrichthyan, the homogametic sex is female (XX) and males are heterogametic (XY). In most cases of parthenogenesis in chondrichthyan, the offspring produced are (XX) females. Genetic analysis indicated that the female offspring were largely homozygous (Chapman et al., 2008; Feldheim et al., 2010, 2017; Harmon et al., 2016; Portnoy et al., 2014; D. P. Robinson et al., 2011). As explained in the previous paragraphs, to obtain (XX) females and be homozygous, the unreduced gametes must contain an assortment of sister chromatids. This phenomenon can result from: abortive meiosis II, the fusion of two meiotic products containing sister chromatids or post-meiotic endoreplication but in most cases, the mechanisms are unknown in these species.

Studies on elasmobranchs have been facilitated by the existence of numerous fossils (Carrier et al., 2012). These fossils provide evidence that elasmobranchs have experienced periods of glaciation that were accompanied by bottlenecks in populations. Moreover, the presence of these bottlenecks may have favoured the emergence of facultative parthenogenesis in isolated populations (Dudgeon et al., 2012; Portnoy & Heist, 2012). As parthenogenesis conferred short-term evolutionary advantages, the populations recolonised their environment (Dudgeon et al., 2012).

Gynogenesis/Pseudogamy

Gynogenesis is a form of sperm-dependent parthenogenesis, where oocytes must be fertilised by a sperm cell to generate an embryo. In plants, this mechanism is referenced as pseudogamy. In most species, the sperm is provided by the male of another species. That's why this system is also referenced as sperm-parasitism. As we will see below, auto-pseudogamy has also been described in the nematode from the *Mesorhabditis* genus. Here, both males and females are produced. However, the sperm of males is required for egg activation at fertilization and to provide centrioles but the male genome is not passed on to the females. Therefore, females produce few males that are only needed for the production of more asexual females (Grosmaire et al., 2019; Launay et al., 2020).

In species undergoing gynogenesis, the presence of sperm cells circumvents the mechanical constraints of parthenogenesis, as explained in What is asexuality from a cellular

point of view?**.** Indeed, the sperm carries the centrosomes required for the zygote mitotic cell divisions, can trigger embryo polarization and triggers the completion of meiosis. After fertilisation, the sperm is degraded or set aside, and does not contribute any genetic material to the zygote. Gynogenesis has been described in seven phyla: Chordata, Nematoda, Arthropoda, Mollusca, Plathelmintes and Annelida (Beukeboom & Vrijenhoek, 1998; Galis & van Alphen, 2020). Interestingly in teleosts (Fish) and amphibians, gynogenesis is the only mode of parthenogenetic reproduction reported so far (Lampert & Schartl, 2010).

Gynogenesis has been mainly studied in teleost fishes, nematodes of the *Mesorhabditis* genus and salamanders of the genus *Ambystoma*. Despite the requirement for sperm, systematic pseudogamy can be potentially assigned to strict parthenogenesis. However, because there is a fertilization event in the production of every individual, careful analyses are required to demonstrate that sexual events never occur in pseudogamous species.

In 1932, the Amazon molly, *Poecilia formosa,* was described as the first parthenogenic vertebrate (Hubbs & Hubbs, 1932). Amazon molly is a unisex species with only females. To generate an embryo, *P. formosa* must mate with males of closely related sexual species such as *Poecilia latipunctata* or *Poecilia mexicana* (Joachim & Schlupp, 2012). Later, cytogenetic analyses revealed that the pairing of chromosomes in the females of *P. formosa* is absent and no bivalents are formed. Thus, only the separation of sister chromatids is achieved during meiosis II. Unreduced gametes contain an assortment of non-sister chromatids without the possibility of recombination (Dedukh, da Cruz, et al., 2022). Those observations are consistent with genetic analyses that revealed that the heterozygosity level of *P. formosa* is 10-fold higher than that of close sexual species (Warren et al., 2018). Interestingly studies were conducted on the hybrid offspring of the theoretical ancestor of *P. formosa*, *P. mexicana limantouri* and *P. latipinnaI.* During meiosis of the female hybrid, the two cycles of divisions occur canonically but at the end of the meiosis process, two meiotic products fuse randomly, allowing the production of an unreduced gamete (Lampert et al., 2007). The meiosis processes described in the hybrids is different from the mechanism found in the actual *P. formosa* females but this study nicely illustrates the flexibility of meiosis and the high susceptibility of these species to become asexual.

As with *P. formosa*, the parthenogenetic species of other teleost fishes described so far originate from hybridisation events. Nevertheless, the mechanisms differ according to the species. In *Misgurnus* species, meiosis is preceded by an endoreplication process that doubles

the number of chromosomes entering meiosis (Itono et al., 2006). Whereas in hybrids of *Cobitis elongatoides* and *C. taenia* species, the homologs do not pair during meiosis I, in the same way as in the Amazon molly (Dedukh et al., 2020).

In the amphibians described so far, gynogenesis has evolved only in the salamanders of the genus *Ambystoma* (Elinson et al., 1992). As with teleosts, *Ambystoma* animals undergoing gynogenesis are unisexual and require a sperm donor from another species (*A. laterale, A. jeffersonianum, or A. texanum* (Bogart et al., 2007, 2009). In unisexual *Ambystoma* females, homologous pairing is absent, the offspring possess a set of non-sister chromatids (Elinson et al., 1992).

In nematodes, gynogenesis is widespread in the *Mesorhabidits* genus, is induced and accidental in the *Caenorhabditis* genus, and has been hypothesized in parasitic nematodes belonging to the *Strongyloides* genus (see Cyclic parthenogenesis).

While several species have been described as being gynogenetic in the initial studies by Victor Nigon (Nigon, 1949), only the *Mesorhabditis* genus is currently being investigated because worms have been re-isolated in the wild (Beukeboom & Vrijenhoek, 1998; Grosmaire et al., 2019; Launay et al., 2020; Nigon, 1949; Nigon & Félix, 2018). Among 60 *Mesorhabditis* isolates collected, Delattre's team identified 12 species reproducing by auto-pseudogamy. Phylogenetic reconstruction suggested they most likely derived from a single origin within the *Mesorhabditis* genus (Launay et al., 2020). The details of the reproductive system of *Mesorhabditis* species are discussed in the below section Reproductive System.

Within the *Caenorhabditis* genus, hybridisation can promote gynogenesis. For instance, crosses between *C. nouraguensis* females and *C. becei* males can produce a small fraction of offspring that develop into asexual fertile adults (Lamelza et al., 2019). Th entry of the sperm DNA from *C. becei* triggers the completion of the meiosis in *C. nouraguensis* females. However, the sperm DNA does not fuse with the female DNA. Moreover, meiosis I is aborted and the unreduced gamete is diploid. Here, the *C. becei* sperm cells induce defects in female meiosis but at the same time, the DNA of this sperm is badly segregated during embryogenesis. The majority of hybrids produced are lethal because of aneuploidy, but for a small fraction,

when the sperm DNA is properly set aside, diploid asexual individuals can be produced. This study illustrates how asexuality can emerge from a hybridisation event.

Mesorhabditis belari

Mesorhabditis belari belongs to the same family as *C. elegans*

Nematodes (roundworms) are part of the most widespread phylum of metazoan and account for 82% of the human biomass on Earth (van den Hoogen et al., 2019). Most nematodes measure between 100 µm and 2,500 µm long, although the parasitic worm, in the placenta of sperm whales (*Placentonema gigantissima*), is 8 m long (Carlton et al., 2022). They are subdivided into five clades (Figure 10). Dorylaimia (Clade I) is mainly composed of vertebrate parasites, Enoplia (Clade II) of microbivores, Spirurina (Clade III) of invertebrate and vertebrate parasites, Tylenchina (Clade IV) of microbivores and plant parasites, and Rhabditina (Clade V) of microbivores and vertebrate parasites (Carlton et al., 2022). In 1883, the parasitic nematode *Parascaris equorum* was found in horses (Clade III). Edouard van Beneden first studied its chromosomes and cell divisions, but the parasitic lifestyle of this species complicated the laboratory studies (Van Beneden Edouard, 1883). More descriptions have been made on free-living species in the 40, including the seminal work of Victore Nigon (Nigon, 1949). In 1963, Sydney Brenner found in nematodes an ideal system to study development and the nervous system and opted for *C. elegans* (Clade V) as a model (Brenner, 1988). Whereas the natural environment of this species consists of rotten fruit and litter, this tiny free-living nematode can be easily grown on a petri dish and can be fed with *Escherichia coli*. Their generation time lasts about three days and the strains can be frozen for storage and conservation. The population is mainly hermaphrodite however males are produced at low frequency (<0.2%, (Corsi et al., 2015). *C. elegans* can be grown from 12°C to 25°C. Moreover, they have transparent bodies that facilitate microscopic analysis. *C. elegans* is currently considered one of the most studied models in both behavioural and evolutionary studies, with over 1,200 scientific papers published each year (www.wormbase.org). Indeed, *C. elegans* is extensively studied and serves as a model for the study of cell biology, neurobiology, developmental biology, molecular biology, etc.

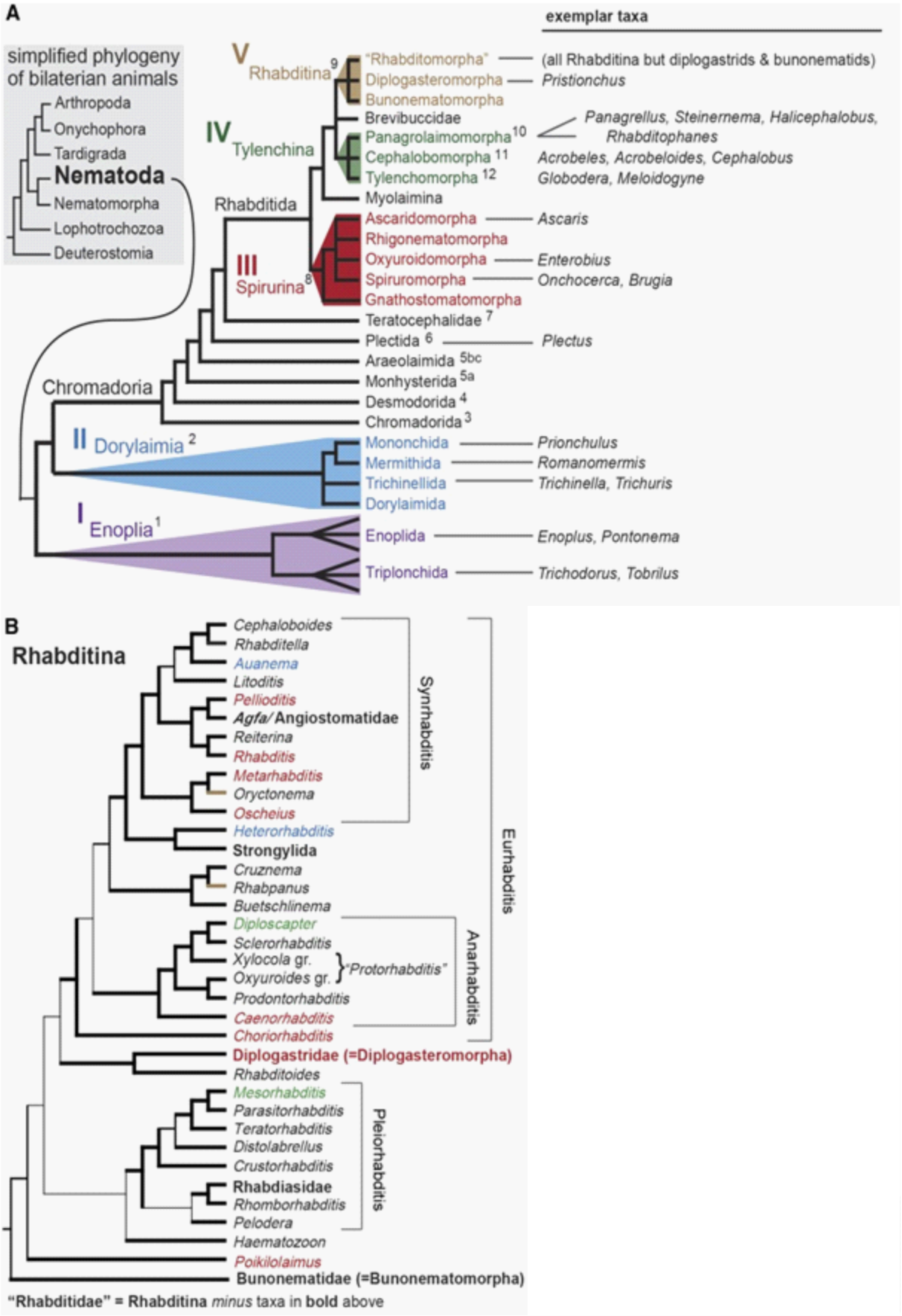
A
exemplar taxa
simplified phylogeny of bilaterian animals
Arthropoda
Onychophora
Tardigrada
Nematoda
Nematomorpha
Lophotrochozoa
Deuterostomia
V Rhabditina 9
"Rhabditomorpha"
Diplogasteromorpha
Bunonematomorpha
(all Rhabditina but diplogastrids & bunonematids)
Pristionchus
IV Tylenchina
Brevibuccidae
Panagrolaimomorpha 10
Cephalobomorpha 11
Tylenchomorpha 12
Panagrellus, Steinernema, Halicephalobus, Rhabditophanes
Acrobeles, Acrobeloides, Cephalobus
Globodera, Meloidogyne
Rhabditida
Myolaimina
III Spirurina 8
Ascaridomorpha
Rhigonematomorpha
Oxyuroidomorpha
Spiruromorpha
Gnathostomatomorpha
Ascaris
Enterobius
Onchocerca, Brugia
Teratocephalidae 7
Plectida 6
Plectus
Araeolaimida 5bc
Monhysterida 5a
Chromadoria
Desmodorida 4
Chromadorida 3
II Dorylaimia 2
Mononchida
Mermithida
Trichinellida
Dorylaimida
Prionchulus
Romanomermis
Trichinella, Trichuris
I Enoplia 1
Enoplida
Triplonchida
Enoplus, Pontonema
Trichodorus, Tobrilus
B
Rhabditina
Cephaloboides
Rhabditella
Auanema
Litoditis
Pellioditis
Agfa/ Angiostomatidae
Reiterina
Rhabditis
Metarhabditis
Oryctonema
Oscheius
Heterorhabditis
Strongylida
Cruznema
Rhabpanus
Buetschlinema
Diploscapter
Sclerorhabditis
Xylocola gr.
Oxyuroides gr.
"Protorhabditis"
Prodontorhabditis
Caenorhabditis
Choriorhabditis
Diplogastridae (=Diplogasteromorpha)
Rhabditoides
Mesorhabditis
Parasitorhabditis
Teratorhabditis
Distolabrellus
Crustorhabditis
Rhabdiasidae
Rhomborhabditis
Pelodera
Haematozoon
Poikilolaimus
Bunonematidae (=Bunonematomorpha)
Synrhabditis
Anarhabditis
Eurhabditis
Pleiorhabditis
"Rhabditidae" = Rhabditina minus taxa in bold above

Figure 10: Phylogenies of phylum Nematoda. (A) Inset shows the phylogenetic position of Nematoda within a very simplified phylogeny of bilaterian animals. Recent molecular studies place Nematoda together with its sister group Nematomorpha as the closest relatives of Panarthopoda (Arthropoda, Onychophora, Tardigrada) in a clade often called Ecdysozoa. The phylogeny of Nematoda has been derived mainly from ribosomal RNA (rRNA) genes and contains several well-defined clades: clades I–V designated in like-coloured roman numerals, taxon names, and polygons; and clades 1–12 designated in black superscripts to corresponding taxon names. Some taxa have been left out here for simplicity. Taxa other than Rhabditina that are mentioned in this review are listed at the right. Taxa in quotation marks are paraphyletic: "Rhabditomorpha" includes all Rhabditina except Diplogasteromorpha and Bunonematomorpha. (B) Phylogeny of Rhabditina (clade V), almost entirely based on molecular data from rRNA and other loci (Kiontke *et al.* 2007; Ross *et al.* 2010; Kanzaki *et al.* 2017). Thickness of the lineages, as indicated in the key at lower right, indicates the approximate level of confidence estimated from statistical tests. The systematics of "Rhabditidae" was recently revised (Sudhaus 2011) based almost entirely on the molecular phylogeny (Kiontke *et al.* 2007) with some consideration of morphological characters to place taxa only known from literature descriptions (brown lineages). A few, mostly monotypic taxa of uncertain position are not shown. Four named suprageneric clades are shown with brackets. Despite being paraphyletic, "Rhabditidae" is a useful taxon because it includes many free-living (rarely parasitic) species with fairly similar Bauplan and excludes three specialized parasitic taxa (Angiostomatidae/*Agfa*, Strongylida, Rhabdiasidae) and Diplogastridae, a clade of species morphologically distinguished from "Rhabditidae" that have undergone an extensive adaptive radiation. *Pristionchus pacificus* and its relatives are included in the Diplogastridae. The "Rhabditidae" sister taxa to each of these special groups provide important resources for investigating the evolutionary origins of parasitism and other specializations that have resulted in adaptive radiations. Colored fonts indicate taxa in which reproductive mode has evolved from gonochorism to hermaphroditism, heterogonism or parthenogenesis (see key at lower right). Taxon names in bold font are at higher levels than the genera otherwise depicted. For more complete information, see RhabditinaDB at rhabditina.org (Haag et al., 2018)

Concerning *M. belari*, the species is for the first time described by Belar in 1923as *Rhabditis monhystera* (Clade V) (Figure 10, 11) (Belar, 1923). It is a free-living nematode, also found in rotten fruits and leaves. In 1949, after more investigations on the tail of males, Victor Niggon attests to the dissimilarity with *R. monhystera* and renamed the species *Rhabditis belari, after Belar's name* (Nigon, 1949). Only in the 1990s were phylogenetic studies and in-depth morphological analyses conducted. Finally, the species belongs to the Rhabditina family, as *C. elegans* does, and does not belong to the *Rhabditis* genus (Félix & Sternberg, 1996). Within the

*Mesorhabditi*s genus, auto-pseudogamous species have been described as well as regular sexual species, producing 50% males and 50% females (Launay et al., 2020).

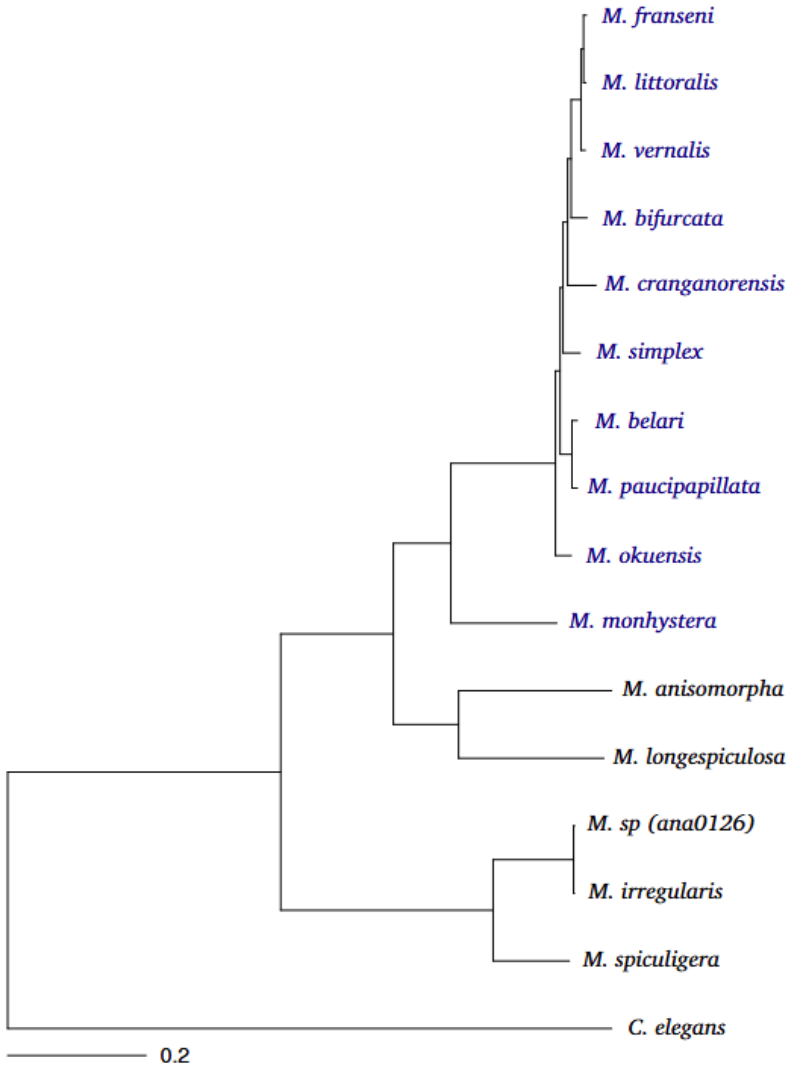

Figure 11: Phylogenetic tree of documented *Mesorhabditis* species. Auto-speudogamous are in blue and sexual species are in black. *Caenorhabditis elegans* is used as the root of the tree. Scale = substitution rate per site

Morphology of *M. belari*

M. belari is 700 µm long and 30 µm wide (Figure 12A). *M.belari* animals have a generation time of about one week and can survive more than two weeks at an optimal growth temperature of 20°C. *M. belari* generates both male and female individuals. Females and males possess only one gonadal arm (Figure 12B) (Félix & Sternberg, 1996). In contrast to the majority of nematodes, the vulva in *Mesorhabditis* females is posteriorly positioned in this species, close to the rectum (Félix & Sternberg, 1996). This morphological characteristic simplifies the identification of species from this genus during sampling campaigns. In *M. belari* females, the gonad is not a syncytium, since all germ cells are cellularized (personnal Observation, Figure 12C, 13 and 18). The female gonad contains about 60 germ cells at a given time, but in total, during its reproductive lifetime, one female can lay up to 100 eggs. The uterus usually contains a single egg, which is often laid soon after fertilisation. The females generally lay one egg per hour. In contrast, males have a syncytial gonad. On fixed specimens, we counted approximately 300 germ cells, including mature sperm cells.

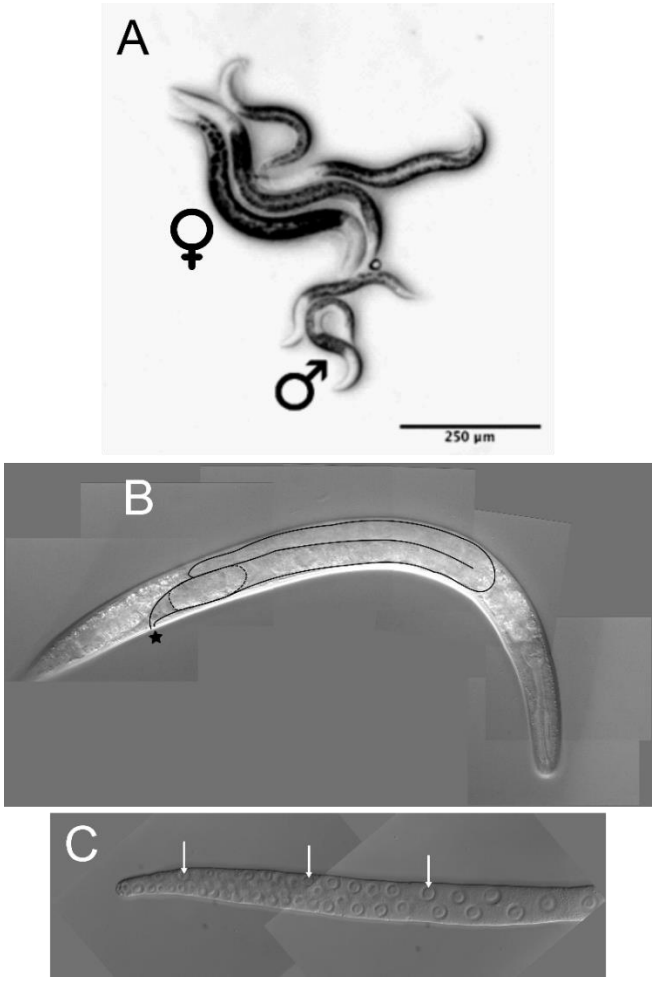

Figure 12: Image of *M. belari* adults. A) Images of female and male adults on a Petri dish. B) DIC picture of a *M. belari* female. The gonad is represented by the solid line, the mature oocyte by a dotted

line and the star shows the position of the vulva. C) DIC image of cellularized gonad of *M. belari* female. Arrows show germ cell nuclei.

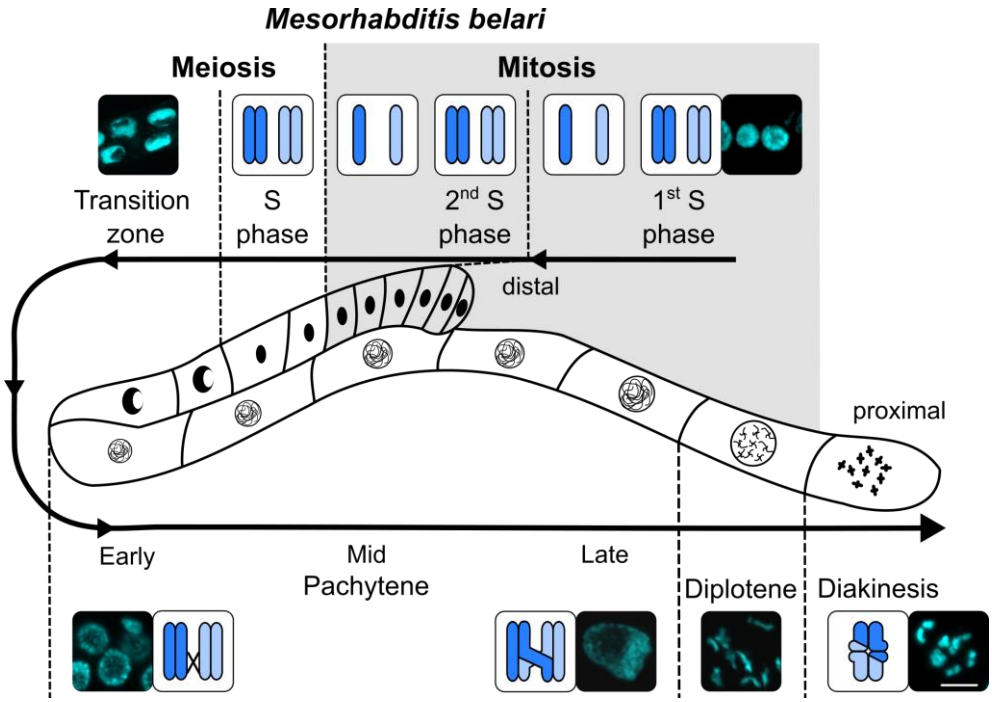

Figure 13: Schematic representation and staining of the gonad of *M. belari* females.

M. belari has 20 holocentric chromosomes (2n=20). After two mitotic phases (as demonstrated in Chapter 2), the oocytes enter meiosis. In prophase I, homologous chromosomes pair in the transition zone and recombine during the pachytene. The bivalents can be observed during the last stage of prophase I, diakinesis, as 10 DNA-stained units. The sister chromatids are schematized in dark and light blue, corresponding to homologous chromosomes.

The reproductive system of *M. belari*

The reproductive system of *M. belari* was analysed for the first time by Belar in 1923. Belar noted the very low proportion of males in the species, which is also stable over time (Belar, 1923). Later, Nigon characterized the cytology of this species in more details (Nigon, 1949). Auto-pseudogamy was later established by the team Delattre (Grosmaire et al., 2019).

In the population of *M. belari*, both females and males are produced (Figure 14). The sex ratio is far from being balanced. Indeed, there are 90% females against only 10% males, but males are essential for reproduction (Belar, 1923; Grosmaire et al., 2019; Nigon, 1949). A single female is not able to produce offspring, because embryos must be fertilised by a sperm.

Nevertheless, in the majority of eggs, the sperm DNA is not used after fertilisation and the embryo will develop as a female, as in pseudogamous species. In 10% eggs, the sperm DNA fuses with the female DNA after fertilisation. These amphimictic eggs will develop as males. Hence, females are produced asexually and males are produced sexually. The males are therefore produced at low frequency but their genes are never transferred to the female gene pool. This reproductive system has been called auto-pseudogamy because the females are produced by pseudogamy and males do no come from another species. The males are produced only for the production of asexual females. A game theory model explained that the production of 10% males maintains a good fitness for the species (Grosmaire et al., 2019). Below, I detail the cellular mechanisms behind this unusual reproductive system, described by the team before I joined the lab.

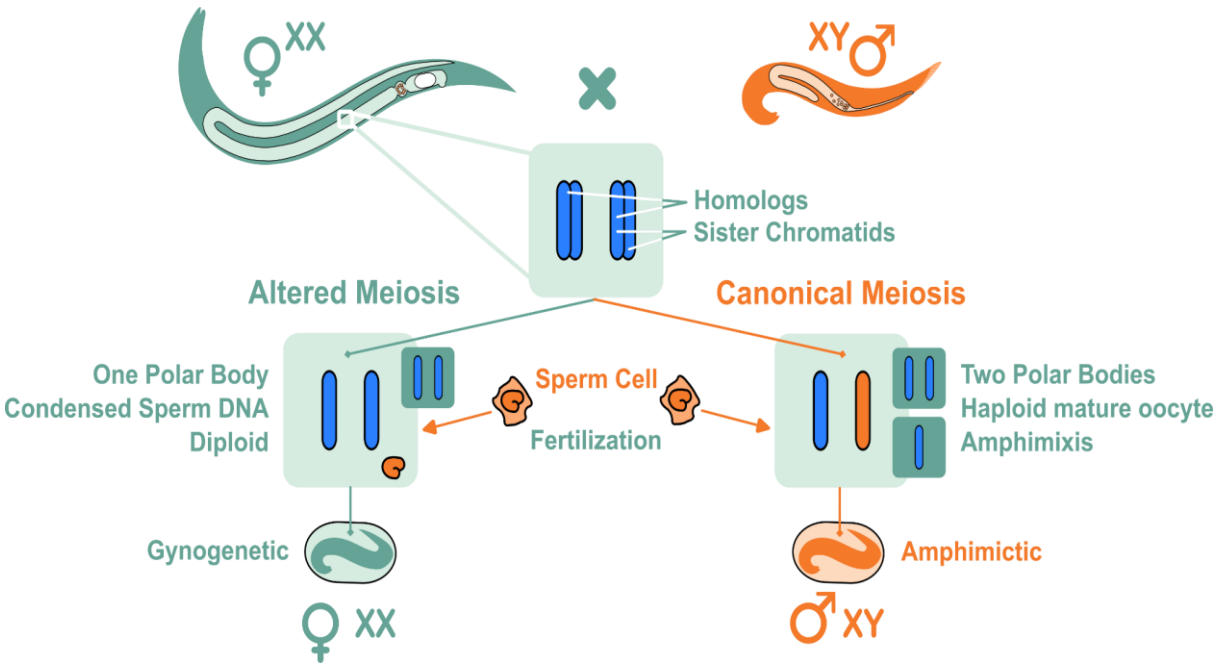

Figure 14: Reproductive system of the auto-pseudogamous species *Mesorhabditis belari*. Males (amphimictic, XY, in orange) are produced sexually. The meiosis that generates the males is canonical and results in the formation of a haploid oocyte and two polar bodies. In amphimictic eggs, fusion with male sperm DNA restores diploidy. Females (gynogenetic, XX, in green) are produced asexually. Female meiosis is modified, only one polar body is produced and the unreduced gamete is diploid. The DNA of the male (in orange) is not used in the female offspring.

All *M. belari* individuals are diploid. During female meiosis, a diploid cell replicates so that all homologs have two chromatids. In nematodes, oogenesis is arrested at the end of

prophase I of meiosis. Meiosis resumes at fertilisation when the sperm cell fuses with the plasma membrane of the oocyte. Meiosis that produces amphimictic oocytes is canonical. The early oocyte undergoes two successive divisions and excludes two polar bodies. Therefore, it contains a haploid genome with only 10 chromatids. After fertilisation in those oocytes, the haploid sperm DNA decondenses and fuses with the maternal genetic DNA, restoring the initial diploidy (Figure 15). The team has shown that *M. belari* possesses an (XY) sex-determining system where females are (XX) and males are (XY). The meiosis in males (XY) is also canonical and males generate two types of sperm cells: 50% bearing the X chromosome and 50% bearing the Y chromosome. Surprisingly, in more than 97% embryos, the sperm DNA that fertilized the eggs carry the Y chromosome. For this reason, amphimictic oocytes only give rise to males (XY). The team genotyped over 1,000 females and none of them was found to be sexually produced. Thus, the fertilization by sperm bearing the X chromosomes (3%) must be lethal. This is consistent with the low proportion of lethal embryos (<5%) quantified in the lab (Grosmaire et al., 2019).

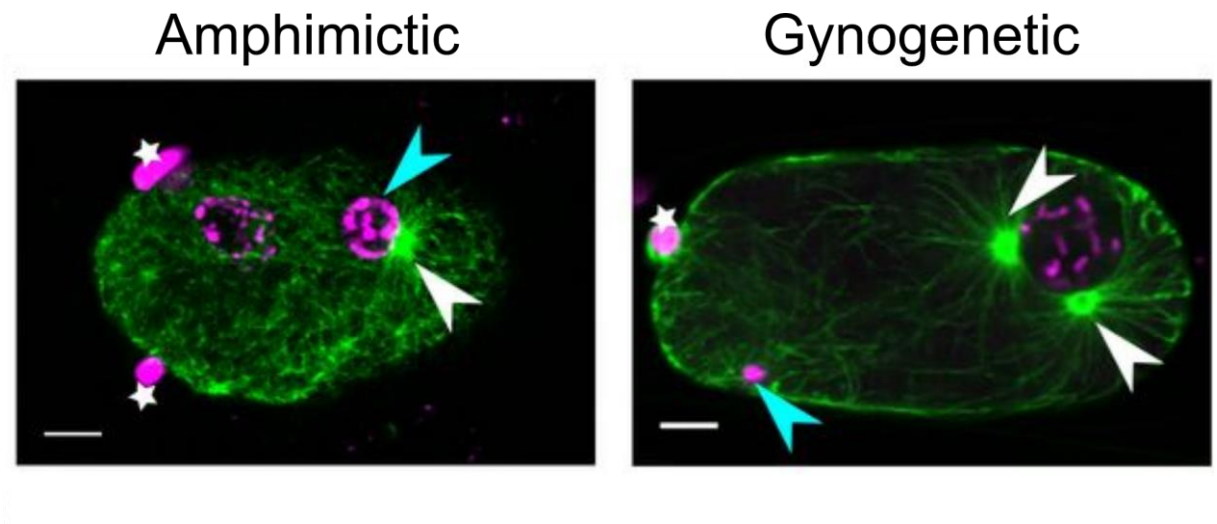

Figure 15: Two types of embryos are produced by *Mesorhabditi*s belari females. Images from a representative amphimictic and a gynogenetic embryo during the reformation of the pronuclei fixed and stained with an anti-tubulin antibody (in green) and with Hoechst to label DNA (in magenta). White arrows heads point towards the centrosomes. Note that in the amphimictic embryo, only one aster is visible on this focal plane. Polar bodies are shown with a white star and male DNA is shown with a blue arrowhead. (Grosmaire et al., 2019)

By contrast, the meiosis that will produce females, i.e. gynogenetic oocytes, is altered compared to a canonical meiosis. Here, only one polar body is extruded and the unreduced gamete remains diploid, with 20 chromatids. Therefore, only one complete division stage of meiotic occurs in gynogenetic oocytes. In these oocytes, fertilisation by the sperm remains essential, otherwise, meiosis does not resume. Also, the sperm contributes the first centrosomes to the zygote (Figure 15). However, the sperm DNA remains condensed after fertilisation and is set aside. Consequently, the zygote develops only from the maternal genome. Even if the sperm DNA carries a Y chromosome, the embryo will develop as a female because it has inherited the two X chromosomes from its mother and does not use the male DNA.

From a cellular perspective, this reproductive system raises many questions. In particular, which step of meiosis is modified in order to produce asexual females? How can a given female switch from the production of regular oocytes (to produce males) to the production of unreduced gametes (to produce females)? How is the coordination between the type of female meiosis and fate of the sperm DNA achieved? Why are Y-bearing sperm so competent at fertilisation compared to X-bearing sperm?

Interestingly, a female produces males throughout her life. Indeed, although young females generate more males than older ones (see Chapter 3), old females still produce both males and females. This means that during oogenesis, the meiotic process can be switched from altered to canonical and vice versa. In this species, modification of meiosis is therefore constrained by the necessity to also produce oocyte through regular meiotic divisions.

Meiosis in the nematode model species *Caenorhabditis elegans*

In order to analyse the meiosis in *M. belari*, I used a lot of analogies with *C. elegans*. Indeed, the two species share a lot of features. In the below section, I describe the meiosis of *C. elegans*.

Nematodes described so far, present the particularity to have holocentric chromosomes. Holocentric organisms have evolved in several plants and animals namely arthropods and nematodes (Heckmann et al., 2014; Mandrioli & Manicardi, 2020). In holocentric species, the centromeres span along the chromosomes instead of being located on a single site as in

monocentric organisms. Centromeres allow attachment of microtubules to chromosomes during all cell divisions. Since they are found throughout the chromosomes, during mitosis the chromosomes move in a straight line perpendicular to the metaphase plate instead of the "V" shaped structure observed in monocentric species.

Another distinction between holocentric and monocentric species is the orientation of chromosomes during meiosis. In meiosis I, sister chromatids must be co-oriented to the same pole to separate only the homologs and not the sister chromatids. In contrast, during meiosis II, in order to separate the sister chromatids, they are oriented towards each of the two poles. This generates a conflict for holocentric species during meiosis. Because centromeres extend along the entire length of the chromosomes, co-orientation of sister chromatids cannot be achieved for all attachment sites to microtubules (Figure 15). To solve this problem, chromosomes are remodelled into a conformation that enables the sequential segregations of chromatids. The homologous pairs display a specific cruciform structure where one axis of the cross separates the homologs and the other one corresponds to the limit between the sister chromatids (Schvarzstein et al., 2010) (Figure 17).

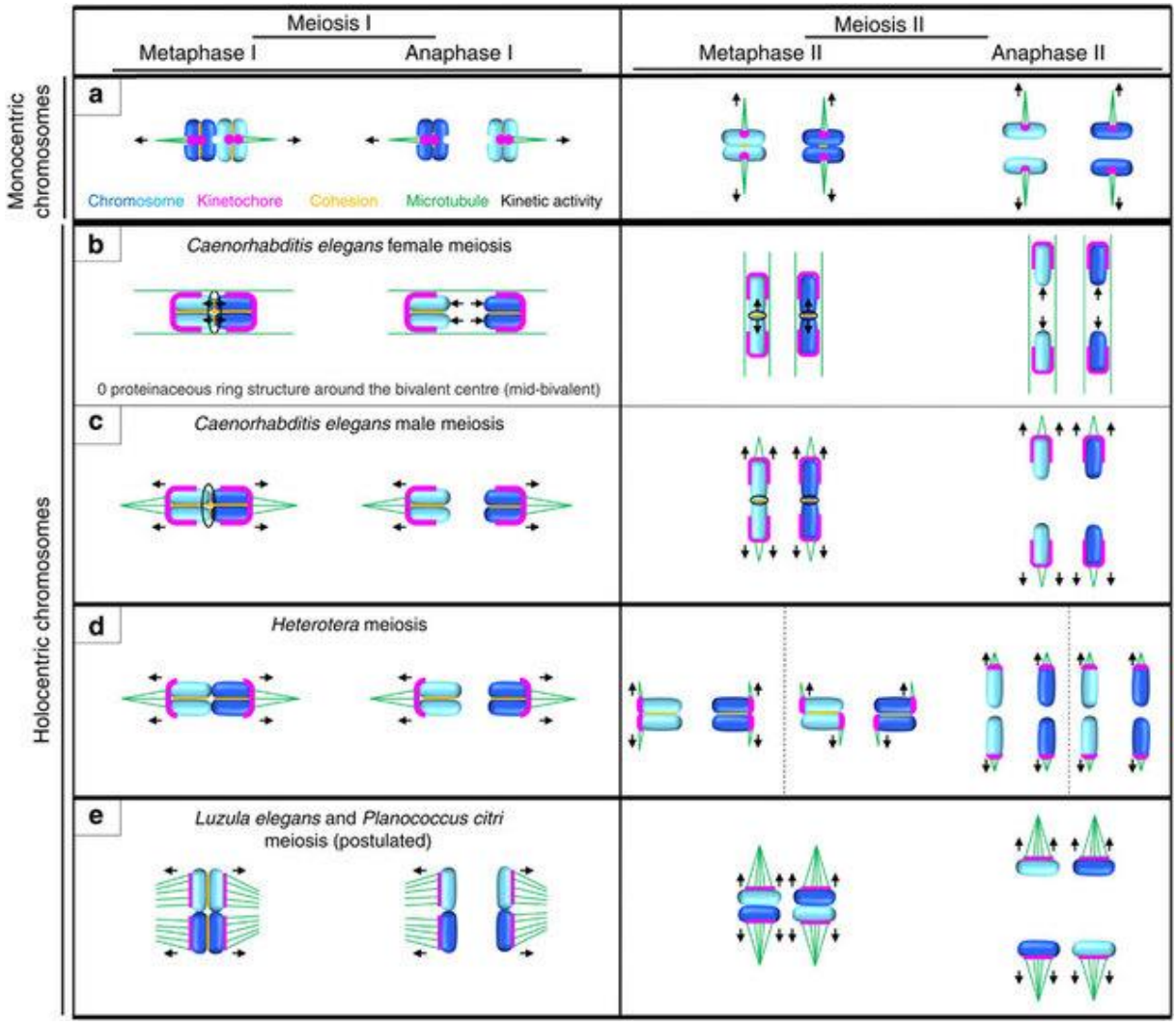

Figure 16: Segregation of monocentric and holocentric chromosomes in meiosis. (a) Monocentric chromosomes: Sister chromatids mono-orient at metaphase I via fused sister kinetochores, allowing homologous chromosome segregation, whereas at metaphase II sister chromatids bi-orient and segregate from each other. Note a ring bivalent configuration of metacentric chromosomes. (b–e) Holocentric chromosomes: Several options exist to deal with a holocentric chromosome architecture and meiosis: (b, c) chromosome remodelling, (d) functional monocentric chromosomes and (e) 'inverted chromatid segregation'. Note that rod-shaped bivalents are shown (b–e), recombination events are not indicated (a–e) and sister chromatids are of the same colour. (b,c) One typically off-centred crossover leads to a cruciform bivalent with a short and a long arm. Owing to progressive condensation, bivalents occur 'rod-shaped' at metaphase I (short and long arms are not indicated in b, c). The crossover location triggers a distinct spatiotemporal protein distribution, for example, proteins including Aurora B or chromokinesin KLP-19 form a ring around the mid-bivalent, and (outer) kinetochore proteins encase each homologue. This distribution conditions cohesion loss at the mid-bivalent and retention at long arms during anaphase I enabling homologue separation. During meiosis II, proteins including KLP-19 surround the ring-shaped sister chromatid interface while (outer) kinetochore proteins encase individual

sisters. At anaphase II, cohesion gets lost at the sister chromatid interface allowing sister chromatid separation. (b) KLP-19 mediates a pushing force from the mid-bivalent supported by lateral microtubules ensheathing bivalents during meiosis I and linked sister chromatids during meiosis II. (c) Bivalent ends facing polewards are attached by microtubules. (d) Microtubule attachment to a restricted terminal chromosomal region. Holocentric chromosomes become functional monocentric ("telekinetic") enabling separation of homologues during meiosis I and of sister chromatids during meiosis II. Active sister kinetochores can form even at opposite metaphase II chromosome termini. (e) Homologous sister chromatids, attached by microtubules along nearly their entire lengths, face opposite poles during metaphase I and separate from each other at anaphase I. Homologous non-sister chromatids are joined by metaphase II and separate at anaphase II. Hence, an inverted meiotic chromatid segregation sequence compared to the typical meiotic segregation pattern in monocentric chromosome species occurs. (Heckmann et al., 2014)

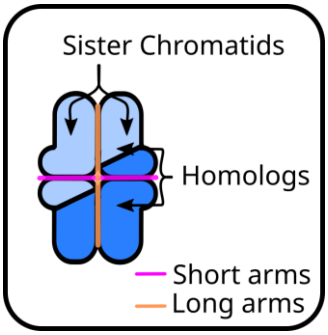

Figure 17: Schematic representation of a bivalent in holocentric species. Axis of the short arms (in pink) separates the sister chromatids. Axis of the long arms (in orange) separates the homologous chromosomes. The sister chromatids are in dark and light blue, corresponding to homologous chromosomes.

C. elegans meiosis has been extensively investigated and is still largely studied today (www.wormbase.org). Indeed, in nematodes, the study of meiosis is convenient because of the following advantages: 1) nematodes have transparent organisms whose gut and gonad are easily identifiable, which facilitates observations and 2) nematodes have an extremely stereotyped gonad: the spatiotemporal stages of meiosis are conserved from an individual to another 3) the different stages of meiosis have a distinct appearance.

C. elegans have two syncytial gonads containing more than 400 germ cells. In the more distal part (from the vulva where eggs are laid once fertilised) are the proliferative germ cells (Figure 18). After a last round of replication these cells enter meiotic prophase. At the beginning of meiosis, the oocytes are not cellularized. Cellularisation is completed when the oocytes reach the last stage of prophase I, the diakinesis. In prometaphase I, meiosis arrests. Once the oocytes pass into the spermatheca, fertilisation is achieved and meiosis resumes. In *C. elegans*, embryos are stored in the uterus before being laid at ~40 cell stage.

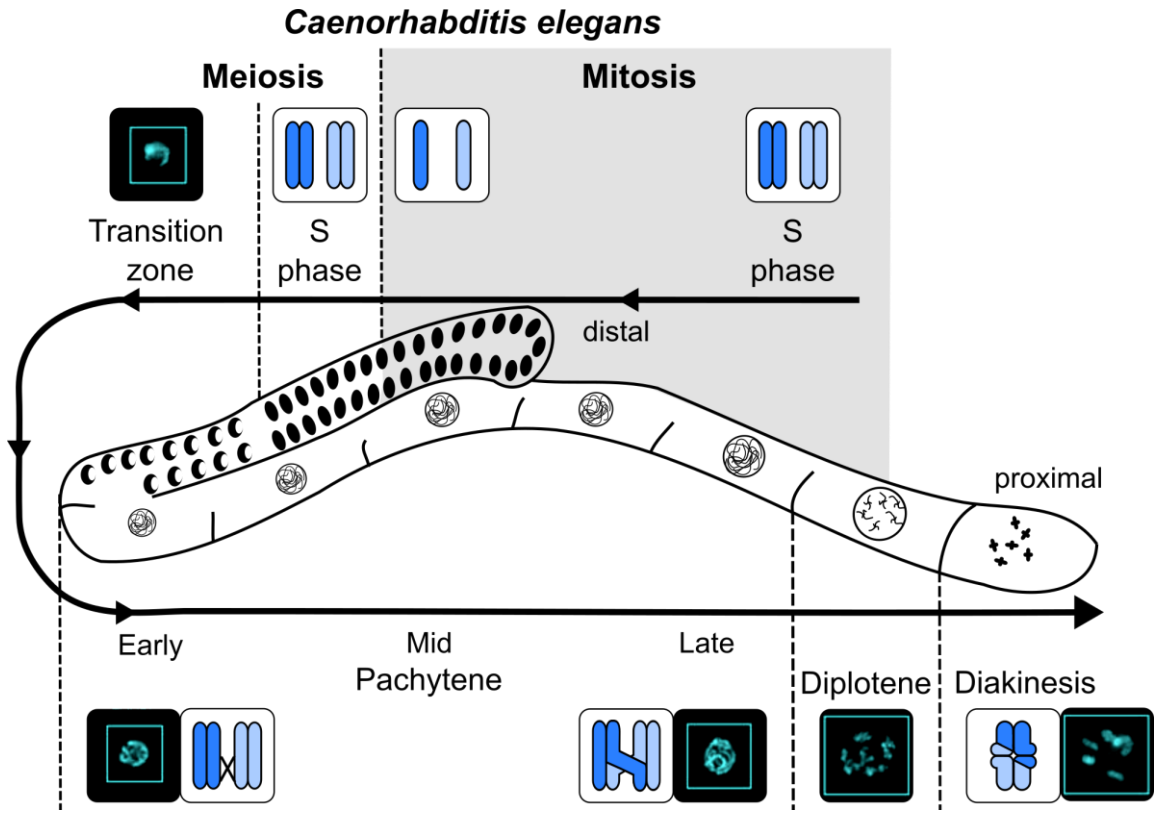

Figure 18: Schematic representation and staining of the syncytial gonad of *C. elegans*. *C. elegans* has 6 chromosomes (2n=12). After a mitotic phase, the oocytes enter meiosis. In prophase I, homologous chromosomes pair in the transition zone and recombine during the pachytene. The 6 bivalents can be observed during the last stage of prophase I, diakinesis. The sister chromatids are in dark and light blue, corresponding to homologous chromosomes.

Meiosis I

Prophase I

Meiosis is preceded by an event of chromosome replication. Hence, after replication, each homolog has two chromatids (4C in total). Meiosis I, and thus prophase I begins with a key event of meiosis, the pairing of homologs during the leptotene/zygotene stage. Pairing is followed by another crucial event: recombination during pachytene. This recombination event generates genetic shuffling but also creates the bivalent which is a chromosomal structure necessary for the proper segregation of homologs. In *C. elegans* the short arms distinguish the junction between homologs (in pink in Figure 16) while the long arms separate the sister chromatids (in orange in Figure 16). The association between sister chromatids are called univalents.

Pairing

In *C. elegans*, the pairing occurs during the leptotene and zygotene stages of prophase I, also referenced as the croissant stage due to the shape of chromosomes or also called the transition zone. In this species, as in female *Drosophila melanogaster* for instance (McKim et al., 1998) double-strand breaks (DSBs) induced by SPO-11 are not required for the synapsis and pairing as is found in *Saccharomyces cerevisiae*, *Danio rerio* or *Mus musculus* (Rubin et al., 2020). A recent study has shown that the nematode *Pristionchus pacificus*, close to *C. elegans*, relies on SPO-11 for synapsis, suggesting rapid evolution in meiosis within the Rhabditina family (Rillo-Bohn et al., 2021).

In *C. elegans*, pairing is ensured by a specific sequence found on each of the six chromosomes called the "pairing center" (MacQueen et al., 2005). Each PC is associated with one of four of the PC proteins (PCs): C2H2 zinc finger proteins (HIM-8, ZIM-1, ZIM-2, ZIM-3). The chromosomes I and IV bind to ZIM-3, the chromosomes II and III to ZIM-1, while ZIM-2 only binds to chromosome V and HIM-8 to the X heterochromosome (Harper et al., 2011; Phillips et al., 2005; Phillips & Dernburg, 2006; Sanford & Perry, 2001). The search of homologs requires chromosome movements along the nuclear envelope (Baudrimont et al., 2010; Sato et al., 2009). These movements are achieved by a protein complex (Figure 19) that links chromosomes to dyneins located on microtubules in the extranuclear region of the cell (Wynne et al., 2012). This complex is formed of inner and outer nuclear envelope proteins, and PCs. After the PCs bond the chromosomes, these PCs nucleoprotein complex are used as

recruitment sites for the polo kinase PLK-2 (Labella et al., 2011). And, PKL-2 induces an organisation of the nuclear envelop. The polo kinase recognizes the inner protein of the nuclear envelope SUN-1 which interacts with the outer proteins of the nuclear envelope ZYG-12. In turn, ZYG-12 connects to dynein and hence to the cytoskeleton of the cell. The complex SUN-1 ZYG-12 forms SUN/KASH interaction, broadly conserved in eukaryotes (Baudrimont et al., 2010 (Malone et al., 2003). Once the homologs have been correctly recognized and matched, the pairing is stabilized by the formation of the synaptonemal complex (SC).

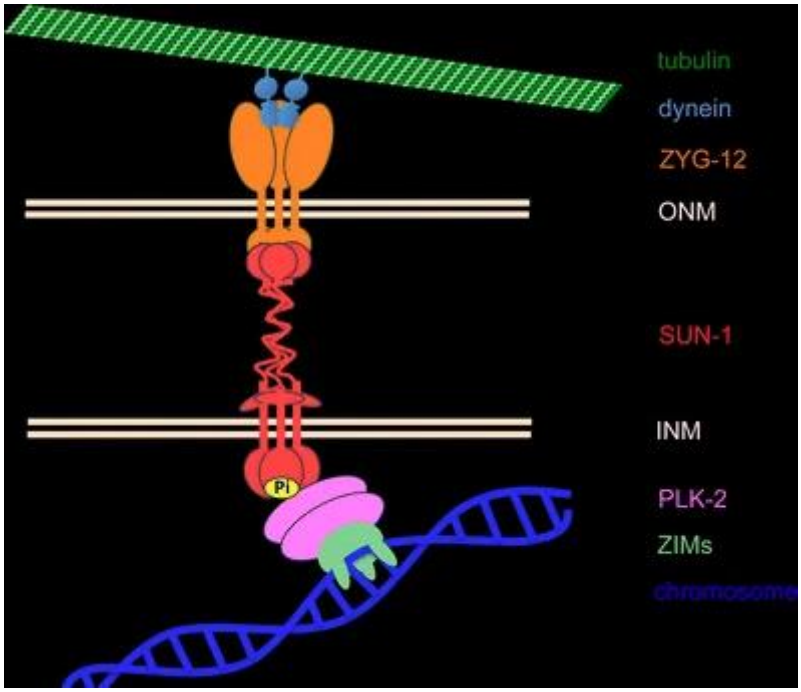

Figure 19: Schematic diagram of the SUN-1/KASH bridge spanning the nuclear membranes. The SUN-1/KASH bridge spans the outer and inner nuclear membranes (ONM and INM). ZYG-12/KASH connects the molecular motor dynein whereas SUN-1 indirectly binds to the chromosomes via the pairing center proteins (ZIMs) and PLK-2. (Woglar & Jantsch, 2014)

The Synaptonemal Complex (SC)

During pachytene, the homologous chromosomes are further linked by the synaptonemal complex (SC). The SC is a tripartite protein complex forming a ladder from a microscopic perspective (Figure 20B). This meiosis-specific complex is universal as it is found in yeast, mammals, and plants (Gillies, 1975; Gillies & Moens, 1984). The SC is composed of two lateral parts, two axial parts and one central part, in between the two homologous

chromosomes (Figure 20A). On each side, the homologs comprise two sister chromatids that are held together by the cohesin COH-3/-4 and REC-8 (Köhler et al., 2017; Pasierbek et al., 2001; Severson et al., 2009; Severson & Meyer, 2014).

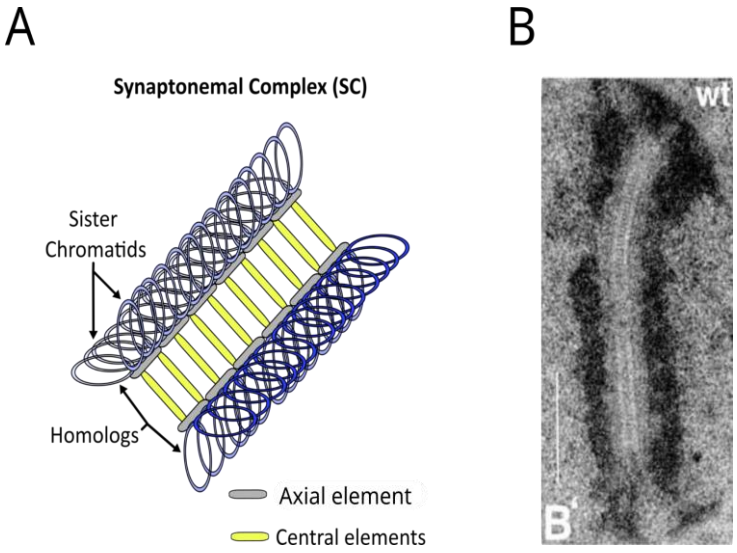

Figure 20: The ladder structure of the synaptonemal complex (SC) in *C. elegans*. A) schematic representation of the SC. The sister chromatids are in dark and light blue, corresponding to homologous chromosomes. Chromatids constitute the lateral element. Central elements are in yellow and axial elements are in grey. B) Transmission electron microscopy image of nuclei from the late pachytene regions of a wild-type germ line. Scale bars equal 500 nm (Colaiácovo et al., 2003)

The axial parts of the SC are constituted of four HORMA domain proteins (Figure 20) (HTP-1, HTP-2, HTP-3 and HIM-3) and the cohesins previously cited (Woglar et al., 2020; Zetka et al., 1999). The HORMA domain is able to recognise a specific chromatin state. Those proteins can be distinguished biochemically and structurally, except for, HTP-1 and HTP-2 which share 82% identity at the amino acid level and cannot be differentiated by antibody staining (Martinez-Perez & Villeneuve, 2005). Furthermore, meiosis can be accomplished in the absence of HTP-2, suggesting the function of HTP-2 overlaps those of HTP-1. For these reasons authors usually reference HTP-1 and HTP-2 proteins as the paralogs HTP-1/-2. HTP-3 is a scaffolding protein which recruits HTP-1/-2 and HIM-3 (Figure 21) (Köhler et al., 2017). In vitro experiments show that HTP-1/-2 is recruited on the HTP-3 long tail and in the C-terminal region of HIM-3. While HIM-3 binds to a more central site of HTP-3 than HTP-1/-2.

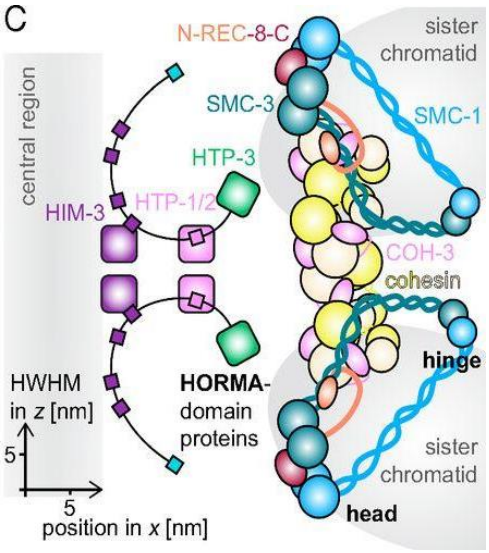

Figure 21: Model of synapsed chromosome axes in cross-sectional view. The positions from the midline of the SC in x and the HWHM in z of components within the chromosome axes measured by STORM. These positions were used to construct a model of the synapsed chromosome axis. (Köhler et al., 2017)

Cohesins are protein complexes that hold together the sister chromatids. Cohesins are composed of two parts: the head and the hinge. In meiosis or mitosis, the head always consists of the same two SMC (Structural Maintenance of Chromosomes) proteins: SMC-1 and SMC-3. The hinge differs according to the type of kleisin subunit present: SCC-1 ensures sister chromatids holding in mitosis while REC-8 and COH-3/-4 are found only in meiosis (Figure 22, 23 and 25). REC-8 is the most common meiotic cohesin (Morgan et al., 2022; Sakuno & Hiraoka, 2022). And various species such as budding and fusion yeasts, REC-8 is only cohesin present. In these species, the sequential removal of REC-8 is ensured by Sungoshin (Morgan et al., 2022; Sakuno & Hiraoka, 2022).

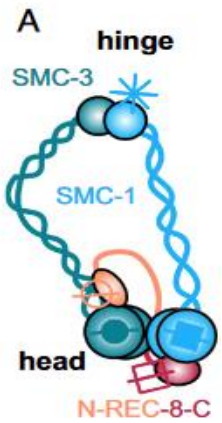

Figure 22: A schematic of the putative cohesin ring complex structure of REC-8 cohesins. (Köhler et al., 2017)

Furthermore, REC-8 and COH-3/-4 have distinct roles in *C. elegans* meiosis. REC-8 is present during the replication phase and maintains the sister chromatids together. While the role of REC-8 is well-defined, those of COH-3/-4 is still unclear. COH-3/-4 kleisin and hence COH-3/-4 cohesin hold sister chromatids together but also hold the homologs together via the synaptonemal complex. Indeed, in absence of REC-8, some univalents are tethered together by the COH-3/-4 cohesin complex (Castellano-Pozo et al., 2022; Severson & Meyer, 2014). COH-3/-4 kleisin and hence COH-3/-4 cohesin hold homologs together via the synaptonemal complex. The meiotic cohesins are essential to SC assembly (Severson & Meyer, 2014). The central part of SC is constituted of SYP-1, SYP-2, SYP-3 and SYP-4 proteins (Colaiácovo et al., 2003; MacQueen et al., 2005; Smolikov et al., 2007, 2009). These proteins have long helical domains and form transverse filaments that connect the axial elements of paired homologs. Once the SC is assembled and stabilized, the homologous recombination occurs during pachytene. The SC is disassembled after homologous recombination: only the proteins in the centre (SYP-1, SYP-2, SYP-3 and SYP-4) are removed. Thus COH-3/-4 controls a higher structure of chromatin than sister-sister cohesion, hypothetically by acting in the extrusion loop. Moreover, the COH-3/-4 complex can be only detected when cells enter meiotic prophase whereas REC-8 is detected from the mitotic S-phase (Severson et al., 2009). In prometaphase, the two cohesins have distinct localization: REC-8 is found only between sister chromatids on the long arms of the bivalent, while COH-3/-4 localize only between homologs on the short arms (Figure 23) (Castellano-Pozo et al., 2020, 2022; Severson & Meyer, 2014).

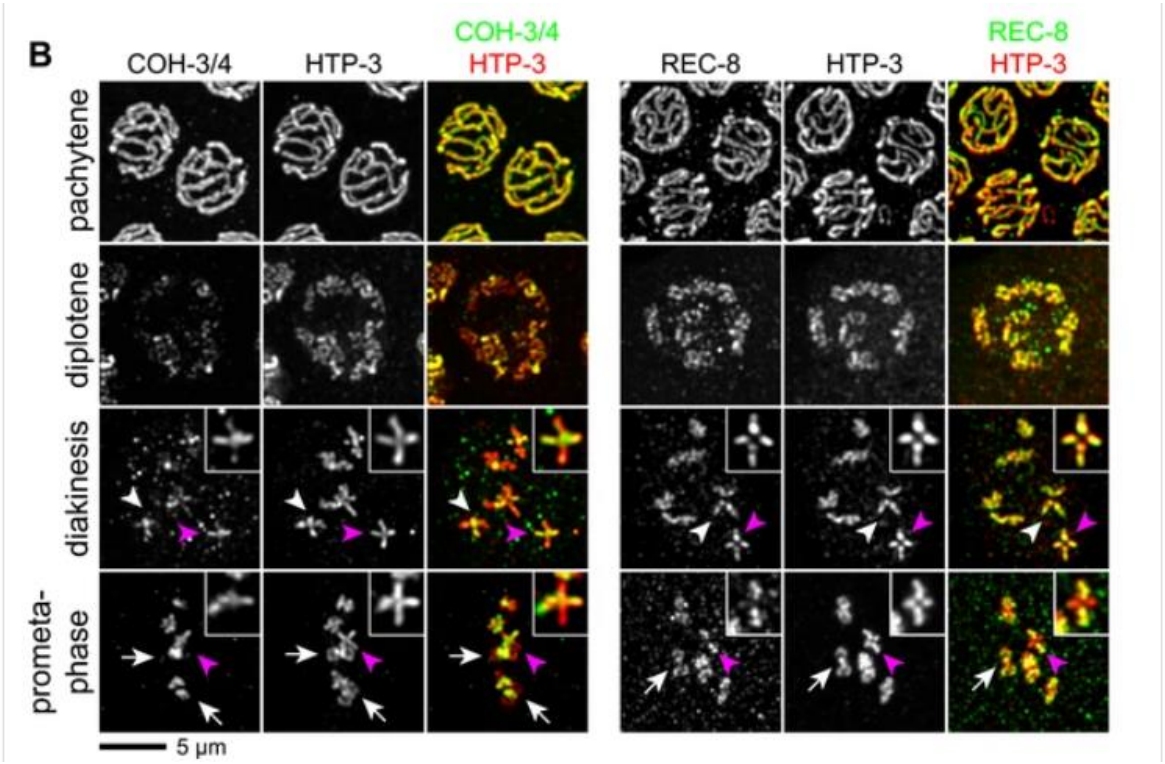

Figure 23: Confocal micrographs showing REC-8 and COH-¾ localization in prophase I and metaphase I. REC-8 and COH-3/4 adopt complementary patterns on meiotic chromosomes by metaphase. In pachytene, REC-8 and COH-3/4 overlap with HTP-3 along the entire meiotic axis. In diplotene, HTP-3 and REC-8 persist along the length of the axis, but COH-3/4 staining diminishes at long arms. By diakinesis, COH-3/4 levels are substantially reduced at long arms but not at short arms. In contrast, REC-8 levels usually remain equal at long and short arms until late diakinesis or prometaphase. Diakinesis nuclei shown are from the third oldest oocyte. In prometaphase/metaphase I, REC-8 and COH-3/4 occupy reciprocal domains. REC-8 is reduced or undetectable at short arms, while COH-3/4 is detectable only at short arms. Arrowheads indicate bivalents viewed from the 'front', that is with both long and short arms in the image plane. In these bivalents, HTP-3 staining is cruciform and long and short arms can usually be distinguished by their relative lengths. Pink arrowheads indicate the bivalent shown at higher magnification in the inset. Arrows indicate bivalents viewed from the 'side', that is with short arms perpendicular to the image plane. In these bivalents, HTP-3 staining resembles a 'figure 8', with two loops of uniform staining (the long arms) meeting at a region of more intense staining (the short arms). (Severson & Meyer, 2014)

Homologous Recombination

During the pachytene stage homologous recombination occurs. Here, I first describe the general process of homologous recombination, which is highly conserved (Haber, 1999). It is first initiated by multiple DSBs along all chromosomes, induced by the highly conserved

topoisomerase, SPO-11 (Figure 24). Subsequently, the exonuclease MRE-11 resects the DNA ends to generate long 3' ssDNA tails which are then coated with RAD-51 (Z. Yu et al., 2016). The RAD-51 nucleofilament promotes homology search and strand invasion into the homologous duplex DNA to form the displacement loop or D loop. The D-loop is a DNA loop where the two dsDNA strands are separated by the invasion of a third strand and DNA. After the D loop DNA formation, RAD-51 is removed by RAD-54, RFS-1 and HELQ-1/HEL308. Then the DNA synthesis begins on the two resected strands. To generate cross-over, the D loop has to stabilize and form a more complex structure: the double Holliday junctions. Holliday junctions are DNA structures formed of four strands of DNA. In absence of Holliday junctions, D loops will result in synthesis-dependent strand annealing (SDSA) pathway. This leads to a cross-over (CO) in which part of one homolog has invaded one of the chromatids of its corresponding homolog. The SDSA thus leads to a local homogenisation of the homologs through gene conversion. In *C. elegans*, the CO designation is reinforced by COSA-1, a cyclin-like protein. Therefore, the enumeration of COSA-1 foci under the microscope is a good proxy for estimating the number of COs (Bohr et al., 2018; Cahoon et al., 2019; Crawley et al., 2016). Finally, the junctions must be cleaved to resolve the double Holliday junctions into mature CO products. This function is ensured by the scaffolding protein SLX-4/HIM-18 joined by the resolvase XPF-1, with HIM-6/BLM helicase, and the resolvase SLX-1/MUS-81.

In *C. elegans*, there is only CO per chromosome pair. Indeed, in this species, the presence of CO is ensured on the six chromosomes. The phenomenon is known as CO assurance (Hillers et al., 2018). If there is no CO, the bivalents are not stabilized and cannot be properly segregated during the first meiotic division. Moreover, a mechanism of CO interference prevents the establishment of more than one CO per chromosome. While the underlying mechanistic is still unclear, the partial depletion of the central element of SC such as SYP-1 or inhibition of phosphorylation of SYP-4 results in increasing the number of CO (Láscarez-Lagunas et al., 2022; Libuda et al., 2013). In addition, interference operates over the entire length of *C. elegans* chromosomes (Libuda et al., 2013). Here again, double CO destabilize the bivalents and leads to aneuploidy (Dernburg et al., 1998). It has been suggested that the presence of one and only one CO per chromosome is a constraint due to holocentricity (Mandrioli & Manicardi, 2020).

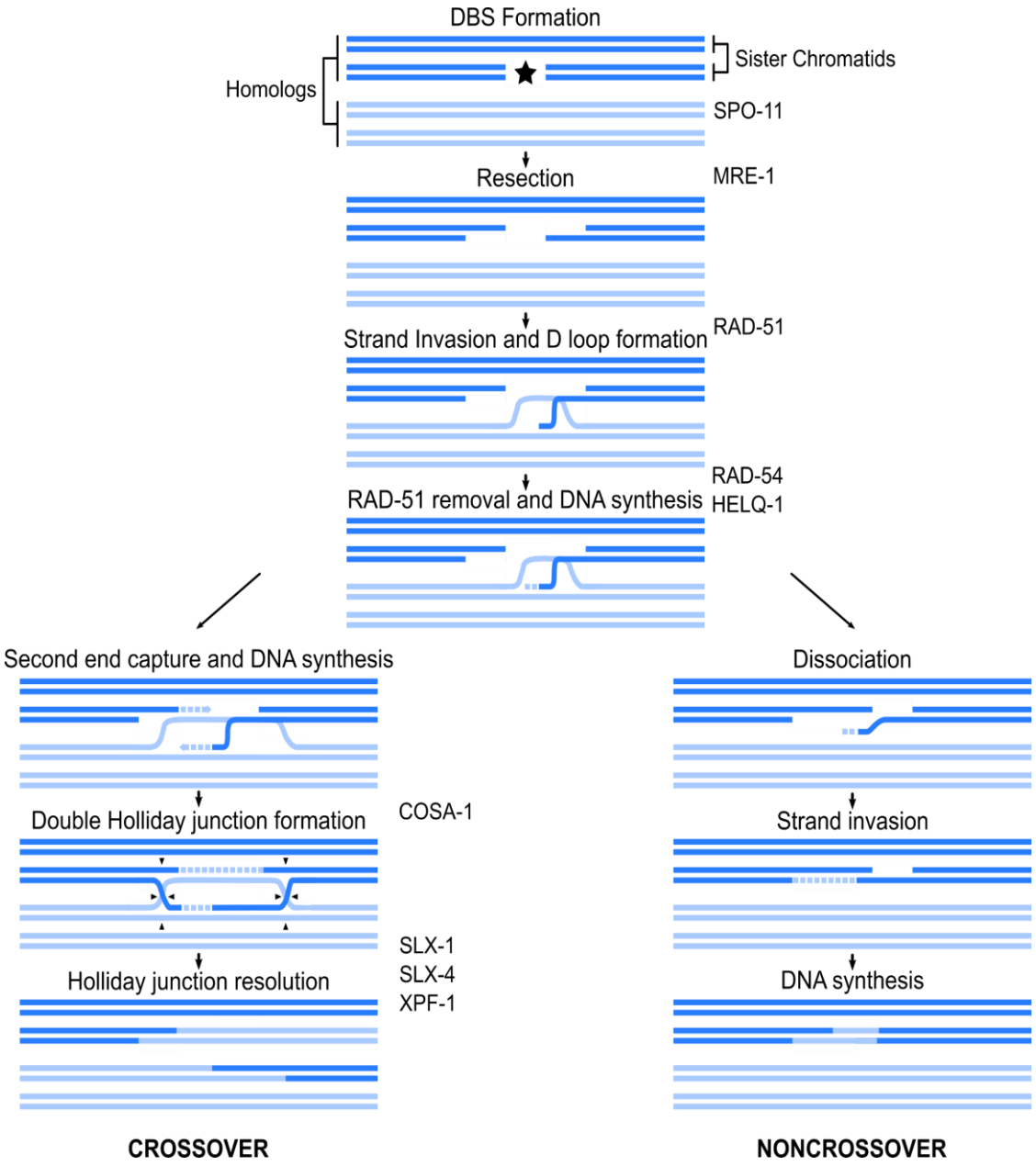

Figure 24: Model depicting the key events of meiotic recombination. Homologous chromosomes are represented in light and dark blue, and the two sister chromatids of each homolog are represented as pairs of double stranded DNA molecules indicated by two parallel lines in close proximity. Proteins required for specific steps of meiotic recombination are indicated on the left and right hand side of the diagram. Note that the existence of some of the recombination intermediates represented in this diagram has not been directly demonstrated in *C. elegans*, but are inferred from studies in yeast. The molecular events of recombination are represented in temporal progression starting at the top of the diagram with the formation of a DSB by SPO-11 in a single chromatid of one of the homologs. Resection of DNA ends and RAD-51 loading promote the invasion of a chromatid from the homologous chromosome, the formation of a D loop, and the start of DNA synthesis. These intermediates can be destabilized and leads to repair as NCO products, or they can be stabilized by CO promoting factors that promote the formation

of double Holliday junctions. Note that although COSA-1 is required for CO formation and eventually become associated with CO-fated recombination events, there are clear differences in the timing of loading of these proteins that are not depicted in this model. The asymmetric cleavage of double Holliday junctions by different endonucleases promotes the formation of inter-homolog CO events. (Adapted from Hillers et al., 2018).

Diakinesis and formation of bivalents

After homologous recombination in pachytene, the paired and recombinant chromosomes condense in diplotene (Figure 25). Condensation is completed in diakinesis and six bivalents are observed. At diakinesis the bivalents have a very characteristic shape in in holocentric species. Indeed, during this stage, the resolution of the unique CO lead to the formation of a cross-shaped structure of chromosomes. This structure cannot be formed in the absence of CO or the existence of more than one CO on the chromosome. This bivalent structure is composed of two axes: the short arm that separates the homologs and the long arm that lies along the SCs. Interestingly, HORMA domain proteins are not equitably maintained along the chromosomes (Figure 20). HTP-3 and HIM-3 are found on both axes while antibody against HTP-1, which stained the paralogs HTP-1/-2 is located only between sister chromatids along the long arms of the bivalents. Similarly, as we have seen before, COH-3/-4 cohesins are located on the short arms, between the homologs, while REC-8 cohesins which are initially found on both axes, become restricted to the long axis (Figure: 23) (Severson & Meyer, 2014).

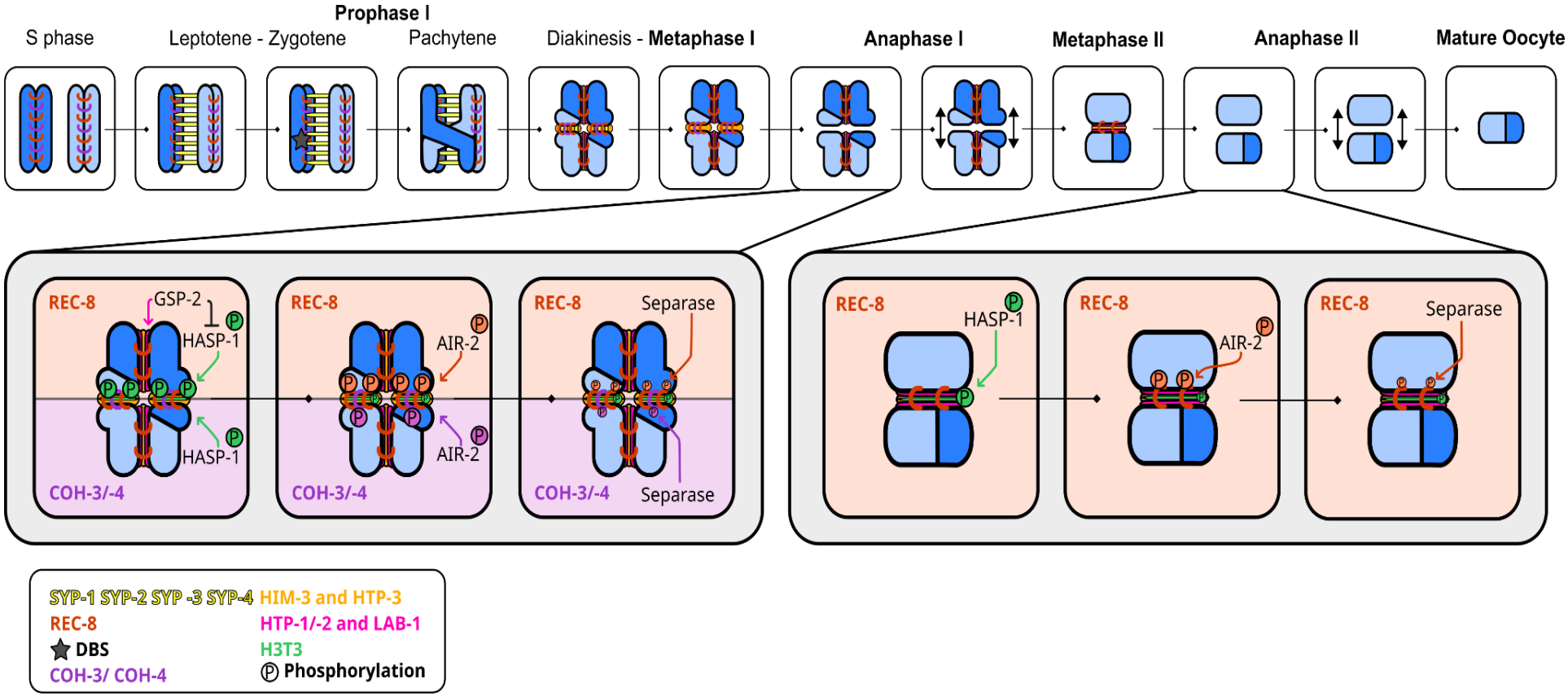
Prophase I
S phase
Leptotene - Zygotene
Pachytene
Diakinesis - Metaphase I
Anaphase I
Metaphase II
Anaphase II
Mature Oocyte
REC-8
GSP-2
HASP-1
COH-3/-4
AIR-2
Separase
SYP-1 SYP-2 SYP -3 SYP-4
HIM-3 and HTP-3
REC-8
HTP-1/-2 and LAB-1
DBS
H3T3
COH-3/ COH-4
Phosphorylation

Figure 25: Model of chromosomes pairing and segregation during *C. elegans* meiosis. Homologous chromosomes are represented in light and dark blue, and the two sister chromatids of each homologous chromosome by the same blue shade. In the S phase, chromosomes replicate and REC-8 (red rings) and COH-3/-4 (purple rings) hold the sister chromatids together. The Synaptonemal Complex (SC) forms during the leptotene/zygotene stage. SYP-1, SYP-2, SYP-3 and SYP-4 (yellow) are found in the central part of the SC. After DBS (black star) recombination occurs in pachytene. Chromosome remodelling generates the bivalent structure in diakinesis (see Figure 16). In diakinesis, HTP-1/-2 (pink), HIM-3 (light orange), HTP-3 (light orange) and REC-8 localise on the long arms, while HIM-3, HTP-3, COH-3/-4 and REC-8 are found on the short arms. In anaphase I, HASP-1 phosphorylates H3T3 (green) only on the short arms. On the long arms, phosphorylation of H3T3 is antagonised by GSP-2 which is recruited by LAB-1. The kinase AIR-2 then phosphorylates COH-3/-4 and REC-8 of the short arms allowing the Separase to cleave the cohesins. The cleavage of cohesin releases the cohesion between the homologous chromosomes. In metaphase II, HTP-1/-2, HIM-3, HTP-3 and REC-8 are found between the univalent. As in anaphase I, in anaphase II, H3T3 is phosphorylated and AIR-2 phosphorylates REC-8 allowing the cleavage of the cohesins by the Separase.

Prometaphase and metaphase I

The transition from diakinesis to metaphase is ensured by numerous checkpoints such as the maturation-promoting factor (MPF) CDK-1 and cyclin B (Dorée & Hunt, 2002). Prometaphase and metaphase I, are the meiotic stages where the bivalents align to be correctly positioned on the metaphase plate. Each bivalent must be placed perpendicular to the metaphasic plate (equatorial plate, Figure 26) to have each homolog on one side of the metaphasic axis and hence ensure their correct segregation in anaphase I.

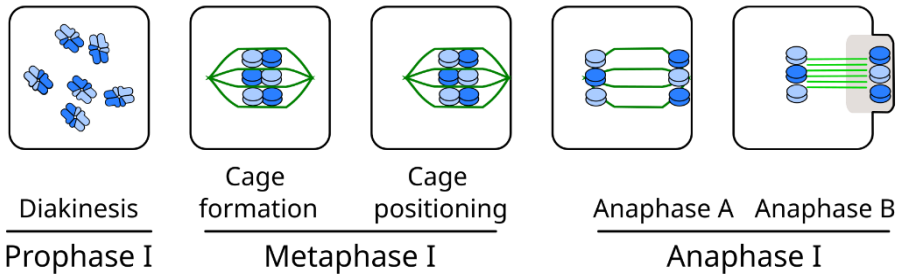

Figure 26: Schematic representation of *C. elegans* meiosis I. Homologous chromosomes are represented in light and dark blue, and the two sister chromatids of each homologous chromosome by the same blue shade. In diakinesis, six bivalents are observable. In metaphase I, the microtubule cage

(green) forms and surrounds the bivalents. The bivalents are aligned on the metaphasic plate and the cage is positioned near the cell cortex. In anaphase A of meiosis I, two sets of univalents segregate until reaching the poles of the microtubule cage. In anaphase B, the spindle elongates, the cage is disassembled and half of the univalents is pushed into the first polar body (grey).

In addition, meiotic divisions are asymmetric: divisions occur close to the cortex of the cell to generate tiny polar bodies and thus maintain the size integrity of the oocyte. Microtubules are necessary to align the chromosomes on a metaphase plate. Microtubules then form a cage around the chromosomes. The microtubule cage is repositioned near the cortex of the cell which will create a very asymmetric division (McNally et al., 2016).

The microtubules cage

In prometaphase, the nuclear envelope disappears, and microtubules polymerise into tapered and elongated bipolar spindles. In female nematodes, mice, or drosophila (Albertson & Thomson, 1993; Gueth-Hallonet et al., 1993; Theurkauf & Hawley, 1992), meiotic divisions are acentriolar: centrioles are dissociated and disappeared during oocyte maturation. In the absence of centrioles, and hence of microtubule organizing centres, other mechanisms are necessary to organize the meiotic spindles. These mechanisms are not yet fully understood in *C. elegans*. However, evidences show that KLP-15 /16 kinesins stabilise the microtubule bundle and act during the clustering of minus-ends (Mullen & Wignall, 2017). The assembling of the microtubule cage also requires the microtubule-severing enzymes MEI-1 and MEI-2 (Srayko et al., 2000; H. Yang et al., 2003), the KLP-18 kinesin (Connolly et al., 2014; Segbert et al., 2003; Wolff et al., 2022), one of the three CLASPS protein, CLS-2; (Maton et al., 2015) the stabilizing protein ZYG-9 (ZYGote defective: embryonic lethal (Cavin-Meza, Mullen, et al., 2022) whereas the pole of the cage is marked by ASPM-1 at the microtubule ends (Connolly et al., 2015). This cage forms individual spindle channels for each bivalent, permitting the univalents to slide through these channels towards the poles. Once the microtubule cage is formed and positioned near the cell cortex, the alignment of chromosomes is completed by a brief shortening of the microtubule cage toward the metaphase plate (McNally et al., 2016).

Kinetochores in holocentric species

Kinetochores are protein complexes that bind microtubules to chromosomes. While kinetochores are essential for correct bivalent alignment in metaphase, kinetochores are not

required for the segregation of chromosomes in anaphase of meiosis in *C. elegans* (Dumont et al., 2010).

Kinetochores are bipartite protein complexes constituted of the inner and the outer kinetochore (Figure 27) (reviewed in (Oegema & Hyman, 2006; Pintard & Bowerman, 2019).

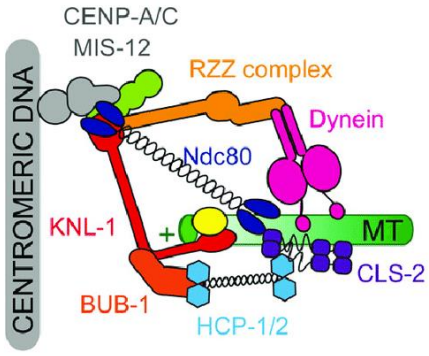

Figure 27: Schematics of the kinetochore protein complex. (Pintard & Bowerman, 2019)

The inner proteins of the kinetochores are directly in contact with DNA. DNA attachment is only achievable when a specific histone H3 variant is present, the CENP-A/HCP-3 protein (Buchwitz et al., 1999; Monen et al., 2005). HCP-4/CENP-C and KNL-2 are the two other inner proteins. Outer proteins of the kinetochores bind the inner proteins to the microtubules. The KMN complex formed by the association of Ndc80, KNL-1 and Mis12, constitutes the core of the microtubule-binding site of the kinetochore (Cheeseman et al., 2006). Independently of the KMN complex, two other structures connect the inner kinetochore to microtubules. The conserved protein KNL-1 also forms a complex with the kinase BUB-1, the CENP-F (centromeric protein-F)-like proteins HCP-1/-2 and CLS-2. While the RZZ complex (ROD-1/rough deal, CZW-1/Zeste-white and ZWL-1/Zwilch) forms a lateral attachment to microtubules. The RZZ complex interacts with a dynein adaptor, SPDL-1 that in turn recruits dynein-dynactin to kinetochores (Gassmann et al., 2008).

Anaphase I

Once the bivalents and the microtubule cage are correctly positioned, i.e. the bivalents on the metaphase plate and the microtubule cage in the vicinity of the cell cortex, anaphase I begins (Figure 26). Anaphase I consists of the segregation of homologs, also called reductional meiosis. As *C. elegans* has 6 bivalents (2n=12), in anaphase I two rosettes of six DNA bodies are observable. At the end of anaphase I, the homologs dissociate into two sets of univalents. The univalent is an association of two sister chromatids. One set of six univalents is extruded into the first polar body. Anaphase I is subdivided into three phases. First, the cohesins that tether the homologs together must be removed, second, the two sets of univalents must reach the poles of the microtubule cage (anaphase A defined chromosome displacement in the absence of spindle elongation) and finally, the microtubule polymerise between the two sets of univalents, which ensures their complete segregation (anaphase B defines chromosome displacement together with spindle elongation).

The precise removal of cohesins between homologs

As seen previously in prophase I, different protein complexes are established which distinguishes the associations between homologs from sister chromatid associations. This distinction enables the designation of the junction between homolog-homologs from the sister-chromatids association. At this stage, the bivalents are held together by cohesins (Figure 25). To allow segregation of homologs, the cohesins REC-8 and COH-3/-4 must be cleaved only on the short arms, while the cohesion of REC-8 on the long arms between sister chromatids must be maintained. Indeed, the complete removal of REC-8 leads to the dissociation of univalents and sister chromatids at once, leaving the oocyte with 24 DNA stained bodies instead of 6 (Castellano-Pozo et al., 2022; Crawley et al., 2016; Pasierbek et al., 2001). The stepwise removal of REC-8 depends on the presence of HTP-1/-2 (from the synaptonemal complex) on the long arms of the bivalents (Figure 25).

The removal of the cohesins REC-8 and COH-3/-4 is achieved by their cleavage, through the action of the Separase enzymes. Separases recognize the cohesins only if they are phosphorylated. This happens in several steps. First, the phosphorylation of the Tyrosine 3 of the Histone 3 (H3pT3) is mediated by the kinase Haspin (Figure 28) (HASP-1, (Dai et al., 2005).

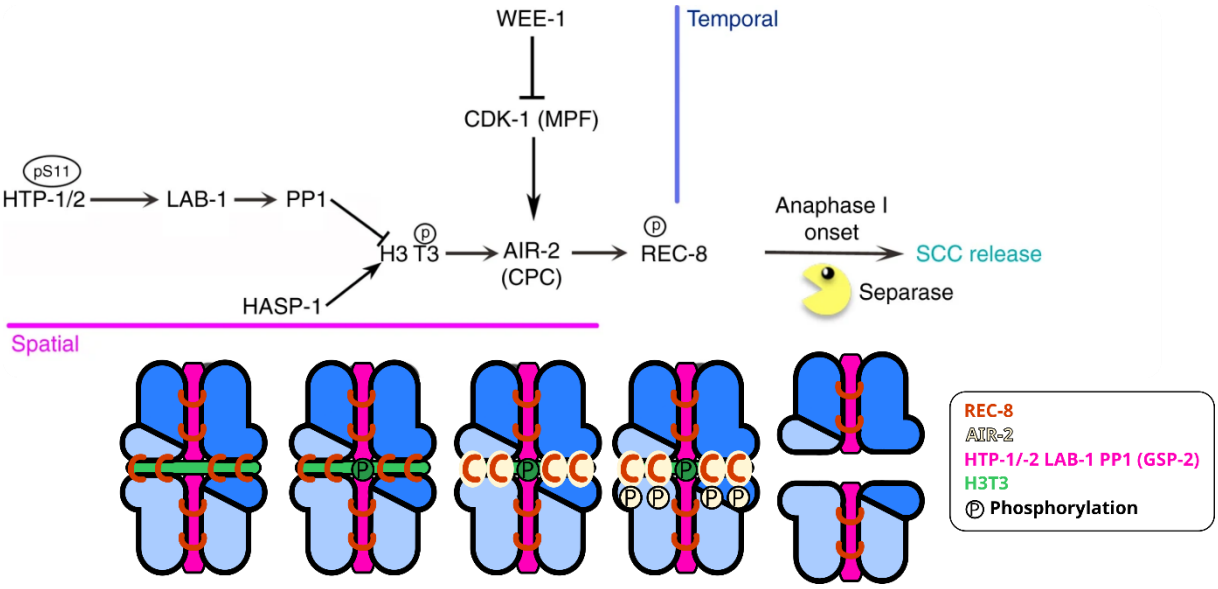

Figure 28: Model of the events that control the release of SCC in *C. elegans* oocytes. Homologous chromosomes are represented in light and dark blue, and the two sister chromatids of each homologous chromosome by the same blue shade. H3 T3 phosphorylation mediated by Haspin and antagonized by PP1 (GSP-2) regulates the spatial recruitment of AIR-2 to diakinesis bivalents, while CDK-1 exerts temporal control. Bivalents shown at the bottom of the model indicate the sequential recruitment of different proteins and phosphorylation events between late diakinesis and the onset of anaphase I. Abbreviations: MPF (Maturation Promoting Complex), CPC (Chromosome Passenger Complex), encircled "p" indicates phosphorylation even. (Adapted from Ferrandiz et al., 2018)

Once H3T3 is phosphorylated and CDK-1 (MPF) is present, Aurora B kinase (AIR-2) positions itself on the short arms of the bivalents and phosphorylates REC-8 and putatively COH-3/-4 (Rogers et al., 2002). AIR-2-mediated phosphorylation must be specific to the REC-8 cohesins present on the short arms of bivalents in meiosis I, in order to remove only the cohesion between chromosomes. This is tightly regulated also because on the long arms, between sister chromatids, HTP-1/-2 promotes the recruitment of LAB-1 (Long Arms of the Bivalent protein) which interacts with GSP-1, the homolog of the protein phosphatase 1 (Figure 25 and 28) (PP1, (Kaitna et al., 2002; Nadarajan et al., 2021; Rogers et al., 2002; Siomos et al., 2001). The phosphatase GSP-1 antagonises REC-8 phosphorylation on the long arms and therefore prevents its cleavage. In *lab-1* mutants, REC-8 is phosphorylated on both short and long arms despite the presence of HTP-1/-2 on the long arms (de Carvalho et al., 2008; Ferrandiz et al., 2018; Tzur et al., 2012).

AIR-2 has a central role because it also belongs to the Chromosomal Passenger Complex (CPC) with survivin, the inner centromeric protein ICP-1 (INCENP) and borealin.

CPC is a master controller of both meiosis and mitosis (Carmena et al., 2012). AIR-2 is an essential kinase of meiosis and in turn, AIR-2 also phosphorylates Histone 3 Serine 10 (Divekar et al., 2021; Ferrandiz et al., 2018; J.-Y. Hsu et al., 2000). Depletion of AIR-2 induces defects in the assembly of the microtubule cages in prometaphase I (Divekar et al., 2021) as well as improper chromosome segregation (Ferrandiz et al., 2018; Kaitna et al., 2002).

Anaphase A

Anaphase A or early anaphase consists of the segregation of the two sets of homologs from the metaphase plate to each pole of the microtubule cage (Figure 26) The two rosettes of six univalents slide along the spindle channels until reaching the poles. The mechanisms by which the univalents slide along the spindles are still debated. However, several experiments suggest that it is a combination of two mechanisms. The first mechanism involves pushing forces generated by the microtubules of the central spindle. As the microtubules would polymerise, they would push on the two sets of homologs toward the pole of the spindle (Dumont et al., 2010; Laband et al., 2017; Pelisch et al., 2019). A second mechanism involves pulling forces from the poles of the spindle. Multiple models for pulling have been proposed. According to the first model, the pulling forces would be generated by the lateral attachment of short, overlapping microtubules to the kinetochores. The spindles are oriented with minus ends towards the poles, therefore the depolymerisation of the plus ends of the spindles tracks the chromosomes towards the poles (Danlasky et al., 2020). The second model implies that dyneins, minus end-directed motors, mediate chromosome movement in microtubule (Muscat et al., 2015). However, doubts have been raised about the involvement of dyneins in anaphase A (Danlasky et al., 2020).

At the beginning of anaphase A, the kinetochores are structured in two cup structures on each bivalent (Dumont et al., 2010). Although kinetochores are necessary for the correct alignment of the bivalent on the metaphase plate, their absence does not interfere with chromosome segregation in anaphase. A gap is formed between the two cups of kinetochores in the middle of each bivalent (mid-bivalent region). This mid-bivalent region is enriched in CPC components. Together with the chromokinesin KLP-19, this region forms the Ring Complex or Ring-Shaped Domain. KPL-19 facilitates the bivalent congression in metaphase I by generating forces on the plus ends of the microtubules (Wignall & Villeneuve, 2009).

Interestingly, the kinetochore proteins BUB-1 and CLS-2 also localise to the ring complex in anaphase (Figure 29). Those two proteins are spindle checkpoints with MDF-1 (MAD (yeast Mitosis arrest DeFicient) related, (Pelisch et al., 2019). The Ring complex is stabilized by the action of the SUMO complex. SUMO complex creates a transient covalent bond between the RC and SMO-1 protein (Sumo ubiquitin-related homolog) via a hierarchical action of an E1 activating enzyme, an E2 conjugating enzyme (UBC-9), and SUMO-specific E3 ligases (GEI-17) (Broday, 2017).

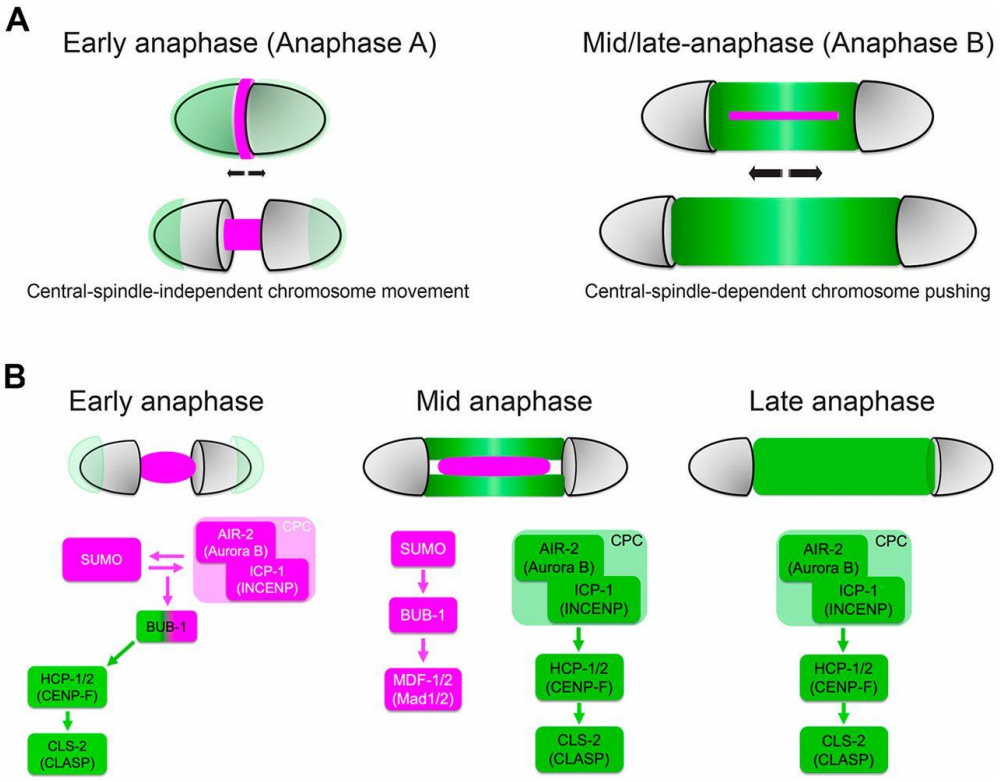

Figure 29: Two-step chromosome segregation model and the role of SUMO. (A) During early anaphase, chromosomes begin to separate without MTs being present between them. This area is filled with BUB-1 and SUMO, among other proteins, suggesting that these proteins could play a role during this early segregation step. As anaphase progresses, MTs populate the region between segregating chromosomes leading to the CLS-2-dependent stage. (B) The dynamic composition of the ring domain and central spindle throughout anaphase is depicted.

And the end of Anaphase A, the kinetochores are disassembled and the kinases BUB-1 and AIR-2 (CPC) relocate exclusively on the RC (Davis-Roca et al., 2017; Dumont et al., 2010; Hattersley et al., 2016; McNally et al., 2016; Pelisch et al., 2019). The presence of BUB-1 on the RC enables the targeting of the protease ULP-1 (Ubiquitin-Like Protease) to the RC (Davis-Roca et al., 2018). ULP-1 in turn removes SUMO modifications and the removal of SUMO triggers RC disassembly to enter in anaphase B.

Anaphase B

Anaphase B, or late anaphase is characterised by a global reorganisation of the microtubules: the cage of microtubules and RC are disassembled and, central spindles polymerise to ensure the completion of the homologs segregation (Figure 26) (Chuang et al., 2020; Davis-Roca et al., 2018; Pelisch et al., 2019; C.-H. Yu et al., 2019). In anaphase B, the two sets of six univalents are separated by the central spindles, which proliferate in the midzone (localization of the metaphase plate, and RC) and exert pushing forces on the chromosomes (Figure 26) (Maddox et al., 2004; C.-H. Yu et al., 2019). Anaphase B is also subdivided into several processes. First, the RCs are disassembled, resulting in the closure of the central spindle channels through which the chromosomes slide in anaphase A. The closure of the channels then enables the central spindles to elongate.

The mechanisms of central spindle polymerisation in the midzone are mainly studied in mitosis but few authors have focused on anaphase B of meiosis in *C. elegans*. In mitosis, the midzone is essential for central spindle polymerisation and chromosome segregation (Nahaboo et al., 2015). Furthermore, the midzone via kinesin-5 activity constrains the speed of chromosome movement (Grill et al., 2001; Saunders et al., 2007).

The RCs disassembling is initiated by BUB-1 which is modified by SUMO complex and more specifically by GEI-1 (E3 ligase). BUB-1, in turn, mediates the deconjugation of ULP-1 protease. UBC-1/E2 and GEI-17/E3 are removed from the RC and the deconjugated ULP-1 disassembles the RC. AIR-2 kinase localises on the entire spindles between the two sets of univalents (Figure 30) (Davis-Roca et al., 2017, 2018) while SMO-1 remains on the RC. In the absence of AIR-2 and under the action of ULP-1, the RC flatten and collapses (Davis-Roca et al., 2018; Pelisch et al., 2019). The degradation of the RC enables the microtubule channels to close and trigger the central spindle polymerisation pathway at the previous location of the RC in the midzone. The AIR-2 signal fades on the spindle and disappears completely. The CLS-2 protein is recruited by another protein from the kinetochore, HCP-1/-2, to the central spindles

and promotes antiparallel microtubule assembly (Davis-Roca et al., 2018; Dumont et al., 2010). And KLP-18 (kinesin-12 family) acts as a microtubule spacer while ZYG-8 (ZYGote defective: embryonic lethal), the inducer of microtubule polymerization, supports the overlap of the central spindles (Cavin-Meza, Kwan, et al., 2022; McNally et al., 2016).

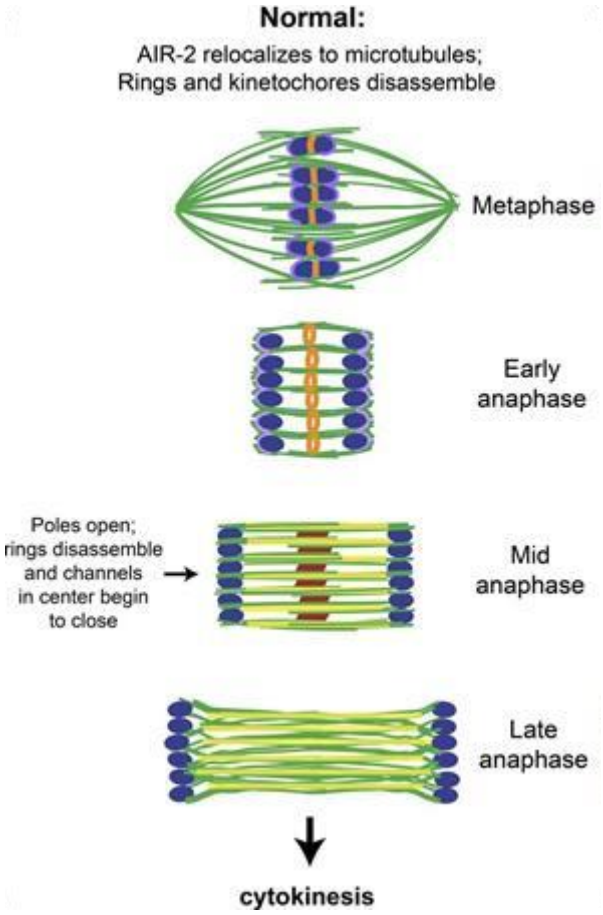

Figure 30: Model for kinetochore and AIR-2/Aurora B–mediated anaphase regulation. Model depicting DNA (blue), microtubules (green), the ring complexes with AIR-2 (orange), AIR-2 (yellow), the ring complexes without AIR-2 (red), and kinetochores (purple). In normal meiosis (left), kinetochores are removed from chromosomes, AIR-2 relocalizes to the microtubules, and the ring complexes begin to disassemble by mid-anaphase. (Davis-Roca et al., 2017)

Intriguingly, KLP-18 depletion does not interfere with the movement of chromosomes in anaphase B, suggesting that KLP-18 is not involved in the driving forces of the central spindles and other motor proteins that are certainly the main agents (Cavin-Meza, Kwan, et al., 2022; Wignall & Villeneuve, 2009; Wolff et al., 2022). The anaphase and the meiosis I are

completed by the extrusion of one set of six univalents into the first polar body. The first polar body contains 2n chromosomes, as the extruded homologs each possess two sister chromatids.

Meiosis II

Meiosis II is the separation of sister chromatids. Through meiosis, the chromosomes change from 2C to 1C. For this reason, meiosis II is usually called equational division. Few descriptions of meiosis II divisions are available in the literature because meiosis II is supposed to follow the same dynamics as meiosis I divisions (Figure 31). However, as meiosis II is less documented than meiosis I, some of the steps may differ from the theory. For instance, condensins are required for the segregation of sister chromatids in anaphase II but are dispensable in anaphase I (Hagstrom et al., 2002), demonstrating specific differences between these two cell divisions.

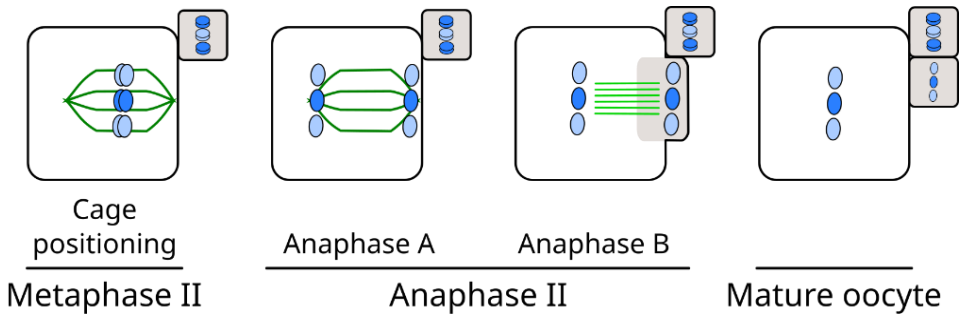

Figure 31: Schematic representation of *C. elegans* meiosis II. The two sister chromatids of each univalent are represented by the same blue shade. At the beginning of meiosis II, six univalents are observable. In metaphase I, the microtubule cage (green) formed and surround the bivalent. The univalents are aligned on the metaphasic plate and the cage is positioned near the cell cortex. In anaphase A of meiosis II, two sets of chromatids segregate until reaching the poles of the microtubule cage. In anaphase B, the cage is disassembled and half of the chromatids is pushed into the second polar body (grey), generating a haploid oocyte.

In the first stage of meiosis II, the univalents must be oriented perpendicular to the metaphase plate to ensure the segregation of half of the sister chromatids into the second polar body. The other set of sister chromatids is retained in the mature oocyte. In monocentric

organisms, the sister chromatids are segregated via a modification of the microtubule attachment. In meiosis I, they are co-oriented towards the same microtubule pole. While in meiosis II, sister chromatids are bioriented towards each of the two poles. In holocentric species, and in particular, in *C. elegans*, the mechanism of the chromatid bi-orientation is not known. Some authors suggested that the REC-8 cohesin is removed from one side of the chromatids to allow alignment of the chromatids along their longitudinal axis ("Reattachment Hypothesis", Figure 31) (Schvarzstein et al., 2010). Another possibility is to switch the orientation of the univalents by rotating them ("Rotation Hypothesis", Figure 31).

Here, sister chromatids would be separated along their short axes. Regardless of the mechanism, at the beginning of meiosis II, the kinetochores reassemble around the univalents to ensure their alignment on the metaphase plate and the spindle is positioned near the cell cortex.

Anaphase II proceeds in a similar manner to anaphase I. The HORMA domain proteins, HIM-3, and HTP-1/-2 persist between the sister chromatids but LAB-1 is not recruited (de Carvalho et al., 2008; Martinez-Perez et al., 2008; Severson et al., 2009). In the absence of LAB-1, GSP-1 does not exert phosphatase activity and thus enables the phosphorylation of H3T3 between sister chromatids. The mechanism of LAB-1 recruitment release is not known. H3pT3, therefore, localizes to the mid-univalent region. Phosphorylation of H3T3 allows REC-8 phosphorylation by AIR-2. Phosphorylation of REC-8 releases the cohesion between sister

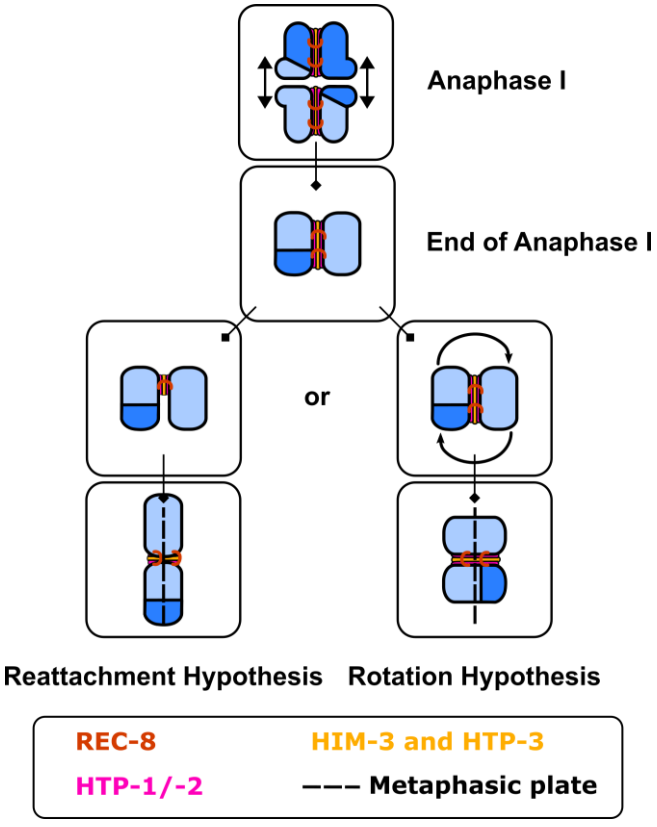

Figure 32: Models of events leading to univalents reorientation in *C. elegans* meiosis II. Homologous chromosomes are represented in light and dark blue, and the two sister chromatids of each homologous chromosome by the same blue shade. According to Reattachment Hypothesis, REC-8 cohesins (red rings) with HTP-1 (pink), HIM-3 and HTP-3 (orange) are removed along the long axis of chromatids and replaced on the short axis. The Rotation Hypothesis stipulates that no protein rearrangement occurs. The univalents rotate to align their short axis parallel to the metaphasic plate. The dotted line represents the metaphase plate.

chromatids. SUMO, BUB-1, CLS-2, AIR-2 and KLP-19 follow the same dynamic as in meiosis I (Figure 33) (Monen et al., 2005; Pelisch et al., 2019; Schvarzstein et al., 2010). For this reason, it is plausible that the dynamics of microtubules are comparable between anaphase I and anaphase II.

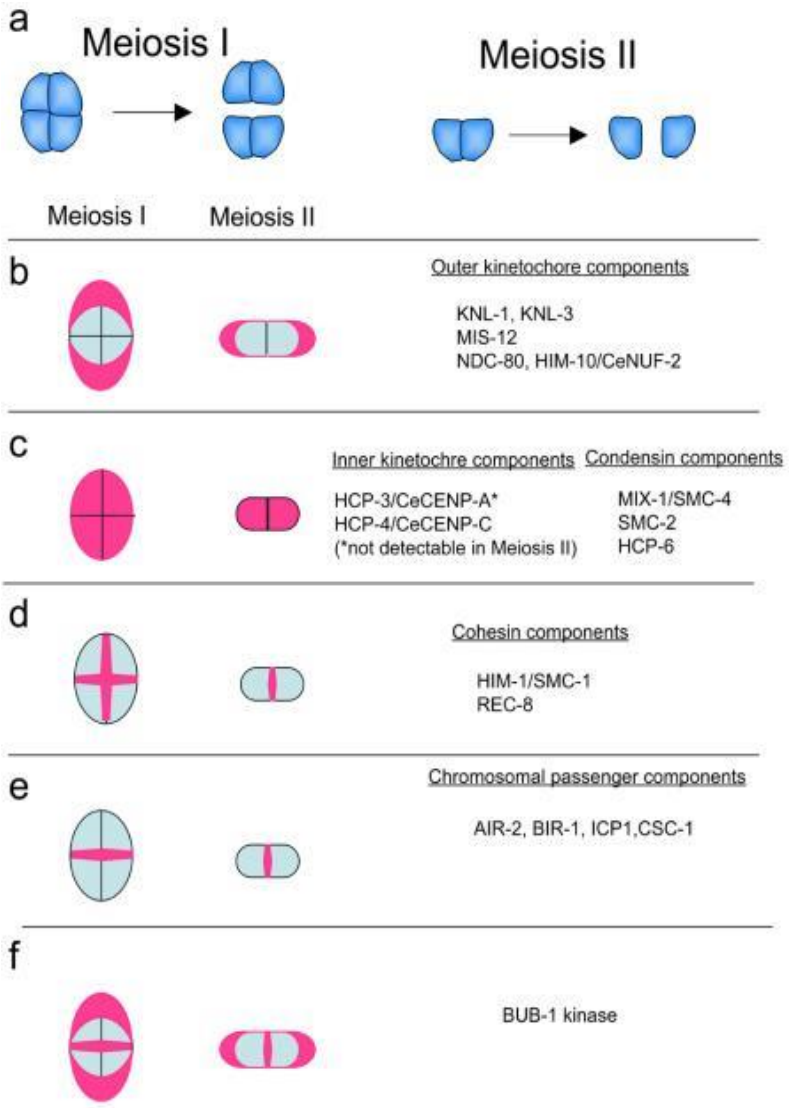

Figure 33: Localization of kinetochore components on meiotic chromosomes. Schematic showing the meiotic chromosomes during meiosis I and II division. Each bivalent, from diakinesis to metaphase

of meiosis I, consists of a pair of homologous chromosomes comprising a pair of sister chromatids. Homologous chromosomes separate during meiosis I division and sister chromatids separate during meiosis II division (b – f). The localization pattern of indicated proteins on bivalents during meiosis I and meiosis II divisions are illustrated in red. The meiotic chromosomes (a pair of homologous chromosomes during meiosis I and a pair of sister chromatids during meiosis II) are shown in blue. (b) Outer kinetochore components localize to a cup-like structure (red) surrounding the bivalent. (c) In contrast, inner kinetochore components and condensin components localize through the chromosomal DNA. HCP-3/Ce.CENP-A is removed from chromosomes prior to meiosis II division. (d) The cohesin complex (red), which localizes to cohesion between homologous chromosomes (blue) and between sister chromatids (blue), forms a cruciform shape. (e) The chromosomal passenger complex (red) colocalizes with cohesin only to be resolved. (f) BUB-1 (red) colocalizes with outer kinetochore components (shown in red in b) and chromosomal passenger proteins (shown in red in e). (Kitagawa, 2009; Monen et al., 2005)

Objectives of my thesis

In the introduction, we saw that the nematode *M. belari* has an intriguing reproductive system where males are amphimictic and sexually produced while females are gynogenetic and therefore asexually produced. In gynogenetic oocytes, meiosis consists of only one complete meiotic division, with the extrusion of a single polar body. In amphimictic oocytes, there is a two-step division producing two polar bodies. Thus, during its life, a given female will undergo two types of meiotic divisions, to generate either males or females. My thesis is subdivided into three main parts. I) the characterisation of the meiotic division of gynogenetic oocytes, II) the determination of factors defining the kind of meiosis in oocytes and III) the development of tools in a new model species.

The first objective of my thesis was to describe and characterise the missing step in the meiosis of gynogenetic oocytes. To this end, I used cytology. I proceeded by analogy with the well-described meiosis of the closest species to *M. belari*, *C. elegans*. In parallel, our collaborators at ISEM (Montpellier) characterized the genome of *M. belari*. Our results were in contradiction, which pushed me to further explore the mechanism of meiosis in *M. belari*. With this first project, we discovered a new type of chromosome segregation, involving biased segregation of recombinant chromatids. This work is presented in Chapter 2.

The second objective of my thesis was to identify how a given female can produce two different meiotic programs. The team had previously shown that the proportion of males varied with the age of the mothers: a young female produces more males than an older one. This suggested that alternation in the meiotic process could be under maternal control. We, therefore, hypothesized that a differential maternal deposit of mRNA or proteins could determine the fate of the oocytes and the execution of meiosis. Before my arrival in the team, single embryo transcriptomes had been obtained and analysed. I developed the smiFISH (single molecule inexpensive Fluorescent In Situ Hybridization) technique in *M. belari* as well as RT-qPCR on single oocytes and performed functional analysis with RNAi and CRISPR/Cas9 to validate some candidates. This project, still ongoing, is presented in Chapter 3.

All my projects would have benefited from efficient tools to manipulate the genome of *M. belari*, namely RNAi, knock-out and transgenic lines expressing fluorescent reporters. As described below, RNAi is not very efficient in *M. belari*. I, therefore, concentrated my efforts to implement the CRISPR/Cas9-based editing techniques in this species. Although I succeeded

in establishing knock-out lines this way, I failed to generate knock-in lines, despite many attempts. This work is summarised in Chapter 4.

Finally, I decided to summarize my readings on asexuality and cytology, including my findings on *M.* belari, in a review. This part of my work is presented in Chapter 1 because it complements the information presented in the introduction. Nevertheless, it is not necessary to read this first Chapter to understand the results presented in Chapters 2 to 4.

Material and Methods

Nematode strains and culture

M. belari species JU2817 (PRJEB30104, genome ID: UZWA01000816.1) is maintained at 20°C on NGM plates seeded with *E. coli* OP50, following *C. elegans* protocols, as described in (Grosmaire et al., 2019).

Immunostainings on gonads and embryos

Immunostainings were performed as described in Grosmaire (2019). Gravid females were dissected on slides coated with 0.25%poly-lysine in 0,5X M9 (diluted 1:1 in H2O). After freeze-cracking, the fixation of samples was performed by immersing slides into methanol at -20°C for at least 5 min. Commercial primary antibodies (dilution indicated in brackets) were as follows: mouse anti-$\boldsymbol{\alpha}$-tubulin (Sigma DM1A, 1:2000), rabbit anti-H3pT3 (07-424; Merck Millipore, 1:1000) and mouse anti-SMO-1 (DSHB SUMO 6F2, 1:3000). Mb.HORMA and Mb. BUB-1 were used at 1:700 and 1:3000 dilution, respectively. As secondary antibodies, we used a Cy3 donkey anti-rabbit secondary antibody (Jackson ImmunoResearch #711-165-152, 1:1000) and an Alexa488 donkey anti-mouse secondary antibody (Jackson ImmunoResearch, #715-545-150, 1:2000). All antibodies were incubated at room temperature for 45 min except for Mb.HORMA when co-stained with H3pT3 which was incubated at +4°C overnight. DNA was stained using Hoechst 33258 at 0,5 ul/ml (Merck Sigma-Aldrich, 94403). For co-staining of Mb.HORMA and H3pT3, we used the FlexAble kit (anti-mouse, CoraLite Plus 488, Proteintech) to directly label Mb.HORMA, which was used as a pure solution. Images were acquired using a confocal microscope (Oil immersion 63X objective - LSM800 Airyscan, Zeiss). Z-stacks of embryos were acquired every 0.15 µm. Finally, acquired images were treated using ImageJ 1.53t software.

Antibody production

Specific antibodies against Mb.HORMA (g17455) were produced by injecting rabbits with two synthetic peptides: KTYRLDKSTPGV and YDTKTLYDFDHPDAL (Covalab, Lyon). Polyclonal antibodies were affinity purified from serum obtained at day 74 post-injection. Specific antibodies against BUB-1 (g12204) were produced as described above, also

using two synthetic peptides: SPIVEDQDHENSTNG and SLMAQNQQEEQKGTA (Covalab, Lyon).

EdU Pulse/Chase

EdU sample preparation

The protocol was adapted from Almanzar (Almanzar et al., 2021). In order to obtain diakinesis oocytes and early embryos for which only one chromatid per chromosome was labeled with EdU, we had to optimize the protocol for *M. belari*. First, we synchronized worms using axenization, as described in (Grosmaire et al., 2019). Briefly, worms were collected and treated with bleach and NaOH in order to dissolve all individuals except the embryos. The embryo pellet was then washed and placed on plates without bacteria, allowing L1 larvae to hatch. Without food, all L1s were arrested at the same stage after 2 days. L1s were then placed back on food and allowed to grow for 72h at 20°C. At this stage, young L4 syncrhonized worms were collected and washed in 1X PBS, 0.1% Triton X . A pellet of ~300ul of worms was transferred into 200 ul of 10 mM EdU diluted in water (ThermoFisher A10044) to obtain a final concentration of 4 mlM EdU. The tube was transferred on a tube rotator for 4h at room temperature. After whashes[c3] in M9, animals were plated onto fresh NGM plates seeded with *E. Coli* and placed at 25°C for 48 before embryos were collected for fixation.

EdU Click-it labeling

Cytology was performed following the instructions provided by the EdU Click-it kit (ThermoFisher C10337). Embryos and adults were collected and treated with bleach and NaOH, as described above, to recover only embryos. Embryos were placed on poly-lysine coated slides, freeze-cracked and fixed in -20°C methanol. Samples were incubated with BSA 2% for 20 min. The slides were then washed twice with PBS. Following the instructions of the kit, slides were washed for 30 min at room temperature in PBS with 1% Triton X (v/v) and labelled with Alexa 488-azide for 30 min at room temperature. Samples were washed twice with PBS and incubated in a tank with Hoechst 33258 for 20 min at RT. Finally, slides were mounted using ProLong™ Diamond Antifade Mountant (ThermoFisher P36965) and sealed with nail polish.

SmiFISH

SmiFISH protocol is adapted from Tsvanov's and Hubsentenberger's protocol (Hubstenberger et al., 2017; Tsanov et al., 2016).

Design of the probes

Probes are designed using the R script Oligostan (Nucleic Acids Res. 2016 Dec 15; 44(22): e165): https://bitbucket.org/muellerflorian/fish_quant/src/master/Oligostan/. We used the following parameters: GC content, 40% ≤ X≤ 60%, dG37 probes Minimum score ScoreMin ≤ 0.9; PNAS option #1 #2 and #4; Size of the probes, 24 ≤ X ≤32.

Labelling with smiFISH probes

On the first day, gravid females were dissected in 15 µl of PB buffer (Na_2HPO_4 1N, NaH_2PO_4 1N, H_2O) on slides coated with poly-K. Dissection enables the release of the embryo from the gonad. After dissection, 15 µl of PFA 8% was added to the slides. And the slides were freeze-cracked and fixed in -20°C methanol. After adding 4% PFA to the slides, they were incubated in 70% EtOH at +4°C overnight. On day 2, slides were washed in 1X PBS and two times washes in 1X SSC with 15% of formamide. Then, slides were incubated in a dark tank overnight at +37°C in the Hybridization buffer that contains probes and Stellaris buffer. Probes were prepared as in the Tsanov protocol (Tsanov et al., 2016). On day 3, slides were washed successively in 1XSSC with 25% of formamide and 1XSSC. DNA was labeled using Hoechst 33258 at 0,5 ul/ml (Merck Sigma-Aldrich, 94403). Finally, slides were mounted in ProLong® Diamond Antifade Mountant (Merck Sigma-Aldrich, P36965) and sealed with nail polish.

Airyscan and ImageJ 3D analysis

As with immunostaining, confocal airyscan images were acquired using the Zeiss LSM800 Airyscan and LSM980 Airyscan using a 63x oil objective and 0.15µm interval between slides. Images were subsequently processed by the airyscan processing method (Blue 2., 3D analysis, automatic low stringency). Processed images were treated using ImageJ 1.53t software. For EdU experiments, in the inset chromosome images, Z-projection was adjusted for each chromosome in a given image using the maximum intensity. Analyses were combined with the 3D projection on the Y-axis using the brightest point method and interpolation.

RT-qPCR

Quantitative PCR experiments were performed on single embryos. Embryos lysis, reverse transcription and pre-amplifaication were achieved following the Smart-Seq2 protocol (Picelli et al., 2014). Samples were purified on beads using JetSeq™ Clean (Bioline, BIO-68031). Two housekeeping genes were used (g22258 and g8248) and validated using BestKeeper and geNorm (Mestdagh et al., 2009; Pfaffl et al., 2004)). The concentration samples were normalized according to the target gene. The probes were designed to generate amplicons of 150 to 200 bp. Quantitative PCRs were performed as presented in the protocol for Takyon™ No Rox SYBR® MasterMix dTTP Blue (Eurogentec, UF-NSMT-B0701). Standard curves were obtained of population cDNA and ranged from 1µg to 0.064 ng (1:5 dilution). Deposit of samples was performed in duplicates and duplicates with delta CT> 0.3, were excluded from the analyses. All genes were normalized with the two housekeeping genes and analysed using the 2 (-Delta Delta C(T)) method (Livak & Schmittgen, 2001). Boxplots of the relative expression of each gene were achieved using ggplot2 3.4.1, Rstudio (Wickham, 2016).

Microinjections of antibody or CRISPR complex

Antibody injections

Females were injected with a secondary antibody conjugated with Cy3 (λ emission: 565 nm, Jackson ImmunoResearch, approximately 160 kDa, #711-165-152). The antibody was either diluted in water at a final concentration of 0.7 mg/ml or diluted in HEPES (20 mM final concentration) at a final concentration of 0.56mg/mL. After injection, worms were immediately placed on an agar pad 2% in M9 and images were taken with a fluorescent Zeiss A1 microscope, equipped with a Kappa camera.

CRISPR editing in *C. elegans* and *M. belari*

All CRISPR-Cas9 components (crRNAs, tracrRNAs, IDTE #11-05-01-05, IDT Duplex buffer #11-05-01-03 Cas9, Cat#1081058) were furnished by IDT DNA (Coralville, IA). The guides were designed to be 20nt long and have a GC content between 50 and 75%. We favored PAMs with GGNGG or GNGG sequences. To target the *M. belari unc-22* gene (mbelari.g26112), two guides separated by 85nt (distance between PAMs) were designed on the forward strand of the 96th exon (5'-GAGAGCTGCCGGAAAGCACG-3' and 5'-TGGAGAGTACACATGTGAGG-3'). To target *dpy-1* in *C. elegans*, only one guide was designed on the reverse strand (5'-GCTACCATAGGCACCACGAG-3') on the third exon, as described in (Paix et al., 2014, 2015)

Species	Gene	Guide #1 strand forward or reverse	Guide #2 strand forward or reverse	Distance between guides	N° exon
C. elegans	*dpy-10*	5'-GCTACCATAGGCACCACGAG-3' reverse	-	-	3
M. belari	*unc-22* (mbelari.g26112)	5'-GAGAGCTGCCGGAAAGCACG-3' forward	5'-TGGAGAGTACACATGTGAGG-3' forward	85nt	96
	g1126	5'-TGCGGATCCGCGATTTGTTG-3' reverse	CGCGGATCCGCATGATGTCG forward	26 nt	1
	g4082	5'-TATCTACCTCTGAACGAGGG-3' reverse	5'-GTGAGCGTCGAGCTGATCGG-3' reverse	89nt	2 and 3
	g14314	5'-TCTCCATTGGGGCTTCTCGG-3' forward	5'-TGAACCAAGAGAAATCGGGG-3' reverse	5nt	7
	g23490	5'-CTCCATCAACTCCAAATCGG-3' reverse	5'-GCCCGATCGATCACTGGAAG-3' reverse	233nt	2 and 3

	g3341	5'-ATGTGGGAGTCATCGGCCGG-3' forward	5'-AGTGGAGGACGAAGAAGCGG-3' forward	247nt	1 and 2

Table 1: Guides RNAs used for the CRISPR technique in *C. elegans* and *M. belari*.

CRISPR injections

Firstly a guide RNA was prepared by mixing 3µL of tracrRNA (100 µM, Sigma) and 3µL of crRNA (100 µM) in 4 µL of Duplex Buffer (IDT) and placed 5 min at 95°C followed by 45min at RT (20-25°C). Before the injections, 2.5 µL of the guide RNA was added to 0.5µL Cas9 purified protein (10mg/mL, Sigma CAS9PROT-50UG) and the mix was heated for 10 min at 37°C. In the case of *C. elegans* knock-in, 1 µL of ssDNA repair (250 µM) was added to the mix before being placed for 20 min on ice. To increase the HEPES concentration, 1 µl of 80 mM HEPES was added to the mix, to obtain a final concentration of 20mM. In all cases, the final mix was centrifuged for 10 min at 4°C before filling the needle. Importantly, without the addition of HEPES to the mix, the basal concentration of HEPES is 18mM because it is already present in the Duplex buffer and the Cas9 buffer.

Microinjections

Microinjections of *C. elegans* and *M. belari* were conducted using a FemtoJet 4i and an InjectecMan 4 Manipulator (Eppendorf). Gravid adult females were placed on a dried agarose pad 3% (w/v) and covered with Halocarbon oil 700 (Merck Sigma-Aldrich, 9002-83-9) to avoid worm desiccation (as described in (Evans, 2006). Femtotips II microcapillary needles (Eppendorf) were filled with 2 µL of CRISPR or antibody mix (see above). Depending on the experiments, females were injected either towards the gonad in the case of *M. belari*, inside the syncytial gonad in the case of *C. elegans*, or near the pharynx using an input gas pressure of 900 hPa balance pressure. After injection, animals were transferred to a fresh NGM plate seeded with OP50 by pipetting the worms in a small amount of M9 buffer. For each injected animal, the F1 progeny was screened for the appearance of DPY or UNC/twitching worms, depending on the targeted gene.

RNAi

To produce target-specific dsRNA, we used a gene-specific PCR product as templates. For each gene with performed PCR with primers containing the T7 promoter sequence (5′-TAATACGACTCACTATAGGG-3′) at the 5′ end of the primer sequences. PCRs were performed with TaqPolymerase and purified on a column with NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, 740609.50). In vitro transcriptions were achieved using MAXIscript™ T7 Transcription Kit (ThermoFisher, AM1312). To anneal the transcript into dsDNA, samples were heated for 10 min at 72°C and 30 min at 37°C in a thermocycler. The microinjection mixture was produced by incubating the dsRNA and 12% Lipofectamine®RNAiMax reagent (ThermoFisher, 13778150) at room temperature for 20 min. Animals were microinjectied as explained in Microinjections.

Chapter 1: Alteration of meiosis in asexual animals

Introduction

This chapter is devoted to the study of the different types of meiosis encountered in parthenogenetic species. Meiosis is an extremely conserved mechanism present in every species described to date. As explained in Introduction, while the main steps of meiosis (reductional and equational division) are conserved, the underlying mechanisms that enable these steps to be achieved vary. It is these variations that might have permitted the production of unreduced gametes, which in turn contributed to the emergence of asexuality in parthenogenetic species. Indeed, in parthenogenetic species, the offspring possess the same ploidy than their progenitor. During canonical meiosis, the number of copies of each homologue is reduced to a single copy in the gamete. In parthenogenetic species, female meiosis must be modified to maintain ploidy from the progenitor to the progeny. But what alterations to meiosis are found in nature?

In the Introduction, we also saw that among these variations, some species have lost the ability to recombine. Recombination is a determinant event that promotes mixing by generating new allelic combinations. In parthenogenic species, if recombination occurs, it will result in some local homogenisation of the genomes, also known as loss of heterozygosity (LOH). The question, therefore, arises as to the impact on the genome of the different types of alteration of meiosis. By studying the meiotic process, we are able to infer the theoretical consequences of a given reproductive system on the genome of the species. While heterogeneity is assessed by genomic analyses, what could be the contributions of cytology in the analyses?

In this chapter, we define the different categories of meiosis alterations in parthenogenetic species. We also discuss the impact of a given type of meiosis on the genome of the species concerned. Furthermore, we highlight the importance of the contribution of cytology in the study of parthenogenetic systems.

Results

When cytology and genomics cooperate to elucidate the mechanistic constraints at the origin of asexuality

A cell biologist journey in the meiosis of asexual animals

Authors

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Abstract

Asexuality is a derived character and asexual species have emerged multiple times independently over the course of evolution. In animals, asexuality, whereby a zygote inherits only its mother genome, requires modification of female meiosis, to produce unreduced gametes. A wide range of changes in the meiotic steps has been described so far. Upon maintenance of recombination, most of these modifications should theoretically lead to loss of heterozygosity (LOH). However, a lot of asexuals avoid LOH, strongly suggesting that a necessary step in the emergence of asexuals is the ability to modify meiosis in a way that maintains heterozygosity. Deciphering these mechanisms of LOH avoidance is required to understand the constraints at the origin of asexuality. With this review, we describe some of these mechanisms. We emphasize that for most species the mechanisms are unknown and there is a clear need to revisit, with state-of-the-art imaging techniques, the old literature. For that, as a community, we also need to find a way to convince cell biologists to explore meiosis in asexuals. Overall, we speak for a simplification of the nomenclature in the field, because in its current form, it has led to many misinterpretations and may constitute a barrier to further cooperation between disciplines.

Glossary

Ameiosis, Apomixis, Parameiotic division, Mitotic-like division or Mitotic parthenogenesis: Meiosis with a single complete division consisting of the separation of the sister chromatids.

Amphimixis: Sexual reproduction where the gametes of two individuals fuse to generate a genetically new zygote.

Automixis: Mechanism of meiosis in parthenogenetic species where the ploidy is maintained from a progenitor to its progeny.

Equational division: Second stage of meiotic division where sister chromatids are segregated.

Reductional division: First stage of meiotic division where homologous chromosomes are separated.

Parthenogenesis: Asexual mode of reproduction in which a new zygote is generated from a single gamete of a single individual.

Ploidy: Number of copies of each homologous chromosome found in a species.

Introduction

Meiosis is a universal and specific type of cell division that allows all sexual eukaryotes to reproduce. This mechanism is highly conserved as it is found in animals, protists and plants. Meiosis results in the production of haploid gametes. In most cases, the ploidy is re-established upon fertilization by the fusion of the haploid gametes of two individuals, thus generating an individual with a mixed genome.

In this review, we will talk about asexual animals. Here, asexuality is defined by the absence of mixing of the parental genomes. We will discuss only about asexual females which produce asexual females. Other forms of asexuality exist, for instance, haplo-diploidy and hybridogenesis (Kennedy, 2021; Lavanchy & Schwander, 2019). It is now well established that asexuality is a derived character and that sexuality is the ancestral state in eukaryotes (Bell, 1982). Also, asexuals are rare, they represent less than 0.1% of vertebrates and insects (Avise, 2008; Normark, 2014). This scarcity is a paradox in evolutionary biology because, for many reasons, which have been very clearly exposed in other reviews, asexuality confers a lot of advantages over sexuality (Maynard Smith, 1971, 1978, 1986; Neiman et al., 2014).

None of the asexual females described so far are haploid, demonstrating that the process of meiosis has been modified in these species in order to produce unreduced gametes. These modifications have made the rise of asexuality possible. Which modifications were possible and which ones were favored? Because asexuality has emerged multiple times independently, it offers an ideal opportunity to explore the mechanistic and genomic constraints in meiosis.

Although many aspects of sexual meiosis are tightly constrained, other steps in meiosis show large variations even within sexual species, already showing that changes are possible, at least in some clades. For instance, in *Saccharomyces cerevisiae*, *Danio rerio* or *Mus musculus*, Double-Strand Breaks (DSBs) are required to pair homologous chromosomes, whereas DSBs occur after homologous pairing in *Caenorhabditis elegans* or females *Drosophila melanogaster* (reviewed in (Rubin et al., 2020). In other species, as in males of *D. melanogaster*, homologous chromosomes pair but do not recombine (Morgan, 1914). This shows that recombination is not a necessary step for meiosis (Haag et al., 2017) and that for instance evolution in the recombination machinery could be at the root of the emergence of asexuality. Nevertheless, not all variations in the process of meiosis are possible and many may cause direct or indirect lethality of the progeny. If modifications in the meiotic program are not so simple, this might explain the scarcity of asexual species (Engelstädter, 2008; Neiman et al., 2014).

Suomailanen in its 1987 opus has given an exhaustive list of asexual species, and the accompanying modifications of meiosis, when available (Suomalainen et al., 1987). These many types of modifications can nevertheless be grouped into a handful of categories, which we will define later. Importantly, the genetic prediction is that, except if recombination is totally abolished or in other modifiyng the recombination, all types of modifications occurring with recombination will lead to a total or partial loss of heterozygosity (LOH) in the genomes of asexuals.

Some parthenogenetic species are indeed homozygous genome-wide, which is puzzling because homozygosity is expected to reveal deleterious mutations and therefore be detrimental to fitness (Maynard Smith, 1978). Surprisingly, the parthenogenetic species of the stick insect genus *Timema* are entirely homozygous but do not exhibit lower average fitness, moreover, the species undergo positive selection at a lower rate than closely related sexual *Timema* (Bast et al., 2018; Jaron et al., 2022). The reason why some species tolerate becoming homozygous while others are detrimental has not yet been identified. In the crustacean *Daphnia pule*x and *Daphnia obtusa*, during the asexual phase, the emergence of high LOH levels was recorded (Omilian et al., 2006; Xu et al., 2011). In the same manner, a drastic homogenisation of the genome has been reported in sharks (Chapman et al., 2008; Feldheim et al., 2010, 2017; Harmon et al., 2016; Portnoy et al., 2014; Robinson et al., 2011). However, *Daphnia* are cyclical parthenogens and sharks exhibit facultative parthenogenesis, so those species are temporarily parthenogenetic. Therefore, such transient LOH may be compensated by sexual events, leaving the species with a reasonable level of heterozygosity (Omilian et al., 2006).

A real paradox arises from the existence of obligate parthenogenetic species that nevertheless carry widely heterozygous genomes, despite meiotic modification expected to lead to substantial LOH. Strikingly, few mechanistic explanations exist to reconcile these contradictions. For a long time, the literature has propagated the idea that most asexual species are “ameiotic” or mitotic-like” suggesting they do not undergo meiotic recombination anymore, which indeed would naturally preserve heterozygosity. However, a recent revisit of some iconic asexual species has demonstrated that recombination is at work (Omilian et al., 2006; Simion et al., 2021; Terwagne et al., 2022). As we will show in this review, a lot of these assumptions and classifications are based on old cytological observations which are often unclear and require re-investigations. Therefore, although asexuality probably arose with mechanisms of LOH avoidance, such mechanisms have most often not been identified.

In order to start solving this long-lasting question with modern tools, it is necessary to combine state-of-the-art cytological techniques developed by cell biologists and state-of-the-art genomic approaches developed by evolutionary biologists. Such cooperation between disciplines will greatly benefit from standardization and simplification of the nomenclature, which has evolved and often drifted from the initial definitions, leading to misleading descriptions. Consequently, the concepts are not simple for cell biologists and the mechanisms are difficult to understand for evolutionary biologists.

The outline of our review is: 1) describe why meiosis and mitosis are different and why ameiotic sex does not exist, *i.e.* all species undergo meiosis, 2) which categories of modifications have been described, and propose a simple way to classify them. Show the paradox because LOH is theoretically expected for most of them but LOH is in fact rare. 3) show, through examples, why a revisit of the old cytology is necessary, also highlight the few known mechanisms of LOH avoidance that have been uncovered so far. Throughout the text, we show why paying attention to the nomenclature is important, and is not only a semantic point.

Mitosis and Meiosis are two very different types of cell divisions; “ameiosis” does not exist in animals, unless otherwise proven

Mitosis is the somatic cell division that ensures the proliferation and maintenance of the somatic cells. Upon mitosis, sister chromatids (SCs) are segregated into the two daughter cells. For this purpose, the SCs are attached to the microtubules and bi-oriented towards each of the two poles of the mitotic spindles. Consequently, daughter nuclei inherit one chromatid per homologous chromosome, *i.e.* an assortment of non-sister chromatids (NSCs). In most cases, mitotic divisions generate two daughter cells of equal size because the spindle is positioned in the center of the cell. Asymmetric mitosis exists although the asymmetry in size remains limited in most cases (Betschinger & Knoblich, 2004).

Meiosis is a very different type of division. It occurs only in germline cells and generates gametes. Meiosis involves two successive divisions with no replication in between (Figure 1). During meiosis I, the homologous chromosomes (homologs) which have been paired during meiotic prophase, separate from each other. During meiosis II, the sister chromatids separate from each other. The orientation of the attachment to the microtubules of the SCs changes according to the stage of meiosis to specifically separate homologs in meiosis I and SCs in meiosis II. In meiosis I, within a given pair of homologs, the SCs are co-oriented to the same pole, whereas in meiosis II, SCs are bi-orientated to both poles. Thus, in females, meiosis produces one haploid cell (containing 1 chromatid per homolog) while mitosis generates two diploid daughter cells each carrying an assortment of NSCs. As we will see below, asexual species undergo a modified meiosis, which may affect the number of divisions and/or the assortment of chromatids. For some asexual species, the production of gametes is achieved after a single cell division and leads to the assortment of non-sister chromatids, exactly as found after mitosis. However, we argue that these types of meiosis cannot be assigned to mitosis, because meiosis is different from mitosis for two other essential cellular features.

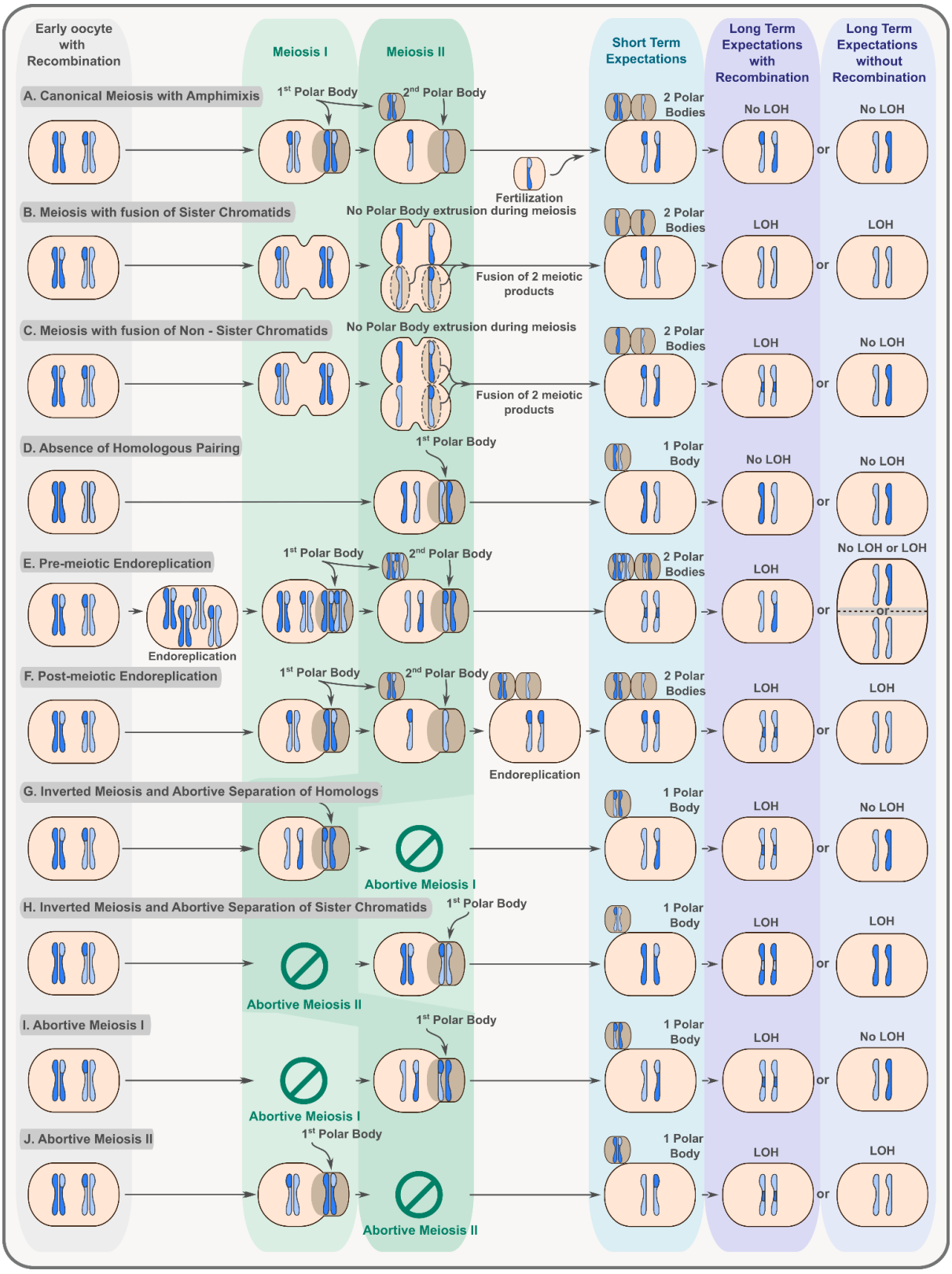

Figure 1: List of 20 possible alterations of meiosis and its theoretical consequences on the genome.

The count includes each type of meiosis with or without recombination. The first line refers to canonical

meiosis. The two columns on the right show the genomic expectations in the long term with and without recombination. In the case of pre-meitotic endoreplication (E), the assortment of two sister chromatids with recombination would also result in LOH. The sister chromatids are in dark and light blue, corresponding to homologous chromosomes. Loss of Heterozygosity (LOH)

First, in contrast to mitosis, meiotic divisions in females do not lead to cell size reduction of the oocyte (Figure 1). In some species, this is achieved by a very asymmetric positioning of the meiotic spindle. Hence, after cytokinesis and asymmetric cell divisions, tiny polar bodies and one large oocyte are formed. In other species, the segregation of chromatids during the meiotic divisions is not accompanied by cell cleavages, leaving the large size of the oocyte intact. The four products of meiosis align and only one of those will become the oocyte nucleus. These are mechanisms allowing the production of large female gametes, full of maternally contributed material (such as proteins, RNAs and yolk). A second main difference resides in the composition and organization of the meiotic chromosomes compared to the mitotic chromosomes. At the early stage of meiosis in prophase I, specific cohesins are loaded on the chromosomes to ensure sister chromatids association (Severson & Meyer, 2014). The distinction between mitotic and meiotic cohesins is based on the kind of kleisin present. Kleisin forms the hinge part of cohesin, the phosphorylation of kleisin results in the opening and removal of cohesin from the DNA. The most conserved meiotic kleisin is the protein REC-8, it is found between the two homologs and sister chromatids only during meiosis. Premature removal of REC-8 hinders the integrality of the meiotic process. Another specificity of meiosis is the pairing of homologous chromosomes. Without pairing the first meiotic division leads to aneuploidy. Indeed, because the sister chromatids are co-oriented, each chromosome (pair of chromatids) can move in either direction, instead of segregating away from its homolog. Successful division in the absence of pairing is only possible provided a change in the orientation of the sister chromatids. Depending on the species, pairing is mediated by different sequences (reviewed in (Rubin et al., 2020). In *C. elegans*, homolog recognition is initiated in sequences called "pairing centers". In *S. cerevisiae, S. pombe* and *D. melanogaster*, the synapse originates at the centromeres. Whereas, in males *M. musculus* and *D. rerio*, the recognition is initiated at the telemoric and subtelomeric regions. After correct recognition, homologous pairing is achieved by the formation of another meiosis-specific element, the synaptonemal complex. The synaptonemal complex is a ladder-like tripartite structure that physically connects the homologs. The lateral parts of the synaptonemal complex are attached with meiotic cohesins

(Woglar et al., 2020). In absence of the synaptonemal complex, pairing is prevented. In many organisms, the pairing of homologs before meiosis I goes along with recombination. Recombination is the reciprocal exchange of part(s) of chromatids between homologs, upon Double Strand Breaks (DSB) induced by the meiotic specific, and highly conserved, topoisomerase-like protein, Spo11. This exchange creates a cohesive structure, the crossing-over, that stabilizes the association between homologs. The cytological manifestation of the crossing-over is the chiasmata. In the absence of crossing overs, as found when chromosomes fail to pair, the segregation of homologs during meiosis I is abnormal and leads to aneuploidy. Depending on the species, DSBs are induced before or after homologous pairing. In *S. cerevisiae, Clytia hemisphaerica*, *D. rerio* or *M. musculus*, for instance, DSBs are necessary for initiating the pairing of homologs (Munro et al., 2023; Rubin et al., 2020). In contrast, in *C. elegans* or *S. pombe*, homologous chromosomes are first paired, and next DSB and crossing overs are initiated. The number of recombination events per chromosome is also highly variable. Indeed, some species, such as *C. elegans*, *Drosophila*, mouse, and humans, one or two recombination events per homolog pair are found, whereas others, such as *S. cerevisiae* present an average of six crossing-overs per pair (Chakraborty et al., 2017). Some species nevertheless show achiasmatic bivalents, i.e. chromosomes that are able to pair but do not undergo crossing over. Hence, although pairing is essential for proper segregation of homologous chromosomes during meiosis I, crossing over are dispensable in some cases (Lenormand & Dutheil, 2005).

In species with holocentric chromosomes, differences between mitotic and meiotic chromosomes are even more striking. Chromosomes of monocentric species have a single attachment site to the microtubules. Therefore, in order to separate only the homologs during meiosis I and not the sister chromatids, the latter must be co-oriented to the same pole. In contrast, during meiosis II, in order to separate the sister chromatids, they are oriented towards each of the two poles. In holocentric species, there is a conflict during meiosis. Because centromeres extend along the entire length of the chromosomes, co-orientation of sister chromatids cannot be achieved for all attachment sites to microtubules. To solve this problem, chromosomes are remodelled into a conformation that enables the sequential segregations of meiosis. The homologous pairs display a specific cruciform structure where one axis of the cross separates the homologs and the other corresponds to the limit between SCs. Thus, in metaphase I, chromatids are not all oriented on the same axis and only the homolog pairs are perpendicular to the metaphase plate. Of note, holocentricity is compatible with an inversion in

the order of segregation events: sister chromatids can separate first, followed by a segregation homolog during the second meiotic division. Such inverted meiosis has been clearly demonstrated for instance in the plant *Rhynchospora pubera* (Cabral et al., 2014).

Some asexual species, as shown below, have lost pairing and homologous recombination. Consequently, a single division segregating away sister chromatids leads to the production of an unreduced oocyte. Because such a modified meiosis is genetically identical to mitosis, these animals have been often categorized as "ameiotic", "mitotic parthenogens". We claim that this nomenclature is really misleading because it suggests the meiotic program has been lost in these asexuals. However, such species have meiotic-like chromosomes and produce the oocyte through a typical meiotic-like asymmetric cell division. Switching from meiosis to a mitotic program during gamete production sounds very unlikely, because meiosis is a very ancient and overly sophisticated multi-step program. We want to stress that this is not only a semantic point (see Box 1). This is an important distinction when it comes to deciphering the mechanistic changes at the origin of asexuality. Hence, unless proven otherwise, all asexual animals described so far undergo meiosis, although meiosis is modified to various extent depending on the species, as described below.

All asexual animals undergo meiosis, most modifications theoretically lead to LOH

In asexual animals, ploidy is maintained, despite the lack of paternal genome contribution, by the production of unreduced gametes. Many modifications to meiosis have been described, in particular in exquisite detail by Suomalainen (Suomalainen et al., 1987) (Figure 1). From a cytological point of view, all these modifications fall into three broad categories. From the least modification compared to the regular two-step meiosis found in sexual species to the most number of changes, we find: i) two-step meiotic divisions compensated by chromosome doubling prior to divisions or after divisions (Figure 1 - E and F), ii) two-step meiotic divisions followed by a final fusion of two of the meiotic products (Figure 1- B and C), iii) one-step meiotic division due to abortion of one division (Figure 1- D, G, H, I and J). In each case, recombination between homologous chromosomes may, or may not be conserved. Below, we briefly describe these "cytological" categories and in doing so,

emphasize that the theoretical genetic expectation for most of these modifications is a long-term loss of heterozygosity (LOH) (Figure 1).

Two-step meiotic divisions with chromosomes endoreplication

One more round of DNA replication can occur in some species, before meiosis (Figure 1 - E, F). This has been called pre-meiotic endoreplication (Luthes 2010, Newton Development 2016, Cuellar 1971, Dedukh et al 2022). Each chromosome being in two copies, the end product of the two-step meiosis is a gamete with two chromatids per chromosome. Depending on how the four chromosomes have formed the two pairs, the gamete will inherit two identical chromatids (SCs) or one chromatid per homolog (NSCs). Upon recombination of the chromosomes, and because of the random segregation of chromatids at each chromosome, some chromosomes will have homozygous regions. In the next generation, chromosomes with small parts of LOH recombine again, increasing the size of LOH. Hence this mechanism progressively leads to LOH. Note however that if pairing occurs only between the two recently duplicated chromosomes (identical chromosomes), the recombinant and non-recombinant chromatids are identical, which is genetically identical as an absence of recombination. In this case, there is no LOH expected, the zygote will be a clone of its mother.

In other cases, the extra replication step occurs after the two-step meiotic divisions, in a process called gamete duplication (Stouthamer & Kazmer, 1994; Zhang et al., 2015) (Figure 1F). This modification results in the presence of two identical chromatids in the gamete. Whether the recombination is maintained or not in the earlier steps, the zygote will immediately be homozygous genome-wide, *i.e.* immediate LOH.

Two-step meiotic divisions followed by fusion of the meiotic products

In some species, the two regular meiotic divisions occur without cell cleavage (no polar bodies are extruded). The four products of meiosis are therefore present, often in a row, within the oocyte, only one of those will fuse with the sperm DNA. This is the case for instance in fungi or insects (see Box 1). Asexual species that have emerged in these groups, can restore ploidy by the fusion of two of these meiotic products, which is facilitated by the absence of cytoplasmic membranes surrounding the four meiotic products. The mechanism of fusion is for now still unknown. If the products of meiosis II fuse, it leads to sister chromatid assortment.

Cytologically, it corresponds to the fusion of the terminal product of meiosis and has been called 'terminal fusion' (Figure 1B). This is found for instance in the insect *Pristiphora pallipes* (Comrie, 1938). If the central products fuse, here the products of the first division, it leads to the assortment of non-sister chromatids, i.e. called "central fusion" (Figure 1C). The term has been first used by Carson in 1961, who described the mechanisms of facultative parthenogenesis in *Drosophia parthenoegentica*, *D. robusta* with *D. mangabeirai* (Carson, 1961; Murdy & Carson, 1959; Stalker, 1954). *Apis mellifera capensis*, *Cerapachys biroi* are other examples of insects undergoing a typical central fusion (Cole-Clar, et al. 2017; Oxley, et al. 2014). If recombination is maintained in these species, LOH is expected distally to the crossing-over in the case of "central fusion" or proximally in the case of "terminal fusion". In the absence of homologous recombination during meiotic prophase, only "terminal fusion" should lead to immediate and genome-wide LOH.

The term "fusion", is extensively used in the current literature on asexuals, whereby the cytological phenomenon is used to describe a genetic content: "terminal fusion" is used in the case of "assortment of sister chromatids" whereas "central fusion" means "assortment of non-sister chromatids". As discussed in Box 1, it is based on initial descriptions made in specific taxa, namely fungi and insects (Doty & Menez, 1960; Stalker, 1954). For many species, as shown below, the products of meiosis are extruded into a polar body (separated from the oocyte by a cell membrane), and most likely cannot undergo fusion to restore ploidy. Beyond the semantic point, the classification based on such a cytological description has generated a lot of confusion in the field. For instance, if a fusion was not observed, authors have concluded that the division was "apomictic", which literally means "without fusion". However, "apomixy" is also associated with "mitotic-like parthenogenesis" based on the plant nomenclature (see Box 1). This way, some species, such as Artemia and Daphnia, have been wrongly classified for a long time as species which have "'lost meiosis".

Abortion of one meiotic division

Abortive Meiosis I

In abortive meiosis I, the segregation of homologous chromosomes fails, and only sister chromatids segregate during a single division. It leads to the assortment of non-sister chromatids

(Figure 1 - D, G and I). There are multiple causes for an abortive meiosis I, such as incorrect segregation of homologs (Dumont et al., 2010; Hollis et al., 2020; Siomos et al., 2001), tripolar spindle poles (Yamashita et al., 1993), incomplete spindle rotation (McNally et al., 2016) or a defect in polar body extrusion (Carmena et al., 1998; Yang et al., 2010). Regardless of the mechanism, abortion of meiosis I in the presence of recombination, will lead to LOH distally to the crossing-over, i.e. only the central region of chromosomes maintains some heterozygosity. If the CO is very close to chromosome ends, LOH is restricted to small regions at the tip of chromosomes (Oldroyd et al., 2021).

Abortive meiosis I is also occurring when chromosomes are unable to pair during the early step of meiotic prophase, or if pairing is unstable in the absence of a crossing-over. Indeed, segregation of chromosomes during meiosis I would lead to aneuploidy if the chromosomes are not initially bound, because of the co-orientation of the sister chromatids. Therefore, species for which pairing is lost can restore ploidy only by skipping meiosis I and segregating sister chromatids away during a single meiotic division. Because an absence of pairing induces an absence of recombination, the assortment of non-sister chromatids ensures a high level of heterozygosity genome-wide. This is expected theoretically and has been empirically confirmed for *Poecillia formosa, Meloidogyne incognita* and *Diploscapter pachys* for instance (Abad et al., 2008; Fradin et al., 2017; Warren et al., 2018). As discussed above, because this type of modified meiosis is genetically identical to mitosis (segregation of sister chromatids in the absence of recombination), it has been often called “ameiosis” or “mitotic-like” (see Box 1). We have seen that this is only an extreme case of modified meiosis, whereby the first steps of meiotic prophase are skipped (pairing of homologs). We prefer using the term “assortment of NSC without recombination” to describe such type of meiosis modification. Cytologically, it is also not very different from an abortive meiosis I due to failure to extrude the first polar body for instance. As illustrated in Box 2, there are ways to distinguish the two, and the cytological distinction is essential to understand the theoretical genetic consequences.

Importantly, the mechanisms of meiosis abortion are generally unknown. Yet, for some species it is clear that the very first step of meiosis during which the synaptonemal complex is loaded on chromosome axis is absent (Dedukh et al., 2022; Fradin et al., 2017; M. Marais, 2012). In other species, the synaptonemal complex is properly assembled but chromosomes still fail to complete pairing (Amazon molly (Dedukh et al., 2022)). More work is needed to understand which molecular changes prevent chromosome pairing in all these species. Other obvious candidates are the meiotic cohesins whose destabilisation could lead to premature

release of chromosomes. REC8 has been associated with transition from cyclic parthenogenesis to obligate parthenogenesis. Three polymorphic REC8 loci are identified in Daphnia species. Interestingly, all asexual females carry the same REC8 insertion upstream of a transposon, which could interfere with REC8 function (Eads et al., 2012; Molinier et al., 2021). The exact mechanism is unclear because loss of REC-8 may lead to a total dissociation of all chromatids, preventing any division. In Cape Honey Bee, a single locus is the major determining factor for parthenogenesis (Oldroyd et al., 2021). Interestingly, this locus shows similarity with SMC proteins, a protein family to which REC-8 belongs. Overall, whether many molecular changes can lead to abortion of meiosis or only very few are tolerated remains an open question.

ex: fish *Carassius auratus*, the crustacean *Procambarus virginalis*, the amphibian *Ambystoma,* in the rotifer *A. ricciae*, *Rotaria macrura*, *R. magnacalcarata*, and the, *M. enterolobii*, and the amphibian *Ambystoma* (Elinson et al., 1992; Kato et al., 2016; M. and de W. K. Marais, 1991; Rebecchi, 1991; Triantaphyllou & Moncol, 1977; Yamashita et al., 1993)

Abortive Meiosis II

In some asexual species (Chapman et al., 2008; Feldheim et al., 2010, 2017; Harmon et al., 2016; Liu et al., 2007; Portnoy et al., 2014; Robinson et al., 2011; Triantaphyllou, 1966; Van Der Beek et al., 1998), homologs are properly segregated during meiosis I but the subsequent separation of SCs fails (Figure 1 - H and J). These phenomena can also be the consequences of incomplete spindle rotation (McNally et al., 2016) or a defect in polar body extrusion (Carmena et al., 1998; Yang et al., 2010), as well as a defect in cohesion release of SCs (Kaitna et al., 2002; Rogers et al., 2002; Siomos et al., 2001). This time, oocytes containing an assortment of SCs will be produced, thus the genome will become rapidly homozygous. However, here, the presence of recombination will slightly slow down LOH in comparison to abortive meiosis II without recombination, leaving stretches of heterozygosity distally to the crossing-over (Figure 1- H and J). Here, again, the mechanisms are unknown.

The isopteran clade of Hexapoda is composed of the orders of cockroaches and termites. In both orders, species have demonstrated the capacity to undergo facultative parthenogenesis. (Khan et al., 2022; Komatsu et al., 2015; Tanaka & Daimon, 2019). Interestingly in termites, studies using genetic markers revealed that the mechanism differ according to the species

(Fournier et al., 2016; Tanaka & Daimon, 2019). In some cases, an association of sister chromatids is observed ("terminal fusion"). In other, association of non-sister chromatids is achieved, but through several mechanisms: abortive meiosis I or "central fusion" This example shows how flexible the meiotic program is in some clades.

The specific case of inverted meiosis

Some sexual species with holocentric chromosomes undergo a two-step meiosis, although the order of events are inverted (Figure 1 - G and H). SCs chromatids are first segregated while homologs are separated in the second round of cell division. Demonstrating an inverted meiosis is a complicated task, which requires the existence of dimorphic chromosomes (Cabral et al., 2014), in order to distinguish the segregation of chromatids to chromosomes at each division. Theoretically, abortion of one meiotic division in the case of inverted meiosis is also possible for an asexual species, generating "assortment of SC " if the first division fails and 'assortment of NSC' if the second division fails. Here again, if recombination is maintained, LOH is expected. Inverted meiosis with abortive meiosis I have so far been demonstrated in asexual Coccids and the plant *Luzula elegans* (Bongiorni et al., 2004; Heckmann et al., 2014). Inverted meiosis with abortive meiosis II has been proposed for Oribatid mites, as discussed below (Archetti, 2022).

Interestingly, the three different categories of meiosis are mediated by distinct mechanisms, but ultimately have similar features in terms of chromatid content in the unreduced gamete. Furthermore, the meiosis of parthenogenetic species can be classified according to their content in chromatids (sister chromatids or non-sister chromatids) and the presence of recombination (Figure 2). Indeed, the presence of recombination results in LOH (Figure 2 – A, B). In contrast, association of non-sister chromatids with the absence of recombination ensures the maintenance of heterozygosity (Figure 2D).

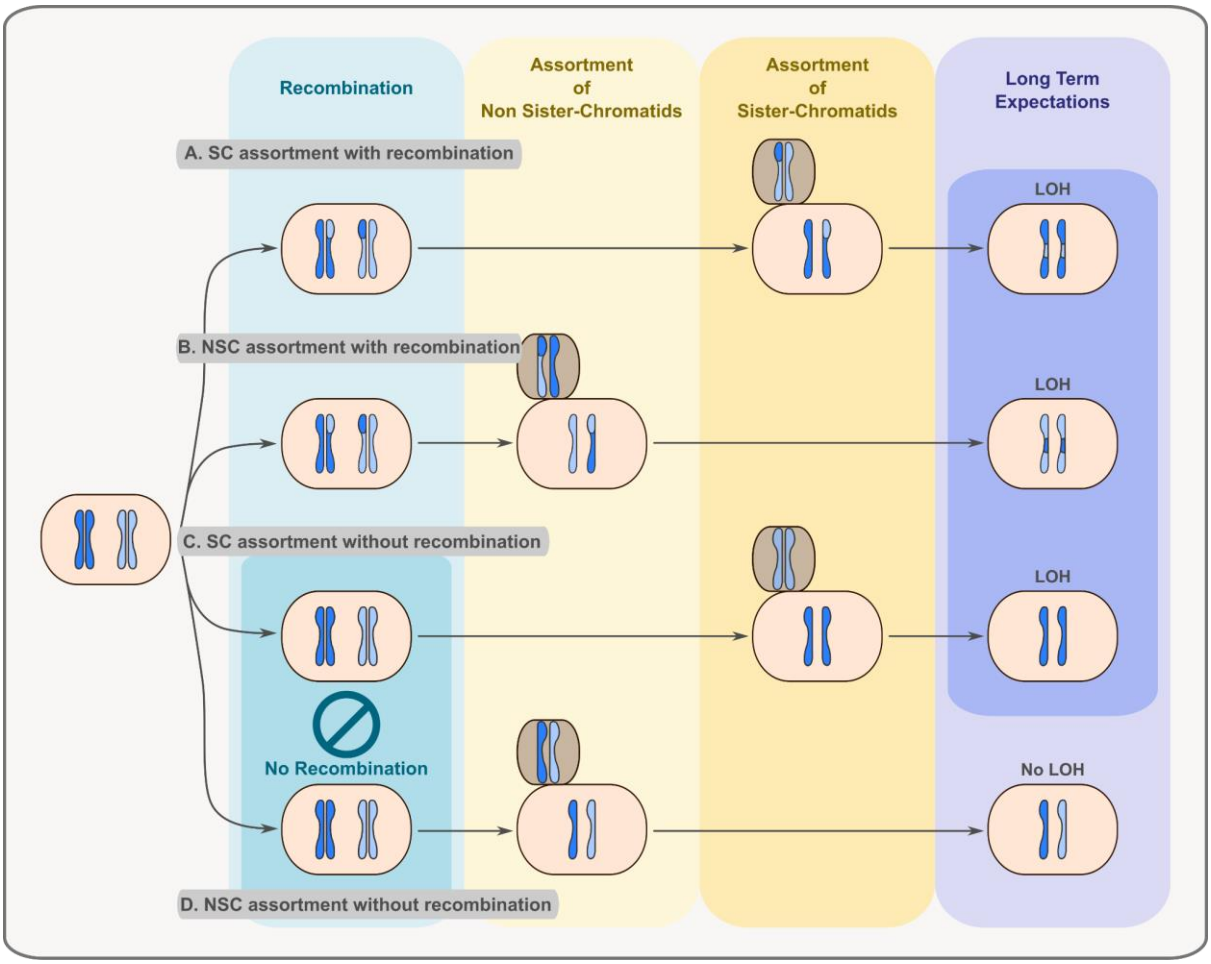

Figure 2: Influence of recombination and assortment of chromatids on the genome. The assortment of sister chromatids leads systematically to LOH, as well as the assortment of non-sister chromatids with recombination.

Mechanisms of LOH avoidance in asexual animals, cytology revisited

We have seen that regardless of the modification of meiosis that has been selected in the different branches leading to asexuality, most are theoretically expected to generate partial or total LOH for the asexual animals. In the absence of recombination however, no LOH is expected and the genome should show a high level of heterozygosity.

LOH is supposedly not good for fitness, and therefore, being an asexual with LOH is probably counter selected in most cases (although exceptions exist), this could explain why asexuals are rare (Avise, 2008; Normark, 2014). In agreement with the expectation, a lot of asexuals show little or no LOH. Is it because most of them have lost recombination? In this case, it would suggest that loss of recombination is one of the few efficient strategies to

transition towards asexuality from a regular meiosis. The literature has propagated this idea for a long time. First because the cytology was often imprecise and has categorized species as "mitotic-like" suggesting they do not undergo recombination. Second, many studies were based on the analysis of genetic and genomic analysis alone, and used circular arguments: because the species is highly heterozygous, it must have lost recombination, but there was no cytological data to support these conclusions. Such lack of confrontation, or not rigorous enough, between the cytological and genomic empirical data has led to many misinterpretations. Recent revisits have shown that many asexuals, even the most famous ones, have not lost homologous recombination. Hence, the question remains: which mechanism allows the maintenance of heterozygosity in recombining asexual animals? The question is essential for the field because it questions at the same time the mechanistic and the genetic constraints in the asexual world.

At the beginning of the 20th century, most asexual species were studied using cytological approaches, as witnessed by Suomalainen (Suomalainen, 1950; Suomalainen et al., 1987). With the development of genomic techniques, many authors then sought to understand how asexuality affects the genome. In many organisms, cytological studies are laborious and have been discontinued over time. For this reason, there is often little contemporary cytological evidence of asexual meiosis exploiting up-to-date techniques. Below we describe some species for which evidence of a lack of pairing and recombination has been clearly demonstrated in the past. We next present some of these recent revisits, as well as few cases for which a mechanistic basis of LOH avoidance has been revealed.

True non-recombining asexual/apomictic

The Amazon molly, *Poecilia formosa*, was described as the first parthenogenic vertebrate (Hubbs & Hubbs, 1932). Cytogenetic analyses revealed that the pairing of chromosomes in the females of *P. formosa* is absent and no bivalents are formed. Thus, only the separation of sister chromatids is achieved during meiosis II. Unreduced gametes contain an assortment of non-sister chromatids without the possibility of recombination (Dedukh, da Cruz, et al., 2022). Those observations are consistent with genetic analyses that revealed that the heterozygosity level of *P. formosa* is 10-fold higher than that of close sexual species (Warren et al., 2018).

This is also true for the nematodes *D. pachys* and *M. incognita*. Conclude by saying that in most other cases the conclusions are not so simple and have led to misinterpretations. For instance, in *D. pachys*, using cytology authors revealed that homologous pairing is absent in meiosis I. In absence of pairing, homologs are unable to segregate and the only division observed consists of the separation of SCs in meiosis II (Fradin et al., 2017). In addition, the lack of pairing prevents recombination is abrogated. The same meiotic process is found in the close species, *D. coronatus* (Hiraki et al., 2017). In nematodes pairing is also absent in *M. incognita* and *M.* arenaria (M. Marais, 2012; Triantaphyllou, 1963, 1981).

Apomixy revisited, recombining asexuals with LOH, no paradox

The emblematic crustacean *Daphnia pulex* nicely illustrates the matter. *D. pulex* is a cyclical parthenogenetic species that can be maintained as an obligate parthenogen. In this species, LOH is observed during the asexual phase and might be counterbalanced by sexual events (Omilian et al., 2006). During the asexual phase, the meiosis of *D. pulex* has long been described as apomictic/ameiotic, *i.e.* non-recombining asexual (Kühn, 1908; Ojima, 1954; Zaffagnini & Sabelli, 1972). This was based on the presence of a single polar body. As explained in Box 1, cytological studies used the observations made in fungi and insects as reference. In these species, the first step of meiosis produces two haploid polar bodies, (because the first polar body also divides into two cells after being extruded) (Tremblay & Caltagirone, 1973), while the second step produces a single polar body. For this reason, the authors concluded that meiosis I was not present in *D. pulex* and only sister chromatids segregated. Therefore, the authors assumed that the mechanism was genetically similar to mitosis and indirectly that chromosomes should not recombine. This led to a contradiction because the absence of recombination should prevent LOH. Reinvestigations using detailed cytology have revealed that during the asexual phase, homologous chromosomes are paired at the onset of meiosis I. Next, the segregation of homologs is initiated but aborted. Consequently, univalent chromosomes align on the metaphase plate and a second meiotic division occurs, segregating sister chromatids away. The unreduced gamete therefore contains an assortment of NSCs and one polar body is produced (Hiruta et al., 2010, 2012). Genomics analysis confirmed that chromosomes recombine (Omilian et al., 2006; Xu et al., 2011). Hence, the contradiction was solved: assortment of non-sister chromatids upon recombination leads to LOH, in agreement with the observed genomic pattern.

Apomixy revisited: recombining and no LOH: an unsolved paradox

Adineta vaga is another interesting example. Here, the old cytology was in agreement with the genomics (Hsu, 1956a, 1956b), but as Simion & al. revisited the cytology, they revealed a paradox (Simion et al., 2021; Terwagne et al., 2022). *A. vaga* is a bdelloid rotifers also known as an “asexual scandal " because despite its obligate asexuality, it is probably asexual for more than 50 million years (Poinar & Ricci, 1992; Tang et al., 2014). Bdelloid rotifers have been also qualified for decades as “apomictic”, meaning pairing is prevented thus no recombination can occur (Melone & Ricci, 1995). Genomic data revealed that *A. vaga* has a largely heterozygous genome which was consistent with an absence of recombination. However, recent cytological investigations have demonstrated that during the meiosis of *A. vaga*, homologous chromosomes form pairs and that a unique meiotic division segregate sister chromatids apart, *i.e.* abortive meiosis I (Simion et al., 2021; Terwagne et al., 2022). Importantly, joined cytology and genomics revealed that the six pairs of homologous chromosomes recombine (Simion et al., 2021). This work has highlighted a paradox because *A. vaga* is an ancient recombining asexual, which nevertheless maintains a highly heterozygous genome. At this stage, the mechanism of LOH avoidance is unknown but hypotheses on the displacement of crossing-overs at the tip of chromosomes or implication of horizontal gene transfer have been proposed (Hespeels et al., 2014; Simion et al., 2021).

Recombining asexual and LOH at the tip: no paradox, displaced CO

A. mellifera capensis, the Cape honey bee, is a facultative parthenogenetic species. When the queen of a colony is lost, workers spontaneously produce queens and workers via parthenogenesis. Joined cytology and genomics revealed that, in *A. mellifera capensis*, diploidy is maintained by assorting NSCs and recombination is present, although the genome is heterozygous (Baudry et al., 2004; Cole-Clark et al., 2017; Goudie et al., 2012). Interestingly, the recombination differs according to the fate of the eggs. In future queens, recombination is comparable to sexually produced queens. In contrast in future workers, the rate of recombination is lower and limited to the tips of chromosomes. Hence, only the subtelomeric regions suffer from LOH without affecting the coding genes. In this species, homologous pairing and chiasmata are required for the popper segregation of chromosomes (Oldroyd et al., 2021). This raises a question: how does the decrease in recombination rate translate

cytologically when chiasmata are necessary for segregation? In this context, cytology and microscopy are valuable tools for deciphering genomic evidence.

R*ecombining asexual and no LOH due to directed chromatid assortment*

In the nematode *Mesorhabditis belari*, asexual females are produced upon abortive meiosis I (Blanc et al., 2023). Here, chromosomes (which are holocentric) are paired and form a cross-shape structure resembling the chiasmata found in other holocentric species. Evidence of recombination was confirmed by the use of EdU, which allowed the labeling of one chromatid out of the two of a given chromosome. If two chromatids of a different color recombine (which statistically happens in 50% of the combination), a bicolor chromatid should be visible. Using this approach, the authors demonstrated that all chromosomes recombine and also that recombination is not restricted to the tip of chromosomes, in the subtelomeric region, but spans all along the chromosomes. Recombination was also confirmed by a genomic approach, using the analysis of linkage disequilibrium between loci. *M. belari* females are therefore recombining asexuals with an assortment of non-sister chromatids. In such a scenario, LOH distally to the crossing-over is theoretically expected. However, the genome of *M. belari* is heterozygous genome-wide with no signs of LOH, even locally. The authors thus hypothesized that the NSCs segregation was not Mendelian, but led to the assortment of chromatids with complementary genetic contents. In other words, that heterozygosity could be maintained is the recombinant chromatids of a given chromosome pair co-segregated, either in the egg or in the polar body. By following the segregation of the recombining EdU bicolor-chromatids, they confirmed such a bias segregation of chromatids. This mechanism, named Directed Chromatid Assortment, prevents LOH in a recombining asexual. How the recombinant chromatids become co-oriented during division is for the moment unknown. Interestingly, distal CO destabilizes the bivalents in the holocentric species *C. elegans* (Dernburg et al., 1998), strongly suggesting that distal CO, the other mechanism preventing LOH, would face difficulties evolving within a holocentric species. Whether DCA is restricted to holocentric species remains to be demonstrated.

A mechanism based on asymmetric segregation of chromatids has also been proposed for the other “asexual scandal” Oribatid mites. Cytological observations have revealed that the species *Platynothrus peltifer* for instance, has holocentric chromosomes, which form bivalents at the onset of meiosis, strongly suggesting the chromosome do recombine. From fixed samples, Taberly has reconstituted the different steps of meiosis and concluded that the second meiotic

division was abortive (Taberly, 1958, 1960). Oribatid mites would therefore belong to the category of recombining asexuals, where SCs are assorted. A largely homozygous genome is expected in this case, although little heterozygosity is expected distally to the CO. However, Oribatid mites show only a small difference in heterozygosity between sexual and parthenogenetic strains (Bergmann et al., 2018; Brandt et al., 2021; Palmer & Norton, 1992; Schaefer et al., 2006)). To reconcile these results, it has been proposed that Oribatid mites may undergo an inverted meiosis (this is possible because these are holocentric species), combined with a biased segregation of chromatids (called Z segregation) whereby the recombinant chromatids cosegregate (Archetti, 2022). In this case, as in the case of *M. belari*, heterozygosity would be maintained. However, at this stage, there is no evidence of an inverted meiosis in Oribatid mites. As mentioned earlier, heterologous chromosomes are needed to formally prove such an inversion in the divisions (Senaratne et al., 2022). Moreover, the descriptions of the sequence of events by Taberly in the 60s are limited to a one page article and a single drawing (Taberly, 1958), so that at this stage, the demonstration that meiosis II is abortive rather than meiosis I is really questionable. Therefore, it could very well be that similar to *M. belari*, Oribatid mites recombine, fail meiosis I and use DCA as a mechanism of LOH avoidance. The case of Oribatid mites illustrates very well how important a revisit of the old cytology is required, although, as pointed out by Taberly himself, exploring meiosis in these tiny animals is a real challenge.

Conclusion

Here we have seen different possibilities for long term maintenance of asexuals: either the species does not suffer from becoming homozygous as in the genus *Timema* (Bast et al., 2018; Jaron et al., 2022) or because they are transient asexuals, or mechanisms of LOH avoidance have been selected. So far, two categories of mechanisms are distinguished: those that influence recombination and those that modify chromatid segregation during meiosis. Heterozygosity can be preserved by completely suppressing recombination as mentioned before, e.g. by preventing homologous pairing, by reducing the rate of recombination which only delays LOH, or by displacing the CO to the tips of the chromosomes. We have seen that maintenance of heterozygosity can also be ensured by selecting the chromatids which are inherited by the zygote. By sorting the two recombinant chromatids or the two non-recombinant chromatids in unreduced gametes using Directed Chromatid Assortment, heterozygosity is

preserved from mother to daughters and over generations (Archetti, 2022); Blanc et al., 2023(Archetti, 2022). For now, this mechanism has been demonstrated only in *Mesorhabditis belari* (Blanc et al., 2023). It remains hypothetical in the case of Oribatid mites (Archetti). DCA, or yet to be discovered other mechanisms may exist in other species. All the recombining asexuals with no signs LOH should be re-examined in this context. This will undoubtedly reveal other fascinating rewiring of the meiotic program.

BOX 1: Why does the current nomenclature refer to apomixis, central fusion and terminal fusion?

In 1973, Tremblay and Caltagirone in “Fate of polar bodies in insects" describe two classes of modified meiosis: *In apomictic (or ameiotic) parthenogenesis the eggs usually undergo only one mitotic or parameiotic division, which is equational, with expulsion of only one diploid polar body. In meiotic (or automictic) parthenogenesis maturation is reductional, and a haploid pronuclei and one or two polar bodies are formed; the somatic number of chromosomes is restored by fusion of two of the haploid nuclei resulting from maturation, or by fusion of two cleavage nuclei*.

These terms and definitions are widely used for describing asexual animals. We argue that they have generated a lot of confusion and misunderstanding in the field. In insects, the two meiotic divisions occur successively without cytokinesis. Hence, the four products of meiosis, or polar bodies, are aligned in a row within the oocyte. Only the most central haploid product of meiosis will become the oocyte nucleus (Figure 1C). In asexuals, ploidy can be restored by the fusion of these meiotic products, by either the assortment of the SCs as for terminal fusion (fusion of the products of the last division) or an assortment of NSCs in the case of central fusion (fusion of the products of each initial division). Here, the term automixis is used because there is indeed a fusion of the meiotic products and after fusion, two products are left aside. However, if one meiotic division is skipped or is abortive, only two polar bodies are generated, one of which will become the diploid oocyte nucleus. Semantically, this modification can be indeed called “apomixis”, because ploidy is not restored by a fusion event. If the division that is abortive is the first one, there is only an equational division, maintaining together the non-sister chromatids, which is genetically equivalent to mitosis. For an obscure reason, over the years, the term “Apomixis”, has been systematically associated with the term “Ameiosis”, although an absence of fusion does not mean that meiosis is lost, it also does not mean that an equational division is expected (if meiosis II is abortive for instance). Importantly also, the assortment of NSC is genetically identical to a mitotic division provided there is no recombination but, in most species, studied, the loss of homologous recombination has not been demonstrated.

BOX 2: Using pairing to assess LOH

The theoretical genetic expectation strongly depends on the presence or not of recombination. In the absence of recombination, the genome of asexuals will not suffer from LOH (Figure 1, 2,3). When recombination is maintained, the absence of LOH is not expected and mechanisms of LOH avoidance must be identified. As demonstrated above, this is not a simple task, because for many species, following the different steps of the meiotic division is a real technical challenge. Nevertheless, there is a way to first identify whether there is a contradiction between the cytology and the genomics, by simply assessing whether recombination is maintained or not. Achiasmatic chromosomes exist, whereby homologous chromosomes are paired in the absence of crossing-over/recombination. Proving the absence of a chiasmata in this context can be a complicated task. However, for sure, if the homologs are not paired, recombination is impossible. Therefore, identifying whether homologs are paired or not in an asexual species is a first step to assess recombination. A simple way to evaluate pairing is to compare the number of chromosomes in mitosis, in any somatic cell, to the number of chromosomes prior to the meiotic division (i.e. diakinesis stage) (Figure 3). If these numbers are similar, the pairing does not occur. Conversely, if there are half as many chromosomes in mitosis as in meiosis, the homologous chromosomes are paired and recombination is possible.

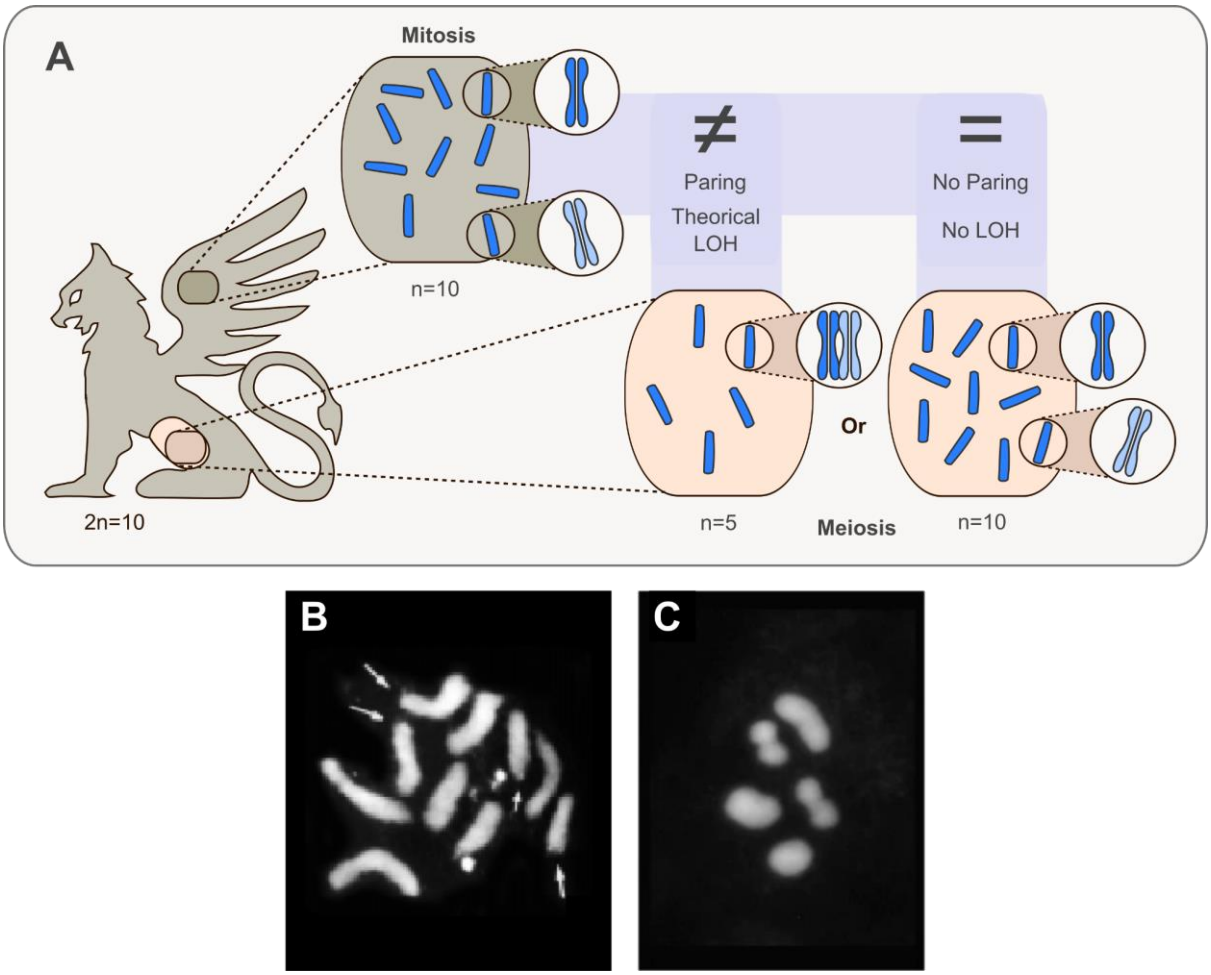

Figure 3: Evaluation and Influence of pairing on the loss of heterozygosity. A) Schematic representation of the difference in the number of chromosomes between mitosis and meiosis and according to pairing. Somatic cells are in grey and germline cells in light orange. Loss of Heterozygosity (LOH). B-C) Chromosomes of the plant *Rhynchospora pubera* are paired in meiosis (2n=10). B shows the 10 chromosomes present during mitosis and C shows the 5 bivalents of meiosis. DNA is in grey. Images adapted from (Cabral et al., 2014; Vanzela & Guerra, 2000)

References

Cabral, G., Marques, A., Schubert, V. *et al.* Chiasmatic and achiasmatic inverted meiosis of plants with holocentric chromosomes. *Nat Commun* **5**, 5070 (2014). https://doi.org/10.1038/ncomms6070

Abad, P., Gouzy, J., Aury, J.-M., Castagnone-Sereno, P., Danchin, E. G. J., Deleury, E., Perfus-Barbeoch, L., Anthouard, V., Artiguenave, F., Blok, V. C., Caillaud, M.-C., Coutinho, P. M., Dasilva, C., De Luca, F., Deau, F., Esquibet, M., Flutre, T., Goldstone, J. V., Hamamouch, N., … Wincker, P. (2008). Genome sequence of the metazoan plant-parasitic nematode Meloidogyne incognita. *Nature Biotechnology*, *26*(8), Article 8. https://doi.org/10.1038/nbt.1482

Archetti, M. (2022). Evidence from automixis with inverted meiosis for the maintenance of sex by loss of complementation. *Journal of Evolutionary Biology*, *35*(1), 40–50. https://doi.org/10.1111/jeb.13975

Avise, J. (2008). *Clonality: The Genetics, Ecology, and Evolution of Sexual Abstinence in Vertebrate Animals*. Oxford University Press, USA.

Bast, J., Parker, D. J., Dumas, Z., Jalvingh, K. M., Tran Van, P., Jaron, K. S., Figuet, E., Brandt, A., Galtier, N., & Schwander, T. (2018). Consequences of Asexuality in Natural Populations: Insights from Stick Insects. *Molecular Biology and Evolution*, *35*(7), 1668–1677. https://doi.org/10.1093/molbev/msy058

Baudry, E., Kryger, P., Allsopp, M., Koeniger, N., Vautrin, D., Mougel, F., Cornuet, J.-M., & Solignac, M. (2004). Whole-Genome Scan in Thelytokous-Laying Workers of the Cape Honeybee (Apis mellifera capensis): Central Fusion, Reduced Recombination Rates and Centromere Mapping Using Half-Tetrad Analysis. *Genetics*, *167*(1), 243–252. https://doi.org/10.1534/genetics.167.1.243

Bell, G. (1982). *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*.

Bergmann, P., Laumann, M., Norton, R. A., & Heethoff, M. (2018). Cytological evidence for automictic thelytoky in parthenogenetic oribatid mites (Acari, Oribatida): Synaptonemal complexes confirm meiosis in Archegozetes longisetosus. *Acarologia*, *58*(2), 342–356. https://doi.org/10.24349/acarologia/20184246

Betschinger, J., & Knoblich, J. A. (2004). Dare to Be Different: Asymmetric Cell Division in Drosophila, C. elegans and Vertebrates. *Current Biology*, *14*(16), R674–R685. https://doi.org/10.1016/j.cub.2004.08.017

Blanc, C., Saclier, N., Faou, E. L., Marie-Orleach, L., Wenger, E., Diblasi, C., Glemin, S., Galtier, N., & Delattre, M. (2023). *Co-segregation of recombinant chromatids maintains genome-wide heterozygosity in an asexual nematode* (p. 2023.03.17.533182). bioRxiv. https://doi.org/10.1101/2023.03.17.533182

Bongiorni, S., Fiorenzo, P., Pippoletti, D., & Prantera, G. (2004). Inverted meiosis and meiotic drive in mealybugs. *Chromosoma*, *112*(7), 331–341. https://doi.org/10.1007/s00412-004-0278-4

Brandt, A., Tran Van, P., Bluhm, C., Anselmetti, Y., Dumas, Z., Figuet, E., François, C. M., Galtier, N., Heimburger, B., Jaron, K. S., Labédan, M., Maraun, M., Parker, D. J., Robinson-Rechavi, M., Schaefer, I., Simion, P., Scheu, S., Schwander, T., & Bast, J. (2021). Haplotype divergence supports long-term asexuality in the oribatid mite Oppiella nova. *Proceedings of the National Academy of Sciences*, *118*(38), e2101485118. https://doi.org/10.1073/pnas.2101485118

Cabral, G., Marques, A., Schubert, V., Pedrosa-Harand, A., & Schlögelhofer, P. (2014). Chiasmatic and achiasmatic inverted meiosis of plants with holocentric chromosomes. *Nature Communications*, *5*(1), Article 1. https://doi.org/10.1038/ncomms6070

Carmena, M., Riparbelli, M. G., Minestrini, G., Tavares, Á. M., Adams, R., Callaini, G., & Glover, D. M. (1998). Drosophila Polo Kinase Is Required for Cytokinesis. *Journal of Cell Biology*, *143*(3), 659–671. https://doi.org/10.1083/jcb.143.3.659

Carson, H. L. (1961). Rare parthenogenesis in Drosophila robusta. *The American Naturalist*, *95*(881), 81–86.

Chakraborty, P., Pankajam, A. V., Lin, G., Dutta, A., Krishnaprasad, G. N., Tekkedil, M. M., Shinohara, A., Steinmetz, L. M., & Nishant, K. T. (2017). Modulating Crossover Frequency and Interference for Obligate Crossovers in Saccharomyces cerevisiae Meiosis. *G3: Genes|Genomes|Genetics*, *7*(5), 1511–1524. https://doi.org/10.1534/g3.117.040071

Chapman, D. D., Firchau, B., & Shivji, M. S. (2008). Parthenogenesis in a large-bodied requiem shark, the blacktip Carcharhinus limbatus. *Journal of Fish Biology*, *73*(6), 1473–1477. https://doi.org/10.1111/j.1095-8649.2008.02018.x

Cole-Clark, M. P., Barton, D. A., Allsopp, M. H., Beekman, M., Gloag, R. S., Wossler, T. C., Ronai, I., Smith, N., Reid, R. J., & Oldroyd, B. P. (2017). Cytogenetic basis of thelytoky in Apis mellifera capensis. *Apidologie*, *48*(5), 623–634. https://doi.org/10.1007/s13592-017-0505-7

Comrie, L. C. (1938). Biological and Cytological Observations on Tenthredinid Parthenogenesis. *Nature*, *142*(3602), Article 3602. https://doi.org/10.1038/142877a0

Dedukh, D., da Cruz, I., Kneitz, S., Marta, A., Ormanns, J., Tichopád, T., Lu, Y., Alsheimer, M., Janko, K., & Schartl, M. (2022). Achiasmatic meiosis in the unisexual Amazon molly, Poecilia formosa. *Chromosome Research*, *30*(4), 443–457. https://doi.org/10.1007/s10577-022-09708-2

Dernburg, A. F., McDonald, K., Moulder, G., Barstead, R., Dresser, M., & Villeneuve, A. M. (1998). Meiotic Recombination in C. elegans Initiates by a Conserved Mechanism and Is Dispensable for Homologous Chromosome Synapsis. *Cell*, *94*(3), 387–398. https://doi.org/10.1016/S0092-8674(00)81481-6

Doty, M. S., & Menez, E. G. (1960). Tiffaniella, a New Genus in the Ceramiales. *Transactions of the American Microscopical Society*, *79*(2), 135. https://doi.org/10.2307/3224079

Dumont, J., Oegema, K., & Desai, A. (2010). A kinetochore-independent mechanism drives anaphase chromosome separation during acentrosomal meiosis. *Nature Cell Biology*, *12*(9), 894–901. https://doi.org/10.1038/ncb2093

Eads, B. D., Tsuchiya, D., Andrews, J., Lynch, M., & Zolan, M. E. (2012). The spread of a transposon insertion in Rec8 is associated with obligate asexuality in Daphnia. *Proceedings of the National Academy of Sciences*, *109*(3), 858–863. https://doi.org/10.1073/pnas.1119667109

Elinson, R. P., Bogart, J. P., Licht, L. E., & Lowcock, L. A. (1992). Gynogenetic mechanisms in polyploid hybrid salamanders. *Journal of Experimental Zoology*, *264*(1), 93–99. https://doi.org/10.1002/jez.1402640114

Engelstädter, J. (2008). Constraints on the evolution of asexual reproduction. *BioEssays*, *30*(11-12), 1138–1150.

Feldheim, K. A., Chapman, D. D., Sweet, D., Fitzpatrick, S., Prodöhl, P. A., Shivji, M. S., & Snowden, B. (2010). Shark Virgin Birth Produces Multiple, Viable Offspring. *Journal of Heredity*, *101*(3), 374–377. https://doi.org/10.1093/jhered/esp129

Feldheim, K. A., Clews, A., Henningsen, A., Todorov, L., McDermott, C., Meyers, M., Bradley, J., Pulver, A., Anderson, E., & Marshall, A. (2017). Multiple births by a captive swellshark Cephaloscyllium ventriosum via facultative parthenogenesis. *Journal of Fish Biology*, *90*(3), 1047–1053. https://doi.org/10.1111/jfb.13202

Fournier, D., Hellemans, S., Hanus, R., & Roisin, Y. (2016). Facultative asexual reproduction and genetic diversity of populations in the humivorous termite Cavitermes tuberosus. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1832), 20160196. https://doi.org/10.1098/rspb.2016.0196

Fradin, H., Kiontke, K., Zegar, C., Gutwein, M., Lucas, J., Kovtun, M., Corcoran, D. L., Baugh, L. R., Fitch, D. H. A., Piano, F., & Gunsalus, K. C. (2017). Genome Architecture and Evolution of a Unichromosomal Asexual Nematode. *Current Biology: CB*, *27*(19), 2928-2939.e6. https://doi.org/10.1016/j.cub.2017.08.038

Goudie, F., Allsopp, M. H., Beekman, M., Oxley, P. R., Lim, J., & Oldroyd, B. P. (2012). MAINTENANCE AND LOSS OF HETEROZYGOSITY IN A THELYTOKOUS LINEAGE OF HONEY BEES (APIS MELLIFERA CAPENSIS). *Evolution*, *66*(6), 1897–1906. https://doi.org/10.1111/j.1558-5646.2011.01543.x

Haag, C. R., Theodosiou, L., Zahab, R., & Lenormand, T. (2017). Low recombination rates in sexual species and sex–asex transitions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1736), 20160461.

Harmon, T. S., Kamerman, T. Y., Corwin, A. L., & Sellas, A. B. (2016). Consecutive parthenogenetic births in a spotted eagle ray Aetobatus narinari. *Journal of Fish Biology*, *88*(2), 741–745. https://doi.org/10.1111/jfb.12819

Heckmann, S., Jankowska, M., Schubert, V., Kumke, K., Ma, W., & Houben, A. (2014). Alternative meiotic chromatid segregation in the holocentric plant Luzula elegans. *Nature Communications*, *5*(1), Article 1. https://doi.org/10.1038/ncomms5979

Hespeels, B., Flot, J.-F., Derzelle, A., & Van Doninck, K. (2014). Evidence for Ancient Horizontal Gene Acquisitions in Bdelloid Rotifers of the Genus Adineta. In P. Pontarotti (Ed.), *Evolutionary Biology: Genome Evolution, Speciation, Coevolution and Origin of Life* (pp. 207–225). Springer International Publishing. https://doi.org/10.1007/978-3-319-07623-2_10

Hiraki, H., Kagoshima, H., Kraus, C., Schiffer, P. H., Ueta, Y., Kroiher, M., Schierenberg, E., & Kohara, Y. (2017). Genome analysis of Diploscapter coronatus: Insights into molecular peculiarities of a nematode with parthenogenetic reproduction. *BMC Genomics*, *18*(1), 478. https://doi.org/10.1186/s12864-017-3860-x

Hiruta, C., Nishida, C., & Tochinai, S. (2010). Abortive meiosis in the oogenesis of parthenogenetic Daphnia pulex. *Chromosome Research*, *18*(7), 833–840. https://doi.org/10.1007/s10577-010-9159-2

Hiruta, C., Tochinai, S., Hiruta, C., & Tochinai, S. (2012). How Does the Alteration of Meiosis Evolve to Parthenogenesis?-Case Study in a Water Flea, Daphnia pulex -. In *Meiosis—Molecular Mechanisms and Cytogenetic Diversity*. IntechOpen. https://doi.org/10.5772/29558

Hollis, J. A., Glover, M. L., Schlientz, A. J., Cahoon, C. K., Bowerman, B., Wignall, S. M., & Libuda, D. E. (2020). Excess crossovers impede faithful meiotic chromosome segregation in C. elegans. *PLOS Genetics*, *16*(9), e1009001. https://doi.org/10.1371/journal.pgen.1009001

Hsu, W. S. (1956a). Oogenesis in Habrotrocha tridens (Milne). *The Biological Bulletin*, *111*(3), 364–374.

Hsu, W. S. (1956b). Oogenesis in the Bdelloidea rotifer Philodina roseola Ehrenberg. *Cellule*, *57*, 283–296.

Jaron, K. S., Parker, D. J., Anselmetti, Y., Tran Van, P., Bast, J., Dumas, Z., Figuet, E., François, C. M., Hayward, K., Rossier, V., Simion, P., Robinson-Rechavi, M., Galtier, N., & Schwander, T. (2022). Convergent consequences of parthenogenesis on stick insect genomes. *Science Advances*, *8*(8), eabg3842. https://doi.org/10.1126/sciadv.abg3842

Kaitna, S., Pasierbek, P., Jantsch, M., Loidl, J., & Glotzer, M. (2002). The Aurora B Kinase AIR-2 Regulates Kinetochores during Mitosis and Is Required for Separation of Homologous Chromosomes during Meiosis. *Current Biology*, *12*(10), 798–812. https://doi.org/10.1016/S0960-9822(02)00820-5

Kato, M., Hiruta, C., & Tochinai, S. (2016). The Behavior of Chromosomes During Parthenogenetic Oogenesis in Marmorkrebs Procambarus fallax f. Virginalis. *Zoological Science*, *33*(4), 426–430. https://doi.org/10.2108/zs160018

Kennedy, P. (2021). Haplodiploidy. In C. K. Starr (Ed.), *Encyclopedia of Social Insects* (pp. 477–489). Springer International Publishing. https://doi.org/10.1007/978-3-030-28102-1_56

Khan, Z., Khan, M. S., Bawazeer, S., Bawazeer, N., Suleman, Irfan, M., Rauf, A., Su, X.-H., & Xing, L.-X. (2022). A comprehensive review on the documented characteristics of four *Reticulitermes* termites (Rhinotermitidae, Blattodea) of China. *Brazilian Journal of Biology*, *84*. https://doi.org/10.1590/1519-6984.256354

Komatsu, N., Kawakami, Y., Banzai, A., Ooi, H. K., & Uchida, A. (2015). Species clarification of Ogasawara cockroaches which inhabit Japan. *Tropical Biomedicine*, *32*(1), 98–108.

Kühn, A. (1908). *Die Entwicklung der Keimzellen in den parthenogenetischen Generationen der Cladoceren" Daphnia pulex"(De Geer) und" Polyphemus pediculus"(De Geer), Inaugural-Dissertation... Von Alfred Kühn...* W. Engelmann.

Lavanchy, G., & Schwander, T. (2019). Hybridogenesis. *Current Biology*, *29*(1), R9–R11. https://doi.org/10.1016/j.cub.2018.11.046

Lenormand, T., & Dutheil, J. (2005). Recombination Difference between Sexes: A Role for Haploid Selection. *PLOS Biology*, *3*(3), e63. https://doi.org/10.1371/journal.pbio.0030063

Liu, Q. L., Thomas, V. P., & Williamson, V. M. (2007). Meiotic Parthenogenesis in a Root-Knot Nematode Results in Rapid Genomic Homozygosity. *Genetics*, *176*(3), 1483–1490. https://doi.org/10.1534/genetics.107.071134

Marais, M. (2012). *The cytogenetics of some South African root-knot nematodes (Heteroderidae: Nematoda)*.

Marais, M. and de W. K. (1991). The cytogenetics of some South African root-knot nematodes (Heteroderidae: Nematoda). *Phytophylactica*, *23*(4), 265–272.

Maynard Smith, J. (1971). What use is sex? *Journal of Theoretical Biology*, *30*(2), 319–335. https://doi.org/10.1016/0022-5193(71)90058-0

Maynard Smith, J. (1978). *The evolution of sex.*

Maynard Smith, J. (1986). Evolution: Contemplating life without sex. *Nature*, *324*, 300–301.

McNally, K. P., Panzica, M. T., Kim, T., Cortes, D. B., & McNally, F. J. (2016). A novel chromosome segregation mechanism during female meiosis. *Molecular Biology of the Cell*, *27*(16), 2576–2589. https://doi.org/10.1091/mbc.e16-05-0331

Melone, G., & Ricci, C. (1995). Rotatory apparatus in Bdelloids. *Hydrobiologia*, *313*(1), 91–98. https://doi.org/10.1007/BF00025935

Molinier, C., Lenormand, T., & Haag, C. R. (2021). *No support for a meiosis suppressor in Daphnia pulex: Comparison of linkage maps reveals normal recombination in males of obligate parthenogenetic lineages* (p. 2021.12.09.471908). bioRxiv. https://doi.org/10.1101/2021.12.09.471908

Morgan, T. H. (1914). NO CROSSING OVER IN THE MALE OF DROSOPHILA OF GENES IN THE SECOND AND THIRD PAIRS OF CHROMOSOMES. *The Biological Bulletin*, *26*(4), 195–204. https://doi.org/10.2307/1536193

Munro, C., Cadis, H., Pagnotta, S., Houliston, E., & Huynh, J.-R. (2023). Conserved meiotic mechanisms in the cnidarian Clytia hemisphaerica revealed by Spo11 knockout. *Science Advances*, *9*(4), eadd2873. https://doi.org/10.1126/sciadv.add2873

Murdy, W. H., & Carson, H. L. (1959). Parthenogenesis in Drosophila mangabeirai Malog. *The American Naturalist*, *93*(873), 355–363.

Neiman, M., Sharbel, T. F., & Schwander, T. (2014). Genetic causes of transitions from sexual reproduction to asexuality in plants and animals. *Journal of Evolutionary Biology*, *27*(7), 1346–1359.

Normark, B. B. (2014). Modes of reproduction. *The Evolution of Insect Mating Systems.*, 1–19.

Ojima, Y. (1954). Some Cytological Observations on Parthenogenesis in Daphnia pulex (de Geer)(With 3 Plates). **北海道大學理學部紀要**, *12*(1–2), 230–235.

Oldroyd, B. P., Yagound, B., Allsopp, M. H., Holmes, M. J., Buchmann, G., Zayed, A., & Beekman, M. (2021). Adaptive, caste-specific changes to recombination rates in a thelytokous honeybee population. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1952), 20210729. https://doi.org/10.1098/rspb.2021.0729

Omilian, A. R., Cristescu, M. E. A., Dudycha, J. L., & Lynch, M. (2006). Ameiotic recombination in asexual lineages of Daphnia. *Proceedings of the National Academy of Sciences*, *103*(49), 18638–18643. https://doi.org/10.1073/pnas.0606435103

Palmer, S. C., & Norton, R. A. (1992). Genetic diversity in thelytokous oribatid mites (Acari; Acariformes: Desmonomata). *Biochemical Systematics and Ecology*, *20*(3), 219–231. https://doi.org/10.1016/0305-1978(92)90056-J

Poinar, G. O., & Ricci, C. (1992). Bdelloid rotifers in Dominican amber: Evidence for parthenogenetic continuity. *Experientia*, *48*(4), 408–410. https://doi.org/10.1007/BF01923444

Portnoy, D. S., Hollenbeck, C. M., Johnston, J. S., Casman, H. M., & Gold, J. R. (2014). Parthenogenesis in a whitetip reef shark *Triaenodon obesus* involves a reduction in ploidy: PARTHENOGENESIS AND PLOIDY REDUCTION IN *T. OBESUS*. *Journal of Fish Biology*, *85*(2), 502–508. https://doi.org/10.1111/jfb.12415

Rebecchi, L. (1991). *Karyological analysis of. Macrobiotus pseudohufelandi*.

Robinson, D. P., Baverstock, W., Al-Jaru, A., Hyland, K., & Khazanehdari, K. A. (2011). Annually recurring parthenogenesis in a zebra shark Stegostoma fasciatum. *Journal of Fish Biology*, *79*(5), 1376–1382. https://doi.org/10.1111/j.1095-8649.2011.03110.x

Rogers, E., Bishop, J., Waddle, J., Schumacher, J., & Lin, R. (2002). The aurora kinase AIR-2 functions in the release of chromosome cohesion in Caenorhabditis elegans meiosis. *The Journal of Cell Biology*, *157*, 219–229. https://doi.org/10.1083/jcb.200110045

Rubin, T., Macaisne, N., & Huynh, J.-R. (2020). Mixing and Matching Chromosomes during Female Meiosis. *Cells*, *9*(3), Article 3. https://doi.org/10.3390/cells9030696

Schaefer, I., Domes, K., Heethoff, M., Schneider, K., Schön, I., Norton, R. A., Scheu, S., & Maraun, M. (2006). No evidence for the 'Meselson effect' in parthenogenetic oribatid mites (Oribatida, Acari). *Journal of Evolutionary Biology*, *19*(1), 184–193. https://doi.org/10.1111/j.1420-9101.2005.00975.x

Senaratne, A. P., Cortes-Silva, N., & Drinnenberg, I. A. (2022). Evolution of holocentric chromosomes: Drivers, diversity, and deterrents. *Seminars in Cell & Developmental Biology*, *127*, 90–99. https://doi.org/10.1016/j.semcdb.2022.01.003

Severson, A. F., & Meyer, B. J. (2014). Divergent kleisin subunits of cohesin specify mechanisms to tether and release meiotic chromosomes. *ELife*, *3*, e03467. https://doi.org/10.7554/eLife.03467

Simion, P., Narayan, J., Houtain, A., Derzelle, A., Baudry, L., Nicolas, E., Arora, R., Cariou, M., Cruaud, C., Gaudray, F. R., Gilbert, C., Guiglielmoni, N., Hespeels, B., Kozlowski, D. K. L., Labadie, K., Limasset, A., Llirós, M., Marbouty, M., Terwagne, M., … Van Doninck, K. (2021). Chromosome-level genome assembly reveals homologous chromosomes and recombination in asexual rotifer Adineta vaga. *Science Advances*, *7*(41), eabg4216. https://doi.org/10.1126/sciadv.abg4216

Siomos, M. F., Badrinath, A., Pasierbek, P., Livingstone, D., White, J., Glotzer, M., & Nasmyth, K. (2001). Separase is required for chromosome segregation during meiosis I in Caenorhabditis elegans. *Current Biology*, *11*(23), 1825–1835. https://doi.org/10.1016/S0960-9822(01)00588-7

Stalker, H. D. (1954). PARTHENOGENESIS IN DROSOPHILA. *Genetics*, *39*(1), 4–34. https://doi.org/10.1093/genetics/39.1.4

Stouthamer, R., & Kazmer, D. J. (1994). Cytogenetics of microbe-associated parthenogenesis and its consequences for gene flow in Trichogramma wasps. *Heredity*, *73*(3), Article 3. https://doi.org/10.1038/hdy.1994.139

Suomalainen, E. (1950). Parthenogenesis in Animals**Received for publication March 11, 1948. In M. Demerec (Ed.), *Advances in Genetics* (Vol. 3, pp. 193–253). Academic Press. https://doi.org/10.1016/S0065-2660(08)60086-3

Suomalainen, E., Saura, A., & Lokki, J. (1987). *Cytology and evolution in parthenogenesis*. CRC Press.

Taberly, G. (1958). [The cytology of parthenogenesis in Platynothrus peltifer (Koch) (Acarien, Oribate)]. *Comptes Rendus Hebdomadaires Des Seances De l'Academie Des Sciences*, *247*(19), 1655–1657.

Taberly, G. (1960). *La régulation chromosomique chez Trhypochthonius tectorum (Berlese) espèce parthénogénétique d'Oribate (Acarien) un nouvel exemple de mixocinèse*.

Tanaka, M., & Daimon, T. (2019). First molecular genetic evidence for automictic parthenogenesis in cockroaches. *Insect Science*, *26*(4), 649–655. https://doi.org/10.1111/1744-7917.12572

Tang, C. Q., Obertegger, U., Fontaneto, D., & Barraclough, T. G. (2014). SEXUAL SPECIES ARE SEPARATED BY LARGER GENETIC GAPS THAN ASEXUAL SPECIES IN ROTIFERS. *Evolution*, *68*(10), 2901–2916. https://doi.org/10.1111/evo.12483

Terwagne, M., Nicolas, E., Hespeels, B., Herter, L., Virgo, J., Demazy, C., Heuskin, A.-C., Hallet, B., & Van Doninck, K. (2022). DNA repair during nonreductional meiosis in the asexual rotifer Adineta vaga. *Science Advances*, *8*(48), eadc8829. https://doi.org/10.1126/sciadv.adc8829

Tremblay, E., & Caltagirone, L. E. (1973). Fate of Polar Bodies in Insects. *Annual Review of Entomology*, *18*(1), 421–444. https://doi.org/10.1146/annurev.en.18.010173.002225

Triantaphyllou, A. C. (1963). Polyploidy and parthenogenesis in the root-knot nematode Meloidogyne arenaria. *Journal of Morphology*, *113*(3), 489–499. https://doi.org/10.1002/jmor.1051130309

Triantaphyllou, A. C. (1966). Polyploidy and reproductive patterns in the root-knot nematode Meloidogyne hapla. *Journal of Morphology*, *118*(3), 403–413. https://doi.org/10.1002/jmor.1051180308

Triantaphyllou, A. C. (1981). Oogenesis and the Chromosomes of the Parthenogenic Root-knot Nematode Meloidogyne incognita. *Journal of Nematology*, *13*(2), 95–104.

Triantaphyllou, A. C., & Moncol, D. J. (1977). Cytology, reproduction, and sex determination of Strongyloides ransomi and S. papillosus. *The Journal of Parasitology*, *63*(6), 961–973.

Van Der Beek, J., Los, J., & Pijnacker, L. (1998). Cytology of parthenogenesis of five Meloidogyne species. *Fundam. Appl. Nemawl., 21 (4),393-399*.

Vanzela, A. L. L., & Guerra, M. (2000). Heterochromatin differentiation in holocentric chromosomes of Rhynchospora (Cyperaceae). *Genetics and Molecular Biology*, *23*, 453–456. https://doi.org/10.1590/S1415-47572000000200034

Warren, W. C., García-Pérez, R., Xu, S., Lampert, K. P., Chalopin, D., Stöck, M., Loewe, L., Lu, Y., Kuderna, L., Minx, P., Montague, M. J., Tomlinson, C., Hillier, L. W., Murphy, D. N., Wang, J., Wang, Z., Garcia, C. M., Thomas, G. C. W., Volff, J.-N., … Schartl, M. (2018). Clonal polymorphism and high heterozygosity in the celibate genome of the Amazon molly. *Nature Ecology & Evolution*, *2*(4), Article 4. https://doi.org/10.1038/s41559-018-0473-y

Woglar, A., Yamaya, K., Roelens, B., Boettiger, A., Köhler, S., & Villeneuve, A. M. (2020). Quantitative cytogenetics reveals molecular stoichiometry and longitudinal organization of meiotic chromosome axes and loops. *PLOS Biology*, *18*(8), e3000817. https://doi.org/10.1371/journal.pbio.3000817

Xu, S., Omilian, A. R., & Cristescu, M. E. (2011). High rate of large-scale hemizygous deletions in asexually propagating Daphnia: Implications for the evolution of sex. *Molecular Biology and Evolution*, *28*(1), 335–342. https://doi.org/10.1093/molbev/msq199

Yamashita, M., Jiang, J., Onozato, H., Nakanishi, T., & Nagahama, Y. (1993). A Tripolar Spindle Formed at Meiosis I Assures the Retention of the Original Ploidy in the Gynogenetic Triploid Crucian Carp, Ginbuna Carassius auratus langsdorfii. *Development, Growth & Differentiation*, *35*(6), 631–636. https://doi.org/10.1111/j.1440-169X.1993.00631.x

Yang, K.-T., Li, S.-K., Chang, C.-C., Tang, C.-J. C., Lin, Y.-N., Lee, S.-C., & Tang, T. K. (2010). Aurora-C Kinase Deficiency Causes Cytokinesis Failure in Meiosis I and Production of Large Polyploid Oocytes in Mice. *Molecular Biology of the Cell*, *21*(14), 2371–2383. https://doi.org/10.1091/mbc.e10-02-0170

Zaffagnini, F., & Sabelli, B. (1972). Karyologic observations on the maturation of the summer and winter Eggs of Daphnia pulex and Daphnia middendorffiana. *Chromosoma*, *36*(2), 193–203. https://doi.org/10.1007/BF00285213

Zhang, J., Sun, M., Zhou, L., Li, Z., Liu, Z., Li, X.-Y., Liu, X.-L., Liu, W., & Gui, J.-F. (2015). Meiosis completion and various sperm responses lead to unisexual and sexual reproduction modes in one clone of polyploid Carassius gibelio. *Scientific Reports*, *5*, 10898. https://doi.org/10.1038/srep10898

Conclusion

In this chapter, we saw that in parthenogenetic species there are 18 possible manners in which meiosis can be modified while maintaining the original ploidy. These alterations can be classified according to the existence of recombination and the content of sister chromatids in the reduced gamete. We emphasise that the presence of recombination results in at least a local if not total homogenisation of the genome of the parthenogenetic specie. In addition, we outline the different mechanisms used to maintain heterozygosity. This underlines the importance of cytological studies in the exploration of asexual systems. Indeed, these data are crucial for understanding the cellular and molecular constraints of a given reproductive mode and how these constraints affect the species' genome. We also point out the importance of combining cytology with genomic data and, therefore, the interest in revisiting some cytology performed with old techniques.

Chapter 2: Mechanism of heterozygosity maintenance despite recombination in *M. belari*

Introduction

In this chapter, we will focus on the characterization of the meiosis that produces unreduced oocytes, hence the asexual females. It will be compared to the regular meiosis found in the oocytes giving rise to amphimictic eggs. We will also explore the consequence of this meiosis on the genome of the species. There is a direct impact because the contribution of males to the diversity of the genome is potentially null. Indeed, the males do not contribute any gene to the gene pool of females and at each generation their DNA is diluted two fold in the genome of males (Grosmaire et al., 2019).

In gynogenetic oocytes, one polar body is extruded and the resulting egg is diploid. Thus, several alterations of meiosis can be applied to this system (Figure 33). These alterations are presented in Figure 34. As also illustrated in Chapter 1, the type of meiotic process affects the genome content and thus the evolutionary capacity of the species. For instance, in the absence of pairing, recombination is prevented and initial heterozygosity of the individual is maintained over generations (Figure 34b). For these reasons, it is necessary to characterize the meiosis *M. belari*.

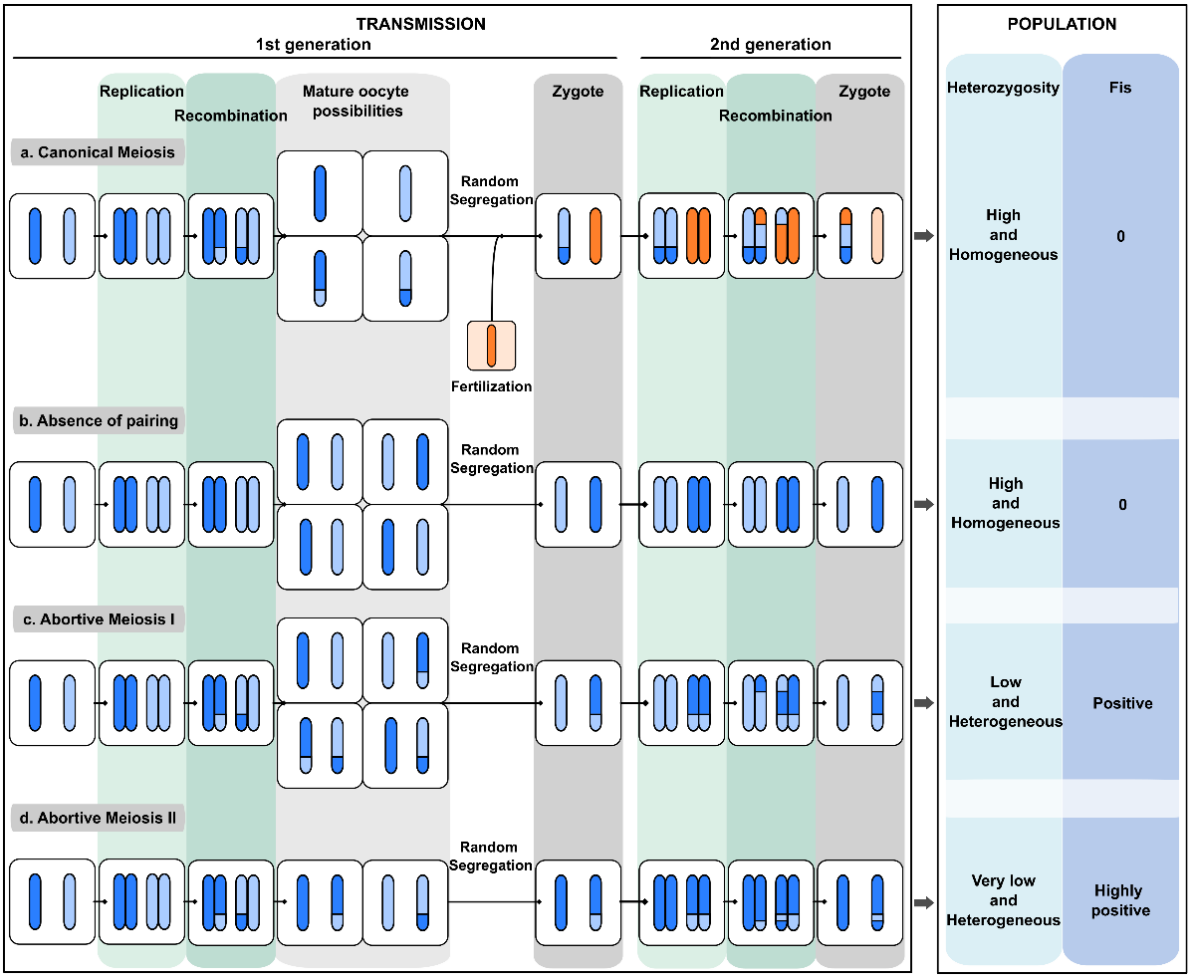

Figure 34: Possible modifications of meiosis in *M. belari*. Description of the expected genetic composition of a zygote, and of a population, upon different types of modifications of the meiotic program (b-d). Canonical meiotic division as found in regular sexual species is shown on the top (a). Each bar represents a chromatid. The paternal chromatid is in orange. The maternal chromatids are in dark and light blue, corresponding to homologous chromosomes. The level of heterozygosity in the population is shown in the right column. Heterogeneous heterozygosity underlines a disparity in the level of heterozygosity along the chromosomes. In contrast, homogeneous heterozygosity demonstrates uniformity in the level of heterozygosity. The coefficient of inbreeding (Fis) corresponds to the expected level of heterozygosity compared to Hardy-Weinberg's expectation (in a randomly mating population). Positive Fis indicates a deficit in heterozygous individuals.

In the first part of this chapter, I present our findings on the characterisation of meiosis in gynogenetic oocytes (Blanc et al., 2023). The cytological results are confronted with the genomic data, performed by our collaborators (Team Galtier, ISEM, Montpellier). This approach allowed us to uncover, and next demonstrate, a new type of chromosome segregation.

While understanding the consequences of unreduced meiosis on the genome of an asexual species is crucial, it is also important to understand the mechanisms at the origin of this modified meiosis. In the second part of this chapter I have tried to identify which specific step of meiosis I, and which molecule is affected in *M. belari* unreduced oocytes. These results are very preliminary and further sampling will be required to validate the conclusions.

Results

Co-segregation of recombinant chromatids maintains genome-wide heterozygosity in an asexual nematode

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One sentence summary

Genome wide heterozygosity in the asexual nematode *Mesorhabditis belari* is achieved by directed assortment of recombinant chromatids during female meiosis

Abstract

In asexual animals, female meiosis is modified to produce diploid oocytes. Associated with recombination, this is expected to lead to a rapid loss of heterozygosity, with adverse effects on fitness. Many asexuals, however, have a heterozygous genome, the underlying mechanisms being most often unknown. Cytological and population genomic analyses in the nematode *Mesorhabditis belari* revealed another case of recombining asexual being highly heterozygous genome-wide. We demonstrated that heterozygosity is maintained because the recombinant chromatids of each chromosome pair co-segregate during the unique meiotic division. A theoretical model confirmed that this segregation bias is necessary to account for the observed pattern and likely to evolve under a wide range of conditions. Our study uncovers a new type of cell division involving Directed Chromatid Assortment.

Introduction

Asexual animal species are composed of females, which produce diploid daughters without paternal genome contribution. Asexuality requires the production of diploid oocytes and hence, a modified female meiosis. Asexuality, which is derived from sexuality, has emerged multiple times and independently over the course of evolution and many routes to producing diploid oocytes have been documented (*1–3*).

Depending on the type of cellular modification, different genetic outcomes are expected (Figure 1A). A common prediction is that most modifications should lead to loss of heterozygosity (LOH). For some species, the entire meiotic program is achieved as in sexual species, however the final haploid nucleus undergoes a duplication event (gamete duplication), which immediately generates a homozygous individual (Figure 1A). When there is homologous recombination and one of the two meiotic divisions fails, either because the division is abortive or because the product of meiosis fuse back, LOH is also expected either distally or proximally to the crossover location (Figure 1A). Hence, maintenance of heterozygosity is theoretically expected only in species for which meiotic recombination is largely reduced or totally abolished (*4*).

LOH is expected to negatively affect fitness due to the exposure of recessive deleterious mutations at diploid state. This has been suggested as a potential cause of the relative scarcity of asexual organisms in nature (*5*), and a selective pressure for reduced recombination rates in asexuals (*6*). Species that maintain some level of heterozygosity do not expose deleterious mutations and as such may circumvent some of the drawbacks of asexuality. In agreement with this expectation, a number of asexual lineages display appreciable amounts of heterozygosity (*7–10*). Yet, except in species for which a total loss of recombination has been demonstrated (for instance (*11*, *12*)), the mechanisms of heterozygosity maintenance are still debated (*7*). Therefore, there is still a need to confront the cytological description and empirical genome data to reach a clear understanding of the genomic and cellular constraints in asexual animals.

We explored the mechanism of meiosis in the auto-pseudogamous nematode *Mesorhabditis belari* (Figure 1B) (*13*). In this species, a female produces a majority of diploid oocytes, which although fertilized by a sperm, develop only from the maternal DNA and become females (gynogenetic embryos). The same female also produces ~10% of haploid oocytes through regular meiosis, which once fertilized undergo fusion of the parental genomes. These amphimictic diploid embryos will give rise to males because active sperm cells always

carry a Y chromosome (*13*). Hence, this species produces 90% asexual females and 10% sexual males. In *M. belari* females, which most likely have maintained recombination for the production of regular oocytes (for the rare males), we asked which modification of meiosis has been selected to produce the asexual females and with which genomic consequence for the species.

Materials and Methods

Nematode strains and culture

Mesorhabditis species are maintained at 20°C on NGM plates seeded with *E. coli* OP50, following *C. elegans* protocols, as described in (*13*).

Immunostainings on gonads and embryos

Immunostainings were performed as described in (*13*). Gravid females were dissected on slides coated with 0.25%poly-lysine in 0,5X M9. After freeze-cracking, fixation of samples was performed by immersing slides into methanol at -20°C during at least 5 min. We used a mouse anti-tubulin antibody as primary antibody (Sigma DM1A, 1:2000) and an Alexa488 donkey anti-mouse secondary antibody (Jackson ImmunoResearch, #715-545-150, 1:2000). Both antibodies were incubated at room temperature for 45 min. DNA was stained using Hoechst 33258 at 0,5 ul/ml (Merck Sigma-Aldrich, #94403). Images were acquired using a confocal microscope (Oil immersion 63X objective – LSM800 and LSM980 Airyscan, Zeiss). Z-stacks of embryos were acquired every 0.15 µm. Finally, acquired images were treated using the ImageJ 1.53t software.

EdU Pulse/Chase protocol

The protocol was adapted from (*15*). In order to obtain diakinesis oocytes and early embryos for which only one chromatid per chromosome was labeled with EdU, we had to optimize the protocol for *M. belari*. First, we synchronized worms using axenization, as described in (*13*). Briefly, worms were collected and treated with bleach and NaOH in order to dissolve all individuals except the embryos. The embryo pellet was then washed and placed on plates without bacteria, allowing L1 larvae to hatch. Without food, all L1s were arrested at the same stage after 2 days. L1s were then placed back on food and allowed to grow for 72h at 20°C. At this stage, young L4 synchronized worms were collected and washed in 1X PBS, 0.1% Triton X. A pellet of ~300ul of worms was transferred into 200 ul of 10 mM EdU diluted in water (ThermoFisher A10044) to obtain a final concentration of 4 mlM EdU. The tube was transferred on a tube rotator for 4h at room temperature. After washes in M9, animals were plated onto fresh NGM plates seeded with *E. Coli* and placed at 25°C for 48 before embryos

were collected for fixation. As summarized in Figure S2, we deducted that two rounds of S phase precede meiotic prophase during *M. belari* oogenesis.

EdU Click-it labeling

Cytology was performed following the instructions provided by the EdU Click-it kit (ThermoFisher C10337). Embryos were collected after axenization, as described above. Embryos were placed on poly-lysine coated slides, freeze-cracked and fixed in -20°C methanol. Samples were incubated with BSA 2% for 20 min at room temperature. Slides were then washed twice with 1X PBS. Following the instructions of the kit, slides were washed for 30 min at room temperature in 1X PBS with 1% Triton X (v/v) and labeled with Alexa 488-azide for 30 min at room temperature. Samples were then washed twice with 1X PBS and were incubated in a tank with Hoechst 33258 for 20 min at room temperature. Finally, slides were mounted using ProLong™ Diamond Antifade Mountant (ThermoFisher P36965) and sealed with nail polish.

Airyscan and ImageJ 3D analysis of EdU-labelled recombinant chromatids

As with immunostaining, confocal airyscan images were acquired using the Zeiss LSM800 Airyscan and LSM980 Airyscan using a 63x oil objective and 0.15µm interval between slides. Images were subsequently processed by the airyscan processing method (3D analysis, automatic low stringency, Zen Blue 3.3). Processed images were treated using ImageJ 1.53t software. IAnalyses of each chromosome were done using a combination of Z projection and 3D projection on the Y-axis using the brightest point method and interpolation.

DNA and RNA preparation for sequencing

We performed DNAseq on 10 strains of the auto-pseudogamous species *M. belari* coming from different locations in Europe. For each strain, one gravid female was initially collected in the wild and left to lay eggs in a Petri dish. This constituted a single strain, which was frozen in our collection. For sequencing, we amplified the animals and extracted the DNA for each strain. Briefly, mixed stage worms were collected, washed in M9 and a pellet of ~300 ul of worms was frozen in liquid nitrogen. After thawing, 600 ul of Cell Lysis Buffer (Qiagen

Cell Lysis Solution #158906) was added, as well as 6 ul of proteinase K at 17 ug/ul and incubated for 3h at 65°C. We next incubated the mix for 1h at 37°C, supplemented with 40 ul of RNAseA (at 5 mg/ml). 200 ul of Protein Precipitation Solution (Qiagen #158912) was next added and after 5min on ice, the mix was centrifuged for 10 min at 13000 rpm at 4°C. 600 ul of isopropanol was added to the supernatant. After 10 min at room temperature, the mix was centrifuged at maximum speed and the pellet was rinsed twice in ethanol 70°C. The pellet was dried and resuspended in nuclease free water.

For the analysis of genotype inheritance in sisters, we isolated gravid females, for each species, let they lay eggs and after few days isolated 3 virgin daughters. The mRNAs of each single female were extracted using the SmartSeq2 protocol, as described in (*30*).

For all samples, genomic libraries (insert sizes of ~550 bp) were prepared using TruSeqNano and the libraries were sequenced on a HiSeq4000 with 100 bp paired-end read length.

Heterozygosity analysis

For each of the 10 strains of *M. belari*, reads were mapped to the assembled genome of *M. belari* with BWA (*31*). BAM files were produced with SAMtools (*32*) and heterozygosity was estimated for each strain using ANGSD (*18*) using the SFS estimation for a single sample. Recombination at the extremities of the chromosomes could explain a limited decrease in heterozygosity, the rest of the chromosome remaining non-recombining. To test this, heterozygosity was calculated on 5000 bp windows. A decrease in heterozygosity at the ends of the contigs was then looked for, graphically. At first, we detected homozygous portions in the JU2817 strain. But these portions were twice as low in coverage as the rest of the contigs, which could be explained by an assembly error due to too much divergence between the two alleles. A similarity search with blastn allowed to detect the presence of indels between the two alleles, preventing a unique assembly of these regions. This pattern was not found in the other strains and therefore explains the lower heterozygosity of this strain compared to the others (*i.e.* haplotype divergence).

Genotype inheritance

RNAseq was performed for three sisters in each of the two auto-pseudogamous species *M. belari* and *M. monhystera* and the two sexual species *M. spiculigera* and *M. longespiculosa*. For each species, reads from the three individuals where pooled to assemble a transcriptome: adapters were clipped from the sequences, low-quality read ends were trimmed (phred score <30) and low quality reads were discarded (remaining length <36bp) using trimmomatic (v0.39 (*33*)). Paired-end transcriptomes were de novo assembled using Trinity v2.13.2 (*34*).

Reads were mapped on their respective assembled transcriptome with BWA (*31*), BAM files were produced with SAMtools (*32*) and SNPs were called using reads2SNP (*35*) focusing on sufficiently covered contigs (minimum contig average coverage = 15X) and positions (minimum = 20X). In each species, we selected positions in which not only the called genotypes but also read frequencies varied significantly among sisters. Specifically, for each position, two multinomial models were fitted to read counts. Model M0 (three degrees of freedom) assumed a common frequency of A, C, G and T in the three sisters. Model M1 (9 degrees of freedom) rather allowed each of the three sisters to have its own frequencies of A, C, G and T reads. A likelihood ratio test was performed and we only positions in which M0 was rejected (p-val<1.e-8). This was intended to exclude positions for which the genotype varied among sisters due to uncertainty in genotype calling.

Measure of linkage disequilibrium

To test for the existence of recombination, we estimated the linkage disequilibrium using the ten strains of *M. belari*. For each of the 10 strains reads were mapped to the assembled genome with BWA (*31*). BAM files were produced with SAMtools (*32*) and SNPs were called with reads2SNP (*35*). To phase haplotypes, we first used WhatsHap (*36*) to extract the phase information contained in reads. Phasing was then completed using Beagle V5.3 (*37*). Linkage disequilibrium was computed on 5000 pb windows with LDhelmet V1.10 (*20*) using recommended parameters.

Table S1: Measure of heterozygosity in 10 strains of *M. belari*

Strain	Country	Location	Heterozygosity
JU2856	France	Angles-sur-l'Anglin, Indre	0,01381808
JU2859	United Kingdom	Cambridge	0,01531046
JU2817	France	Orsay	0,00735625
JU3129	United Kingdom	Coventry	0,01419700
JU3151	France	Saint-Aigny, Indre	0,01280673
JU3152	France	Saint-Aigny, Indre	0,01240229
JU3157	Germany	Heidelberg	0,01400762
JU3158	Germany	Heidelberg	0,01366134
JU3159	Germany	Postdam	0,01459582
JU3388	Ireland	Inis Mor, Aran Islands	0,01361719

Results

Diploid oocytes of M. belari are formed after failure of the first meiotic division

We first asked which steps of meiosis were modified to produce diploid oocytes in *M. belari*. We followed meiosis, making use of the spatio-temporal organization of the gonad, as found in the well-studied *Caenorhabditis elegans* species and other Rhabditidae nematodes (Figure 2A). We had previously shown that this species is diploid and carries 2n=20 holocentric chromosomes (*13*). First, analysis of oocytes in diakinesis revealed that the 20 chromosomes were always paired into 10 units (n > 200 oocytes). Moreover, many bivalents had a crossed-shape structure (Figure 2A), which resemble the chiasmata of holocentric chromosomes found in *C. elegans* (*14*, *15*), strongly suggesting that chromosomes undergo homologous recombination in *M. belari*.

Next, we reconstructed the different steps of meiotic divisions (Figure 2B, C). We found, as is the case for *C. elegans*, that oocytes are arrested at prometaphase of meiosis I and meiosis resumes after fertilization. The long axis of the bivalents is oriented parallel to the spindle axis, as expected for holocentric chromosomes in regular meiosis (Figure 2C) (*14*). In

the Rhabditidae sexual species studied so far, a polar body is extruded at each meiotic division. The polar bodies are easily recognizable as tiny cells at the edge of the embryo (*16*). We had previously shown that all gynogenetic embryos in *M. belari* had only one polar body demonstrating that one meiotic division is suppressed (*13*). We reasoned that if meiosis I was abortive, the 10 bivalents should dissociate into 20 univalents and no polar body should be detected at this stage. These univalents should next enter anaphase of meiosis II, showing two times 20 DNA stained bodies (Figure 2B). On the contrary, if meiosis I was successful, a polar body would be extruded, and the 10 univalents would disassemble into 20 units corresponding to 20 sister chromatids after failure of meiosis II (Figure 2B). We found many cells showing a metaphase plates containing 20 DNA stained bodies. We also detected anaphase figures with two times 20 DNA stained bodies (Figure 2C). Importantly, none of these cells had produced a polar body. Of note, we also found images of metaphase with only 10 DNA stained bodies, which we interpret as being either figures of regular meiosis (~10% are expected) or the initial step of meiosis I before abortion. From these results, we concluded that diploid oocytes in *M. belari* are formed after failure of the first anaphase of meiosis I. This modification will lead to the assortment of non-sister chromatids in the oocytes (equational division only). In the presence of recombination for all chromosomes, this pattern of inheritance should progressively lead to LOH, distally to the crossing-over.

M. belari has a widely heterozygous genome

We analyzed the level and the distribution of heterozygosity in the genome of *M. belari* females, from our lab strain JU2817 and nine other wild strains, which had been sampled in different locations around the world (*17*). We sequenced mixed stage animals from each strain and mapped the short reads on the assembled genome of *M. belari* JU2817. Genome-wide heterozygosity was computed by counting the proportion of heterozygous positions relative to the total number of positions using ANGSD (*18*). Each strain being isofemale (see Material and Methods), the genotype of a strain corresponds to the genotype of a single individual.

We found that all ten strains had approximately the same level of heterozygosity of about 1,3% [sd = 0.2] (*i.e.* one residue every 75nt is heterozygous), demonstrating that the strains behaved similarly in the wild and in the lab (Figure S1 and Table S1). This level of heterozygosity is unexpectedly high for a meiotic asexual experiencing regular recombination; it is 10 times as high as in the self-fertilizing nematode *Caenorhabditis elegans* (*19*) and similar

to natural populations of the fruitfly *Drosophila melanogaster* (*20*), for instance. Heterozygosity could be maintained in most parts of the chromosomes and lost only in subtelomeric regions if crossing-overs were restricted to chromosome ends but we found that heterozygosity was uniform along all contigs (Figure S1).

To further confirm the maintenance of heterozygosity, we performed a genome-wide analysis of genotype inheritance, from mother to daughters in *M. belari* JU2817. We performed this analysis on the transcriptome which can be easily obtained from single worms. We analyzed three female individuals descended from the same mother. RNAseq reads were mapped to the previously assembled *M. belari* JU2817 transcriptome, genotypes were called, focusing on sufficiently covered contigs and positions. Under the assumption of active recombination and random segregation of chromatids, large chromosomal segments - and therefore a substantial number of SNPs and contigs - are expected to be homozygous in some of the females (Table 1). In contrast to this prediction, we found only a small minority of SNPs for which at least one daughter had a homozygous genotype (Table 1). These SNPs were most often surrounded by SNPs, for which all three daughters were heterozygous, suggesting there was no real stretch of homozygosity in any female. Among the >3200 contigs with more than two SNPs, only 9 contigs carried > 2 SNPs homozygous in the same daughter. Similar proportions were found when the same analysis was performed in another autospeudogamous species, *M. monhystera*. As a control, we also analyzed two sexual *Mesorhabditis* species *M. longespiculosa* and *M. spiculigera*. In these species, we found a large fraction of SNPs with homozygous genotypes (Table 1), as expected under random mating of gametes, with 737 (out of 1423, ~52%) and 1371 (out of 2953, ~46%) contigs, respectively, carrying >2 SNPs homozygous in the same daughter. This analysis indicates that the modified meiosis in autopseudogamous species of *Mesorhabditis* (almost) entirely preserves heterozygosity, from one generation to the next, and in the population overtime.

These results seem to contradict our initial cytological observations. We therefore asked if despite the presence of structures, which resemble chiasmata, homologous recombination might be absent, which would then explain the maintenance of heterozygosity in the short and long term upon assortment of non-sister chromatid.

All homologous chromosomes recombine during female meiosis

We wished to directly visualize crossing-over as a formal proof that homologous recombination occurs during *M. belari* female meiosis. To this end, we used the thymidine analog EdU, which is incorporated into replicating DNA during oogenesis and can be fluorescently labelled. *M. belari* females were bathed in EdU (pulse phase) and then allowed to recover (chase phase) so that cells next divided and replicated without EdU. We optimized the pulse and chase periods to obtain chromosomes harboring only one EdU-labelled chromatid (Figure S2). Upon recombination, we then expected a strain exchange between one EdU-labelled (shown in pink in Figure 3) and one non EdU-labelled chromatid (shown in blue in Figure 3, labelled with Hoechst) in 50% of cases, generating bicolor chromatids (blue/pink) (Figure S2, Figure 3). We first analyzed the color of chromatids in diakinesis oocytes, in which homologous chromosomes form chiasmata, *i.e*. bivalents. The expected figures of crossing-over in holocentric chromosomes has been described in (*21*) and is depicted in Figure 3A. From 8 oocytes, we identified 26 bivalent chromosomes whose orientation allowed us to unambiguously distinguish the chromatids within the chiasma. For 12 of them, the two opposed chromatids had the same color and could not be analyzed. Among the 14 showing opposed chromatids of different colors, 13 bivalents, showed an exchange of chromatids and only one showed no exchange (Figure 3A). We also analyzed chromosomes in the female pronuclei of gynogenetic eggs during the first or second cell-cycle, when chromosomes are condensed and chromatids clearly visible. Cycles of DNA replication and mitosis had occurred in the absence of EdU in embryos, generating many chromatids devoid of EdU. Nevertheless, we counted 49 bicolor chromatids from 12 embryos (one representative embryo shown in Figure 3B). This analysis also revealed that chromatid exchange is not restricted to chromosome ends, as many chromosomes show large portions of EdU positive chromatids (Figure 3B). These results demonstrate that exchanges of strands between homologous chromosomes during meiosis are frequent, and that recombination- not restricted to telomeric regions- does occur in *M. belari* during the production of unreduced oocytes.

As another evidence that recombination is maintained in *M. belari*, we analyzed patterns of linkage disequilibrium (LD) across loci. In the absence of sex and recombination, alleles are expected to be strongly associated among loci, with haplotype blocks extending over long stretches of DNA. Recombination, if at work, breaks allele associations, leading to a decay of LD as the physical distance between SNPs increases (*22*). To assess the extent of LD in *M. belari*, we analyzed the genomic data previously obtained from the ten strains. We called SNPs and used the LDHelmet program to estimate the genome-wide distribution of the effective

population recombination rate ρ. This analysis indicated that in both species the estimated ρ was homogeneously high across the genome (Figure S1), with a point estimate of the average ρ of 0.038 per base pair. This implies that LD is lost as the distance between the considered loci exceeds ~100 bp. The average estimated ρ in *M. belari* was similar to estimates reported in sexual species of arthropods, such as *Drosophila melanogaster* (*20*), and indicative of a high effective population recombination rate in this auto-pseudogamous species.

Chromatid segregation is biased during the unique meiotic division of M. belari embryos

Our cytological and genomic data are contradictory because recombination and random assortment of non-sister chromatids should lead to LOH. We reasoned that maintenance of heterozygosity from mother to daughters, and at the population level, can be achieved despite recombination, if either the two recombinants, or the two non-recombinant chromatids of a given chromosome pair co-segregate into the egg during the unique division of meiosis. We validated this hypothesis using our EdU experiment. Because *M. belari* chromosomes cannot be distinguished cytologically (*i.e.* pairs cannot be recognized), we used a statistical approach. We reasoned that under the hypothesis of co-segregating recombinant chromatids, we should always find an even number of recombinant chromatids in the nuclei of one-cell stage embryos, before the first mitosis. Such a pattern would be obtained very rarely in the case of random segregation of the 20 chromatids (p-value 0.00048, binomial test, n=11, p=0.5). We reanalyzed a new set of one-cell embryos, selecting only those in which chromosomes were well spread out at prometaphase, so that chromosome axis was unambiguously identified. In the 11 embryos analyzed, we always found an even number of bicolor chromatids, ranging from 4 to 8 (Figure 3C and Figure S3). This result strongly supported that the single meiotic division of *M. belari* females is unique, as it leads to Directed Chromatid Assortment (DCA).

Modeling the reproductive strategy of M. belari and Directed Chromatid Assortment during meiosis

To assess whether DCA could reconcile cytological and genomic data, we developed a population genetics model. As output parameters, we considered the level of heterozygosity and the Linkage Disequilibrium LD (as measured above) but also the inbreeding coefficient

Fis. In a randomly mating species (Hardy-Weinberg expectation), Fis equal 0. Fis is negative if there is an excess of heterozygosity compared to the Hardy-Weinberg expectation, as found in asexuals that have lost recombination, and positive if there is a deficit of heterozygosity, as in selfers for instance (*23*, *24*). By analyzing the heterozygosity found in the 10 independent wild strains of *M. belari*, we revealed a Fis close to 0, *i.e.* Fis=0.019 throughout the genome. Such a value was unexpected for a species with a rate of sexual reproduction close to 0. We had previously shown that out of 1000 females, no sexual females were produced (*13*).

We modeled the life cycle of *M. belari*, including the production of sexual males and asexual females with a biased sex ratio, the inbreeding mating structure (brother-sister mating or strong family structure as proposed by (*13*)), and the modified meiosis with variable segregation bias (Figure 4A-B). We also allowed for rare production of sexual females. Although no sexual females have been observed under laboratory conditions, they may exist at low rate in natural populations and it is an intermediate stage that necessarily occurred in the transition from sexuality to asexuality. We looked for conditions that could explain the observed genomic pattern: high heterozygosity, Fis~0, low LD.

First, analytical results and multilocus simulations confirmed that DCA is needed to explain the absence of LOH. Second, considering deleterious mutations throughout the genome broaden the conditions that can explain the observed genomic patterns: strict DCA is not required and sexual females can be produced at low rate (Figure 4C and Supp. Text). Actually, the production of sexual females at very low rate better explains the low and flat LD pattern than pure asexuality (Supp. Text). The comparison of results without and with deleterious mutations also illustrates the central role that recessive deleterious mutations likely play in the system. Highly homozygotes individuals that should be produced by imperfect DCA or leaky sex (that should lead to Fis > 0, Figure 4C neutral) are selected against, maintaining Fis close to zero for a large range of conditions (Figure 4C with deleterious mutations). This also supports the idea that LOH should be costly and suggests that DCA could be selected as a LOH-preventing mechanism.

We tested this hypothesis via a modification of the initial model whereby the proportion of biased chromatid segregation can evolve. We first simulated a sexual species with no segregation bias, and added a locus controlling the proportion of asexual females produced. We assumed one mandatory crossover per chromosome. Hence, the asexual females experienced LOH with associated fitness reduction due to the expression of recessive deleterious mutations

in homozygotes, a form of inbreeding depression. If inbreeding depression was higher than 0.5 it compensated for the advantage of not producing males and pseudogamy could not evolve. If inbreeding depression was lower than 0.5, pseudogamy rapidly evolved. Next, we introduced mutations at a second locus controlling DCA during asexual meiosis (in both directions: recombinants could be more positively or more negatively associated than at random). We found that mutations leading to positive association between recombinants were selected for and that the population rapidly evolved towards complete DCA, preventing the deleterious effects of LOH. Interestingly, we also found that when mutations affecting pseudogamy and chromatid assortment were introduced at the same time, the two mechanisms co-evolved, speeding up and broadening the conditions for the evolution of pseudogamy (Figure 4 and Supp. Text). On the one hand, the occurrence of some asexual females enabled the evolution of DCA. On the other hand, once DCA started to evolve, it partly prevented the deleterious effect of LOH, favoring the evolution of pseudogamy, even when inbreeding depression was higher than 0.5 (Figure 4D).

Our modeling approach thus confirmed that all a priori contradictory observations can be reconciled by the mechanism of DCA during the unique meiotic division of females and proposed a selective explanation for the evolution of such a peculiar mechanism from a sexual ancestor.

Discussion

In this study, we found that *M. belari* asexual females are produced in the presence of recombination and assortment of non-sister chromatids, which should lead to rapid LOH, distally to the crossing-over. Our genomic analysis, however, revealed a surprisingly high level of heterozygosity throughout the genome and no sign of LOH, even locally. Using a combination of cytological, genomic and modeling approaches, we demonstrated that this pattern is possible provided the recombinant chromatids of each chromosome pair are not randomly assorted but instead co-segregate during the unique meiotic division. We named this new type of non-Mendelian inheritance Directed Chromatid Assortment (DCA). With DCA, specific pairs of chromatids are chosen during cell division such that the whole set of maternal alleles is transmitted to offspring (Figure 5).

Most asexual animal models have been characterized either by cytology or genomics, rarely both, whereas such a combination of approaches was here decisive. We suggest that DCA may exist in other asexuals displaying highly heterozygous genomes. For instance, DCA is compatible with the absence or very reduced LOH found in the recombining parthenogenetic water flea *Daphnia magna* (*25*), the asexual females produced by Cape Honey Bee workers (*26*) or the Rotifer *Adineta vaga* (*9,46*).

During their reproductive life time, *M. belari* females produce 10% reduced oocytes via regular meiotic divisions, which develop into sexual males, males being essential for sperm-dependent parthenogenesis (*13*). Hence, the meiotic program is intact in this species. This is a constraint for the evolution of asexuality because upon recombination, asexual females should experience LOH. We discovered DCA, as a new mechanism of LOH avoidance despite recombination. Another mechanism of LOH avoidance have been previously proposed for recombining asexuals, which relies on distal crossing-over location (*6, 26*). However, distal crossing-overs are very unstable for the *C. elegans* holocentric chromosomes and often lead to aneuploidy (*27*), suggesting that such mechanism of LOH avoidance could not have been selected in a holocentric species such as *M. belari*. A mechanism involving inverted meiosis (which is compatible with holocentricity), failed meiosis II and biased chromatid segregation has been proposed for the maintenance of heterozygosity in Oribatid mites (*28*, *29*). Although such biased segregation of chromatids remains hypothetical in the absence of further cytological description, it is conceptually similar to the DCA we describe in our study.

How did DCA emerge mechanistically during the evolution of auto-pseudogamy in *Mesorhabditis*? One hypothesis is that the abortion of meiosis I was first fixed in the population. If sexual females were initially still produced, it compensated for LOH in the asexuals. Once DCA appeared, LOH was prevented, next allowing the loss of most sexual females. Alternatively, a modification of the meiotic program could have generated simultaneously a defect in anaphase I and a biased segregation of chromatids. At this stage, it is difficult to speculate on a molecular mechanism, and on a sequence of events, because to our knowledge no such phenotype has been described in mutants of model species. Yet, it is not inconceivable that some of the proteins that are loaded on chromatids during recombination remain specifically attached to the recombinant chromatids, contributing to a directed orientation during division, or that chromosomes experience an incomplete resolution of the crossing-overs. More work on the mechanistic basis of meiosis in *M. belari* is required to address this question.

References

1. E. Suomalainen, A. Saura, J. Lokki, *Cytology and Evolution in Parthenogenesis* (CRC Press, Florida, Boca Raton., 1987).
2. M. Neiman, T. F. Sharbel, T. Schwander, Genetic causes of transitions from sexual reproduction to asexuality in plants and animals. *Journal of Evolutionary Biology*. 27, 1346–1359 (2014).
3. T. Lenormand, J. Engelstädter, S. E. Johnston, E. Wijnker, C. R. Haag, Evolutionary mysteries in meiosis. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 371 (2016), doi:10.1098/rstb.2016.0001.
4. M. Archetti, Complementation, genetic conflict, and the evolution of sex and recombination. *J Hered*. 101 Suppl 1, S21-33 (2010).
5. J. Engelstädter, Constraints on the evolution of asexual reproduction. *Bioessays*. 30, 1138–1150 (2008).
6. C. R. Haag, L. Theodosiou, R. Zahab, T. Lenormand, Low recombination rates in sexual species and sex-asex transitions. *Philos Trans R Soc Lond B Biol Sci*. 372, 20160461 (2017).
7. K. S. Jaron, J. Bast, R. W. Nowell, T. R. Ranallo-Benavidez, M. Robinson-Rechavi, T. Schwander, Genomic Features of Parthenogenetic Animals. *J Hered*. 112, 19–33 (2021).
8. A. Brandt, P. Tran Van, C. Bluhm, Y. Anselmetti, Z. Dumas, E. Figuet, C. M. François, N. Galtier, B. Heimburger, K. S. Jaron, M. Labédan, M. Maraun, D. J. Parker, M. Robinson-Rechavi, I. Schaefer, P. Simion, S. Scheu, T. Schwander, J. Bast, Haplotype divergence supports long-term asexuality in the oribatid mite Oppiella nova. *Proc Natl Acad Sci U S A*. 118, e2101485118 (2021).
9. P. Simion, J. Narayan, A. Houtain, A. Derzelle, L. Baudry, E. Nicolas, R. Arora, M. Cariou, C. Cruaud, F. R. Gaudray, C. Gilbert, N. Guiglielmoni, B. Hespeels, D. K. L. Kozlowski, K. Labadie, A. Limasset, M. Llirós, M. Marbouty, M. Terwagne, J. Virgo, R. Cordaux, E. G. J. Danchin, B. Hallet, R. Koszul, T. Lenormand, J.-F. Flot, K. Van Doninck, Chromosome-level genome assembly reveals homologous chromosomes and recombination in asexual rotifer Adineta vaga. *Sci Adv*. 7, eabg4216 (2021).
10. P. Tran Van, Y. Anselmetti, J. Bast, Z. Dumas, N. Galtier, K. S. Jaron, K. Martens, D. J. Parker, M. Robinson-Rechavi, T. Schwander, P. Simion, I. Schön, First annotated draft genomes of nonmarine ostracods (Ostracoda, Crustacea) with different reproductive modes. *G3 (Bethesda)*. 11, jkab043 (2021).
11. H. Fradin, K. Kiontke, C. Zegar, M. Gutwein, J. Lucas, M. Kovtun, D. L. Corcoran, L. R. Baugh, D. H. A. Fitch, F. Piano, K. C. Gunsalus, Genome Architecture and Evolution of a Unichromosomal Asexual Nematode. *Current Biology*. 27, 2928-2939.e6 (2017).
12. R. Guidetti, M. Cesari, R. Bertolani, T. Altiero, L. Rebecchi, High diversity in species, reproductive modes and distribution within the Paramacrobiotus richtersi complex (Eutardigrada, Macrobiotidae). *Zoological Lett*. 5, 1 (2019).
13. M. Grosmaire, C. Launay, M. Siegwald, T. Brugière, L. Estrada-Virrueta, D. Berger, C. Burny, L. Modolo, M. Blaxter, P. Meister, M.-A. Félix, P.-H. Gouyon, M. Delattre, Males as somatic investment in a parthenogenetic nematode. *Science*. 363, 1210–1213 (2019).
14. D. G. Albertson, J. N. Thomson, Segregation of holocentric chromosomes at meiosis in the nematode, Caenorhabditis elegans. *Chromosome Res*. 1, 15–26 (1993).
15. D. E. Almanzar, S. G. Gordon, O. Rog, Meiotic sister chromatid exchanges are rare in C. elegans. *Curr Biol*. 31, 1499-1507.e3 (2021).
16. A.-C. Valfort, C. Launay, M. Sémon, M. Delattre, Evolution of mitotic spindle behavior during the first asymmetric embryonic division of nematodes. *PLoS Biol.* 16, e2005099 (2018).
17. C. Launay, M.-A. Félix, J. Dieng, M. Delattre, Diversification and hybrid incompatibility in auto-pseudogamous species of Mesorhabditis nematodes. *BMC Evol Biol*. 20, 105 (2020).
18. T. S. Korneliussen, A. Albrechtsen, R. Nielsen, ANGSD: Analysis of Next Generation Sequencing Data. *BMC Bioinformatics*. 15, 356 (2014).
19. E. C. Andersen, J. P. Gerke, J. A. Shapiro, J. R. Crissman, R. Ghosh, J. S. Bloom, M.-A. Félix, L. Kruglyak, Chromosome-scale selective sweeps shape Caenorhabditis elegans genomic diversity. *Nat. Genet.* 44, 285–290 (2012).
20. A. H. Chan, P. A. Jenkins, Y. S. Song, Genome-wide fine-scale recombination rate variation in Drosophila melanogaster. *PLoS Genet.* 8, e1003090 (2012).
21. M. Mandrioli, G. C. Manicardi, Holocentric chromosomes. *PLoS Genet*. 16, e1008918 (2020).
22. M. P. H. Stumpf, G. A. T. McVean, Estimating recombination rates from population-genetic data. *Nat Rev Genet*. 4, 959–968 (2003).
23. S. Glémin, N. Galtier, Genome evolution in outcrossing versus selfing versus asexual species. *Methods Mol. Biol.* 855, 311–335 (2012).
24. F. Balloux, L. Lehmann, T. de Meeûs, The population genetics of clonal and partially clonal diploids. *Genetics*. 164, 1635–1644 (2003).
25. M. Dukić, D. Berner, C. R. Haag, D. Ebert, How clonal are clones? A quest for loss of heterozygosity during asexual reproduction in Daphnia magna. *J Evol Biol*. 32, 619–628 (2019).
26. B. P. Oldroyd, B. Yagound, M. H. Allsopp, M. J. Holmes, G. Buchmann, A. Zayed, M. Beekman, Adaptive, caste-specific changes to recombination rates in a thelytokous honeybee population. *Proc Biol Sci*. 288, 20210729 (2021).
27. E. Altendorfer, L. I. Láscarez-Lagunas, S. Nadarajan, I. Mathieson, M. P. Colaiácovo, Crossover Position Drives Chromosome Remodeling for Accurate Meiotic Chromosome Segregation. *Curr Biol*. 30, 1329-1338.e7 (2020).
28. G. Taberly, [The cytology of parthenogenesis in Platynothrus peltifer (Koch) (Acarien, Oribate)]. *C R Hebd Seances Acad Sci*. 247, 1655–1657 (1958).

29. M. Archetti, Evidence from automixis with inverted meiosis for the maintenance of sex by loss of complementation. *J Evol Biol*. 35, 40–50 (2022).
30. L. Serra, D. Z. Chang, M. Macchietto, K. Williams, R. Murad, D. Lu, A. R. Dillman, A. Mortazavi, Adapting the Smart-seq2 Protocol for Robust Single Worm RNA-seq. *Bio Protoc*. 8, e2729 (2018).
31. H. Li, R. Durbin, Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 25, 1754–1760 (2009).
32. H. Li, B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, R. Durbin, 1000 Genome Project Data Processing Subgroup, The Sequence Alignment/Map Format and SAMtools. *Bioinformatics (Oxford, England)*. 25, 2078–2079 (2009).
33. A. M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 30, 2114–2120 (2014).
34. M. G. Grabherr, B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, Q. Zeng, Z. Chen, E. Mauceli, N. Hacohen, A. Gnirke, N. Rhind, F. di Palma, B. W. Birren, C. Nusbaum, K. Lindblad-Toh, N. Friedman, A. Regev, Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat Biotechnol*. 29, 644–652 (2011).
35. P. Gayral, J. Melo-Ferreira, S. Glémin, N. Bierne, M. Carneiro, B. Nabholz, J. M. Lourenco, P. C. Alves, M. Ballenghien, N. Faivre, K. Belkhir, V. Cahais, E. Loire, A. Bernard, N. Galtier, Reference-free population genomics from next-generation transcriptome data and the vertebrate-invertebrate gap. *PLoS Genet.* 9, e1003457 (2013).
36. M. Patterson, T. Marschall, N. Pisanti, L. van Iersel, L. Stougie, G. W. Klau, A. Schönhuth, WhatsHap: Weighted Haplotype Assembly for Future-Generation Sequencing Reads. *J Comput Biol*. 22, 498–509 (2015).
37. B. L. Browning, X. Tian, Y. Zhou, S. R. Browning, Fast two-stage phasing of large-scale sequence data. *Am J Hum Genet*. 108, 1880–1890 (2021).
38. D. E. Reich, S. F. Schaffner, M. J. Daly, G. McVean, J. C. Mullikin, J. M. Higgins, D. J. Richter, E. S. Lander, D. Altshuler, Human genome sequence variation and the influence of gene history, mutation and recombination. *Nat Genet*. 32, 135–142 (2002).
39. L. S. Stevison, A. E. Woerner, J. M. Kidd, J. L. Kelley, K. R. Veeramah, K. F. McManus, Great Ape Genome Project, C. D. Bustamante, M. F. Hammer, J. D. Wall, The Time Scale of Recombination Rate Evolution in Great Apes. *Mol Biol Evol*. 33, 928–945 (2016).
40. A. F. Shanfelter, S. L. Archambeault, M. A. White, Divergent Fine-Scale Recombination Landscapes between a Freshwater and Marine Population of Threespine Stickleback Fish. *Genome Biol Evol*. 11, 1573–1585 (2019).
41. D. L. Halligan, F. Oliver, A. Eyre-Walker, B. Harr, P. D. Keightley, Evidence for pervasive adaptive protein evolution in wild mice. *PLoS Genet*. 6, e1000825 (2010).
42. T. R. Booker, R. W. Ness, P. D. Keightley, The Recombination Landscape in Wild House Mice Inferred Using Population Genomic Data. *Genetics*. 207, 297–309 (2017).
43. S. Singhal, E. M. Leffler, K. Sannareddy, I. Turner, O. Venn, D. M. Hooper, A. I. Strand, Q. Li, B. Raney, C. N. Balakrishnan, S. C. Griffith, G. McVean, M. Przeworski, Stable recombination hotspots in birds. *Science*. 350, 928–932 (2015).
44. S. H. Martin, M. Möst, W. J. Palmer, C. Salazar, W. O. McMillan, F. M. Jiggins, C. D. Jiggins, Natural Selection and Genetic Diversity in the Butterfly Heliconius melpomene. *Genetics*. 203, 525–541 (2016).
45. S. H. Martin, J. W. Davey, C. Salazar, C. D. Jiggins, Recombination rate variation shapes barriers to introgression across butterfly genomes. *PLoS Biol*. 17, e2006288 (2019).

46. Terwagne, M., Nicolas, E., Hespeels, B., Herter, L., Virgo, J., Demazy, C., Heuskin, A.-C., Hallet, B., & Van Doninck, K. (2022). DNA repair during nonreductional meiosis in the asexual rotifer Adineta vaga. *Science Advances*, *8*(48), eadc8829. https://doi.org/10.1126/sciadv.adc8829

Table 1. Patterns of shared heterozygosity among sisters in four species of *Mesorhabditis*.

	pseudogamous	**pseudogamous**	**sexual**	**sexual**
species	*M. belari*	*M. monhystera*	*M. spiculigera*	*M. longespiculosa*
#contigs	8381	8632	7099	7564
mean coverage	37X	28X	42X	40X
#polymorphic sites	34,867	29,664	43,543	20,243
one-heterozygote[a]	1.44 %	0.42 %	45.3 %	23.5 %
two-heterozygote[b]	0.89 %	0.99 %	22.1 %	27.6 %
three-heterozygote[c]	97.5 %	98.5 %	22.4 %	39.4 %
other[d]	0.1 %	0.06 %	10.2 %	9.5 %

[a] proportion of polymorphic sites at which exactly one of the three sisters was heterozygous

[b] proportion of polymorphic sites at which exactly two of the three sisters were heterozygous

[c] proportion of polymorphic sites at which all three sisters were heterozygous

[d] no heterozygote or unexpected allele call

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Authors contributions: MD, NG and SG designed the experiments. SG designed the model. ELF developed the simulation code, run and analyzed the simulations with LMO and SG. CD, NS and NG analyzed the genomic data. CB, EW and MD performed the experimental work. NG, SG and MD wrote the manuscript. We acknowledge the contribution of the imaging plateform PLATIM from SFR Biosciences Lyon, the sequencing plateform Genomeast at IGBMC Strasbourg. We also thank Carine Rey for help with the treatment of the NGS raw data. We thank Raphaelle Dubruille and Benjamin Loppin for critical reading of the manuscript.

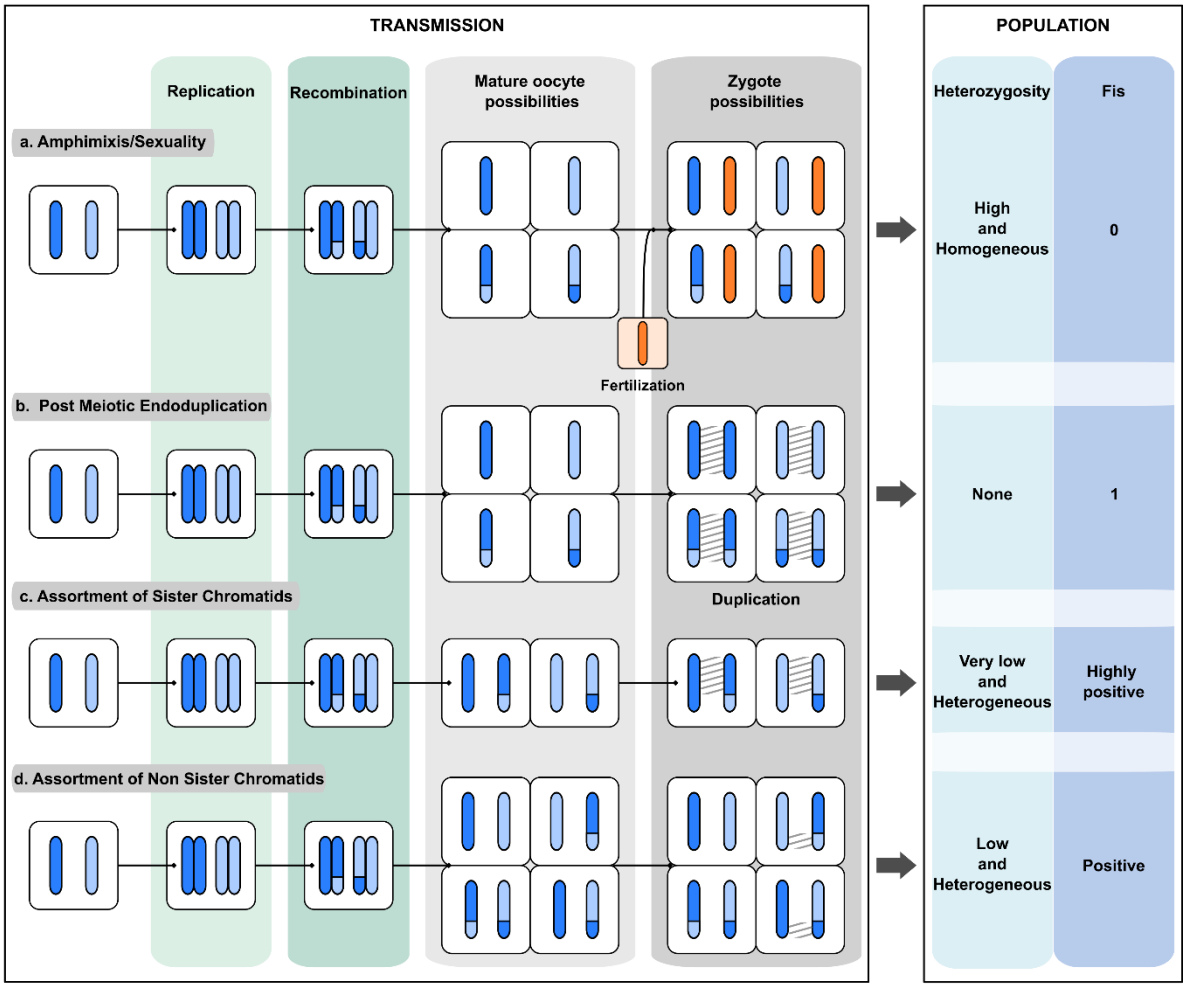
Competing interests: All the authors declare having no competing interests.

Data and materials availability: all data is available in the manuscript or the supplementary materials except data related to genome sequencing, which can be found on ebi.ac.uk/ena accession number PRJEB30104)

Figures

Figure 1

A


TRANSMISSION
POPULATION
Replication
Recombination
Mature oocyte possibilities
Zygote possibilities
Heterozygosity
Fis
a. Amphimixis/Sexuality
High and Homogeneous
0
Fertilization
b. Post Meiotic Endoduplication
None
1
c. Assortment of Sister Chromatids
Duplication
Very low and Heterogeneous
Highly positive
d. Assortment of Non Sister Chromatids
Low and Heterogeneous
Positive

B

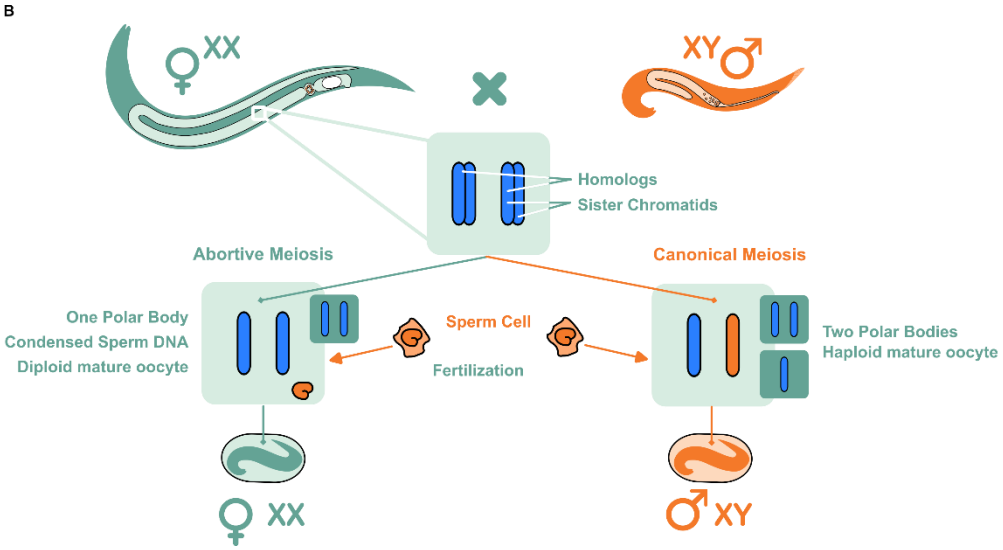

♀XX
XY♂
Homologs
Sister Chromatids
Abortive Meiosis
Canonical Meiosis
One Polar Body
Condensed Sperm DNA
Diploid mature oocyte
Sperm Cell
Fertilization
Two Polar Bodies
Haploid mature oocyte
♀XX
♂XY

Figure 1: **Genetic expectation upon modification of meiosis in asexuals and reproductive system of *Mesorhabditis belari*.** A) Description of the expected genetic composition of a zygote, and of a population, upon different types of modifications of the meiotic program (b-d). Canonical meiotic division as found in regular sexual species is shown on the top (a). Each bar represents a chromatid. The paternal chromatid is in orange. The maternal chromatids are in dark and light blue, corresponding to homologous chromosomes. Upon recombination, assortment of chromatids after modification of meiosis generate stretches of homozygosity, shown with hatching. Consequently, the level of heterozygosity in the population (right column) is decreased. Heterogeneous heterozygosity underlines a disparity in the level of heterozygosity along the chromosomes. In contrast, homogeneous heterozygosity demonstrates uniformity in the level of heterozygosity. The coefficient of inbreeding (Fis) corresponds to the expected level of heterozygosity compared to Hardy-Weinberg expectation (in a randomly mating population). B) Schematic representation of the reproductive system found in *M. belari* as described in (*13*). Females (in green) produce two types of oocytes. Through canonical meiosis, a single chromatid per chromosome is transmitted to the oocyte (in blue). The sperm provides in single chromatid (in orange). The resulting diploid individuals give rise to males. 90% of the oocytes are however diploid (incomplete meiosis, on the left) in which case the sperm does not contribute DNA. The individuals give rise to females.

Figure 2

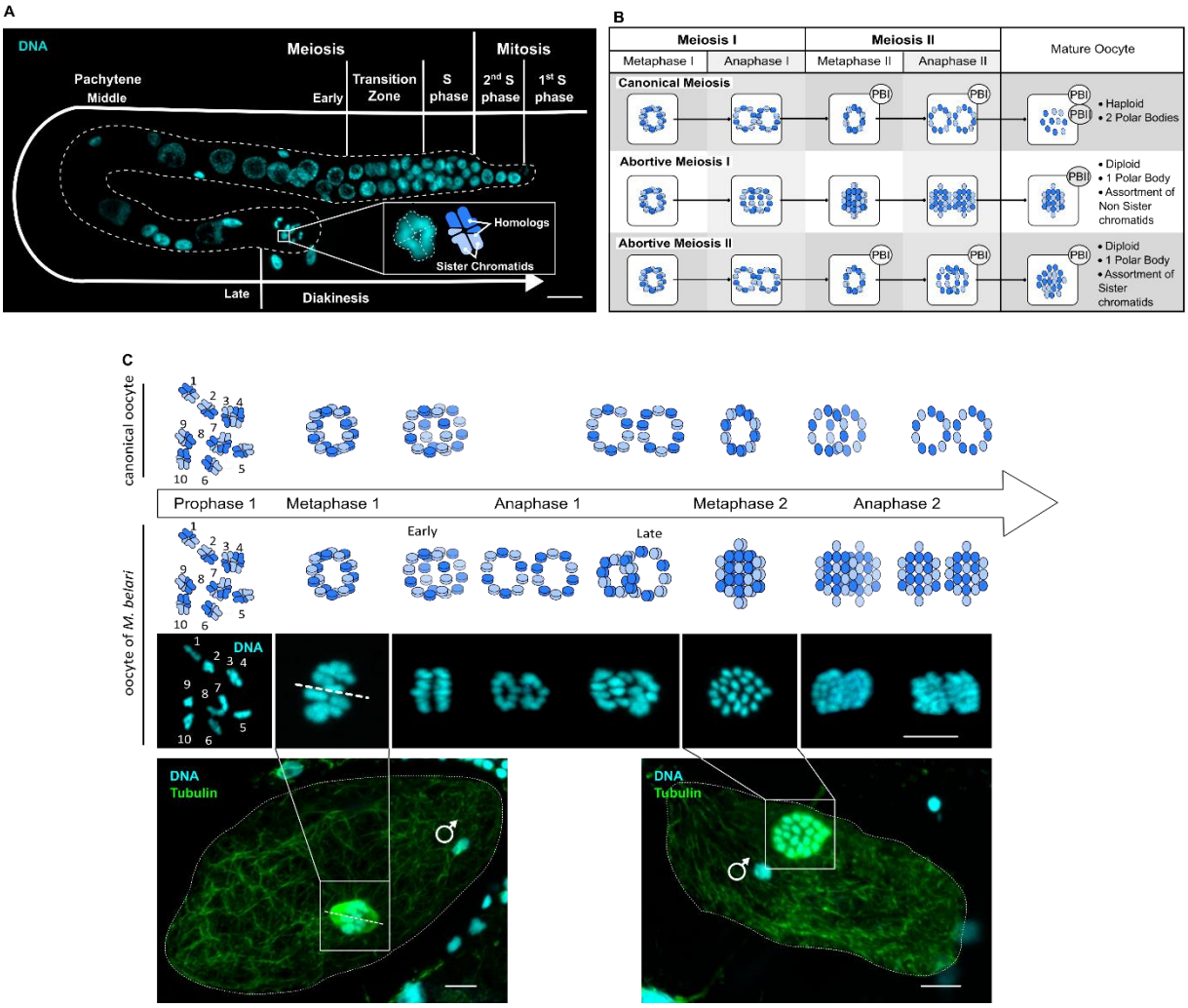
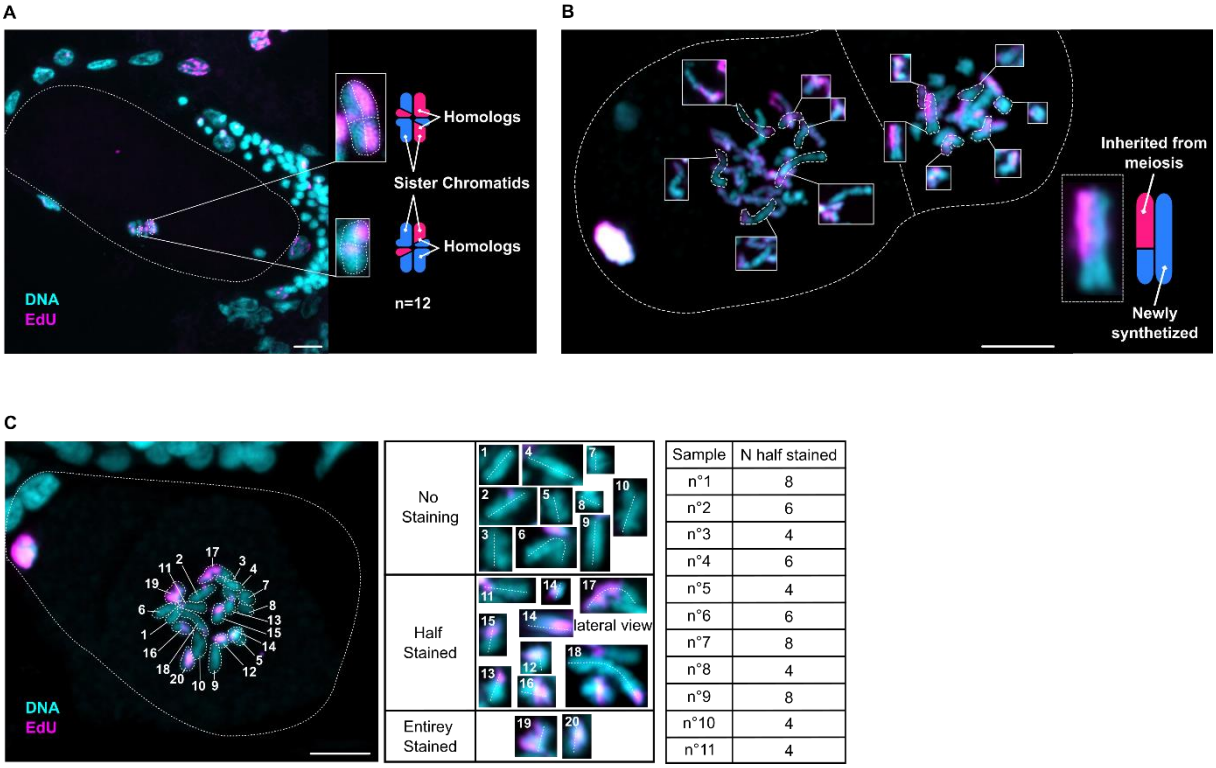


Figure 2: **Cytological evidence of abortive meiosis I in *M. belari* females**. A) Gonad of a *M. belari* female stained for DNA, showing the progression of meiotic cells along the tract. Oocytes in diakinesis are found on the distal part, with chiasmatic chromosomes. DNA is in blue. Scale bar is 20 µm. The holocentric bivalent chromosomes show a typical cross shape. The bivalents are schematized in blue, one chromosome is in dark blue and its homolog is shown in light blue. B) Expected figures of chromosome organization upon failure of meiosis I or meiosis II in *M. belari*. A canonical meiosis is shown on the top. At metaphase, chromosomes orient as a ring. During anaphase, chromosomes (Anaphase I) or chromatids (Anaphase II) segregate as two rings. PB represent the polar body. C) Reconstitution of *M. belari* meiosis in amphimictic (canonical meiosis) and gynogenetic (incomplete meiosis) embryos from fixed samples. On the bottom, representative gynogenetic embryos, from which the images are taken, are show. A sperm DNA is visible although it will remain condensed and will fuse with the female DNA. Tubulin is in green and DNA is in blue. *M. belari* is diploid carrying 2=20 chromosomes. The dotted line represents the long axis of the meiotic spindle along which the bivalent chromosomes align. Scale bar is 5 µm.

Figure 3.



Sample	N half stained
n°1	8
n°2	6
n°3	4
n°4	6
n°5	4
n°6	6
n°7	8
n°8	4
n°9	8
n°10	4
n°11	4

Figure 3: **Evidence of recombination and Directed Chromatid Assortment**. Fixed embryos after an EdU experiment, where one chromatid out of the two is stained with EdU (in pink). DNA is in blue. Scale bar is 5 µm. A) Embryo in metaphase of meiosis I (*i.e.* no polar body is extruded). The metaphase plate is perpendicular to the glass slide. Few chromosomes are visible on this lateral view. The expected exchange of chromatids, as described in (*21*) is shown as well as the actual images in the inset. B) Two-cell stage embryo during prometaphase. Most chromosomes have a bicolour chromatid (half blue/half pink, demonstrating recombination) and an unlabelled chromatid because it has replicated during the previous S phase in the absence of EdU (entirely blue). C) One representative one-cell embryo in prometaphase. All 20 chromosomes are shown in the insets. 8 chromosomes are bicolour (#14 is shown twice). The table summarizes the count of recombinant chromatids from eleven embryos (also shown in Figure S3).

Figure 4

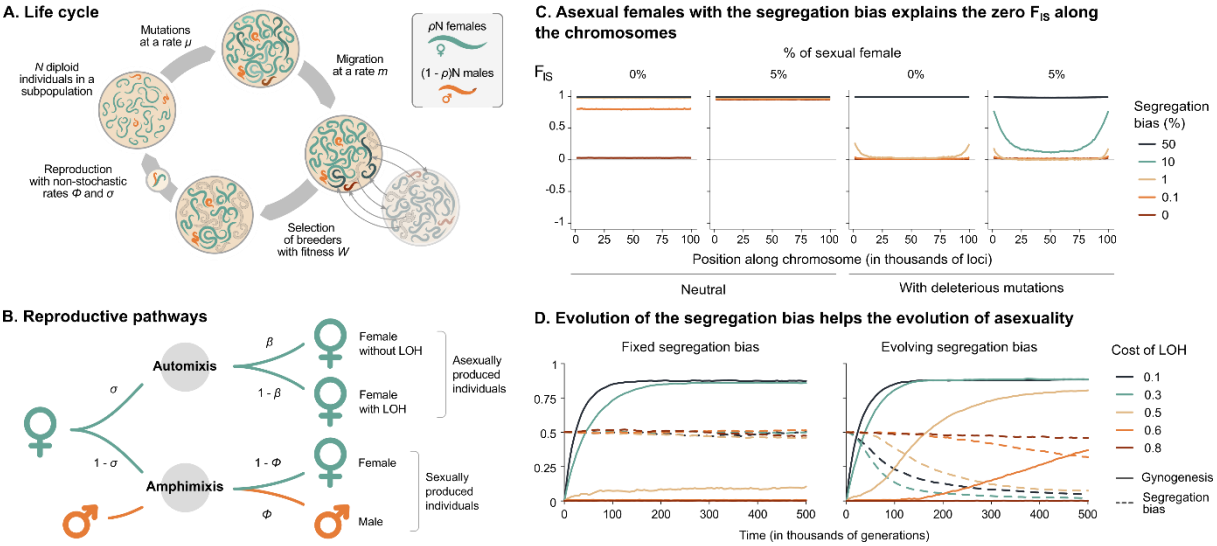


Figure 4: **Modeling the genomic consequences and evolution of DCA in *M. belari*** A) Simulated life cycle with associated parameters. The generations are discrete and non-overlapping and we assumed an island model to simulate the high level of consanguineous mating of natural population of *M. belari*: the lower the local population size (N) and the migration rate (m), the higher the level of consanguineous mating. B) Reproduction can occur either by automixis at rate s or sexually by amphimixis at rate 1 – s. During automixis, the rate of LOH is b. b = ½ corresponds to random assortment of chromatids whereas b = 0 corresponds to strict DCA. Under sexual reproduction, females are produced at rate f. f is close to zero in natural population. s and f determine the sex-ratio, r, in the population. C) Fis along a chromosome as a function of the level of consanguineous mating, with or without sexually produced females and with or without deleterious mutations. The total population size is 1000 and the migration rate is $m = 0.0005$. $N = 1000$ corresponds to a single population, $N = 200$ to five large breeding groups, and $N = 20$ to 50 small breeding groups. D) Evolution of pseudogamy from an initially sexual population with different costs of LOH when the segregation bias is fixed or allowed to evolve.

Figure 5

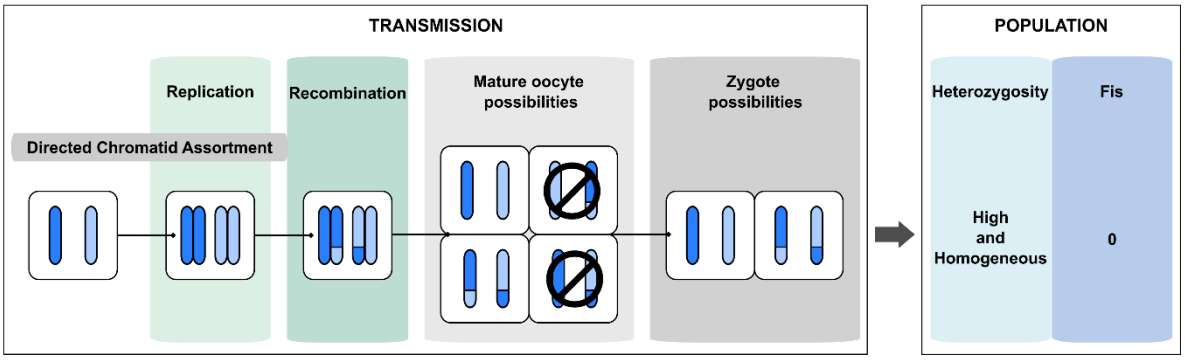


Figure 5: Mechanism of heterozygosity maintenance via DCA in *M. belari*. Schematic representation of meiosis and DCA during the production of diploid oocytes in *M. belari*. Homologous chromosomes (in blue) are initially heterozygous in the mother (shown in nuances of blue). After recombination and failure of meiosis I, non-sister chromatids do not segregate randomly during meiosis II. Instead, co-segregation of recombinant chromatids (in orange) maintains heterozygosity in the progeny and in the population.

Figure S1

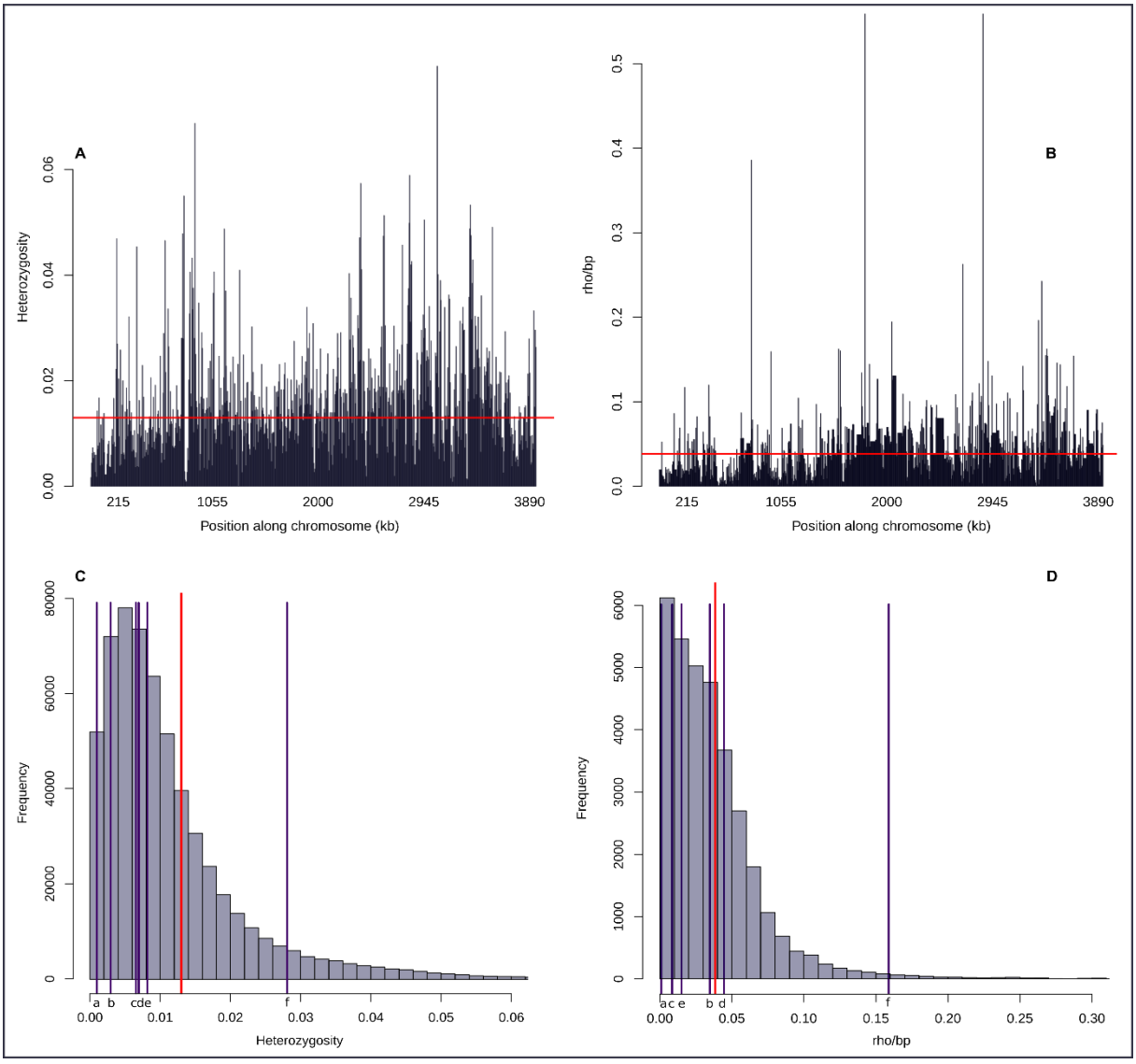


Figure Supplementary 1: Quantification of heterozygosity and estimation of r. Heterozygosity (A) and recombination (B) estimated along a representative contig (contig 110) and distribution of heterozygosity (C) and recombination rates (D) computed on 5000bp windows. Genome-wide average heterozygosity and recombination rate r are represented by a red bar. Other sexual species, taken from the literature, are depicted

with a blue bar: a) *Homo sapiens* (38, 39); b) *Gasterosteus aculeatus* (40); c) *Mus musculus* (41, 42); d) *Drosophila melanogaster* (20); e) *Taenopygia guttata* (43); f) *Heliconius melpomene* (44, 45).

Figure S2

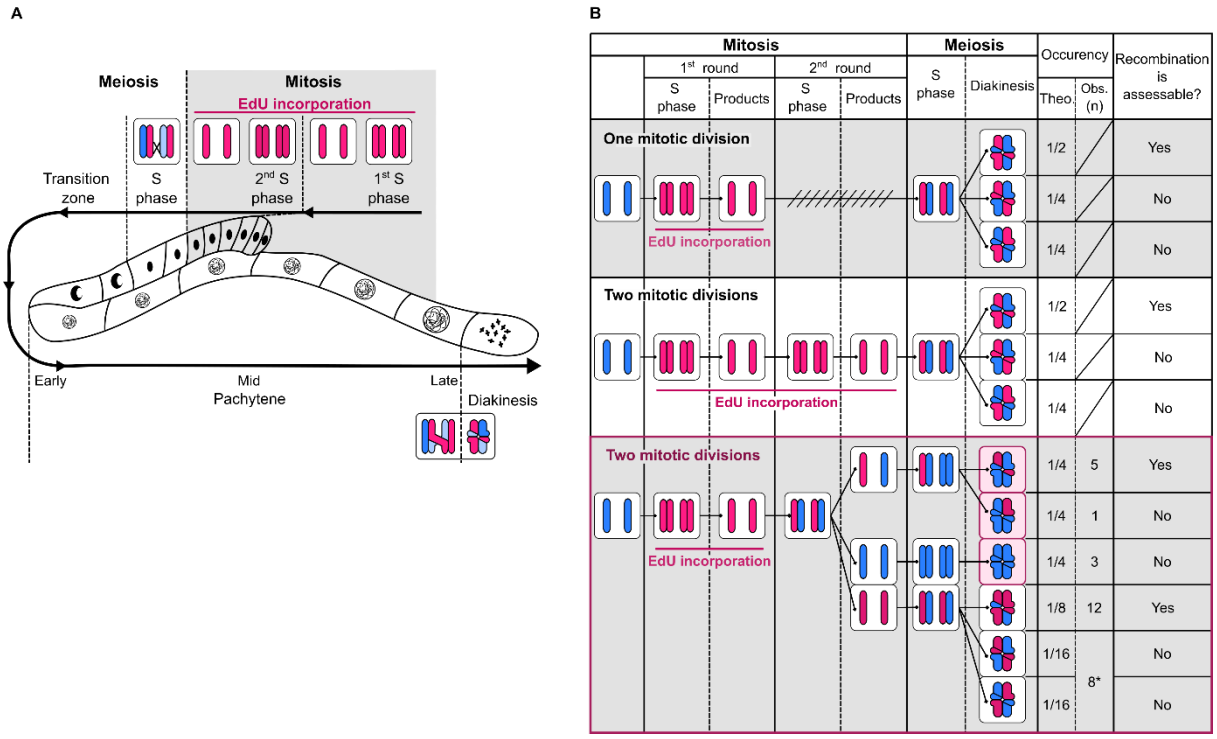


Figure Supplementary 2: Design and expectations for the Edu experiment. A) Design of EdU pulse-chase method. Sketch of M. belari gonad showing the number of homologs present at each stage of oogenesis and optimal EdU incorporation during mitotic S phase (replication phase). B) Expectations for EdU labelling on the bivalents according to the number of miotic S phases and the duration of EdU incorporation (pulse phase). An account of the different EdU labels is provided on the right. EdU is pink and DNA is blue. The observed pattern of EdU incorporation is consistent with EdU exposure during 2 rounds of S phase.

Figure S3

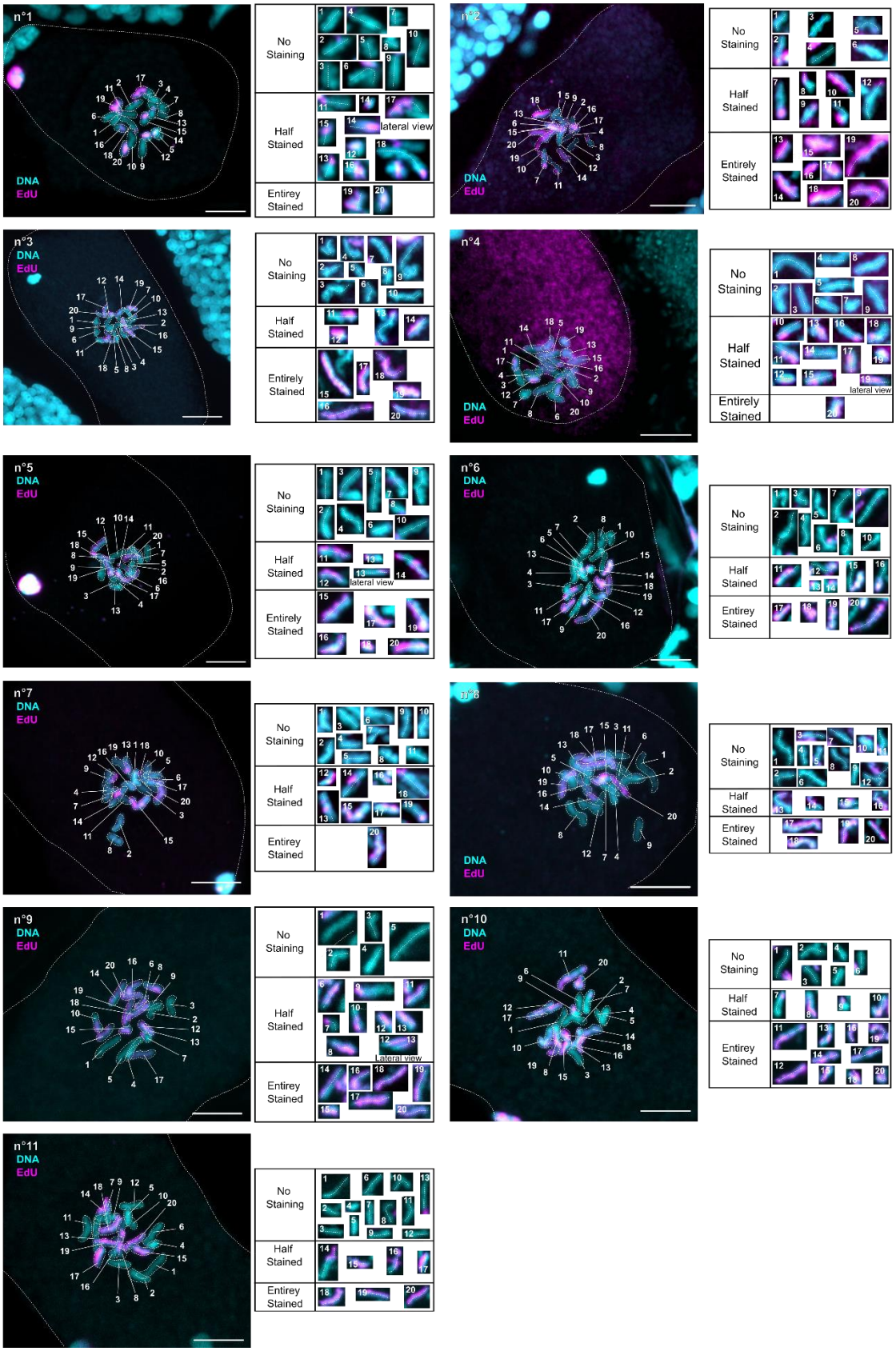


Figure Supplementary 3: Evidence of Directed Chromatid Assortment. 11 images of fixed embryos at one-cell stage. EdU is in pink and DNA is in blue. The count of bicolored chromosomes is summarized in

Figure 3. The dotted line shows the chromosome longest axis. Scale bar is 5 µm. Some chromosomes are shown twice, on the transverse and lateral view to better show the chromatid axis.

Supplementary Text Population genomics and evolution of reproduction in Mesorhabditis

Population genetic structure under the *Mesorhabditis* life cycle

Model

General presentation and definition of parameters

The aim of the model is to predict population genomic patterns expected under the *Mesorhabditis* life cycle. We consider a single neutral locus with an infinite allele model (IAM) of mutation. We consider a subdivided population with K demes, each of size N. The total population is thus $N_T = KN$. The life cycle is as follows (Figure 1 redrawn from the main text.). The generations are discrete and non-overlapping. Migration occurs before reproduction according to the island model at rate m. This simple population structure allows modelling the breeding structure of *Mesorhabditis* with high level of consanguineous mating: the lower the deme size and the migration rates, the higher the level of consanguineous mating.

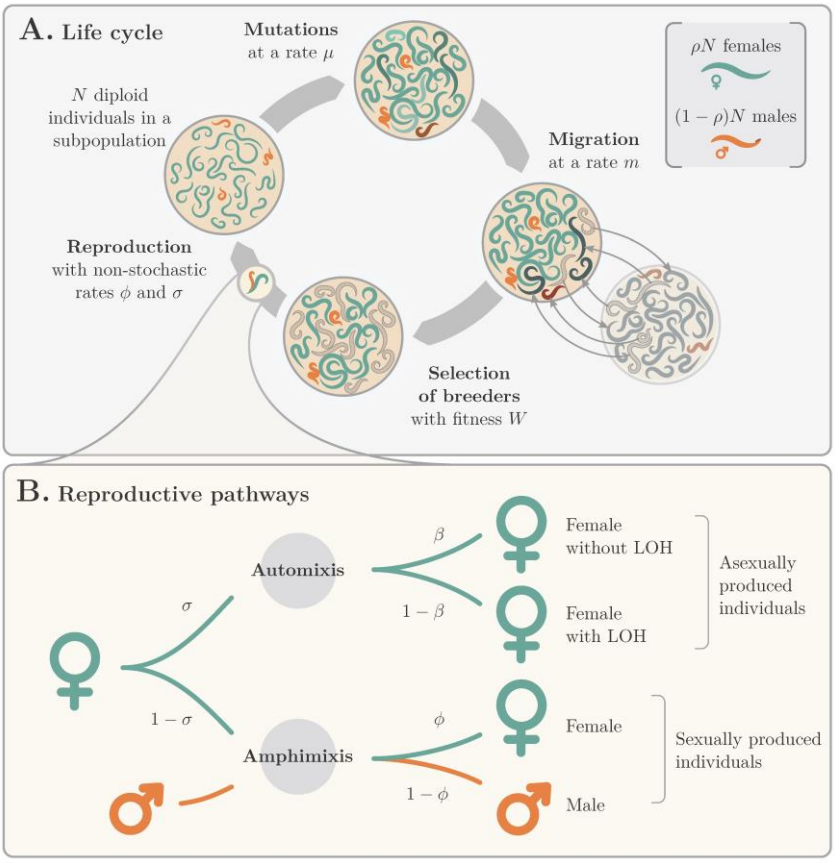


Figure 1: *Mesorhabditis* life cycle and parameters of the model.

Automictic reproduction that leads to gynogenetic females occurs in proportion σ and sexual reproduction $1-\sigma$. Under automixis, the rate of loss of heterozygosity (LOH) is β and depends on the underlying mechanism. With standard central fusion $\beta = 1/2$ after one crossover and random assortment of chromatids, and $\beta = 0$ if there is no recombination or in the proposed model of directed chromatid assortment (DCA, hereafter). Under sexual reproduction, the proportion of females and males is φ and $1-\varphi$, respectively. So far, no sexually-produced female has been observed (corresponding to φ=0). However, it is possible that they are produced at low rate in natural populations. If we set $\sigma = 1$ and $\beta = 0$, this is equivalent to a fully clonal model. If we set $\sigma = 0$ and $\varphi = 1/2$, this is equivalent to a fully sexual model.

To obtain measures of genetic diversity and population structure (F-statistics) we derive recursions on a set of probabilities of identity by descent (IBD), Q_i, which leads to heterozygosity and F-statistics measure of the form [5]:

$$H_i = 1 - Q_i \tag{1}$$

and

$$F_{i,j} = \frac{Q_i - Q_j}{1 - Q_j} \tag{2}$$

To fully describe the model, we need to follow eight probabilities of IBD, noted Q_0^k when the two genes are sampled in the same individual, Q_1^k when they are sampled in two individuals of the same population, and Q_2^k, when they are sampled in two individuals from different populations. The superscript *k* stands for the sex of sampled individuals (see Figure 2).

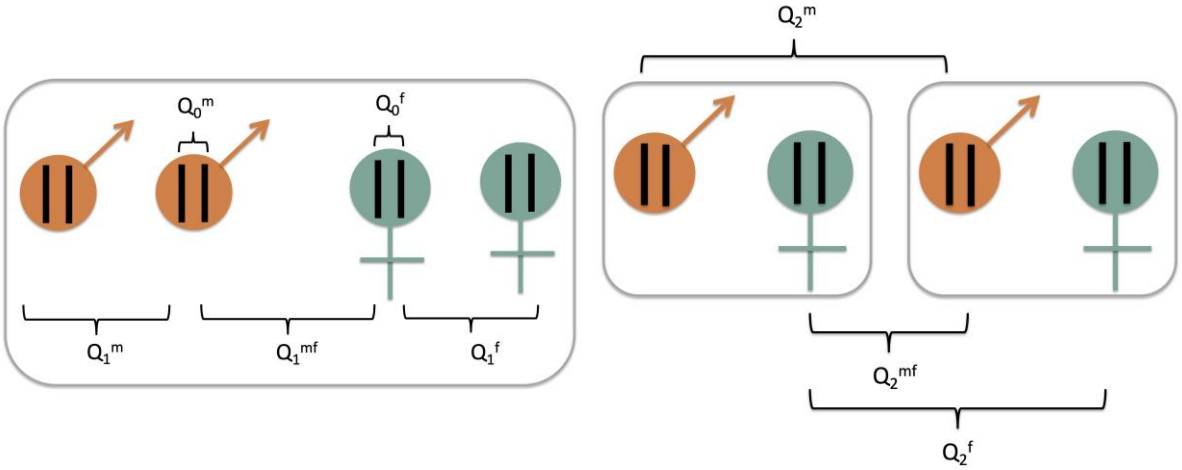


Figure 2: Definition of the eight probabilities of IBD. The grey boxes corresponds to demes.

From the parameters describing reproductive pathways (Figure 1) we obtain the proportion of males, sexual and gynogenetic females as:

$$\rho = (1 - \sigma)(1 - \varphi) \tag{3a}$$

$$x_f = (1 - \sigma)\varphi \tag{3b}$$

$$x_g = \sigma \tag{3c}$$

from which we obtain the number of males and females in the populations and the proportions of gynogenetic females among females

$$\begin{aligned} N_m &= \rho N \\ N_f &= (1 - \rho)N \\ R_g &= \frac{x_g}{x_g + x_f} \end{aligned} \tag{4a) (4b) (4c) (4d)}$$

We also need to introduce the following compound parameters:

$$\Upsilon = (1 - u)^2 \tag{5a}$$

$$a = (1-m)^2 + \frac{m^2}{K-1} \qquad \text{(5b) (5c)}$$
$$b = \frac{1-a}{K-1}$$

Υ corresponds to the probability that the two sampled genes have not mutated in one generation, a, respectively b, corresponds to the probabilities that two individuals sampled in a same population, respectively in two different populations, were in the same population before migration.

Recursions

We can now write the recursions of the $Q_i(t+1)$ as a function of the $Q_i(t)$. At equilibrium $Q_i(t+1) = Q_i(t)$ so we remove the time subscript and directly give the equations at equilibrium:

$$Q_0^f = \Upsilon\left(R_g\left(\beta + (1-\beta)Q_0^f\right) + (1-R_g)Q_1^{mf}\right) \qquad \text{(6a)}$$

$$Q_0^m = \Upsilon Q_1^{mf} \qquad \text{(6b)}$$

$$\begin{aligned} Q_1^f = \Upsilon\Bigg(& a\left(R_g^2\left(\frac{1}{N_f}\left(\frac{1+Q_0^f}{2}\right) + \left(1-\frac{1}{N_f}\right)Q_1^f\right)\right. \\ & + 2R_g(1-R_g)\left(\frac{1}{2}\left(\frac{1}{N_f}\left(\frac{1+Q_0^f}{2}\right) + \left(1-\frac{1}{N_f}\right)Q_1^f\right) + \frac{Q_1^{mf}}{2}\right) \\ & \left. + (1-R_g)^2\left(\frac{1}{4}\left(\frac{1}{N_f}\left(\frac{1+Q_0^f}{2}\right) + \left(1-\frac{1}{N_f}\right)Q_1^f\right) + \frac{1}{4}\left(\frac{1}{N_m}\left(\frac{1+Q_0^m}{2}\right) + \left(1-\frac{1}{N_m}\right)Q_1^m\right) + \frac{1}{2}Q_1^{mf}\right)\right) \\ & + \left(1-a\right)\left(R_g^2 Q_2^f + R_g(1-R_g)(Q_2^f + Q_2^{mf}) + (1-R_g)^2\left(\frac{Q_2^f}{4} + \frac{Q_2^m}{4} + \frac{Q_2^{mf}}{2}\right)\right)\Bigg) \end{aligned} \qquad \text{(6c)}$$

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$$Q_1^m = \Upsilon\Bigg(a\Bigg(\frac{1}{4}\Big(\frac{1}{N_f}\Big(\frac{1+Q_0^f}{2}\Big)+\Big(1-\frac{1}{N_f}\Big)Q_1^f\Big)+\frac{1}{4}\Big(\frac{1}{N_m}\Big(\frac{1+Q_0^m}{2}\Big)+\Big(1-\frac{1}{N_m}\Big)Q_1^m\Big)+\frac{1}{2}Q_1^{mf}\Big)\Bigg)$$
$$+\big(1-a\big)\Big(\frac{Q_2^f}{4}+\frac{Q_2^m}{4}+\frac{Q_2^{mf}}{2}\Big)\Bigg)$$

$$Q_1^{mf} = \Upsilon\Bigg(a\Bigg(R_g\Bigg(\frac{1}{2}\Big(\frac{1}{N_f}\Big(\frac{1+Q_0^f}{2}\Big)+\Big(1-\frac{1}{N_f}\Big)Q_1^f\Big)+\frac{Q_1^{mf}}{2}\Bigg)$$
$$+(1-R_g)\Bigg(\frac{1}{4}\Big(\frac{1}{N_f}\Big(\frac{1+Q_0^f}{2}\Big)+\Big(1-\frac{1}{N_f}\Big)Q_1^f\Big)+\frac{1}{4}\Big(\frac{1}{N_m}\Big(\frac{1+Q_0^m}{2}\Big)+\Big(1-\frac{1}{N_m}\Big)Q_1^m\Big)+\frac{Q_1^{mf}}{2}\Bigg)\Bigg)$$
$$+\big(1-a\big)\Bigg(R_g\Big(\frac{Q_2^f}{2}+\frac{Q_2^{mf}}{2}\Big)+(1-R_g)\Big(\frac{Q_2^f}{4}+\frac{Q_2^m}{4}+\frac{Q_2^{mf}}{2}\Big)\Bigg)\Bigg) \tag{6e}$$

$$Q_2^f = \Upsilon\Bigg(b\Bigg(R_g^2\Bigg(\frac{1}{N_f}\Big(\frac{1+Q_0^f}{2}\Big)+\Big(1-\frac{1}{N_f}\Big)Q_1^f\Bigg)$$
$$+2R_g(1-R_g)\Bigg(\frac{1}{2}\Big(\frac{1}{N_f}\Big(\frac{1+Q_0^f}{2}\Big)+\Big(1-\frac{1}{N_f}\Big)Q_1^f\Big)+\frac{Q_1^{mf}}{2}\Bigg)$$
$$+(1-R_g)^2\Bigg(\frac{1}{4}\Big(\frac{1}{N_f}\Big(\frac{1+Q_0^f}{2}\Big)+\Big(1-\frac{1}{N_f}\Big)Q_1^f\Big)$$
$$+\frac{1}{4}\Big(\frac{1}{N_m}\Big(\frac{1+Q_0^m}{2}\Big)+\Big(1-\frac{1}{N_m}\Big)Q_1^m\Big)+\frac{Q_1^{mf}}{2}\Big)\Bigg)\Bigg)$$
$$+\big(1-b\big)\Bigg(R_g^2Q_2^f+R_g(1-R_g)(Q_2^f+Q_2^{mf})+(1-R_g)^2\Big(\frac{Q_2^f}{4}+\frac{Q_2^m}{4}+\frac{Q_2^{mf}}{2}\Big)\Bigg)\Bigg) \tag{6d}$$

(6f)

$$Q_2^m = \Upsilon\Bigg(b\Bigg(\frac{1}{4}\Big(\frac{1}{N_f}\Big(\frac{1+Q_0^f}{2}\Big)+\Big(1-\frac{1}{N_f}\Big)Q_1^f\Big)$$
$$+\frac{1}{4}\Big(\frac{1}{N_m}\Big(\frac{1+Q_0^m}{2}\Big)+\Big(1-\frac{1}{N_m}\Big)Q_1^m+\frac{Q_1^{mf}}{2}\Bigg)+\big(1-b\big)\Big(\frac{Q_2^f}{4}+\frac{Q_2^m}{4}+\frac{Q_2^{mf}}{2}\Big)\Bigg) \tag{6g}$$

$$Q_2^{mf} = \Upsilon\Bigg(b\Bigg(R_g\Bigg(\frac{1}{2}\Big(\frac{1}{N_f}\Big(\frac{1+Q_0^f}{2}\Big)+\Big(1-\frac{1}{N_f}\Big)Q_1^f\Big)+\frac{Q_1^{mf}}{2}\Bigg)$$
$$+(1-R_g)\Bigg(\frac{1}{4}\Big(\frac{1}{N_f}\Big(\frac{1+Q_0^f}{2}\Big)+\Big(1-\frac{1}{N_f}\Big)Q_1^f\Big)+\frac{1}{4}\Big(\frac{1}{N_m}\Big(\frac{1+Q_0^m}{2}\Big)+\Big(1-\frac{1}{N_m}\Big)Q_1^m\Big)+\frac{Q_1^{mf}}{2}\Bigg)\Bigg)$$
$$+\big(1-b\big)\Bigg(R_g\Big(\frac{Q_2^f}{2}+\frac{Q_2^{mf}}{2}\Big)+(1-R_g)\Big(\frac{Q_2^f}{4}+\frac{Q_2^m}{4}+\frac{Q_2^{mf}}{2}\Big)\Bigg)\Bigg) \tag{6h}$$

As an example, the rationale of the derivation is given for $Q_0^f(t+1)$. The two copies sampled in a female are IBD first if none has mutated (Υ). Then we must consider that this female is gynogenetic (R_g) or sexual ($1-R_g$). If it is gynogenetic, if heterozygozity has been lost (β) the two copies are IBD with probability one, otherwise ($1-\beta$) the probability of IBD is the same as for the mother, so $Q_0^f(t)$. If the female come from sexual reproduction, the two

copies are IBD with the same probability as of a random male/female pair at the previous generation, Q_1^{mf}. The terms in $1/N_m$ and $1/N_f$ that appear in equations for the Q_1^k and Q_2^k correspond to the probability that two different individuals have the same father or mother, respectively.

This system of recursions can be written in the matrix form:

$$\vec{Q} = \Upsilon(\mathbf{G}\vec{Q} + \vec{C}) \tag{7}$$

where $\tilde{Q}$ is the vector of probabilities of IBD, $\mathbf{G}$ is a matrix and $\tilde{C}$ a vector, both depending of the parameters of the model. The solution can be written on the form:

$$\tilde{Q} = (\mathbf{I} - \Upsilon\mathbf{G})^{-1}\Upsilon\tilde{C} \tag{8}$$

where $\mathbf{I}$ is the identity matrix.

The F_{IS} statistics measured on genomic data corresponds to $F_{0,2}$ in our model as we compare the IBD of two gene copies sampled either within an individual or at random over the whole population. It can be defined either for males, females, or for the whole population by weighting as a function of the sex-ratio:

$$F_{IS}^m = \frac{Q_0^m - Q_2^m}{1 - Q_2^m}$$

$$F_{IS}^f = \frac{Q_0^f - Q_2^f}{1 - Q_2^f}$$

$$F_{IS} = x_m \frac{Q_0^m - x_m Q_2^m - (1-x_m)Q_2^f}{1 - x_m Q_2^m - (1-x_m)Q_2^f} + (1-x_m)\frac{Q_0^f - x_m Q_2^m - (1-x_m)Q_2^f}{1 - x_m Q_2^m - (1-x_m)Q_2^f}$$

(9a) (9b) (9c)

Simulations

In addition to analytical derivations, an individual-based, multi-locus model was implemented using the SLiM software [3], where each individual's genome is explicitly defined. It allowed to simulate genomic patterns along a chromosome and to introduce deleterious mutations in the model. Each individual is represented by a unique pair of autosome. This chromosome consist of $L = 10^5$ loci, with a rate of recombination per locus r equal to $1/L = 10^{-5}$, so that on average one recombination event is observed per gametogenesis. Since *M. belari* has holocentric chromosomes (there is no specific centromere), the location of the

chiasma is randomly drawn according to a uniform distribution along the chromosome. Two types of mutations are introduced, either neutral (no effect on fitness) or deleterious ones, with selection coefficient *s*, set to 0.01, and dominance coefficient *h*, set to 0.25, and acting multiplicatively across the genome. The life cycle was the same as described above. Various population sizes and migration rates were explored to modulate the level of inbreeding and we specifically studied the effect of the rate of production of sexual females, φ and the rate of LOH, β.

By adjusting the parameters of the model, we also compared the *M. belari* life cycle with more standard reproductive modes: full sexuality ($\sigma = 0$ and $\varphi = 1/2$), full clonality ($\sigma = 1$ and $r = 0$), and central fusion automixis ($\sigma = 1$, and $\beta = 1/2$).

From the simulations we computed F_{IS} and the linkage-disequilibrium measure r^2 on neutral mutations at the scale of the whole population:

$$F_{IS} = 1 - \frac{H_o}{H_e} \tag{10}$$

with H_o the observed heterozygosity (percentage of heterozygous sites) and H_e the expected heterozygosity :

$$H_e = \frac{2}{L}\sum_{i=1}^{n} f_i(1 - f_i) \tag{11}$$

a sum over all the *n* mutations present in the population with f_i their respective frequencies.

$$r^2 = \frac{D^2}{\pi_A \pi_a \pi_B \pi_b} \tag{12}$$

where $D = \pi_{AB} - \pi_A \pi_B$ with π_A and π_a the allelic frequency at a first locus *A* and π_B and π_b at a second locus *B*. Only mutations in frequency higher than 5% in the population were used. r^2 was calculated for pairs of loci as the function of their distance on the simulated chromosome.

Results

F_{IS}

The general solution of equation (8) can be obtain with the help of *Mathematica* [6] but it is formidable, so useless for direct biological interpretation. We thus performed numerical explorations in the general case. We also obtained approximations under two limit conditions:

φ = 0 (no sexually-produced females) and β = 0 (no LOH). Under these two conditions we also used the standard diffusion limit. First we used the following scaling parameters: $\theta = 4N_T u$, $\Phi = 2N_T \varphi$, $B = 2N_T \beta$. Then, we assumed an infinite number of local populations so $K, N_T \to \infty$ but that the scaled parameters terms tends towards constant: $\theta, \Phi, B \to cte$. We also noted $M = 4Nm$, where migration is scaled with the local, N, which can be small.

Assuming no sexually-produced females we obtained:

$$F_{IS}^{m} \approx \frac{2B + \theta}{2(B + \theta) + M(\sigma B + \theta\sigma + 1)} \quad (13a)$$

$$F_{IS}^{f} \approx \frac{2B + M(\sigma B - 1)}{2(B + \theta) + M(\sigma B + \theta\sigma + 1)} \quad (13b)$$

$$F_{IS} \approx \frac{2B + \theta(1 - \sigma) + \sigma M(\sigma B - 1)}{2(B + \theta) + M(\sigma B + \theta\sigma + 1)} \quad (13c)$$

Assuming no LOH we obtained:

$$F_{IS}^{m} \approx \frac{(1 - \sigma)\Phi + \sigma\theta}{(1 - \sigma)\Phi(1 + M\sigma) + \sigma(2\theta + M(1 + \sigma\theta))} \quad (14a)$$

$$F_{IS}^{f} \approx \frac{(1 - \sigma)\Phi - \sigma M}{(1 - \sigma)\Phi(1 + M\sigma) + \sigma(2\theta + M(1 + \sigma\theta))} \quad (14b)$$

$$F_{IS} \approx \frac{(1 - \sigma)(\sigma\theta + \Phi) - M\sigma^2}{(1 - \sigma)\Phi(1 + M\sigma) + \sigma(2\theta + M(1 + \sigma\theta))} \quad (14c)$$

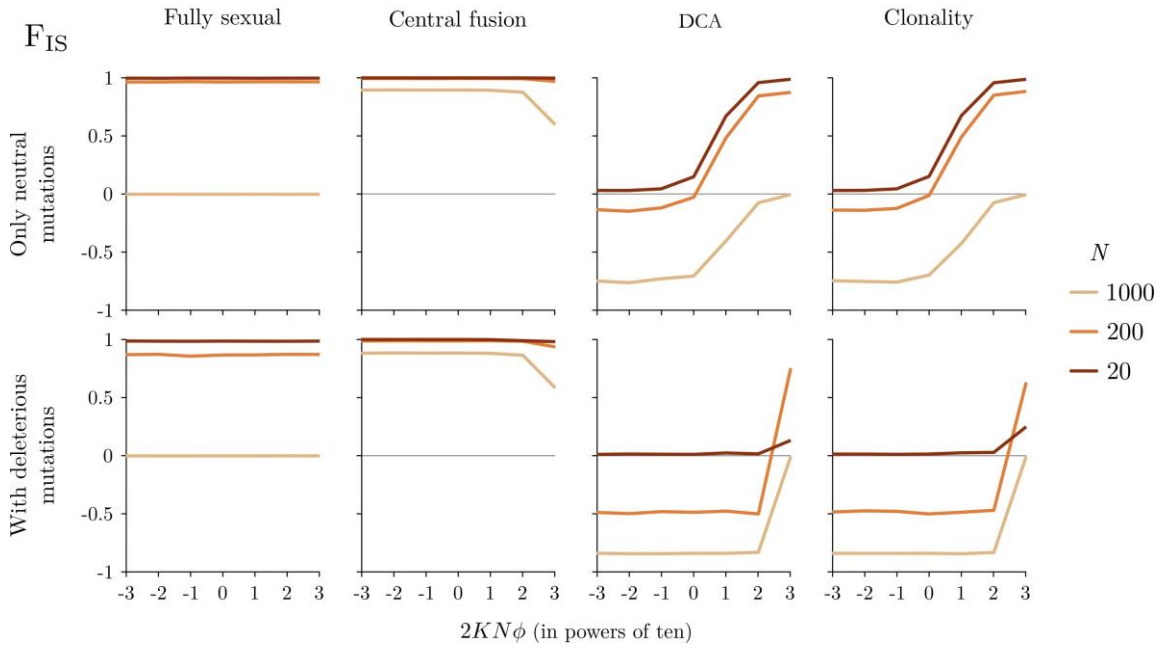


Figure 3: F_{IS} for the different reproductive modes as a function of the rate of sexually produced females ($2KN\varphi$) for different levels of population structure (KN = 1000, so N = 1000 corresponds to a single populations and N =20 to a highly structured one).

We give the three expressions for completeness but we can concentrate on the expression for the average F_{IS} In natural populations, there is a strong family structure with most matings occurring between kins. In our model, it corresponds to low migration between demes, so M close to 0. Then, equations (13c) and (14c) become:

$$F_{IS} \approx \frac{2B + \theta(1-\sigma)}{2(B+\theta)} \tag{15}$$

$$F_{IS} \approx \frac{(1-\sigma)(\sigma\theta + \Phi)}{(1-\sigma)\Phi + 2\sigma\theta} \tag{16}$$

which both reduce to $F_{IS} = (1-\sigma)/2$ when $B = 0$ or $\Phi = 0$. As $\sigma \approx 0.9$ in natural populations it corresponds to $F_{IS} \approx 0.05$, so close to the observed value ($F_{IS} \approx 0.19$, see main text). In an unstructured population ($M \to \infty$, and see $N = 1000$ on figure 3), F_{IS} tends to -1 with no LOH ($B = 0$) (see also [1]). Here, this is compensated by the strong family structure leading to F_{IS}^f close to 0 as illustrated on figure 3 (see also [2]). It is worth noting that just a little bit of sex or LOH (higher than the mutation rate: $\varphi, B > \theta$) rapidly leads to F_{IS}^f close to 1. This is confirmed by simulations as presented (3 and Figure 4 in the main text). This result is thus very sensitive to the occurrence of sexually produced females and to low level of LOH. For comparison,

However, simulations showed that because of deleterious mutations, the range of parameters leading to F_{IS}^f close to 0 can be much wider, even when some females are sexually produced and when the DCA is not complete (Figure 4). This is explained by the selection against highly homozygotes individuals. This is an important result as a non-zero proportion of sexually produced females is required to explain the low level of linkage disequilibrium observed genome wide as presented below.

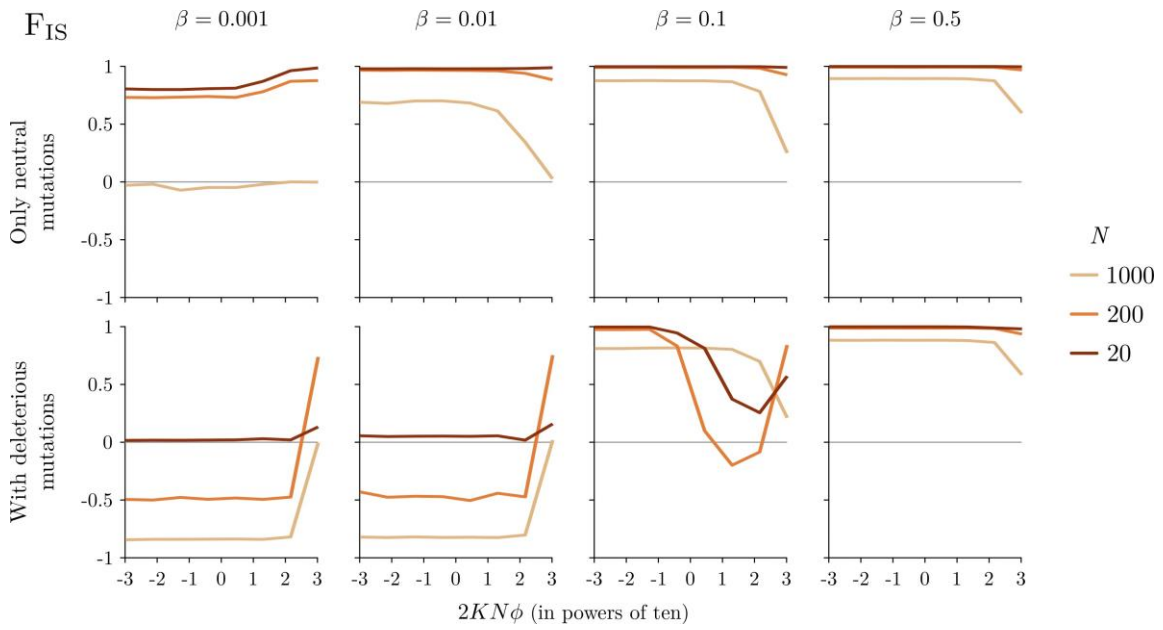


Figure 4: F_{IS} for automixis with different level of DCA (β =0 corresponds to full assortment and β =1/2 to random pairing).

For comparison, figures 3 and 4 also shows the results for full sexuality, full clonality and standard central fusion automixis (without DCA).

Linkage disequilibrium

Analytical derivations for linkage disequilibrium would require recursion equations for 40 IBD probabilities. We thus only relied on simulations.

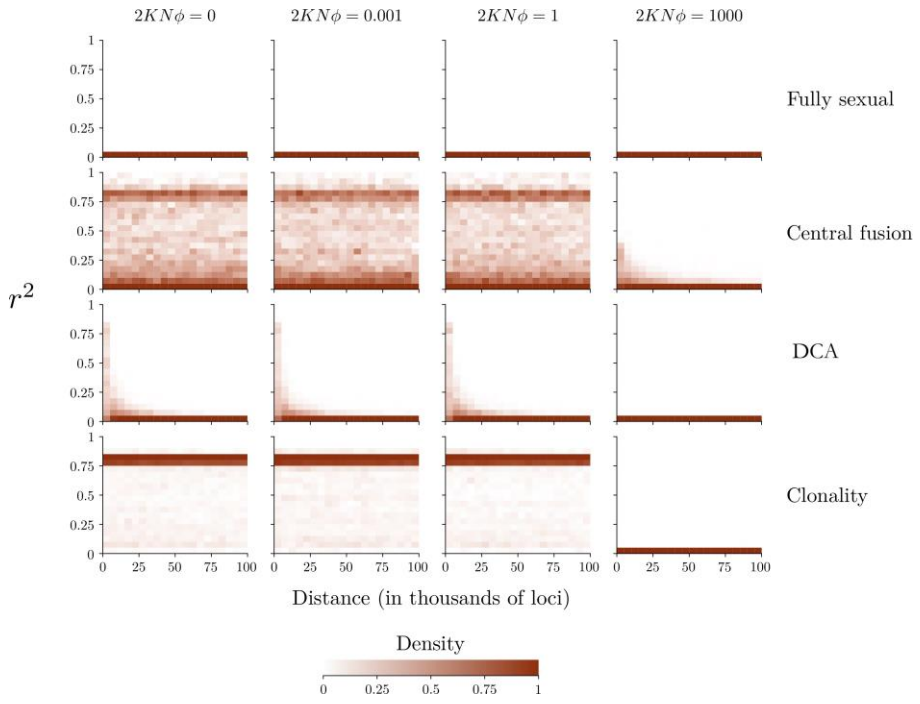


Figure 5: Pattern of linkage disequilibrium as a function of physical distance (in number of loci) for different rates of sexually produced females ($2NK\varphi$ = $0.001,1,100$) and for different reproductive modes.

To understand the specific effect of the DCA we first considered a single unstructured population. As expected, LD is low and rapidly decreases with physical distance under sexual reproduction (Figure 5). In contrast, LD is high and independent of physical distance along chromosome under both full clonality because recombination does not occur, and under standard central fusion because recombination is not efficient as individuals are mostly homozygotes. Under complete DCA and without sexually produced females, LD pattern is intermediate: much lower than under clonality or standard central fusion but higher than under full sexuality, and decreasing with physical distance (Figure 5). The reason is that, although new haplotypes are generated by recombination within individuals, they are never associated together through mating, so recombination is not as efficient as under full sexuality.

When we also consider the effect of population structure, LD is globally higher and the difference among reproductive modes are less clear under pure neutrality. However, when deleterious mutations are added the differences become stronger. In particular a very low rate of sexually produced females is sufficient to make DCA similar to full sexuality whereas much higher rates are necessary to erase the signature of clonality or standard central fusion (Figure 6).

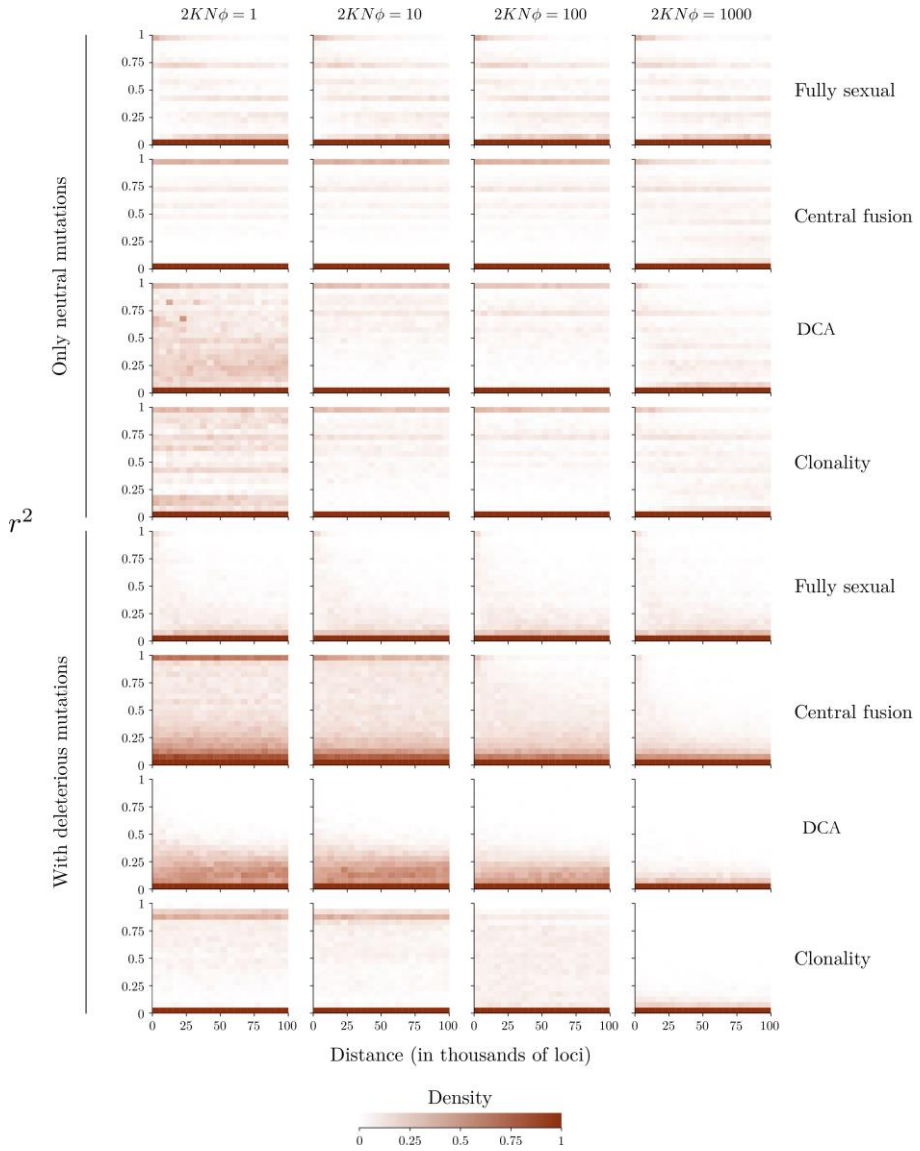


Figure 6: Pattern of linkage disequilibrium as a function of physical distance (in number of loci) for different rates of sexually produced females ($2NK\varphi$ =0.001,1,100) and for different reproductive modes.

Conclusion

Overall, the observed genomic pattern is well predicted by the strong family structure of *M. belari* populations directed chromatif assortment, sexually produced females at low rate (which can be unobserved in natural conditions), and the occurrence of deleterious mutations genome wide.

Evolution of the reproductive system

In the second model, we study the evolution of the atypical reproductive system of *M. belari* from a standard sexual population. Compared to the previous model, the parameters σ (proportion of asexually produced offspring) and β (segregation bias) are no longer fixed but under the control of two independent evolving loci. Among sexually produced individuals the proportion of females is set to $\varphi = 1/2$. Additional simulations where the sex-ratio can also evolve do not change the results and a sex ratio biased towards males evolved in a second step (not shown).

We started with a burn-in period where the population evolved under sexual reproduction. Then mutations are introduced at one or at the two loci controlling the reproductive mode. Each evolving locus is unlinked with any other locus. Transmission from parents to children of an evolving locus follows the same rules as transmission of loci on the focal chromosome, taking into account the potential loss of heterozygosity through chromosome segregation during gynogenesis. It can take two different values, a resident value, g_r, or a mutant value, $g_m = g_r \pm \delta$. We used $\delta = 0.1$ to avoid too long simulations. We assumed additivity between the two alleles so the genotypic value is the sum of the two alleles. At the beginning of simulations, all individuals are homozygous for the resident allele and a mutant allele is randomly introduced in one individual of the population. If this mutant becomes fixed in the population, this mutant allele becomes the resident allele. If the mutant disappears, another mutant is directly reintroduced into the population at random.

Genotype to phenotype map

The value of σ is constrained between 0 and 1 and the value of β between 0 and 1/2. The gynogenesis rate starts at 0 and can theoretically go up to 1, the segregation bias and the segregation bias start at 0.5 and can reach 0 or 1. So, to map allelic values from $]-\infty,+\infty[$ to $[0,1]$ we used the following functions:

$$P_\sigma = f(G_\sigma) \quad ; \quad P_\beta = \frac{1}{2}\left(1 - f(G_\beta)\right) \tag{17}$$

with

$$f(x) = \begin{cases} 1 - \left(1 - \frac{2}{\pi}\tan^{-1}(ax)\right)^b, & \text{if } g > 0 \\ \left(1 - \frac{2}{\pi}\tan^{-1}(-ax)\right) - 1 & \text{, otherwise} \end{cases} \tag{18}$$

The parameters a and b define whether the function tends more or less quickly to 1 as x increases. We used $a = 0.1$ and $b = 15$ but equilibrium results are not sensitive to chosen values. The gynogenesis rate and the segregation bias are controlled by the maternal genotype.

Inbreeding depression

The supposedly strong barrier to the evolution of asexuality (assuming that automixis mechanisms can emerge) is inbreeding depression (ID) due to LOH that leads to the exposure of recessive deleterious alleles. It is calculated as $1 - w_1/w_0$ with w_0 the fitness of sexually produced individuals and w_1 the fitness of asexually produced individuals.

A strong ID should hold back the evolution of automixis if the chromosome segregation bias is not strong, preventing LOH. Since we only modelled a single chromosome and central fusion leads to a LOH in only half of offsrpings, the maximum ID is 50%, which is the minimum value preventing the evolution of asexuality. Asexuality should always evolved under such a conditions. To allow a broader range of ID values we assumed an additional cost of LOH and corrected w_1 to $w_1^{corr} = (1 - c)^{\beta(n-1)} w_1$, where n is the number of chromosomes (fixed to 10) and c is the mean fitness decline caused by LOH per chromosome.

Extinction-recolonisation cycle of subpopulations

In this model, a higher migration rate is set, allowing the evolving loci to be transmitted not too slowly from one population to another. It should also be noted that here the parameters controlling reproduction are no longer fixed rates. It is thus possible that populations may stochastically run out of males, especially if gynogenesis evolves and the percentage of males decreases. If a population has no more males, it becomes extinct. An extinct population is recolonised in the generation following its extinction by a male and at least one female randomly selected from the other population. This pair reproduces and together they form the next generation of the subpopulation.

References

[1] Engelstadter J. 2017. Asexual but Not Clonal: Evolutionary Processes in Automictic Populations. Genetics.

[2] Prugnolle F and De Meus T. 2010. Apparent high recombination rates in clonal parasitic organisms due to inappropriate sampling design. Heredity 104: 135-140.

[3] Haller BC and Messer PW. 2019. SLiM 3: Forward Genetic Simulations Beyond the Wright–Fisher Model. Molecular Biology and Evolution 36: 632–637.

[4] Hartfield M, Wright SI, Agrawal AF. 2016. Coalescent Times and Patterns of Genetic Diversity in Species with Facultative Sex: Effects of Gene Conversion, Population Structure, and Heterogeneity. Genetics 202(1): 297-312.

[5] Rousset, F. 2004. Genetic structure and selection in subdivided populations. Princeton University Press.

[6] Wolfram Research I. 2010. Mathematica. Champaign, Illinois: Wolfram Research, Inc.

The production of asexual females in *Mesorhabditis belari* relies on defective Anaphase B during meiosis I

Authors: Caroline Blanc, Eva Wenger, Marie Delattre

Abstract

The nematode *Mesorhabditis belari* produces parthenogenetic females. In such females, diploidy is maintained by the abortion of one step of meiosis. Here we characterized which aspect of the division is perturbed during the production of the unreduced oocytes. To this end, we used immunostaining of the meiotic spindle with antibodies against proteins known to participate in the meiotic divisions in *C. elegans*. In particular, microtubule staining suggested that the central spindle fails to form at the end of anaphase I in *M. belari*. This was confirmed by the SUMO analysis, which localises between the separating chromosomes in anaphase II, but not in anaphase I. Collectively, these results suggest that chromosomes enter anaphase I without having completed anaphase B. In parallel, our result suggested that *M. belari* possesses a HORMA domain protein, whose dynamics contrast with those of *C. elegans*.

Keywords: asexuality, meiosis, central spindles, H3pT3, BUB-1, SUMO

Introduction

The nematode *Mesorhabditis belari* is an auto-pseudogamous species that undergoes two types of meiotic programs. Canonical meiosis produces reduced oocytes, which will later develop as males after amphimixis. Females however arise from unreduced oocytes which do not inherit a paternal genome (gynogenesis) (Grosmaire et al., 2019). We previously demonstrated that abortive meiosis I maintains diploidy in gynogenetic eggs (Blanc et al., 2023). In the present paper, we investigate which step of meiosis I is interrupted and the possible causes of this interruption.

Canonical meiosis is a two-step cell division that results in the formation of a haploid gamete. After replication, each chromosome has two sister chromatids and the homologous chromosomes (homologs) are paired to form the bivalent. The formation of a ladder-like structure, the synaptonemal complex, stabilises homologous pairing. In *C. elegans*, the synaptonemal complex consists of four SYP (SYnaPsis in meiosis abnormal) proteins, four HORMA domain proteins and cohesins (Colaiácovo et al., 2003; Köhler et al., 2017; MacQueen et al., 2002; Pasierbek et al., 2001; Severson et al., 2009; Severson & Meyer, 2014; Smolikov et al., 2007, 2007; Woglar et al., 2020). In the individual absence of some of these proteins (HTP-1/-2; HTP-3, HIM-3, SYPs), pairing is inhibited and homologous chromosomes are not segregated in meiosis I (Bohr et al., 2016; Cahoon et al., 2019; Severson et al., 2009). Moreover, no recombination occurs if the homologs are not paired.

In *C. elegans*, when pairing is achieved, the bivalents form a cross-shaped structure. The short arms axis delimits the separation of homologs while the long arms axis indicates the separation between sister chromatids. In metaphase, I, the bivalents are aligned so that the axis of the short arms is parallel to the metaphase plate. The positioning of the bivalents is ensured by microtubules that bind to the chromosomes via kinetochores (Dumont et al., 2010)). These microtubules form a cage that encapsulates the chromosomes.

Anaphases (I and II) are subdivided into two main phases. Anaphase A is characterised by a primary separation of the two sets of chromosomes that slide along the microtubule cage until reaching the pole of the microtubule cage (Dumont et al., 2010; Laband et al., 2017; Pelisch et al., 2019b)). This is followed by anaphase B, during which the segregation of chromosomes is completed by the polymerisation of microtubules in the central spindle,

between the two chromosome plates (Chuang et al., 2020; Davis-Roca et al., 2018; Pelisch et al., 2019b; Yu et al., 2019)). The central spindle generates pushing forces capable of relocating a set of chromosomes in the nascent polar body. In addition, in late anaphase I, the cage of microtubules is disassembled and reassembled in metaphase II and finally disappears completely in anaphase II.

During anaphase, homologous separation requires the cleavage of cohesin-holding homologs (Ferrandiz et al., 2018; Kaitna et al., 2002). The kinetochores must also disassemble and some kinetochores proteins are loaded on the Ring Complex (Dumont et al., 2010; Hattersley et al., 2016; Monen et al., 2005). The Ring Complex, also called the ring domain, is a dynamic protein complex positioned at the metaphase plate. It is involved in cohesin cleavage, spindle formation and cytokinesis (Davis-Roca et al., 2018). The Ring Complex is not specific to meiosis and is also present in mitosis. At the end of anaphase B, half of the homologs are extruded into the first polar body. Meiosis II proceeds similarly to the canonical meiosis and results in half of the sister chromatids extrusion into the second polar globule (reviewed in (Kitagawa, 2009).

To address the issue of aborted meiosis in gynogenetic oocytes, we based our analysis on the extent of knowledge about meiosis in *C. elegans*. In addition, *M. belari* undergoes both canonical and altered meiosis. Comparing these two types of division helps to identify the missing steps in gynogenetic oocytes.

Material and Methods

Nematode strain, culture and genome

M. belari species JU2817 (PRJEB30104, genome ID: UZWA01000816.1) is maintained at 20°C on NGM plates seeded with *E. coli* OP50, following *C. elegans* protocols, as described in (Grosmaire et al., 2019).

Antibody production

Specific antibodies against Mb.HORMA (g17455) were produced by injecting rabbits with two synthetic peptides: KTYRLDKSTPGV and YDTKTLYDFDHPDAL (Covalab, Lyon). Polyclonal antibodies were affinity purified from serum obtained at day 74 post-injection. Specific antibodies against BUB-1 (g12204) were produced as described above, also using two synthetic peptides: SPIVEDQDHENSTNG and SLMAQNQQEEQKGTA (Covalab, Lyon).

Immunostainings on gonads and embryos

Immunostainings were performed as described in Grosmaire (2019). Gravid females were dissected on slides coated with 0.25%poly-lysine in 0,5X M9 (diluted 1:1 in H2O). After freeze-cracking, the fixation of samples was performed by immersing slides into methanol at -20°C for at least 5 min. Commercial primary antibodies (dilution indicated in brackets) were as follows: mouse anti-$\boldsymbol{\alpha}$-tubulin (Sigma DM1A, 1:2000), rabbit anti-H3pT3 (07-424; Merck Millipore, 1:1000) and mouse anti-SMO-1 (DSHB SUMO 6F2, 1:3000). Mb.HORMA and Mb. BUB-1 were used at 1:700 and 1:3000 dilution, respectively. As secondary antibodies, we used a Cy3 donkey anti-rabbit secondary antibody (Jackson ImmunoResearch #711-165-152, 1:1000) and an Alexa488 donkey anti-mouse secondary antibody (Jackson ImmunoResearch, #715-545-150, 1:2000). All antibodies were incubated at room temperature for 45 min except for Mb.HORMA when co-stained with H3pT3 which was incubated at +4°C overnight. DNA was stained using Hoechst 33258 at 0,5 ul/ml (Merck Sigma-Aldrich, 94403). For co-staining of Mb.HORMA and H3pT3, we used the FlexAble kit (anti-mouse, CoraLite Plus 488, Proteintech) to directly label Mb.HORMA, which was used as a pure solution. Images were acquired using a confocal microscope (Oil immersion 63X objective - LSM800 Airyscan, Zeiss). Z-stacks of embryos were acquired every 0.15 µm. Finally, acquired images were treated using ImageJ 1.53t software.

Results

H3pT3 dynamics reveal a defect in terminating anaphase I in gynogenetic oocytes

In *C. elegans*, during anaphase I, homologs segregate while sister chromatids' cohesion remains intact (Figure 1). This is made possible by the removal of the cohesin REC-8, through its phosphorylation, specifically on the short arms of the bivalent. The phosphorylation of REC-8 by the kinase AIR-2 is mediated by Histone 3 Tyrosine 3 specific phosphorylation (H3pT3). AIR-2 also phosphorylates Histone 3 Serine 10 (Divekar et al., 2021; Ferrandiz et al., 2018; Hsu et al., 2000). In metaphase of meiosis I, both H3pT3 and H3pS10 are found only on the short arm of the bivalents. Hence, REC-8 is removed only between homologs. During anaphase, the H3pT3 and H3pS10 signals persist in between the separating units but progressively vanish at the end of anaphase. The signal comes back between sister chromatids at metaphase of meiosis II, allowing the separation of sister chromatids during anaphase. Therefore, H3pT3 and H3pS10 have a particular dynamic pattern and both histone phosphorylations are well-conserved histone modifications. However, in *M. belari*, H3pS10 is not specific and is found everywhere on all chromosomes at any stage. Thus, we used only an antibody against H3pT3 to reconstitute the dynamics of events during *M. belari* meiotic divisions.

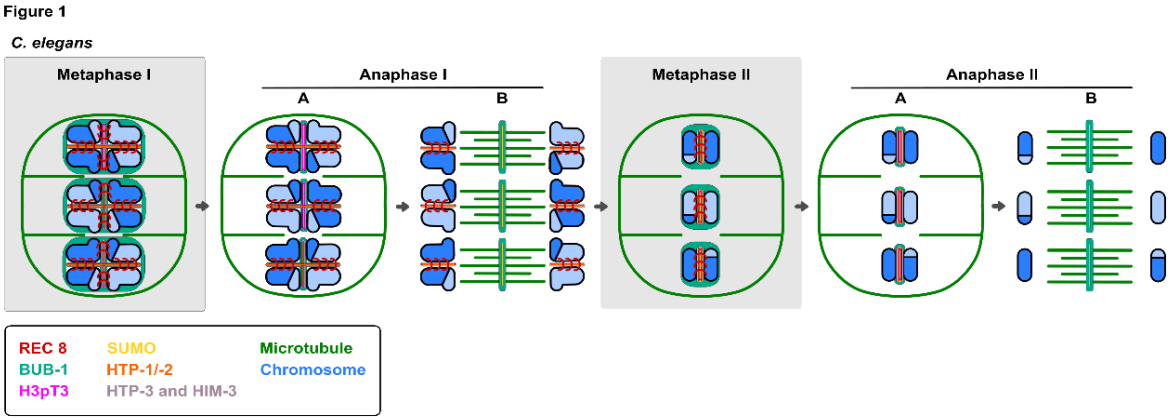


Figure 1: Schematic representation of the dynamics of seven key meiotic proteins of *C. elegans*. REC-8 is in red, BUB-1 is in turquoise, H3T3 phosphorylation is in pink, SUMO (SMO-1) is in yellow HTP-1 and HTP-2 are in orange, HTP-3 and HIM-3 are in grey, microtubules are in green and chromosomes are in blue. Homologs are distinguished by the shades of blue (light and dark). Sister chromatids within a chromosome have the same color.

First, we found that the antibody is very specific in *M. belari*. *M. belari* has 10 chromosomes (2n=20, Figure 2). At the end of prophase I, in diakinesis, 10 units -corresponding to the 10 bivalents- are observable, each unit possessing H3pT3 in the midvalent region (n=22) (Figure 2). In metaphase I, the 10 bivalents are organized in a rosette structure and H3pT3 is aligned parallel to the metaphase plate (n=133 metaphase observed). Anaphase I is defined by the presence of two rosettes of 10 univalents (each being an association of two sister chromatids) and by the absence of a polar body. We had previously found that meiosis I fails because we detect metaphase figures with 20 units forming a ring in the absence of a polar body (Blanc et al., 2023). These 20 units then separate into two rings of 20 units each, corresponding to the segregation of sister chromatids during anaphase II. This division is successful with the production of a polar body containing 20 units. During this last division, the pattern of H3pT3 was consistent with its dynamic localisation in *C. elegans*. Indeed, the signal was initially present at the mid-region of every unit (n=56) and progressively disappeared as anaphase progressed (n=60). During anaphase I however, different H3pT3 localisations were found: i) two rosettes with H3pT3 between the two rosettes plates (n=45), ii) two rosettes with no H3pT3 staining (n=20) and iii) two rosettes with H3pT3 staining on the midvalent region of each univalent (n=55). The two first categories are consistent with the dynamic staining of H3pT3 with a progressive loss of H3pT3 staining between the separating chromosomes. However, the figures showing H3pT3 in the middle of each chromosome strongly suggested that the signal was coming back between sister chromatids, as it would do at the very end of anaphase, although here chromosomes were still close to each other. From this, we concluded that at the end of anaphase I the univalents come back on the metaphase plate instead of being extruded in a polar body in the gynogenetic oocytes. Thus, all 20 univalents participate in meiosis II which results in the segregation and the extrusion of half of the sister chromatids. The failure of the first meiotic division is therefore due to a failure to terminate anaphase.

Figure 2

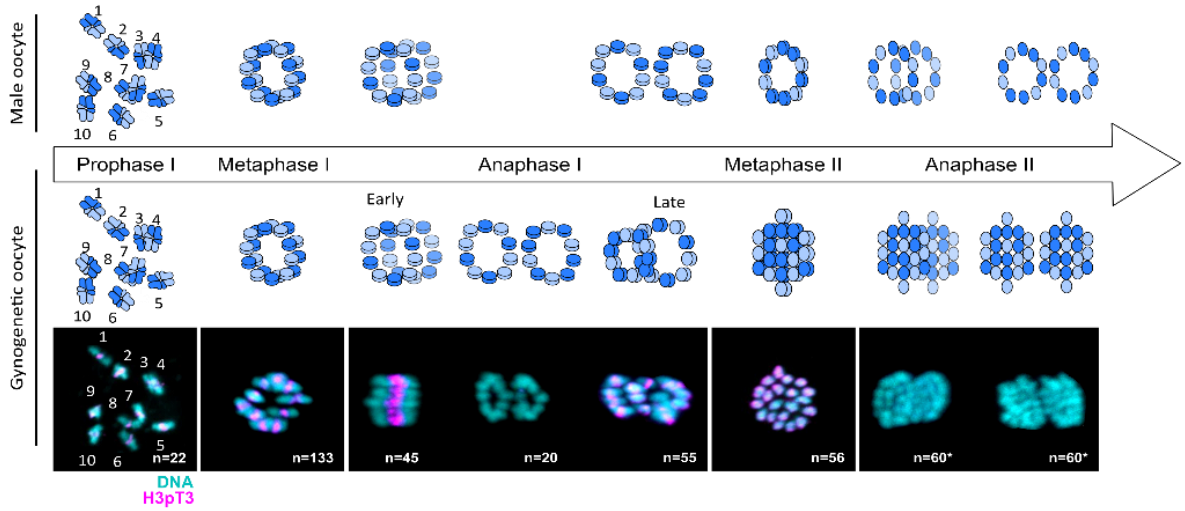


Figure 2: Dynamics of H3pT3 during oogenesis of *M. belari*. The top image corresponds to a schematic representation of the chromosomes in amphimictic and gynogenetic oocytes. The homologs are in light and dark blue and the sister chromatids have the same color. The lower panel shows fixed gynogenetic oocytes at different stages of meiosis. Anaphase I is not initiated but is abortive. DNA is in blue and H3pT3 is in pink. * we found 60 images in total, combining these two steps of anaphase

A HORMA domain protein co-localizes with H3pT3

The synaptonemal complex is a tripartite protein complex that stabilizes homologous pairing in meiosis I. On the lateral sides, sister chromatids are held to the synaptonemal complex by cohesins and the HORMA domain proteins. In *C. elegans*, HORMA domain proteins are loaded on the DNA when the synaptonemal complex is assembled in the transition zone (leptotene/zygotene stages of prophase I). They, therefore, testify to the presence of the synaptonemal complex. The HORMA domain proteins are later removed sequentially from chromosomes during the two successive divisions. We asked if the same dynamics is observed in gynogenetic oocytes of *M. belari*. In *C. elegans*, there are four HORMA domain proteins (Figure 1): HTP-1 (Him-Three Paralog), HTP-2, HTP-3 and HIM-3 (High Incidence of Males). The HORMA domain proteins diverge considerably from one species to another, making it challenging to find orthologs. Using Blast alignments, we identified 10 genes (mbelari.g17455, mbelari.g3100, mbelari.5766, mbelari.g12784, mbelari.16537, mbelari.20214, mbelari.g21106, mbelari.22436, mbelari.25111, mbelari.2546, Figure S1) containing a HORMA domain (Figure

2A). Among those genes, mbelari.g17455.t1 shows the highest identity in amino acid with the HORMA Domain protein of *C. elegans* (respective pairwise Identity with HTP-1: mbelari.g17455: 18%, mbelari.g3100: 13%, mbelari.5766: 12%, mbelari.g12784: 4%, mbelari.16537: 11%, mbelari.20214: 9%, mbelari.g21106: 9%, mbelari.22436: 10%, mbelari.25111: 5%, mbelari.2546: 10%, Figure S1). We named the corresponding protein Mb.HORMA in the rest of the text. We compared this Mb.HORMA protein to the four proteins of *C. elegans*. Pairwise analyses show that the Mb.HORMA protein and the *C. elegan*s proteins share a low amino acid identity (Figure 2A, S2) although HTP-1 remains the best hit. (Figure 2A, S2). We, therefore, raised an antibody against this putative HORMA domain protein in *M. belari*.

We found that the antibody has a very precise signal at the transition zone along the chromosomes, which is expected from a HORMA protein (Figure 3B). The Mb.HORMA protein is loaded before the pachytene stage, in the transition zone, and remains present until metaphase I (Figure 3C). In early prophase I, the Mb.HORMA exhibits the same localisation as the four HORMA domain proteins of *C. elegans.*

In addition, in diakinesis and at metaphase I of *C. elegans*, HTP-1 and HTP-2 which share 82% of acid nucleic similarity, are found only on the long arms between the sister chromatids. HTP-3 and HIM-3 co-localise, and are found on the long and short arms without distinction. HTP-1/-2 staining is not detectable in meiosis II while HTP-3 and HIM-3 persist until anaphase II. The Mb.HORMA protein shows none of these patterns. In diakinesis and metaphase I, the antibody against Mb.HORMA is found strictly on the short arms of the midvalent region (Figure 3C). Also, the Mb.HORMA protein and H3pT3 co-stain in the midline region (Figure 3D). In anaphase I, the Mb.HORMA protein vanishes but is reloaded in metaphase II (Figure 3C). Thus, despite initial similarities, Mb.HORMA protein dynamic is not comparable to those of *C. elegans* HORMA domain proteins.

In conclusion, the Mb.HORMA-like protein is apparently loaded on the synaptonemal complex during the leptotene/zygotene stages, as expected. Nonetheless, the Mb.HORMA protein has unexpected dynamics because it is loaded onto the midvalent region in meiosis I and II metaphases.

Whether this reflects a difference in HORMA protein regulation between species or whether the antibody recognises another protein linked to the synaptonemal complex remains an open question.

Figure 3

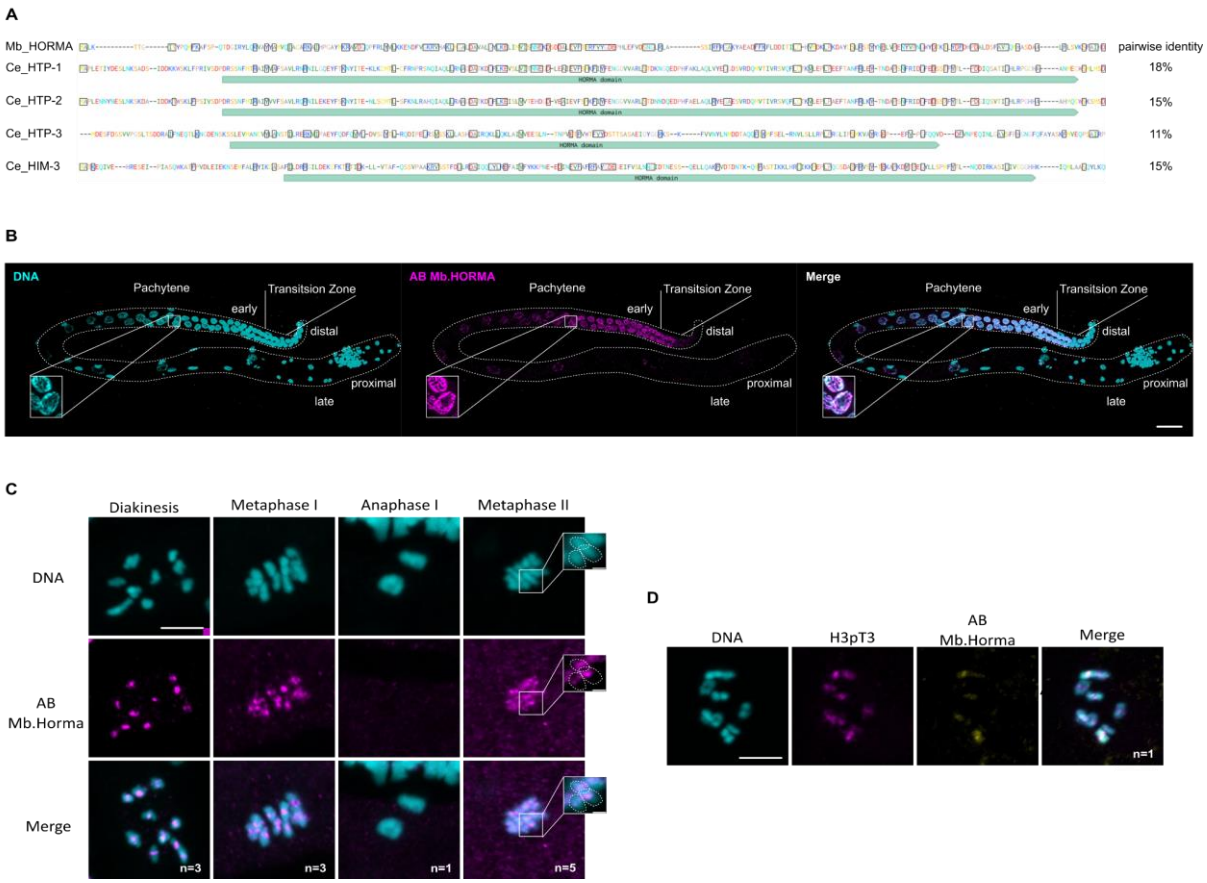


Figure 3: New role of a HORMA domain protein in *M. belari*. A) Nucleic acid alignments in the HORMA region between Mb.HORMA and the four HORMA domain proteins of *C. elegans.* The percentage pairwise identity of all sequences compared to Mb.HORMA is shown on the right of the image. Squares indicate the matches between Mb.HORMA protein and *C. elegans* proteins B) Fixed gonad with the labeling of Mb.HORMA protein. DNA is blue, Antibody against Mb.HORMA is pink. Scale bar = 20µm. C) Fixed oocytes with the labelling of Mb.HORMA protein. In the inset, the dashed lines surround three chromosomes. DNA is blue, Antibody against Mb.HORMA is pink. Main scale bar = 5µm and inset scale bar = 1µm. D) Fixed oocytes with co-labelling of Mb.HORMA protein and H3pT3 in diakinesis. DNA is in blue, H3pT3 is in pink and Antibody against Mb.HORMA is in yellow. Scale bar = 5µm.

In Meiosis I chromosomes are correctly positioned to segregate

In some asexual species (Komaru et al., 2000), and also in mutants of *C. elegan*s (Laband et al., 2017; McNally et al., 2016), anaphase can abort if the meiotic spindle is not properly positioned relative to the cell cortex. Indeed, for the proper extrusion of the tiny polar body, it is essential the spindle is close to the cortex and is oriented perpendicular to the cortex. We then asked whether defects in spindle positioning during anaphase of meiosis I in *M. belari* gynogenetic embryos could explain the abortion of meiosis I. Analyses of seven metaphases I and eight anaphases I show that the meiotic spindle is always located closer to the cell cortex than it is to the centre of the oocyte (Figure 4A and 4B, Table 1). Moreover, for all 15 samples, the pole-to-pole axis of the spindle microtubule cage is always oriented perpendicularly to the cell cortex (Figure 4A and 4B, Table 1). From this result, we concluded that in the gynogenetic oocytes of *M. belari*, the meiotic spindle is correctly oriented to ensure the segregation of homologues in anaphase I. Abortion of meiosis I is therefore not due to defects in spindle positioning.

Figure 4

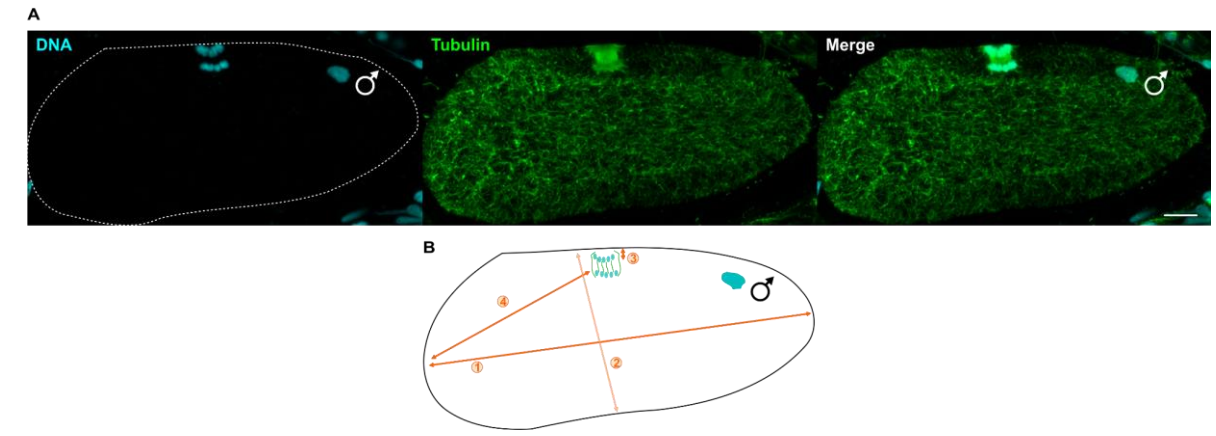


Figure 4: Method of evaluating the positioning of the microtubule cage in gynogenetic oocytes of *M. belari*. A) Image of a fixed oocyte in anaphase I. The oocyte corresponds to "Ana 2" in Table 1. DNA is in blue and microtubules are in green. Scale bar = 5µm. B) Schematic representation of the oocyte above with the four different distances measured in Table 1. The orientation of the spindle relative to the cell cortex is assessed qualitatively, no quantification has been performed.

Table 1

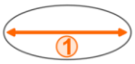
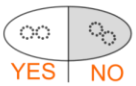
sample n°	long axis length 	short axis length 	closest distance to the cortex 	closest distance to cell tip 	correct rotation 
Meta 1	60	26	2	25	YES
Meta 2	60	26	2	30	YES
Meta 3	56	24	3	28	YES
Meta 4	38	18	0	19	YES
Meta 5	50	22	0	25	YES
Meta 6	60	30	9	25	YES
Meta 7	55	21	0	23	YES
Ana 1	47	16	7	14	YES
Ana 2	50	24	0	20	YES
Ana 3	41	20	7	17	YES
Ana 4	64	31	0.7	30	YES
Ana 5	40	17	6	12	YES
Ana 6	66	29	0	31	YES
Ana 7	43	17	0	21	YES
Ana 8	60	21	1	15	YES

Table 1: Position of meiotic structures in metaphase I and anaphase I of gynogenetic oocytes of *M. belari*. The numbers correspond to the distances shown in Figure 3. All measures are in µm. Meta, metaphase 1; Ana, anaphase I.

Persistence of the microtubule cage in anaphase I

In *C. elegans*, the segregation of chromosomes during anaphase of meiotic division occurs in two phases, during which the microtubules in the spindle have a different organization (Figure 1, 5A). In anaphase A, a cage of microtubules encloses the chromosomes, the spindle does not elongate but chromosomes slide along the microtubules until they reach the pole of the cage. In anaphase B, the cage is dismantled and central spindle microtubules polymerise between the two chromosome plates, generating a pushing force which further displaces the

chromosomes (Danlasky et al., 2020; Dumont et al., 2010; Laband et al., 2017; Maddox et al., 2004; Muscat et al., 2015; Pelisch et al., 2019b; Yu et al., 2019). We asked whether defects in the spindle microtubules could contribute to the abortion of meiosis I in *M. belari* gynogenetic embryos.

In fixed oocytes of *M. belari*, we find a cage of microtubules in all metaphase I figures (n=35, Figure 5B). During anaphase I, where we cannot distinguish amphimictic and gynogenetic eggs, the cage is still detected in both early and late anaphase (n=42) but no central spindles were seen. The cage is also seen in metaphase II of gynogenetic oocytes (showing 20 units) (n=12) and metaphase II amphimictic oocytes (10 units and a polar body) (n=4). In both gynogenetic and amphimictic oocytes, the cage persists in early anaphase II (n=9 and n=2, respectively) but is not detected in late anaphase II (n=16 and n=1, respectively). In addition, at the end of anaphase II, we observe an agglomeration of microtubules between the two chromatid plates that correspond to the central spindles (arrows, n=16 and n=1, respectively).

These results show that in *M. belari*, a microtubule cage persists throughout anaphase I. In contrast, in late anaphase II, the microtubule cage is absent in both amphimictic and gynogenetic oocytes. These results, although preliminary, suggest that in gynogenetic oocytes, the maintenance of a microtubule cage prohibits the completion of anaphase I. However, we should have detected 10% of late anaphase I figures with a regular central spindle, corresponding to the 10% regular meiosis found in this species. We may have missed these images because of undersampling.

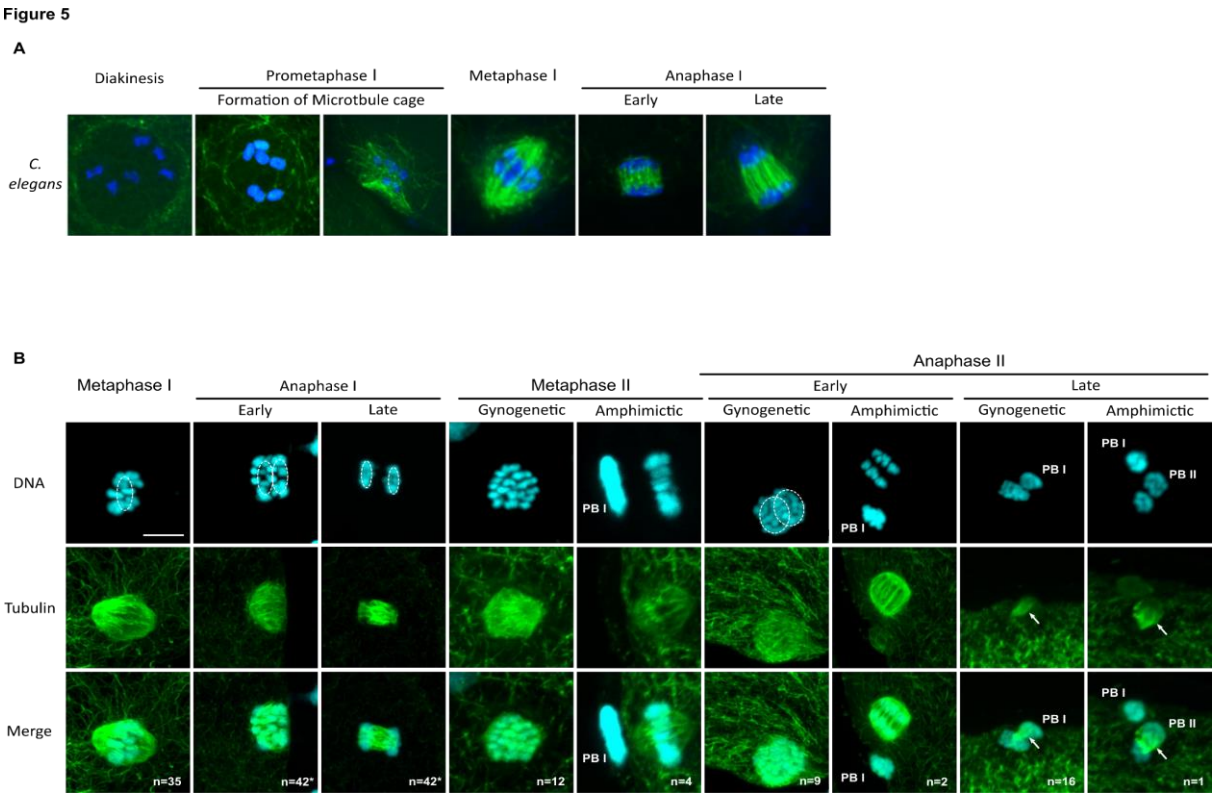


Figure 5: Microtubule cage and central spindles in fixed oocytes of *C. elegans* and *M. belari*. A) Formation of microtubule cage and central spindles in *C. elegans*. Images from (Mullen & Wignall, 2017). B) In gynogenetic oocytes of *M. belari*, the microtubule cage is always present in anaphase I, whereas the cage is disassembled in gynogenetic and amphimictic anaphase II. Dashed circles delineate the plates of chromosomes and the arrows show the central spindle. DNA is in blue and microtubules are in green. PB I, polar body I; PB II, polar body II. Scale bar = 5 μm. * we found 42 images in total, combining these two steps of anaphase

SUMO is absent in meiosis I

The Ring Complex is a transient and dynamic protein complex of meiosis. This complex is required for cohesin cleavage in anaphases and locates the cleavage position of the cell to extrude polar bodies. The assembly, maintenance and stability of the Ring Complex depend on the small ubiquitin-related modifier (SUMO). Indeed, the balance between the conjugation and deconjugation activities of SUMO indirectly and directly mediates the progression of meiosis (Davis-Roca 2018). The ortholog protein of the SUMO complex in *C. elegans* is SMO-1 (referred to as SUMO in the text) and its sequence is highly conserved in nematodes (Al-Yazeedi et al., 2022). In *C. elegans*, the SUMO protein witnesses the progression of meiosis. In metaphase I, SUMO is present only at the midvalent region (Figure 1). In early anaphase I,

SUMO localizes to the Ring Complex until the two plates of univalents reach the microtubule poles. In late anaphase I, the Ring Complex disassembles and SUMO diffuses along the central spindles. The same dynamics occur in meiosis II. In order to assess whether the Ring Complex is properly disassembled during anaphase I of gynogenetic oocytes, we analysed the dynamics of SUMO. A commercial antibody also recognizes the Ring Complex in the nematode species *Auanema rhodensis* (Al-Yazeedi et al., 2022). This species being as distant to *C. elegans* as *M. belari* is, we hypothesized that this antibody could also recognize SUMO in *M. belari*. Using this antibody, we found that in *M. belari*, SUMO shows a very faint signal in meiosis I in the vicinity of chromosomes (Figure 6). In both metaphase I and anaphase I, there is no SUMO signal at the midvalent region (n=1, n=2, respectively). Conversely, in metaphase II, SUMO is present at the midvalent region between sister chromatids (n=3). In anaphase II, SUMO co-localises with the central spindles (arrow, n=3).

These results show that SUMO has a similar dynamic as in *C. elegans* concerning meiosis II. However, during meiosis I, the division which is abortive in *M. belari*, we found a different pattern than those described for *C. elegans*. From these preliminary results, we concluded that defects in Sumoylation during anaphase I might be responsible for abortion of meiosis I of gynogenetic embryos. Whether this is specific to the Ring Complex or whether other targets of SUMO are involved remains to be determined.

Figure 6

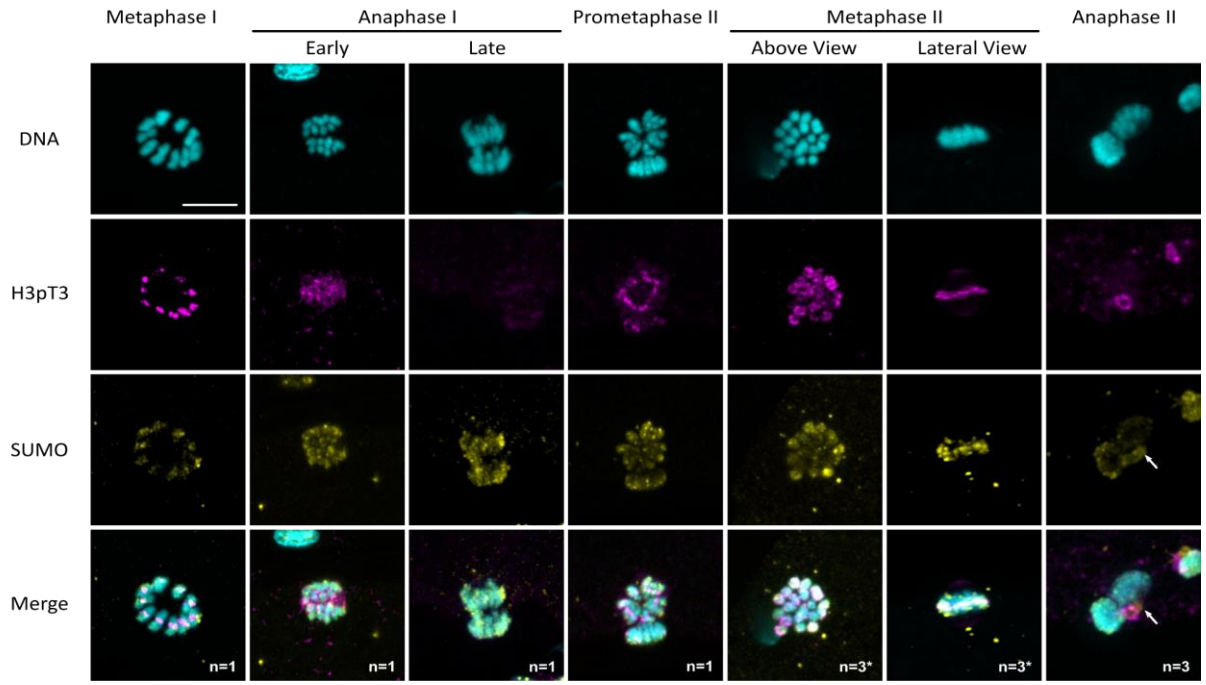


Figure 6: Dynamics of SUMO in gynogenetic oocytes of *M. belari*. SUMO is found at the Ring Complex only in metaphase II. DNA is in blue, BUB-1 is in pink, SUMO is in yellow and microtubules are in green. Scale bar = 5 µm. * we found 3images in total, combining these two steps of metaphase

BUB-1 follows the same dynamics in meiosis I and II

During meiosis, the SUMO complex plays a crucial role by conjugating with the Ring Complex (Pelisch et al., 2019b). SUMO acts on another key protein, BUB-1. SUMO both regulates the localisation of BUB-1 in metaphase I and modifies BUB-1 to relocate it on the central spindle (Pelisch et al., 2019a). The BUB-1 kinase is a structural component of the kinetochores which is involved in chromosome segregation (Dumont et al., 2010; Pelisch et al., 2019b). Kinetochores are protein complexes that connect the microtubules to the chromosomes.

In meiosis, the BUB-1 kinase undergoes a particular dynamic in *C. elegans*. In metaphase I, BUB-1 surrounds each bivalent to form a cup and is also located at the midvalent region. Once the chromosomes are aligned on the metaphase plate, this midvalent determines the position of the Ring Complex that, for instance, establishes the position of the cleavage site of the cell during cytokinesis. In anaphase I, kinetochores are disassembled and BUB-1 is relocated on microtubules between the two univalent plates (Dumont 2010). The BUB-1 signal gradually vanishes during the progression of anaphase. In metaphase II, BUB-1 is reloaded onto

the cup and midvalent region between sister chromatids. Similarly to anaphase I, in anaphase II, BUB-1 relocates to the microtubules between the chromatid plates and progressively fades (reviewed in (Kitagawa, 2009). We asked whether defects in BUB-1 loading may be responsible for the abortion of anaphase I.

The BUB-1 protein is poorly conserved in the nematode order. Using Blast alignment, we identified an ortholog: mbelari.g12204.t1 (referred to as BUB-1 in the text). BUB-1 possesses 22% of amino acid similarity with C. *elegans* BUB-1 (Pairwise analyses, Figure S2). We raised an antibody against this *M. belari* protein and found the expected localisation signal. In mitosis, BUB-1 nicely localizes along chromosomes in a dotted pattern (Figure S2). In metaphase of meiosis I, BUB-1 is found around the bivalent and forms a cup (n=5, Figure 7). However, there is no BUB-1 signal at the midvalent region. In anaphase I, BUB-1 shows two different patterns. The first pattern is the same as in metaphase I where BUB-1 forms a cup around univalents ("#1", n=2). Whereas, in the second, BUB-1 is absent from kinetochores and is only found between the two univalent plates ("#2", n=7). Metaphase II is similar to metaphase I, the BUB-1 signal is found around the univalents and there is no signal in the midvalent region (n=2). In both early and late anaphase II, BUB-1 is only located between the two plates of 20 chromatids (n=1, n=3).

M. belari BUB-1 does not present the same dynamics as the BUB-1 protein of *C. elegans*. In gynogenetic oocytes of *M. belari*, BUB-1 is not found at the midvalent region. However, both BUB-1 of *M. belari* and *C. elegans*, in anaphases, relocate on the microtubules between the chromosome plates. *M. belari* data reveals no distinction in the dynamics of BUB-1 between anaphase I and II. Because we did not find differences in the localisation of BUB-1 between amphimictic and gynogenetic eggs, we concluded that BUB-1 dynamics is probably not involved in the defects in anaphase I found in *M. belari*.

Figure 7

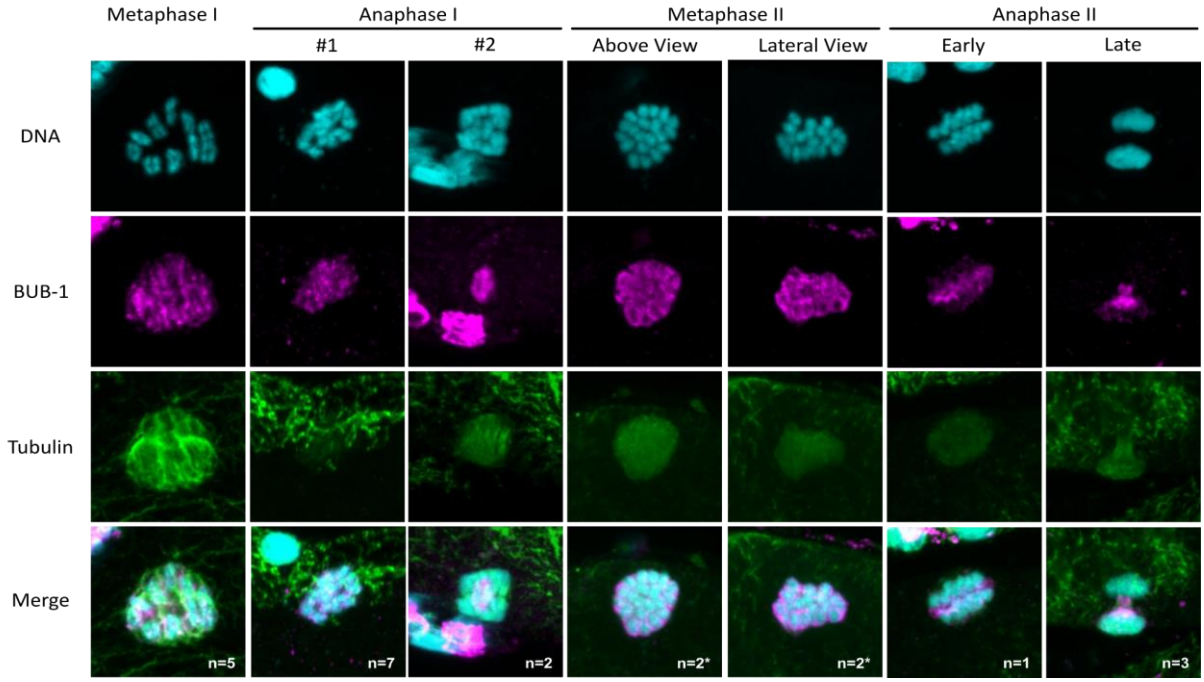


Figure 7: Dynamics of *M. belari* BUB-1. There is no distinction between meiosis I and II. DNA is blue, BUB-1 is pink and microtubules are green. Scale bar = 5 μm.* we found 2 images in total, combining these two steps of metaphase

Discussion

M. belari is a species presenting two types of female meiosis. In this species, the meiosis of amphimictic (future males) oocytes is canonical whereas, the meiosis of gynogenetic (future females) oocytes is altered. In gynogenetic oocytes, the abortion of meiosis I promotes the maintenance of diploidy in females. In this article, we explored which aspect of anaphase I is disrupted in gynogenetic oocytes and maintained in amphimictic oocytes.

In gynogenetic oocytes, anaphase I is incomplete

H3pT3 is a common phosphorylation that causes cleavage of cohesin during meiosis and mitosis. This phosphorylation is highly conserved as it is found in human cells, mice and nematodes (Nguyen et al., 2014; Wang & Higgins, 2013). The presence of H3pT3 thus indicates the site of cohesion removal within chromosomes. Our data show that in gynogenetic oocytes, H3T3 is properly phosphorylated during metaphase and early anaphase I (Figure 1). Indeed, the signal in the midvalent region progressively fades away in the early phases of anaphase until it disappears completely. In late anaphase, the re-emergence of the H3pT3 signal at the midvalent region of the univalents indicates that the chromosomes enter meiosis II, although no polar body has been extruded. Therefore, in gynogenetic oocytes, anaphase I is not completed and all univalents enter meiosis II and are realigned on the metaphase plate. Consequently, there is no reductional meiosis.

Anaphase B is absent during anaphase I

In the second step, we investigated the causes of abortive anaphase I. Anaphase abortions can occur as a result of various abnormalities.

In the closest model species *C. elegans*, defects in the positioning of the meiotic spindle result in the non-extrusion of one-half of homologs (McNally et al., 2016). In prometaphase, a cage of microtubules polymerises and encloses the bivalents (Figure 5A). This structure helps the chromosomes to align on the metaphase plate and, at the same time, positions itself near the cell cortex. The positioning of the meiotic structure is completed by rotation so that the pole-to-pole axis of the cage is perpendicular to the cell edge. Therefore, defects in both positioning and rotations lead to missegregation and non-extrusion of chromosomes. However, we showed that no such defects exist in gynogenetic oocytes (Figure B, Table 1). To define precisely what happens in anaphase I, we then examined which phase of anaphase is problematic. Anaphases are subdivided into anaphase A and B. In anaphase A, cohesins between homologs (in meiosis

I) are cleaved and removed. The loss of cohesion enables the two plates of univalents to slide along the microtubule cage to the poles. Kinetochores disassemble and BUB-1 kinase relocalizes on the Ring Complex with the SUMO complex (Dumont et al., 2010). In anaphase B, SUMO is deconjugated from the Ring Complex which collapses. At this stage, the central spindles polymerise between the two chromosome plates and exert pushing forces that drive the chromosomes into the polar body. Our findings indicate that in gynogenetic oocytes there is no central spindle polymerisation (Figure 3). Thus, we hypothesized that in those oocytes, anaphase B is absent in meiosis I. It is important to note that, we should observe cases with the central spindles in anaphase I. Indeed, the population of *M. belari* is 10% of males (Grosmaire et al., 2019) which are produced via canonical meiosis. Thus, we should at least observe 10% of anaphase I with central spindles. Nonetheless, given the scarcity of males in the population, it is conceivable that among the 42 oocytes screened, we did not find any amphimictic oocytes (Figure 3).

Intriguingly, our data reveal that the dynamics of SUMO is similar between *M. belari* and *C. elegans* only during meiosis II (Figure 6). In *C. elegans,* the depletion of the protease ULP-1 inhibits the deconjugation of SUMO (Davis-Roca et al., 2018). The persistence of SUMO in the Ring Complex leads to the maintenance of the Ring Complex. The cage collapses and meiotic division is impeded. Our data show that in meiosis I, there is no SUMO signal at the midvalent region or in the putative site of the Ring Complex. However, in metaphase II, SUMO labels the midvalent region and is relocated to the central spindles in late anaphase II. Our results are consistent with the absence of anaphase B in gynogenetic oocytes. Indeed, the absence of SUMO in meiosis I can impede the completion of anaphase by preventing anaphase B. Analyses of other proteins involved in anaphase B are needed to validate whether the meiotic process enters anaphase B without completing it or whether only the completion of anaphase is hindered.

As explained above, kinetochores disassembly is required in the transition from anaphase A to B. MEL-28 docks the protein phosphatase I, PP1c, on the chromatids. This docking triggers the disassembly of kinetochores in the middle anaphase (Hattersley et al., 2016). The depletion of *mel-28* prevents the kinetochores disassembly and the polymerization of central spindles in anaphase B. Moreover, in *mel-28* RNAi, all the univalents enter meiosis II. Thus, defects in kinetochores removal could explain abortive anaphase B of gynogenetic oocytes. For these reasons, we investigated the dynamics of the BUB-1 kinase protein during meiosis. First of all, the pattern observed is not similar to *C. elegans*. In both metaphases I and

II, no signal was detected at the midvalent region. Given the rate of divergence between species, it is conceivable that the BUB-1 function may also deviate. However, BUB-1 orthologs are found in both mammals and budding yeast and show a similar function to *C. elegans* BUB-1 (Fernius & Hardwick, 2007; Skoufias et al., 2001). Thus, another possibility in our case, is that the antibody raised for *M. belari* BUB-1 is not able to label the midvalent region. Secondly, in *M. belari,* immunostainings of BUB-1 is similar between aborted meiosis I and complete meiosis II. As only meiosis I is aborted, meiosis II is a reference for complete meiosis. Because the pattern of BUB-1 is the same between meiosis I and II, it is difficult to deduce the influence of the kinetochore on the progression of anaphase. Further analysis of the kinetochore proteins could answer this question.

M. belari has an unconventional HORMA domain protein

Proteins containing the HORMA domain are conserved as they are described in humans, plants, mice, budding yeast, fission yeast and nematodes (reviewed in (Muniyappa et al., 2014). Depending on the species, they are involved in homologous pairing, formation of double-strand breaks, synaptonemal assembly and checkpoint activation. HORMA domain proteins are, thus, necessary to the meiotic process. *C. elegans* possesses four HORMA domain proteins which in metaphase I exhibit distinct patterns. HTP-3 and HIM-3 are located between both sister chromatids and homologs. Immunostainings of those proteins label a cross in the bivalent. Whereas, HTP-1 and HTP-2 are only found between sister chromatids on the long arms. The presence of HTP-1/-2 on the short arms is crucial to prevent early sister chromatid separation. Indeed, HTP-1 recruits the LAB-1 protein which interacts with the GSP-1 phosphatase ((Kaitna et al., 2002; Nadarajan et al., 2021; Rogers et al., 2002; Siomos et al., 2001)). GSP-1 antagonises the phosphorylation of H3pT3 by HASPIN and thus the cleavage of cohesins that maintain sister chromatid association. In the absence of these proteins, sister chromatids are dissociated during anaphase I (de Carvalho et al., 2008; Ferrandiz et al., 2018; Tzur et al., 2012). Our data indicate that Mb.HORMA is the ortholog of HTP-1 (Figure 2A). While the pachytene staining is consistent with this interpretation, the diakinesis and metaphase immunostainings remain intriguing (Figure 2B, 2C). Indeed, at these stages, Mb.HORMA is located in the midvalent region. Therefore, it is located on the short arms between homologs in diakinesis and metaphase I. No HORMA domain proteins of *C. elegans* exhibit this pattern. This raises the question of the identity of Mb.HORMA: is Mb.HORMA the direct ortholog of HTP-1? Why does Mb.HORMA colocalises with H3T3 phosphorylation (Figure 2D)? How are sister chromatids kept associated during meiosis I in the absence of this Mb.HORMA? And

lastly, why is Mb. HORMA reloaded in metaphase II between sister chromatids, again colocalising with H3pT3 (Figure 2C)? If confirmed, this would be the first evidence for the reuse of a HORMA domain protein during meiosis. These findings, although preliminary, suggest an unexpected role for Mb. HORMA in the bimodal reproductive system of *M. belari*.

References

Al-Yazeedi, T., Xu, E. L., Kaur, J., Shakes, D. C., & Pires-daSilva, A. (2022). Lagging X chromatids specify the orientation of asymmetric organelle partitioning in XX spermatocytes of Auanema rhodensis. *Genetics*, *222*(4), iyac159. https://doi.org/10.1093/genetics/iyac159

Blanc, C., Saclier, N., Faou, E. L., Marie-Orleach, L., Wenger, E., Diblasi, C., Glemin, S., Galtier, N., & Delattre, M. (2023). *Co-segregation of recombinant chromatids maintains genome-wide heterozygosity in an asexual nematode* (p. 2023.03.17.533182). bioRxiv. https://doi.org/10.1101/2023.03.17.533182

Bohr, T., Ashley, G., Eggleston, E., Firestone, K., & Bhalla, N. (2016). Synaptonemal Complex Components Are Required for Meiotic Checkpoint Function in Caenorhabditis elegans. *Genetics*, *204*(3), 987–997. https://doi.org/10.1534/genetics.116.191494

Cahoon, C. K., Helm, J. M., & Libuda, D. E. (2019). Synaptonemal Complex Central Region Proteins Promote Localization of Pro-crossover Factors to Recombination Events During Caenorhabditis elegans Meiosis. *Genetics*, *213*(2), 395–409. https://doi.org/10.1534/genetics.119.302625

Chuang, C.-H., Schlientz, A. J., Yang, J., & Bowerman, B. (2020). Microtubule assembly and pole coalescence: Early steps in Caenorhabditis elegans oocyte meiosis I spindle assembly. *Biology Open*, *9*(6), bio052308. https://doi.org/10.1242/bio.052308

Colaiácovo, M. P., MacQueen, A. J., Martinez-Perez, E., McDonald, K., Adamo, A., La Volpe, A., & Villeneuve, A. M. (2003). Synaptonemal complex assembly in C. elegans is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. *Developmental Cell*, *5*(3), 463–474. https://doi.org/10.1016/s1534-5807(03)00232-6

Danlasky, B. M., Panzica, M. T., McNally, K. P., Vargas, E., Bailey, C., Li, W., Gong, T., Fishman, E. S., Jiang, X., & McNally, F. J. (2020). Evidence for anaphase pulling forces during C. elegans meiosis. *The Journal of Cell Biology*, *219*(12), e202005179. https://doi.org/10.1083/jcb.202005179

Davis-Roca, A. C., Divekar, N. S., Ng, R. K., & Wignall, S. M. (2018). Dynamic SUMO remodeling drives a series of critical events during the meiotic divisions in Caenorhabditis elegans. *PLOS Genetics*, *14*(9), e1007626. https://doi.org/10.1371/journal.pgen.1007626

de Carvalho, C. E., Zaaijer, S., Smolikov, S., Gu, Y., Schumacher, J. M., & Colaiácovo, M. P. (2008). LAB-1 antagonizes the Aurora B kinase in C. elegans. *Genes & Development*, *22*(20), 2869–2885. https://doi.org/10.1101/gad.1691208

Divekar, N. S., Davis-Roca, A. C., Zhang, L., Dernburg, A. F., & Wignall, S. M. (2021). A degron-based strategy reveals new insights into Aurora B function in C. elegans. *PLOS Genetics*, *17*(5), e1009567. https://doi.org/10.1371/journal.pgen.1009567

Dumont, J., Oegema, K., & Desai, A. (2010). A kinetochore-independent mechanism drives anaphase chromosome separation during acentrosomal meiosis. *Nature Cell Biology*, *12*(9), 894–901. https://doi.org/10.1038/ncb2093

Fernius, J., & Hardwick, K. G. (2007). Bub1 Kinase Targets Sgo1 to Ensure Efficient Chromosome Biorientation in Budding Yeast Mitosis. *PLOS Genetics*, *3*(11), e213. https://doi.org/10.1371/journal.pgen.0030213

Ferrandiz, N., Barroso, C., Telecan, O., Shao, N., Kim, H.-M., Testori, S., Faull, P., Cutillas, P., Snijders, A. P., Colaiácovo, M. P., & Martinez-Perez, E. (2018). Spatiotemporal regulation of Aurora B recruitment ensures release of cohesion during C. elegans oocyte meiosis. *Nature Communications*, *9*(1), Article 1. https://doi.org/10.1038/s41467-018-03229-5

Grosmaire, M., Launay, C., Siegwald, M., Brugière, T., Estrada-Virrueta, L., Berger, D., Burny, C., Modolo, L., Blaxter, M., Meister, P., Félix, M.-A., Gouyon, P.-H., & Delattre, M. (2019). Males as somatic investment in a parthenogenetic nematode. *Science*, *363*(6432), 1210–1213. https://doi.org/10.1126/science.aau0099

Hattersley, N., Cheerambathur, D., Moyle, M., Stefanutti, M., Richardson, A., Lee, K.-Y., Dumont, J., Oegema, K., & Desai, A. (2016). A Nucleoporin Docks Protein Phosphatase 1 to Direct Meiotic Chromosome Segregation and Nuclear Assembly. *Developmental Cell*, *38*(5), 463–477. https://doi.org/10.1016/j.devcel.2016.08.006

Hsu, J. Y., Sun, Z. W., Li, X., Reuben, M., Tatchell, K., Bishop, D. K., Grushcow, J. M., Brame, C. J., Caldwell, J. A., Hunt, D. F., Lin, R., Smith, M. M., & Allis, C. D. (2000). Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. *Cell*, *102*(3), 279–291. https://doi.org/10.1016/s0092-8674(00)00034-9

Kaitna, S., Pasierbek, P., Jantsch, M., Loidl, J., & Glotzer, M. (2002). The Aurora B Kinase AIR-2 Regulates Kinetochores during Mitosis and Is Required for Separation of Homologous Chromosomes during Meiosis. *Current Biology*, *12*(10), 798–812. https://doi.org/10.1016/S0960-9822(02)00820-5

Kitagawa, R. (2009). Key players in chromosome segregation in Caenorhabditis elegans. *Frontiers in Bioscience : A Journal and Virtual Library*, *14*, 1529. https://doi.org/10.2741/3323

Köhler, S., Wojcik, M., Xu, K., & Dernburg, A. F. (2017). Superresolution microscopy reveals the three-dimensional organization of meiotic chromosome axes in intact Caenorhabditis elegans tissue. *Proceedings of the National Academy of Sciences*, *114*(24), E4734–E4743. https://doi.org/10.1073/pnas.1702312114

Komaru, A., Ookubo, K., & Kiyomoto, M. (2000). All meiotic chromosomes and both centrosomes at spindle pole in the zygotes discarded as two polar bodies in clam Corbicula leana: Unusual polar body formation observed by antitubulin immunofluorescence. *Development Genes and Evolution*, *210*(5), 263–269. https://doi.org/10.1007/s004270050313

Laband, K., Le Borgne, R., Edwards, F., Stefanutti, M., Canman, J. C., Verbavatz, J.-M., & Dumont, J. (2017). Chromosome segregation occurs by microtubule pushing in oocytes. *Nature Communications*, *8*(1), 1499. https://doi.org/10.1038/s41467-017-01539-8

MacQueen, A. J., Colaiácovo, M. P., McDonald, K., & Villeneuve, A. M. (2002). Synapsis-dependent and -independent mechanisms stabilize homolog pairing during meiotic prophase in C. elegans. *Genes & Development*, *16*(18), 2428–2442. https://doi.org/10.1101/gad.1011602

Maddox, P. S., Oegema, K., Desai, A., & Cheeseman, I. M. (2004). "Holo"er than thou: Chromosome segregation and kinetochore function in C. elegans. *Chromosome Research*, *12*(6), 641–653. https://doi.org/10.1023/B:CHRO.0000036588.42225.2f

McNally, K. P., Panzica, M. T., Kim, T., Cortes, D. B., & McNally, F. J. (2016). A novel chromosome segregation mechanism during female meiosis. *Molecular Biology of the Cell*, *27*(16), 2576–2589. https://doi.org/10.1091/mbc.e16-05-0331

Monen, J., Maddox, P. S., Hyndman, F., Oegema, K., & Desai, A. (2005). Differential role of CENP-A in the segregation of holocentric C. elegans chromosomes during meiosis and mitosis. *Nature Cell Biology*, *7*(12), 1248–1255. https://doi.org/10.1038/ncb1331

Mullen, T. J., & Wignall, S. M. (2017). Interplay between microtubule bundling and sorting factors ensures acentriolar spindle stability during C. elegans oocyte meiosis. *PLoS Genetics*, *13*(9), e1006986.

Muniyappa, K., Kshirsagar, R., & Ghodke, I. (2014). The HORMA domain: An evolutionarily conserved domain discovered in chromatin-associated proteins, has unanticipated diverse functions. *Gene*, *545*(2), 194–197. https://doi.org/10.1016/j.gene.2014.05.020

Muscat, C. C., Torre-Santiago, K. M., Tran, M. V., Powers, J. A., & Wignall, S. M. (2015). Kinetochore-independent chromosome segregation driven by lateral microtubule bundles. *ELife*, *4*, e06462. https://doi.org/10.7554/eLife.06462

Nadarajan, S., Altendorfer, E., Saito, T. T., Martinez-Garcia, M., & Colaiácovo, M. P. (2021). HIM-17 regulates the position of recombination events and GSP-1/2 localization to establish short arm identity on bivalents in meiosis. *Proceedings of the National Academy of Sciences*, *118*(17), e2016363118. https://doi.org/10.1073/pnas.2016363118

Nguyen, A. L., Gentilello, A. S., Balboula, A. Z., Shrivastava, V., Ohring, J., & Schindler, K. (2014). Phosphorylation of threonine 3 on histone H3 by haspin kinase is required for meiosis I in mouse oocytes. *Journal of Cell Science*, *127*(Pt 23), 5066–5078. https://doi.org/10.1242/jcs.158840

Pasierbek, P., Jantsch, M., Melcher, M., Schleiffer, A., Schweizer, D., & Loidl, J. (2001). A Caenorhabditis elegans cohesion protein with functions in meiotic chromosome pairing and disjunction. *Genes & Development*, *15*(11), 1349–1360. https://doi.org/10.1101/gad.192701

Pelisch, F., Bel Borja, L., Jaffray, E. G., & Hay, R. T. (2019a). Sumoylation regulates protein dynamics during meiotic chromosome segregation in C. elegans oocytes. *Journal of Cell Science*, *132*(14), jcs232330. https://doi.org/10.1242/jcs.232330

Pelisch, F., Bel Borja, L., Jaffray, E. G., & Hay, R. T. (2019b). Sumoylation regulates protein dynamics during meiotic chromosome segregation in C. elegans oocytes. *Journal of Cell Science*, *132*(14), jcs232330. https://doi.org/10.1242/jcs.232330

Rogers, E., Bishop, J., Waddle, J., Schumacher, J., & Lin, R. (2002). The aurora kinase AIR-2 functions in the release of chromosome cohesion in Caenorhabditis elegans meiosis. *The Journal of Cell Biology*, *157*, 219–229. https://doi.org/10.1083/jcb.200110045

Severson, A. F., Ling, L., van Zuylen, V., & Meyer, B. J. (2009). The axial element protein HTP-3 promotes cohesin loading and meiotic axis assembly in C. elegans to implement the meiotic program of chromosome segregation. *Genes & Development*, *23*(15), 1763–1778. https://doi.org/10.1101/gad.1808809

Severson, A. F., & Meyer, B. J. (2014, August 29). *Divergent kleisin subunits of cohesin specify mechanisms to tether and release meiotic chromosomes*. ELife; eLife Sciences Publications Limited. https://doi.org/10.7554/eLife.03467

Siomos, M. F., Badrinath, A., Pasierbek, P., Livingstone, D., White, J., Glotzer, M., & Nasmyth, K. (2001). Separase is required for chromosome segregation during meiosis I in Caenorhabditis elegans. *Current Biology*, *11*(23), 1825–1835. https://doi.org/10.1016/S0960-9822(01)00588-7

Skoufias, D. A., Andreassen, P. R., Lacroix, F. B., Wilson, L., & Margolis, R. L. (2001). Mammalian mad2 and bub1/bubR1 recognize distinct spindle-attachment and kinetochore-tension checkpoints. *Proceedings of the National Academy of Sciences*, *98*(8), 4492–4497. https://doi.org/10.1073/pnas.081076898

Smolikov, S., Eizinger, A., Schild-Prufert, K., Hurlburt, A., McDonald, K., Engebrecht, J., Villeneuve, A. M., & Colaiácovo, M. P. (2007). SYP-3 restricts synaptonemal complex assembly to bridge paired chromosome axes during meiosis in Caenorhabditis elegans. *Genetics*, *176*(4), 2015–2025. https://doi.org/10.1534/genetics.107.072413

Tzur, Y. B., Egydio de Carvalho, C., Nadarajan, S., Van Bostelen, I., Gu, Y., Chu, D. S., Cheeseman, I. M., & Colaiácovo, M. P. (2012). LAB-1 Targets PP1 and Restricts Aurora B Kinase upon Entrance into Meiosis to Promote Sister Chromatid Cohesion. *PLoS Biology*, *10*(8), e1001378. https://doi.org/10.1371/journal.pbio.1001378

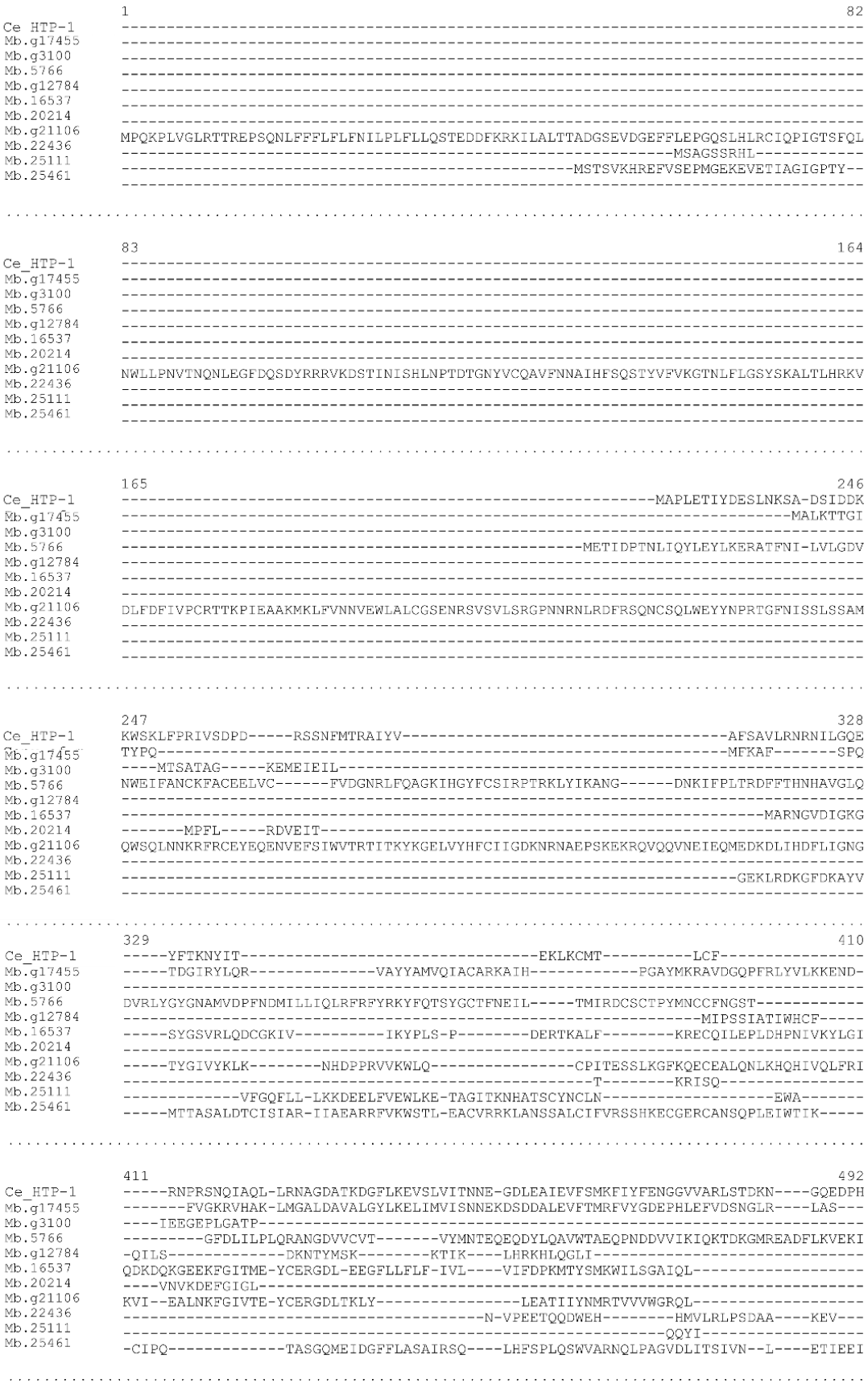
Wang, F., & Higgins, J. M. G. (2013). Histone modifications and mitosis: Countermarks, landmarks, and bookmarks. *Trends in Cell Biology*, *23*(4), 175–184. https://doi.org/10.1016/j.tcb.2012.11.005

Woglar, A., Yamaya, K., Roelens, B., Boettiger, A., Köhler, S., & Villeneuve, A. M. (2020). Quantitative cytogenetics reveals molecular stoichiometry and longitudinal organization of meiotic chromosome axes and loops. *PLOS Biology*, *18*(8), e3000817. https://doi.org/10.1371/journal.pbio.3000817

Yu, C.-H., Redemann, S., Wu, H.-Y., Kiewisz, R., Yoo, T. Y., Conway, W., Farhadifar, R., Müller-Reichert, T., & Needleman, D. (2019). Central-spindle microtubules are strongly coupled to chromosomes during both anaphase A and anaphase B. *Molecular Biology of the Cell*, *30*(19), 2503–2514. https://doi.org/10.1091/mbc.E19-01-0074

Supplemental Figures

Figure S1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----IEEGEPLGATP-----
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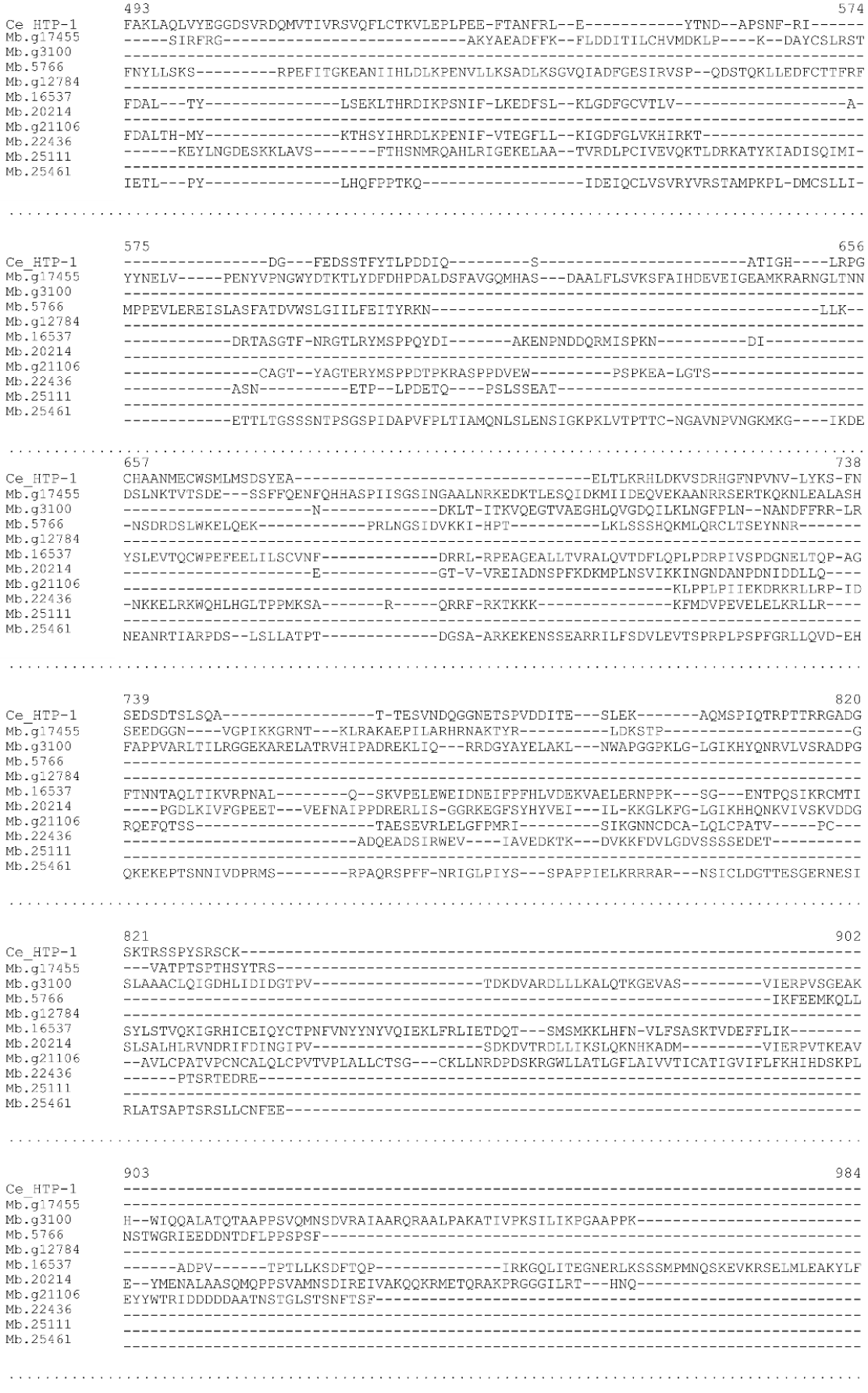
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Figure S1: Amino acid alignments of 10 *M. belari* putative orthologs against *C. elegans* HTP-1.

Figure S2

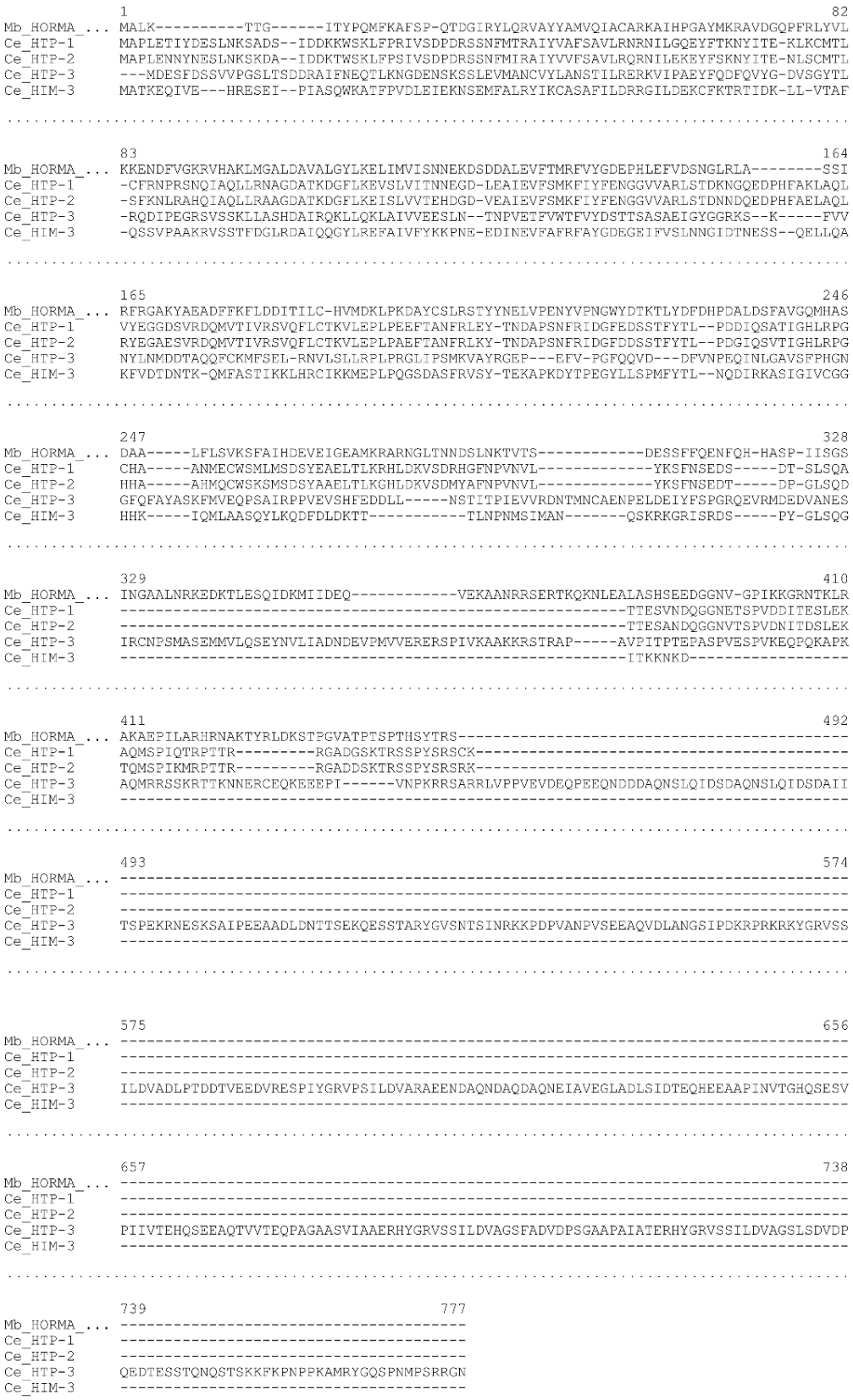


Figure S2: Amino acid alignments of Mb.HORMA (g17455.t1) and the four HORMA domain proteins of *C. elegans*

Figure S3

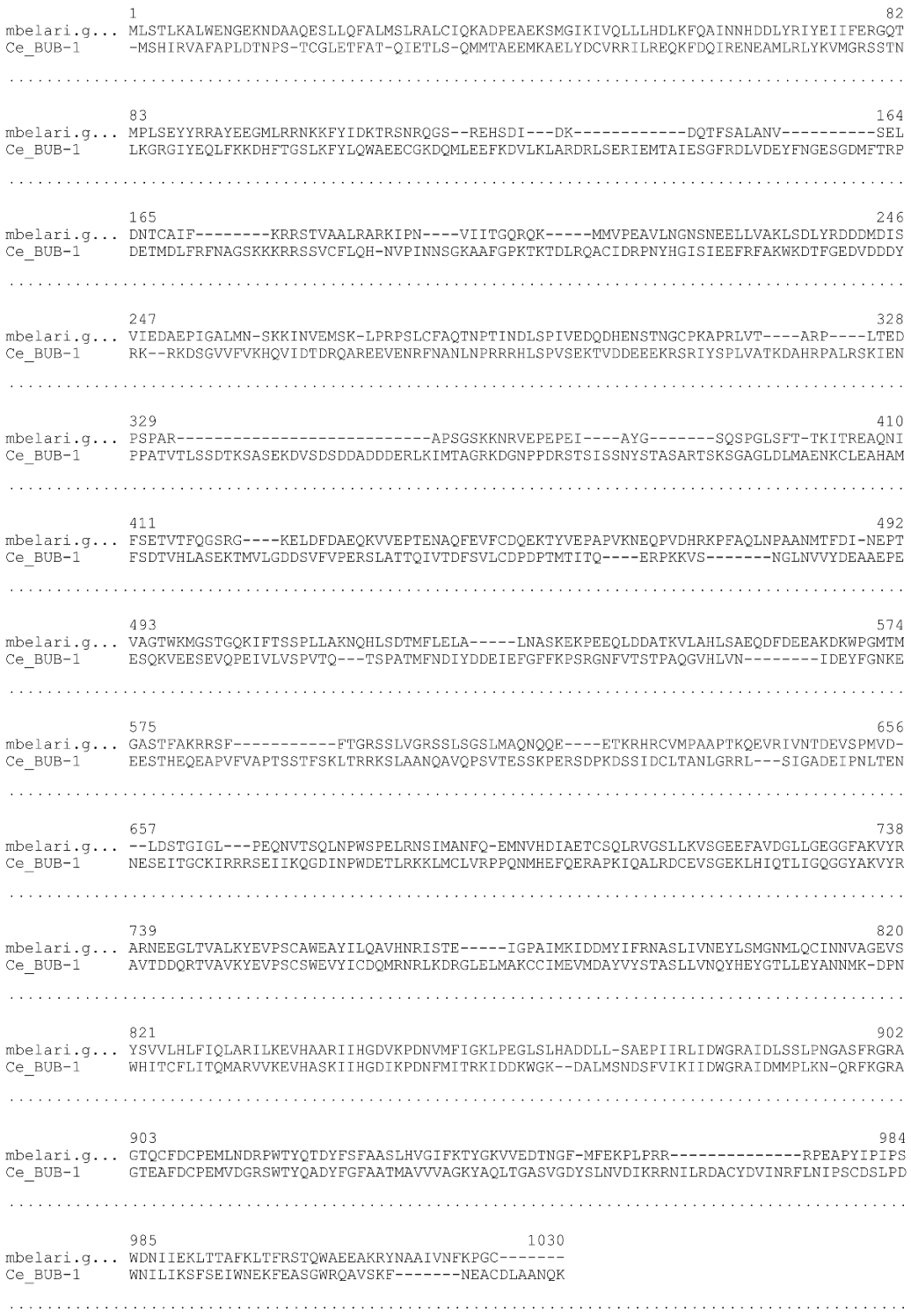


Figure S3: Amino acid alignments of the putative M. belari BUB-1 protein (g12204.t1) and the *C. elegans* BUB-1 protein

Conclusion

I have demonstrated in this Chapter that during meiosis in gynogenetic oocytes of *M. belari*, homologous recombination is preserved but meiosis I is aborted leading to the association of non-sister chromatids in the unreduced gametes. Preliminary results suggest that he abortion of meiosis I is due to defects in anaphase B, maybe because the central spindle is not properly assembled. More work will be required to precisely identify the molecular changes involved. The theory predicts that recombination combined with the association of non-sister chromatids in the unreduced gamete causes homogenisation of the genome. Unexpectedly the genome of *M. belari* is widely heterozygous. We hypothesized and next demonstrated that this is possible provided the recombinant chromatids of a given pair of chromosomes co-segregate during the unique division of meiosis. We named this biased segregation Directed Chromatid Assortment (DCA). DCA, therefore, constitutes a novel mechanism allowing heterozygosity maintenance in recombining asexuals. As discussed in Chapter 1, heterozygosity maintenance has so far been demonstrated only in cases where recombination is totally abolished, or reduced because displaced to the tip of chromosomes. Our results demonstrate a third strategy that allows asexual species to maintain a heterozygous genome on the long term.

Chapter 3: Investigation of the mechanisms allowing the switch from regular to unreduced gamete production in *M. belari*

Introduction

The second objective of my thesis was to identify how a given *M. belari* female can produce two types of gametes. The team had previously shown (unpublished data) that during its life, a given female produces both amphimictic (males) and gynogenetic (females) oocytes. Interestingly, they also reproduced results previously obtained by V. Nigon (Nigon, 1949): young females lay more males than older ones (Figure 35). This result strongly suggests that the meiotic program is more often regular in young females (to produce males) than in older females, which produce mainly unreduced oocytes (females). The observation was made at constant temperature (+20°C) and food availability.

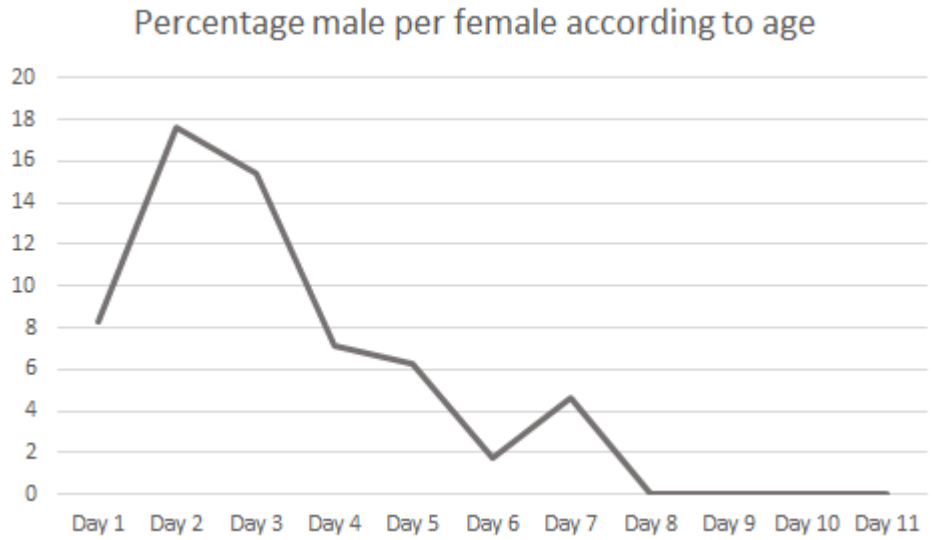


Figure 35: Percentage of males laid according to the age of the mother. n=5 females (mothers) , n=2 males (fathers), n=288 F1 (Grosmaire, PhD thesis, 2018)

Although V. Nigon had shown that the production of males was also higher at a low temperature of 12°C (Nigon, 1949), the team could not reproduce this result. The team noted

that neither food deprivation nor variations in temperature (lower and higher) resulted in changes in male production. Nonetheless, other environmental factors may influence the type of oogenesis: i.e. type of nutriments, crowding, specific pheromones, etc. In any case, the previous results strongly suggest that a factor of maternal origin dictates the meiotic program. Oocytes are large cells that contain all the maternally provided components required for the development of the embryo such as proteins, mRNAs, small RNAs, or simple chemical compounds. Therefore, we tested whether during oogenesis, differential deposits of certain mRNAs could trigger either canonical or altered meiosis.

This chapter is divided into three sub-sections. In the first part, I explain how the team proceeded to identify genes expressed differently between amphimictic and gynogenetic oocytes. Next, I describe the techniques I used to validate the differential expression of a few candidate genes. In the last part, I describe the functional analyses that I have performed on a few candidate genes.

Results

Transcriptomic analyses reveal 15 genes differentially expressed between amphimictic and gynogenetic eggs

Many mRNAs are deposited into the oocytes during oogenesis. We hypothesised that some mRNAs are more expressed in the oocytes destined for an amphimictic development compared to those developing through gynogenesis, or vice versa. Such maternal factors may be progressively degraded as the embryo develops. Thus, the team decided to extract the mRNA of amphimictic and gynogenetic eggs, as early as possible. At the one-cell stage, soon after fertilisation, the two types of eggs are easily recognized because they show a single large pronucleus in the case of gynogenetic eggs. In contrast, two pronuclei are visible in amphimictic eggs (Figure 36).

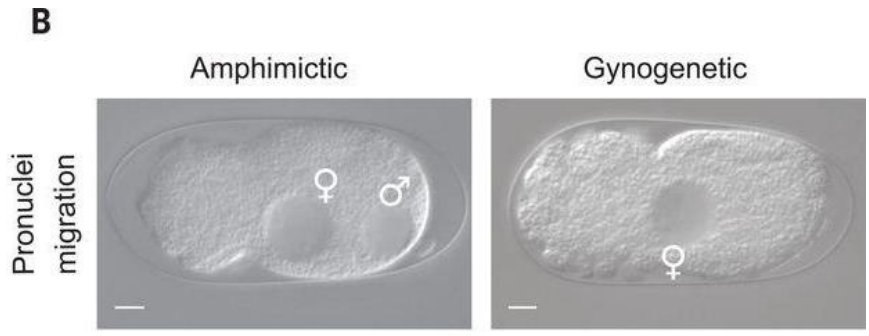
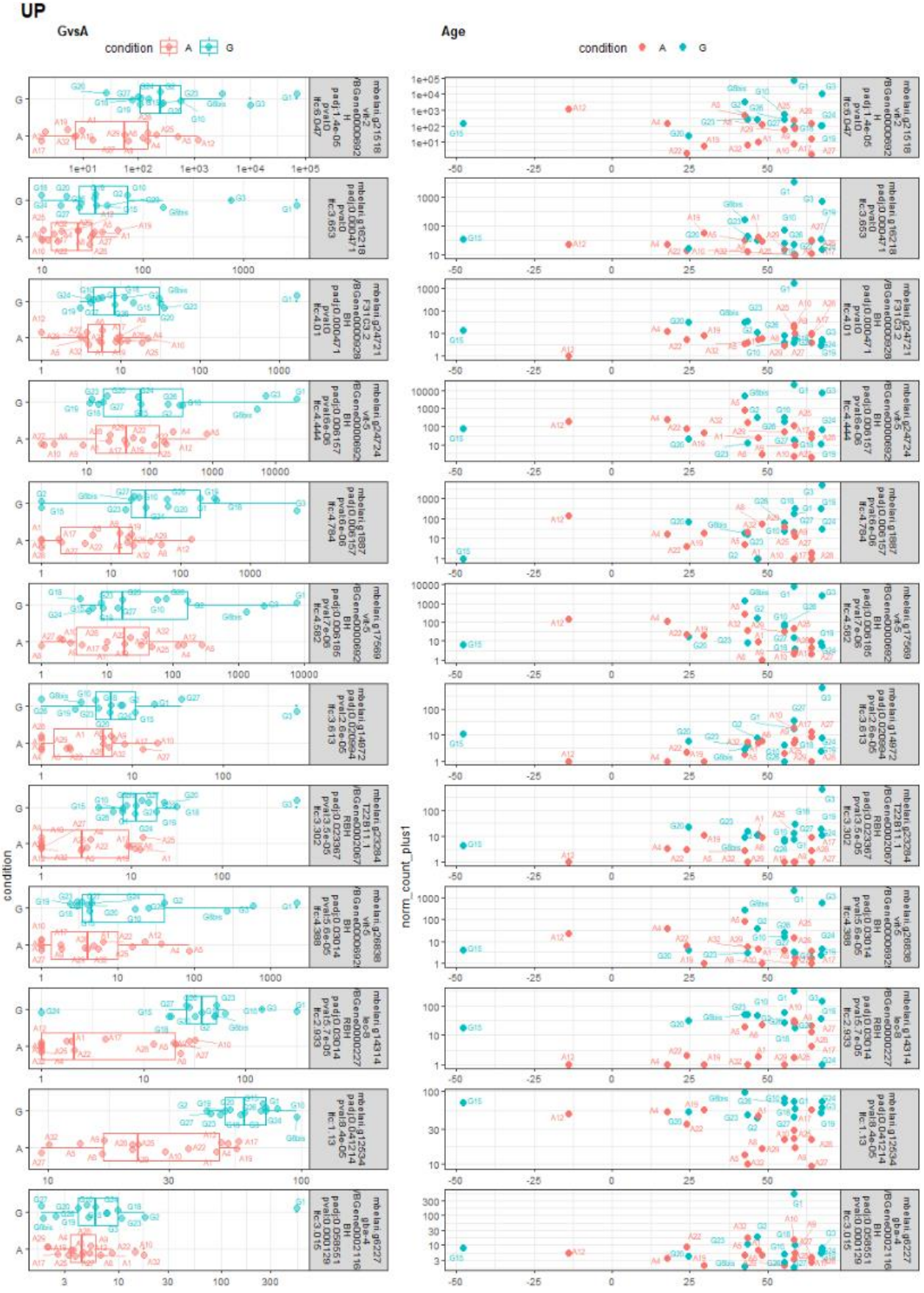


Figure 36: Two types of embryos are produced by *M. belari* females. Representative amphimictic and gynogenetic embryos. Still images from DIC recordings showing the male and female pronuclei before the first cell division of the embryo. Scale bars= 5 μm. (Grosmaire et al., 2019)

After the fusion of the parental nuclei, it is however difficult to distinguish the two phenotypes. For these reasons, the amphimictic and gynogenetic eggs were isolated manually at the one-cell stage. One-cell stage embryos were mounted under a DIC microscope. The embryos were recovered and separated into single tubes before being processed for single-cell RNA extraction and sequencing (adaptation of the Smart Seq2 protocol by an engineer in the team (Picelli et al., 2014). After curation, the data obtained were processed using DESeq2 (Love et al., 2014). Analyses were conducted on 13 gynogenetic samples and 16 samples of amphimictic oocytes. Figure 37 shows the 20 genes with a significantly adjusted p-value. Estimation of the sample age was conducted using RAPToR (Bulteau & Francesconi, 2022).


UP
GvsA
condition A G
Age
condition A G
condition
norm_count_plus1

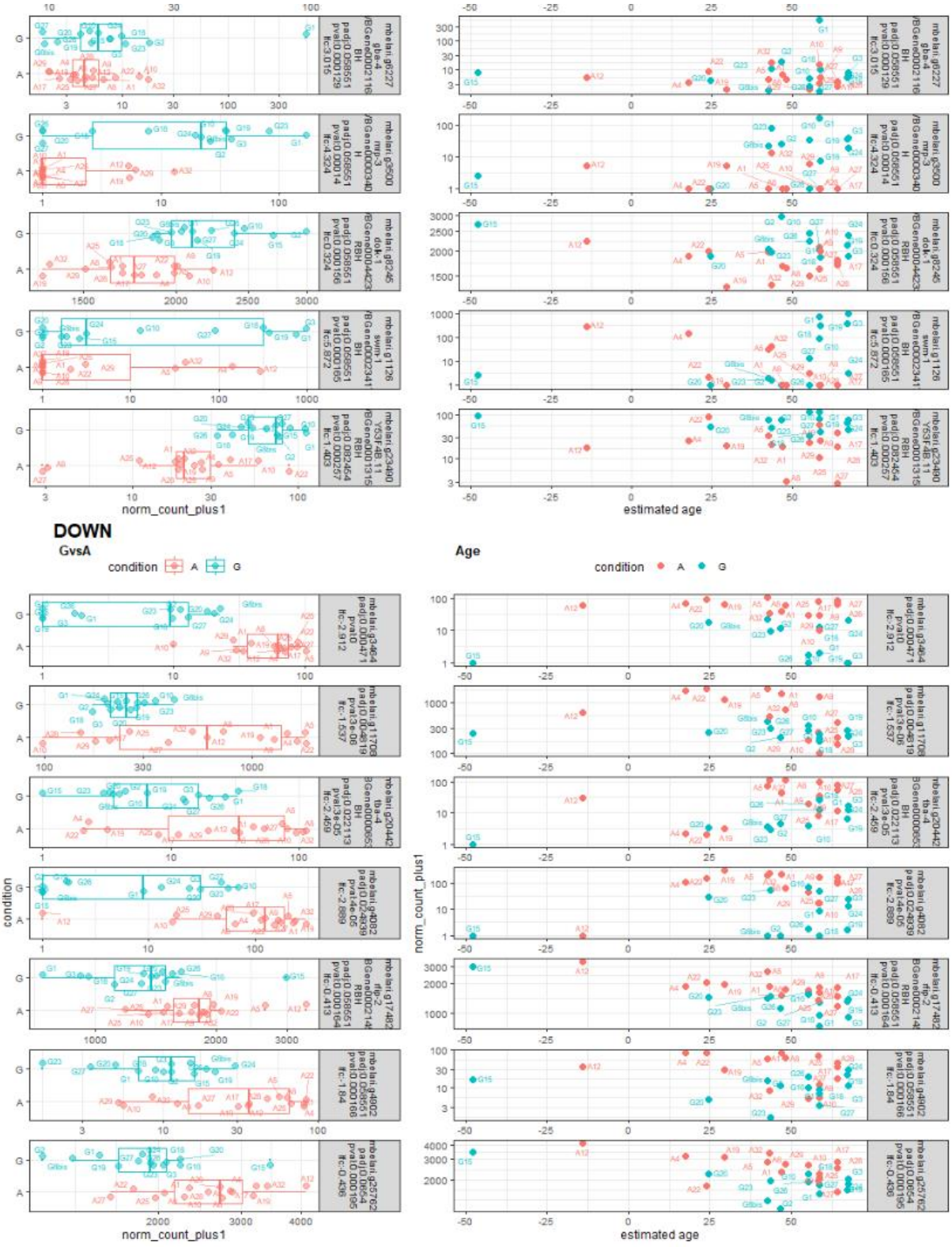


Figure 37: 20 genes differentially expressed between gynogenetic and amphimictic oocytes. The left panels represent the normalized RNA-seq reads according to the type of oocytes. The corresponding ortholog of *C. elegans* is indicated in the facet. On the right panels are represented the normalized RNA-seq reads according to the estimated relative age of the samples. Each dot is an oocyte. Gynogenetic oocytes (G) are blue and amphimictic oocytes (A) are red

Among the 20 genes, 15 genes drew our attention (Table 2) because the differential expression was high (g3500; g12534; g23490; g14314; g17609; g8245; g25532; g4082; g3464; g17482; g4955; g21312; g3341 and g19315; Figure 37) or because the genes involved were ortholog to relevant genes in *C. elegans* (g1126).

N°	Gynogenetic > Amphimictic		Gynogenetic < Amphimictic	
	Candidate gene	*C. elegans* ortholog	Candidate gene	*C. elegans* ortholog
1	g3500	-	g4082	-
2	g12534	-	g3464	-
3	g23490	**Y53F4B.11**	g17482	-
4	g14314	***lec-8***	g4955	***epb-2* family** microtubule end binding
5	g1126	***swm-1* family** Sperm activation without mating	g21312	***gft-2H2C*** General transcription factor
6	g17609	**eif-3.C** eukaryote initation factor	g3341	***Hsp-90*** family
7	g8245	***dolk-1*** involved in co-traductionnal N-glycosylation in the form dolichol pyrophosphate	g19315	***pdi-2*** family Protein disulfide-isomerase 2
8	g25532	***pph-6***		
Total	8		7	

Table 2: 15 genes differentially expressed between amphimictic and gynogenetic oocytes in *M. belari*. The table shows the *C. elegans.* ortholog

The g1126 gene in particular caught our interest. BLAST analysis revealed that *M. belari*g1126 is the ortholog of the *swm-1* gene (Sperm activation Without Mating) of *C. elegans*. In *C. elegans*, *swm-1* is only expressed in males and is involved in the inhibition of the decondensation of the sperm DNA. Depletion of *swm-1* results in premature decondensation of the sperm DNA, which becomes precociously mobile and unable to be transported into the female vulva (Chavez et al., 2018; J. R. Smith & Stanfield, 2011; Stanfield & Villeneuve, 2006). Interestingly in *M. belari*, the g1126 gene is mostly expressed in gynogenetic eggs, in which the sperm DNA does not decondense. This suggested that the sperm DNA fails to decondense in gynogenetic eggs because unexpectedly, an excess of SWM-1 is deposited maternally in the oocytes.

The Mbelari.g14314 gene also caught our attention. This gene is ortholog to the *C. elegans lec-8* gene (gaLECtin), which has glycolipid binding activity and thus is involved in

various processes. This function is not particularly informative but interestingly we found that the first seven exons of the gene were only expressed in gynogenetic eggs (Figure 38). The gene g14314 might thus undergo alternative splicing depending on the type of oocytes.

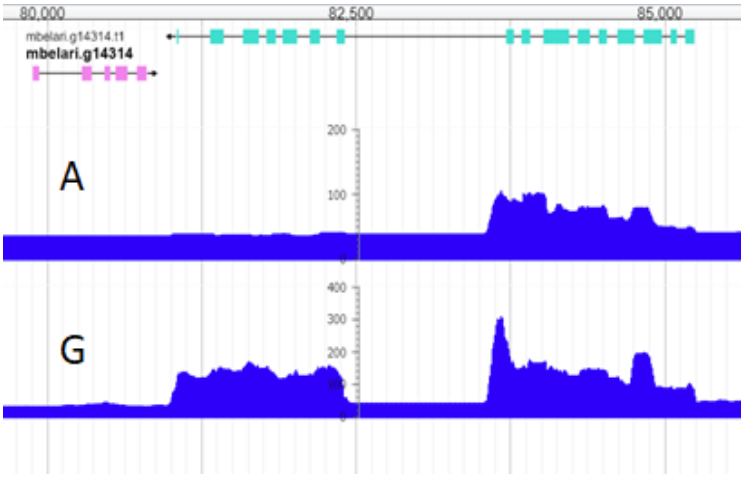


Figure 38: Coverage of RNA-seq reads of g14314 gene. G, gynogenetic; A, amphimictic.

Validation of the 15 candidate genes

Among the 15 genes selected, eight genes were more expressed in gynogenetic than amphimictic eggs ("up" in the text; g3500; g12534; g1126; g23490; g17609; g8245; g25532 and g14314, Table 2) and seven genes were found more expressed in amphimictic than in gynogenetic eggs ("down" in the text; g4082; g3464; g17482; g4955; g21312; g3341 and g19315, Table 2). I first validated the sequence of each candidate by PCR joined to Sanger sequencing. To validate the differential deposit of the maternal mRNAs, I developed two techniques in *M. belari*: smiFISH (single molecule inexpensive FISH) and single embryo RT-qPCR.

The smiFISH is a single molecule RNA FISH technique that labels each targeted molecule of mRNA (Tsanov et al., 2016). Each probe is composed of two subunits: the primary probe which is complementary to a part of the sequence of an mRNA and that possesses a common 5' extension (FLAP sequence), and the secondary probe which matches the FLAP sequence and is coupled to two fluorophores. We design probes that cannot overlap, to have a "single molecule signal", hence, there is no choice as to the number of probes per genes. The protocol by Arnaud Hubstenberger was not adapted for embryo stainings, which in worms is problematic because the eggshell must be removed by freeze-cracking to access the cells. Therefore, I adapted the protocol for the embryos (see Material and Methods). Still, I faced a

major problem which is that embryos must stick on a poly-lysine coated slide for the freeze-cracking method. However, for a reason that I have not been able to solve, the stickiness of embryos was very low after the smiFISH procedure, most likely due to the specific buffers used, and very few embryos were present on the slide at the end of the process. Because I wanted to compare gynogenetic eggs to amphimictic eggs, which are very rare, I often failed to conclude because I lost the amphimictic eggs.

As a control gene to establish the technique, I used the *scc-1* gene (the ortholog of the *C. elegans* mitotic cohesin). I chose this gene because it was highly expressed in our transcriptomic data and because the gene is quite long which allowed me to design 30 probes. As shown in Figure 39, the transcripts are well detected for this gene in young embryos and in the germline, each dot corresponding to one mRNA molecule. Due to the complexity of the experiment, I was able to acquire only a few oocytes per gene, with many genes showing no signal at all (g3500, g14314; g21312, g3464; g23490). A signal was detected in three up-regulated genes (g12534; g1126; g17609; Figure 39) and three down-regulated genes (g3464; g4082; g4955; g17482, Figure 39).

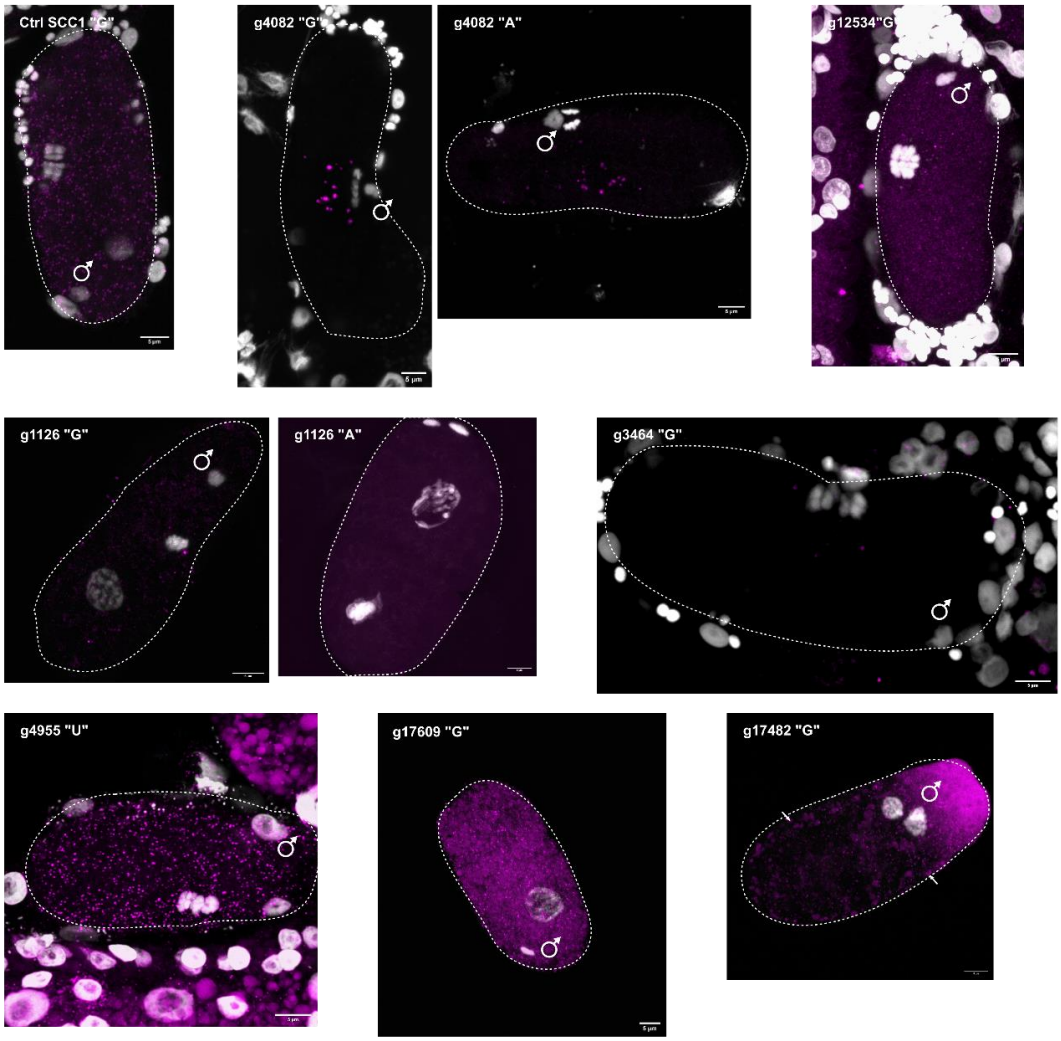


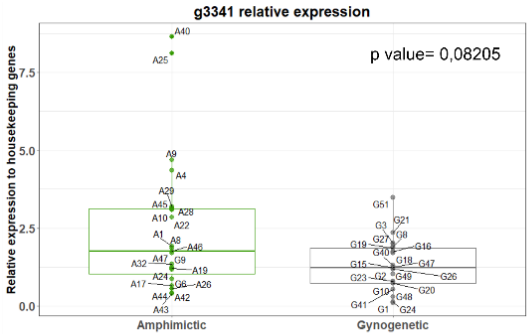
Figure 39: smiFISH of seven candidate genes. Each pink dot is a molecule of mRNA of the concerned gene. *scc-1* is a control gene. Sperm DNA is present in gynogenetic ("G") oocytes. Arrows show the polar body. The category of eggs is determined depending on the number of polar bodies produced and/or the decondensation of the sperm DNA. G, gynogenetic; A, amphimictic, U, undetermined category. mRNA probes are in pink and DNA is in grey.

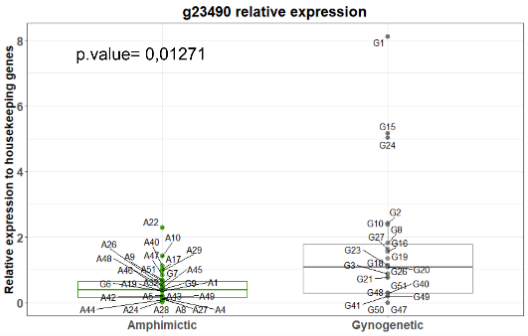
For the gene g1126, gynogenetic oocytes present more mRNAs molecule than amphimictic ones. Therefore, this confirms that those genes are upregulated in gynogenetic oocytes. There were not enough images acquired for g4082 to determine a preferential expression. For the other genes (g12534; g4955), I could not find images of amphimictic eggs, and could not conclude. For genes g17609 and g17482 oocytes, the quantification of the signal was hampered by labelled globules on the edges of the cell membrane (arrows, Figure 38). I

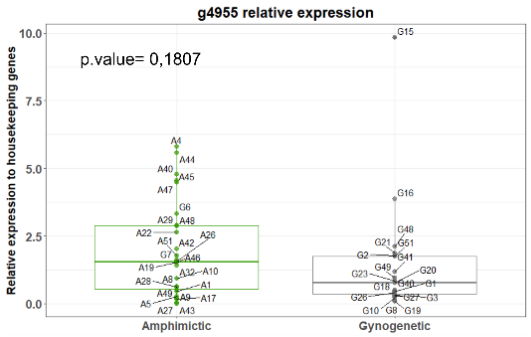
finally discovered that the bleach treatment of the eggs before the experiment was causing this artefact. Unfortunately, I had no time to repeat these experiments (see Material and Methods).

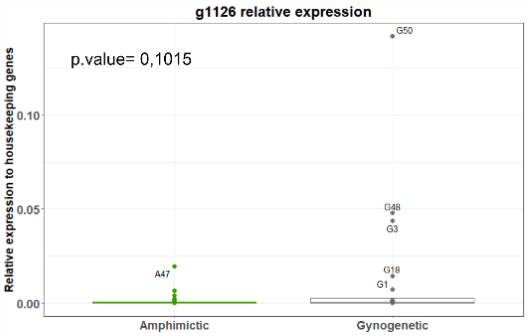
Single embryo RT-qPCR

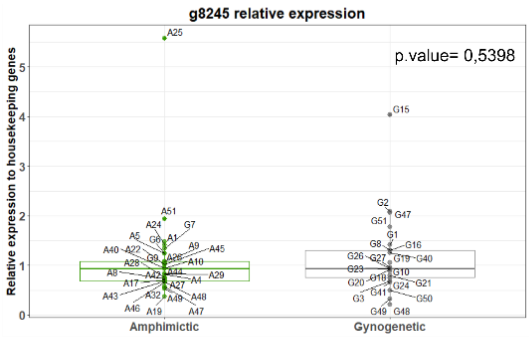
In parallel, I chose to confirm the differential expression of genes by RT-qPCR for four upregulated genes (g1126; g8245; g23490 and g3500; Figure 40) and three down-regulated genes (g17482; g4955 and g3341; Figure 40). To this end, I modified the Smart-Seq2 protocol by removing the tagmentation and the subsequent repair and purification steps (Picelli et al., 2014). Analyses were conducted on 25 single gynogenetic eggs and 26 single amphimictic eggs, which I also manually selected (as described above). I used two housekeeping genes orthologs to the *C. elegans* mitotic cohesin *scc-1* (g22258) and the *C. elegans* cyclin dependent kinase *cdk-12* g8248). After checking the validity of the two housekeeping genes (BestKeeper and geNorm (Mestdagh et al., 2009; Pfaffl et al., 2004), I used them to normalize my experiments. All genes were analysed using the 2 (-Delta Delta C(T)) method (Livak & Schmittgen, 2001). Among the seven genes analysed only g23490 was significantly differently expressed between gynogenetic and amphimictic oocytes (Wilcoxon test p-value = 0.01271 Figure 40). As expected g23490 is more expressed in gynogenetic oocytes than in amphimictic ones. For g3500, the non-significant result may be explained by an outlier “A25” (amphimictic n°25) that is also aberrant for the genes g3341, g8245, g17482 and g1126 (Figure 40).

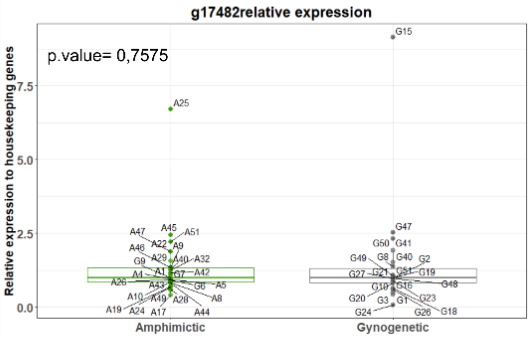

g3341 relative expression
p value= 0,08205
Relative expression to housekeeping genes
Amphimictic
Gynogenetic


g23490 relative expression
p.value= 0,01271
Relative expression to housekeeping genes
Amphimictic
Gynogenetic


g4955 relative expression
p.value= 0,1807
Relative expression to housekeeping genes
Amphimictic
Gynogenetic


g1126 relative expression
p.value= 0,1015
Relative expression to housekeeping genes
Amphimictic
Gynogenetic


g8245 relative expression
p.value= 0,5398
Relative expression to housekeeping genes
Amphimictic
Gynogenetic


g17482relative expression
p.value= 0,7575
Relative expression to housekeeping genes
Amphimictic
Gynogenetic

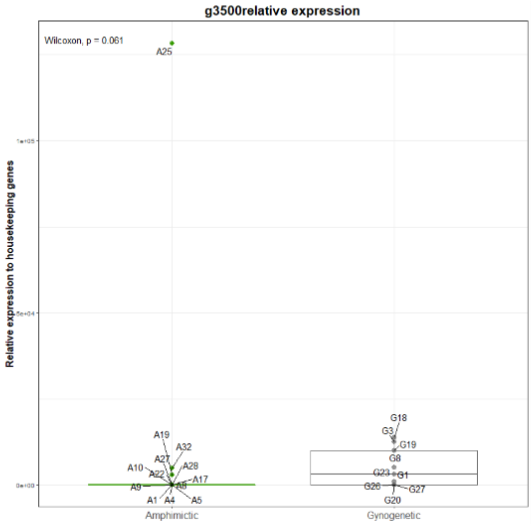

g3500relative expression
Wilcoxon, p = 0.061
Relative expression to housekeeping genes
Amphimictic
Gynogenetic

Figure 40: RT-qPCR of seven candidate genes. The relative expressions are normalized by two housekeeping genes *scc-1* and *cdk-12*. Each dot corresponds to one embryo. Gynogenetic (G) are grey and amphimictic (A) are green. The p-value obtained with a Wilcoxon test.

Functional analysis

Because the validation by smiFISH or RT-qPCR was more complicated than expected, I decided to go on with functional validation, regardless of these results (Table 3).

N°	Gynogenetic > Amphimictic			Gynogenetic < Amphimictic		
	Candidate gene	smiFISH Validation	RT-qPCR Validation	Candidate gene	smiFISH validation	RT-qPCR Validation
1	g3500	No signal	Not confirmed	g4082	Can not evaluate	
2	g12534	Can not evaluate	Not confirmed	g3464	No signal	
3	g23490	No signal	**Confirmed**	g17482	Can not evaluate	Not confirmed
4	g14314	No signal		g4955	Can not evaluate	Not confirmed
5	g1126	**Confirmed**		g21312	No signal	
6	g17609	Can not evaluate		g3341	Not tested	Not confirmed
7	g8245	Not tested	Not confirmed	g19315	Not tested	
8	g25532	Not tested				
Total	8			7		

Table 3: Summary of the results for candidate genes. Two techniques were used: smiFISH and RT-qPCR. The orange colour highlights the seven validated genes: g23490 and g1126

I made the hypothesis that the inactivation of the genes involved in the amphimictic/gynogenetic decision should affect the early embryo development, for instance, because there is no more coordination between the execution of meiosis and the fate of the paternal DNA. Thus, I tested the viability of embryos after the inactivation of the candidate genes. It could also be that the embryos are viable but more (or less) individuals develop as males. Therefore, I also examined the sex ratio after the inactivation of the candidate genes.

In the lab, we had shown that some genes could be easily inactivated by RNAi in *M. belari*. After the microinjection of double-strand RNA in the gonad of females, the F1 progenies showed a mutant phenotype. It was the case for instance for the well-conserved nematode gene *unc-22*, which causes a twitcher phenotype. I could also inactivate very efficiently three genes involved in the formation of the permeability barrier which protects the eggs (the *perm* genes, *perm-1, perm-2 and perm-4*, González et al., 2018; Olson et al., 2012). Of note, the RNAi was

so efficient for these genes that the embryos were dying because of osmotic shocks. I, therefore, started to inactivate the genes of interest by RNAi. I injected adult females with dsRNA and assessed lethality and sex ratio in the F1 generation. Lethality was only observed for RNAi against g12354 (Table 4).

As the experiment was ongoing, other members of the team were testing the inactivation of

Genes		N Injected Animals	N Animal have Laid	phenotype	Concentration of RNAi
Gyno > Amphi	g23490	46	9	None	
	g3500	33	11	None	100ng/ul
	g14314	29	12	None	70ng/ul
	g12534	25	17	Growth delay and lethality	70ng/ul
	g1126	23	14	Virgin F1	27,8 ng/uL
Gyno < Amphi	g4082	26	11	None	100ng/ul

Table 4: Summary of RNAi injection for six differential expressed genes.

other genes of *M. belari* by RNAi. Surprisingly, they found that many highly conserved genes, which had very clear phenotypes in *C. elegans*, showed no phenotype in *M. belari*. This was the case for instance for the *par* genes. This strongly suggested that some genes cannot be inactivated by RNAi in *M. belari*, either because of redundancy between genes, because of the strong stability of the protein or because of some species-specificity of the RNAi machinery. Because of this uncertainty, I did not pursue these RNAi experiments.

As explained in Chapter 4, I developed in parallel the CRISPR/Cas9 technique in *M. belari*. Therefore, I tried to generate knockout mutant lines for five differentially expressed genes: g1126; g14314; g23490; g4082 and g3341. We targeted the first or second exon of each gene to produce hypomorphic genes that have completely lost their function. To control that the CRISPR/Cas9 was operational, we performed co-CRISPR experiments with the *unc-22* gene. Mutations in *unc-22* induce a macroscopic phenotype that is easy to screen (as shown with the RNAi approach above). Females are injected with a mix containing the Cas9 protein, a guide RNA against unc-22 and a guide RNA against the gene of interest. Only females giving rise to UNC-22 F1s are maintained and further analysed because they prove that Cas9 was efficient in these gonads. The expected phenotypes for the genes of interest (embryonic lethality and/or modified sex ratio) were screened in the F2s. In this way, we avoid any maternal effects that might be present in F1, i.e. the deposit of wild type maternal mRNA in oocytes that produce functional proteins. Of the five genes tested, only the g1126 experiments showed a lethal phenotype (Figure 41). Indeed, in several independent lines, we quantified many dead embryos

in F2. Nonetheless, Sanger sequencing of the g1126 in the F1 females generating these dead embryos did not reveal any mutations in the targeted gene. A possible explanation of the phenotype could be that the mutation did not affect the F1 females but the F1 males, generating a paternal effect. This would have been a very interesting result but unfortunately, I did not have time to repeat this experiment to confirm this hypothesis.

For the other genes, it could be that mutations in the genes do not produce the expected macroscopic phenotypes. However, because I did not systematically sequence the F1s in these experiments, I cannot conclude on the phenotypes because I don't even know if the genes have been really inactivated.

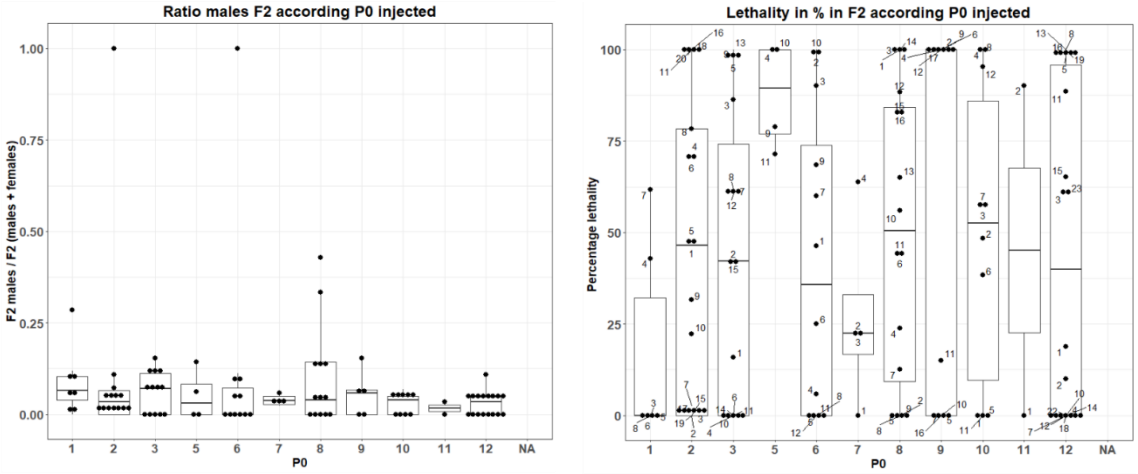


Figure 41: Knock-Out results for g1126 Each dot represents a box where one F1 lays.

Conclusion

In this project, I successfully validated three genes that are differentially expressed in gynogenetic and amphimictic eggs. Indeed, g23490 and g1126 are more expressed in gynogenetic oocytes (Table 3**)**. My first attempt to inactivate g1126/*swm-1* by CRISPR/Cas9 is encouraging but needs to be repeated. This gene is of particular interest because its ortholog in *C. elegans* is normally expressed only in males, and is involved in sperm DNA decondensation. More work will be required to confirm its involvement in controlling paternal DNA in gynogenetic eggs of *M. belari*.

Chapter 4: Establishment of the CRISPR/Cas9 technique in *M. belari*

Introduction

In this chapter, we discuss the importance of developing gene editing tools in non-model species and the difficulties encountered. *M. belari* is a fascinating species. To investigate in depth how female meiosis is modified, we need to be able to modify genes to inactivate them, to replace them, or fuse them with GFP for instance. For that, we need to develop genetic tools. The CRISPR/Cas9 technique is a powerful genome editing technology that proved its efficiency in many model species. However, this technique remains difficult to implement in new species because, for each species, optimization is required.

In this method, the Cas9 protein is coupled to a specific guide RNA to form the Cas9 complex. This complex, once in the cell, allows sequence specific binding (through the guide RNA) and double strand break of the DNA (through the Cas9 enzyme). Repair of the double strand break relies on universal and highly conserved mechanisms: repair by Non-Homologous End Joining (NHEJ) or repair by Homologous Repair (HR). Upon NHEJ, small mutations or indels can be generated, or specific insertions can be made in the case of HR. Therefore, genome editing with the Cas9 complex can be considered universal. However, a major constraint for the technique is the transport of the Cas9 complex into the cell. The transport of the complex differs depending on the species. In Drosophila for instance, the young syncytial embryo can be harvested and microinjected. In other species, the transfer requires electroporation of cells, or microinjection of the fertile adult. In cell culture, the Cas9 complex is often delivered by virus-like particles. In *C. elegans*, the complex is microinjected in the syncytial gonad of the females. Hence, the complex is easily transported inside the germ cell nuclei, each giving rise to a potential mutant progeny. In the nematodes *Auanema rhodensis* and *Pristionchus pacificus*, addition of lipofectamine to the CRISPR Cas9 mix is necessary before microinjection in the gonad, potentially because it helps the transport through cell membranes (S. Adams et al., 2019).

The gonad of *M. belari* females is not syncytial, which should prevent the diffusion of the Cas9/CRISPR complex. Although *M. belari* males have a syncytial gonad, they do not transfer any genes to the females. Therefore, they cannot be used for transgenesis.

I have tested several strategies and finally established the CRISPR/Cas9 technique for gene knock-out in *M. belari* females. The results are presented in the section below.

Results

HEPES: a CRISPR-Cas9 courier in nematodes

Introduction

The CRISPR-Cas9 system is currently the most widely used genome editing tool (Adli, 2018; Doudna & Charpentier, 2014; Gao, 2018). The CRISPR-Cas9 complex is composed of a Cas9 endonuclease, which generates DNA double-strand breaks, and two small RNAs: the CRISPR RNA (crRNA) which is complementary to the target sequence and the trans-activating CRISPR RNA (tracrRNA) that binds to both crRNA and Cas9 protein. This system, which provides sequence specificity of DSB and hence repair, has been successfully used in various organisms, including the model organisms *Caenorhabdits elegans, Drosophila melanogaster, Mus musculus*, in yeasts and *human cells*, but also the squid (*Doryteuthis pealeii*), the butterfly (*Bicyclus anynana*) or the raider ants (*Ooceraea biroi*) (Cho et al., 2013; Cong et al., 2013; Crawford et al., 2020; DiCarlo et al., 2013; Dickinson et al., 2013; Ding et al., 2013; Gratz et al., 2013; Jinek et al., 2013; Mali et al., 2013; Trible et al., 2017, 2017; Wang et al., 2013; Zhang et al., 2017). Initially, researchers used plasmid-based expression of the Cas9 protein in order to facilitate the incorporation of the CRISPR-cas9 complex into the target cell. However, this method is not applicable to all species. Fortunately, the development of injection using purified Cas9 protein and the establishment of in vitro synthesis of guideRNA has allowed the establishment of the CRISPR system in more species such as *Clytia hemisphaerica*, the zebra fish and *Gryllus bimaculatus* (Bai et al., 2023; Bono et al., 2015; Hruscha & Schmid, 2015; Munro et al., 2023). Nevertheless, for some species, delivery is a major issue and has prevented the use of the CRISPR-Cas9 system for genome editing. Collecting young embryos and/or injections inside embryos can be challenging. The gonad is also not often accessible for targeted microinjections. Even in the model species *C. elegans*, microinjection requires specific skills and high precision which has prevented the automatization of microinjection, despite the generalization of the CRISPR-Cas9 technique for genome engineering. In addition, Cas9 endonuclease creates double-stranded Breaks (DSB) that can be repaired by Non-Homologous-End-Joining (NHEJ), a repair mechanism generating random mismatches of small indels, or Homologous Direct Recombination (HDR), a precise repair mechanism (Lieber, 2010). Whereas NHEJ repair occurs at any point in the cell cycle, HDR repair takes place mainly

during the S or G2 phases of the cell cycle (Liu et al., 2019). Hence, genome engineering by CRISPR-Cas9 remains limited to a limited number of species.

In the model species *C. elegans*, microinjections consist in injecting the CRISPR Cas9-guide RNA complex directly into the distal part of the syncytial gonad: guide RNA, purified Cas9 protein and the repair in the case of knockin experiments. Such an approach should theoretically be applicable to other nematode species, except that some species do not have a syncytial gonad. Depending on the species, germ cells are partially or entirely cellularized during meiotic prophase (Fig. 1). Microinjection into these types of gonads leads at best to one injected cell and in the worst case to partial destruction of the gonad.

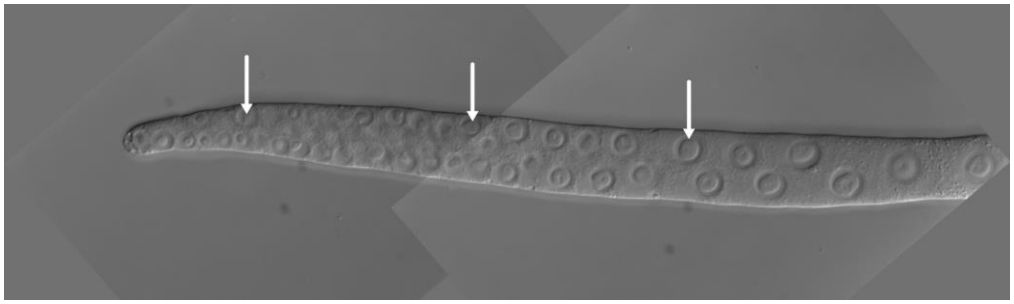


Figure 1: DIC image of cellularized gonad of *M. belari* female. Arrows show some oocytes.

We wished to establish CRISPR-Cas9-based genome editing in the nematode M. belari, whose female gonad is not syncytial. Our first attempt to generate mutants by CRISPR where therefore unsuccessful. Previous work performed on several other nematode species showed that the addition of lipofectamine to the Cas9-guide RNA complex improves the efficiency of genome editing (Adams et al., 2019; Dockendorff et al., 2022). In our hand, however, lipofectamine was ineffective on *Mesorhabditis belari*. Because lipofectamine is a nucleic acid rather than a protein carrier, we proceeded to test other macromolecular carriers, usually used for protein transfection in human cells, in the hope to facilitate the diffusion of the Cas9-guide RNA complex through plasma membranes. Inspired by the works of Chen (Chen et al., 2019)), who showed that a high concentration of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) facilitates protein transfection in human cells, we tested the impact of HEPES in *M. belari* gonads. We found that adding a minimum of 20 mM HEPES to the Cas9-guide RNA complex is necessary and sufficient to generate a CRISPR-based knock-out (KO) mutant in a nematode species with a cellularized gonad. We next tested the HEPES-assisted transport of

the Cas9-guide RNA complex by microinjecting *C. elegans* outside its syncytial gonad. Microinjection of a guide RNA/Cas9 complex near the pharynx of *C. elegans* allowed us to obtain mutants in the F1 progeny, provided 20 mM HEPES was added to the injection mix.

Our work reports strong evidence that a high concentration of HEPES allows a guide RNA/Cas9 complex to cross cell membranes in some nematode species and hence allows genome editing regardless of injection skills and gonad organization. Our findings open the way to the automatisation of microinjection in *C. elegans* and also offer a tool for the generation of CRISPR mutants in other non-model species.

Materials & Method

Strains and Culture

We used *C. elegans* N2 strains and *M. belari* JU2817 (Grosmaire et al., 2019). Both species were grown at 20°C on Nematode Growth Media (NGM) seeded with *Escherichia coli* OP50-1 strain, following standard procedures for *C. elegans* (Stiernagle, 2006).

CRISPR editing in C. elegans and M. belari

All CRISPR-Cas9 components (crRNAs, tracrRNAs, IDTE #11-05-01-05, IDT Duplex buffer #11-05-01-03 Cas9, Cat#1081058) were furnished by IDT DNA (Coralville, IA). The guides were designed to be 20nt long and have a GC content between 50 and 75%. We favored PAMs with GGNGG or GNGG sequences. To target the *M. belari unc-22* gene (mbelari.g26112), two guides separated by 85nt (distance between PAMs) were designed on the forward strand of the 96th exon (5'-GAGAGCTGCCGGAAAGCACG-3' and 5'-TGGAGAGTACACATGTGAGG-3'). To target *dpy-1* in *C.elegans*, only one guide was designed on the reverse strand (5'-GCTACCATAGGCACCACGAG-3') on the third exon, as described in (Paix et al., 2014, 2015)

Microinjections of antibody and of the CRISPR complex

Antibody injections

Females were injected with a secondary antibody conjugated with Cy3 (λ emission: 565 nm, Jackson ImmunoResearch, approximately 160 kDa, #711-165-152). The antibody was either diluted in water at a final concentration of 0.7 mg/ml or diluted in HEPES (20 mM final concentration) at a final concentration of 0.56mg/mL. After injection, worms were immediately placed on an agar pad 2% in M9 and images were taken with a fluorescent Zeiss A1 microscope, equipped with a Kappa camera.

CRISPR injections

Firstly a guide RNA was prepared by mixing 3µL of tracrRNA (100 µM, Sigma) and 3µL of crRNA (100 µM) in 4 µL of Duplex Buffer (IDT) and placed 5 min at 95°C followed by 45min at RT (20-25°C). Before the injections, 2.5 µL of the guide RNA was added to 0.5µL Cas9 purified protein (10mg/mL, Sigma CAS9PROT-50UG) and the mix was heated for 10

min at 37°C. In the case of *C. elegans* knock-in, 1 µL of ssDNA repair (250 µM) was added to the mix before being placed for 20 min on ice. To increase the HEPES concentration, 1 µl of 80 mM HEPES was added to the mix, to obtain a final concentration of 20mM. In all cases, the final mix was centrifuged for 10 min at 4°C before filling the needle. Importantly, without the addition of HEPES to the mix, the basal concentration of HEPES is 18mM because it is already present in the Duplex buffer and the Cas9 buffer.

Microinjections

Microinjections of *C. elegans* and *M. belari* were conducted using a FemtoJet 4i and an InjectecMan 4 Manipulator (Eppendorf). Gravid adult females were placed on a dried agarose pad 3% (w/v) and covered with Halocarbon oil 700 (Merck Sigma-Aldrich, 9002-83-9) to avoid worm desiccation (as described in (Evans, 2006). Femtotips II microcapillary needles (Eppendorf) were filled with 2 µL of CRISPR or antibody mix (see above). Depending on the experiments, females were injected either towards the gonad in the case of *M. belari*, inside the syncytial gonad in the case of *C. elegans*, or near the pharynx using an input gas pressure of 900 hPa balance pressure. After injection, animals were transferred to a fresh NGM plate seeded with OP50 by pipetting the worms in a small amount of M9 buffer. For each injected animal, the F1 progeny was screened for the appearance of DPY or UNC/twitching worms, depending on the targeted gene.

Image acquisition

As with immunostaining, confocal airyscan images were acquired using the Zeiss LSM800 Airyscan and LSM980 Airyscan using a 63x oil objective and 0.15µm interval between slides. Images were subsequently processed by the airyscan processing method (Blue 2., 3D analysis, automatic low stringency). Processed images were treated using ImageJ 1.53t software

Results

HEPES allows the diffusion of large proteins into the cellularized gonad of M. belari females

Secondary antibodies conjugated with fluorochromes have a comparable molecular weight (approximately 150kDa) to that of the Cas9 protein (163kDa). Hence, to evaluate the capacity of HEPES to facilitate the delivery of the RNA-Cas9 complex, we microinjected fluorescent secondary antibodies in *M. belari* females. Because the autofluorescence of *M. belari* worms is strong in the green and blue wavelengths, we choose a secondary antibody coupled to the red Cyanine3 (λ emission: 565 nm). Females were injected either near the gonad or around the pharynx, with a pure solution of antibody (at 0.7 mg/ml in HEPES 0 mM) or with antibodies diluted in HEPES (at 0.56 mg/ml in 20 mM HEPES). In contrast to human cultured cells where transfection requires 4-6 hours, the results of microinjections in nematodes are measurable within minutes. In the absence of HEPES, the red signal from the antibody was detected only at the site of microinjection (Fig. 2A, Fig. 2C). In the presence of HEPES, the antibody signal was detected in the entire worm, regardless of the site of microinjection (Fig. 2B, Fig. 2D). Therefore, the addition of HEPES helps the diffusion of 150 kDa proteins through the cell membranes of *M. belari*.

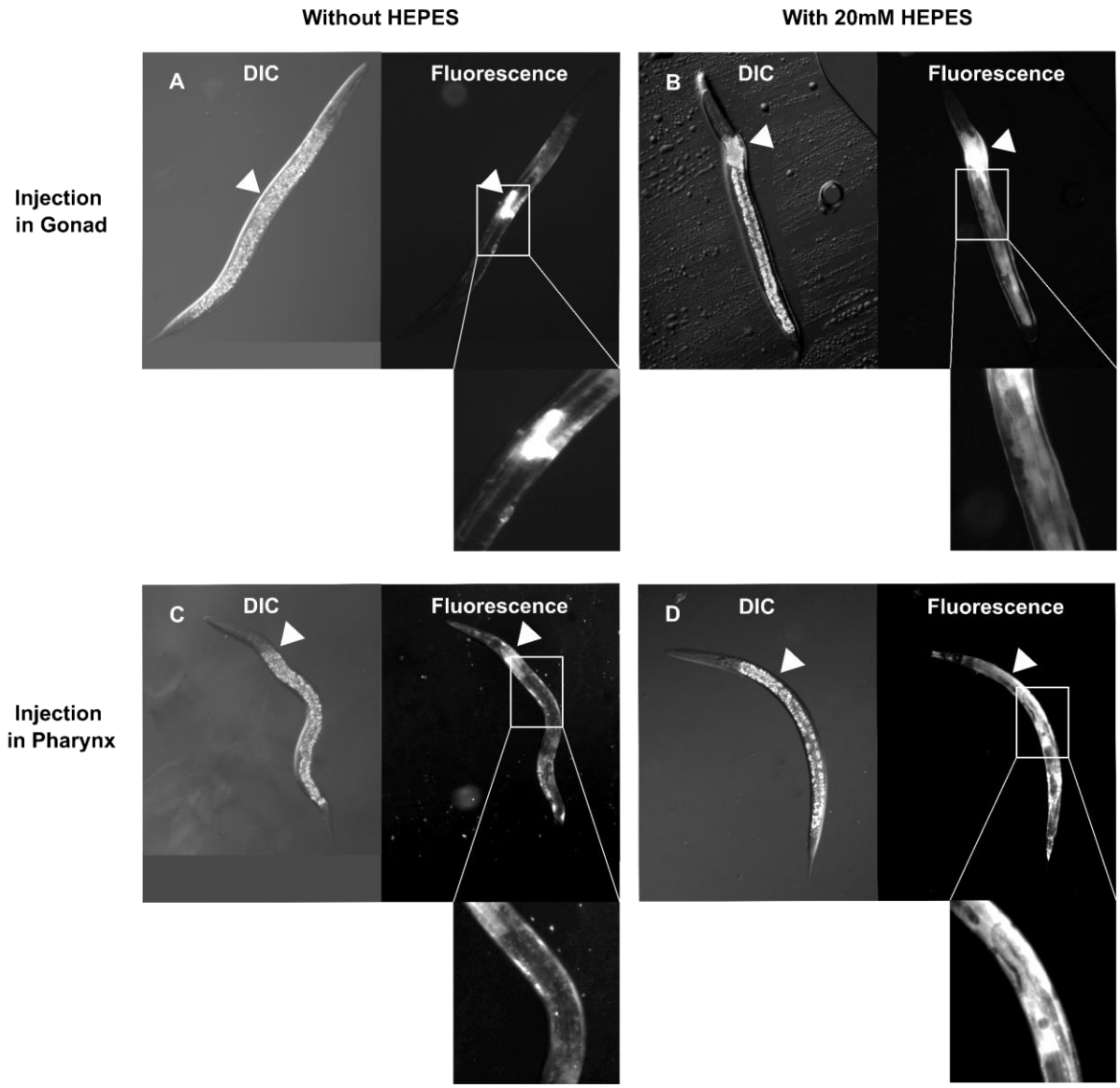


Figure 2: Images of *M. belari* females after microinjection of a fluorescent antibody in the presence or absence of HEPES. The triangles show the microinjection site. Gonads are stained only with the addition of HEPES. The inset in for D) shows an oocyte stained filled with the antibody in the cytoplasm (and not the nucleus)

HEPES is necessary for CRISPR-Cas9-based gene knock-out in M. belari

We next tested the possibility that HEPES could help the delivery of the guide-RNA Cas9 complex inside *M. belari* oocytes, despite the complete cellularisation of the gonad. We choose to target a gene whose knock-out should give a visible locomotion phenotype, by analogy with *C. elegans* and other nematodes. Homozygous mutants for the *unc-22 gene* in *C. elegans* display a characteristic phenotype of twitching worms (Moerman & Baillie, 1979). The

unc-22 gene is largely conserved in nematodes of the Rhabditidae family and twitchers have also been obtained after the knock-down of *unc-22* in both *Auanema rhodensis* and *A. freiburgensis* (Adams, et al. 2019). We thus hypothesized that the knock-out of *unc-22* in *M. belari* should also generate twitcher progenies. We targeted the M. belari putative unc-22 ortholog (mbelari.g26112) with two guideRNA. Injected females were isolated on a plate and the F1 and F2 progenies were observed. Without the addition of HEPES to the guide-RNA Cas9 mix (final concentration of HEPES 38 mM), injection in the vicinity of the gonad or the pharynx did not retrieve any UNC phenotypes in the progeny (34 animal injected, 1539 F1s screened) (Table 1). The absence of mutations was confirmed by randomly sequencing 7 F1s for a 693nt locus around the targeted PAM site. By contrast, after the addition of HEPES to the guide RNA Cas9 mix (final concentration of HEPES 20 mM) we found twitcher F1s in the progeny of 10 injected females, out of 97 injections. We confirmed the specificity of the knock-out by sequencing the *unc-22* locus in some twitcher F1s. We sequenced 17 twitchers that all presented mutations in the vicinity of the targeted PAM, generating a frameshift in the *unc-22* sequence (Fig. S1). Among them, 9 twitchers had two distinct mutations on both chromosomes while 8 presented the same mutation. Hence, twitcher F1s are bi-allelic mutants. For these 10 injected females, 16% of their F1s were twitchers (Table 1), demonstrating the efficiency of CRISPR-Cas9 in *M. belari*. Importantly, in this experiment we underestimated the efficiency of genome editing by referencing exclusively the twitchers in F1s, meaning the animals with biallelic mutations, and not the heterozygote mutants.

	P0 fertile (>10 F1)	**P0 with F1 twitcher**	**F1 twitcher total**	**F1 twitcher in twitcher box**
Without HEPES	34	0	0% (0 out of 1539)	ND
With HEPES	97	10	2.55% (72 out of 2815)	16.10% (72 out of 447)

Table 1: Count of twitchers found in F1s *M. belari*

Our result demonstrates that increasing the concentration of HEPES in the guide RNA Cas9 mix is necessary and sufficient to allow genome editing in a nematode with a cellularized gonad.

HEPES allows CRISPR-Cas9-based knock-out in C. elegans following microinjection in the head

To determine whether the addition of HEPES could facilitate the delivery of the CRISPR-Cas9 complex in *C. elegans* too, we deliberately microinjected a guide-RNA Cas9 mix outside the gonad and searched for mutants in the progeny of the injected animals. Here we choose to target the *dpy-10* gene which encodes a collagen protein and is involved in the development of the cuticle (Levy et al., 1993). D*py-10* mutants show a severe phenotype of dumpy worms and have been used many times as a target gene to improve the CRISPR-Cas9 technique *C. elegans*. We designed the same guide RNA as those described in (Paix et al., 2014, 2015).

First, we performed a control experiment and microinjected the dpy-10 guide-RNA Cas9 mix into the syncytial gonad of *C. elegans* without additional HEPES buffer. We screened the progeny for the presence of DPY animals in F1s (bi-allelic or homozygous mutants) or the F2s (from heterozygous mutants F1s). Forty-eight per cent of the 28 microinjected females produced some DPY progeny (Table 2, 1723 F1s and 6200 F2s scored). Microinjection of the same mix supplemented with HEPES inside the gonad had no impact on the efficacy of gene editing, with 34.7% of the 20 injected females producing DPY progenies. We then microinjected the guide RNA-Cas9 mix near the pharynx with or without the addition of HEPES. We found that in the absence of added HEPES, none of the 28 injected females gave DPY progenies (1660 F1s and 35550 F2 scored). However, injection of the same mix supplemented with HEPES near the pharynx dramatically increased the percentage of dumpy offspring: 18,4% of the 28 females injected gave rise to F1 or F2 mutants (respectively 1.27% and 0.86%, Table 2).

Hence, similar to our results obtained for *M. belari*, we show that HEPES helps the diffusion of the guide RNA-Cas9 complex through the cell membranes in *C. elegans*. This result further demonstrates that precise microinjection inside the syncytial gonad of *C. elegans* is not an absolute requirement to obtain knock-out mutants, provided the injection mix contains a minimum of 20 mM HEPES.

Type of Injection	N injected	% P0 with dpy progeny	% F1 Dpy	% F2 in dpy F1 box	% F2 in non-dpy F1 box	% F2 dpy
Pharynx without Hepes	28	0	0 (out of 1660)	0 (out of 3503)	0 (out of 3503)	0 (0 out of 3503)
Pharynx with Hepes	27	18.42	1,27 (15 out of 1180 F1)	1,05 (47 out of 4489 F2)	2,36 (106 out of 4489 F2)	0,86 (153 out of 1787)
Gonad without Hepes	28	48.39	8.94 (154 out of 1723 F1)	7,69 (477 out of 6200 F2)	1,87 (116 out of 6200 F2)	1,48 (593 out of 39938)
Gonad Hepes	20	34.72	8,11 (71 out of 875 F1)	0,85 (34 out of 4007 F2)	2,10 (84 out of 4007 F2)	1,64 (118 out of 7182)

Table 2: Influence of the presence of HEPES on the CRISPR success in *C. elegans*.

Discussion

HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) is a zwitterionic compound brought to light by Good's laboratory in the 1980s (Good et al., 1966). Ever since this molecule has been used extensively as a buffer in biochemical research or more specifically as a physiological buffer in cell culture because it stabilises the pH. More recently, the Wang lab discovered that HEPES could also be an asset in protein delivery (Chen et al., 2019). They demonstrated that the addition of 20mM to a transfection mix is sufficient to facilitate the transport of large molecules, such as antibodies (approximately 150kDa) across the plasma membrane of mammalian cells. According to their work, HEPES allows the internalization of proteins by modifying the charge of these proteins, thus promoting endocytotic absorption. In agreement with their findings, our results show that HEPES also enables the cellular internalization of antibodies in living animals. We successfully proved that 20 mM HEPES is sufficient to ensure protein incorporation into oocytes, whether the mixture is injected directly into the gonad or completely outside the gonad, in the pharyngeal region.

To go further, we demonstrated that HEPES facilitates not only the delivery of large proteins but also RNA-protein complex to pass the cytoplasmic membranes of living nematodes. We have shown that with the addition of HEPES, knock-outs can be achieved in nematodes whether their gonads are syncytial or fully cellularized from the beginning of gametogenesis. In *M. belari*, which has a completely cellularized gonad, we successfully obtain 16% mutants in F1s (Table 1). Nevertheless, we scored only the F1s presenting a twitcher phenotype. This phenotype is induced by bi-allelic mutations (one mutation for each homologous chromosome). We missed the F1s animals harboring mutations on a single chromosome because we did not score the or did not sequence the non-twitcher F1s. Thus, we underestimated the efficiency of CRISPR Cas9. Therefore, we estimate that the CRISPR yield is superior to 16% of F1 which is comparable to *unc-22* knock-outs in other non-model nematode species, such as *Panagrolaimus* PS1159 (Hellekes et al., 2023) but lower than the *Auanema* species that reached 100% when injected with lipofectamine in Adams' work (Adams, et al. 2019).

In the model species *C. elegans*, we obtained both knock-outs when microinjected outside the gonad. So far, in nematode species with syncytial gonads, microinjections had to target the distal part of the gonads where germ cells are not cellularized. In *C. elegans*, we have shown that injection inside the gonad remains more efficient than injections outside the gonad.

Nevertheless, our results show that it is no longer necessary to have special microinjection skills to obtain a decent yield of mutants in the progeny, provided HEPES is added to the mix.

All these findings presented here, bring us to a simplification of the CRISPR cas9 microinjection methods, making them easier and more efficient. Thus, our discovery opens the way to full automatization of microinjection in *C. elegans*.

In summary, the addition of HEPES is the key factor in the successful delivery of the CRISPR Cas9 complex in both model and non-model nematode species. It remains to be tested whether this technique can also be applied to other species, nematodes and others.

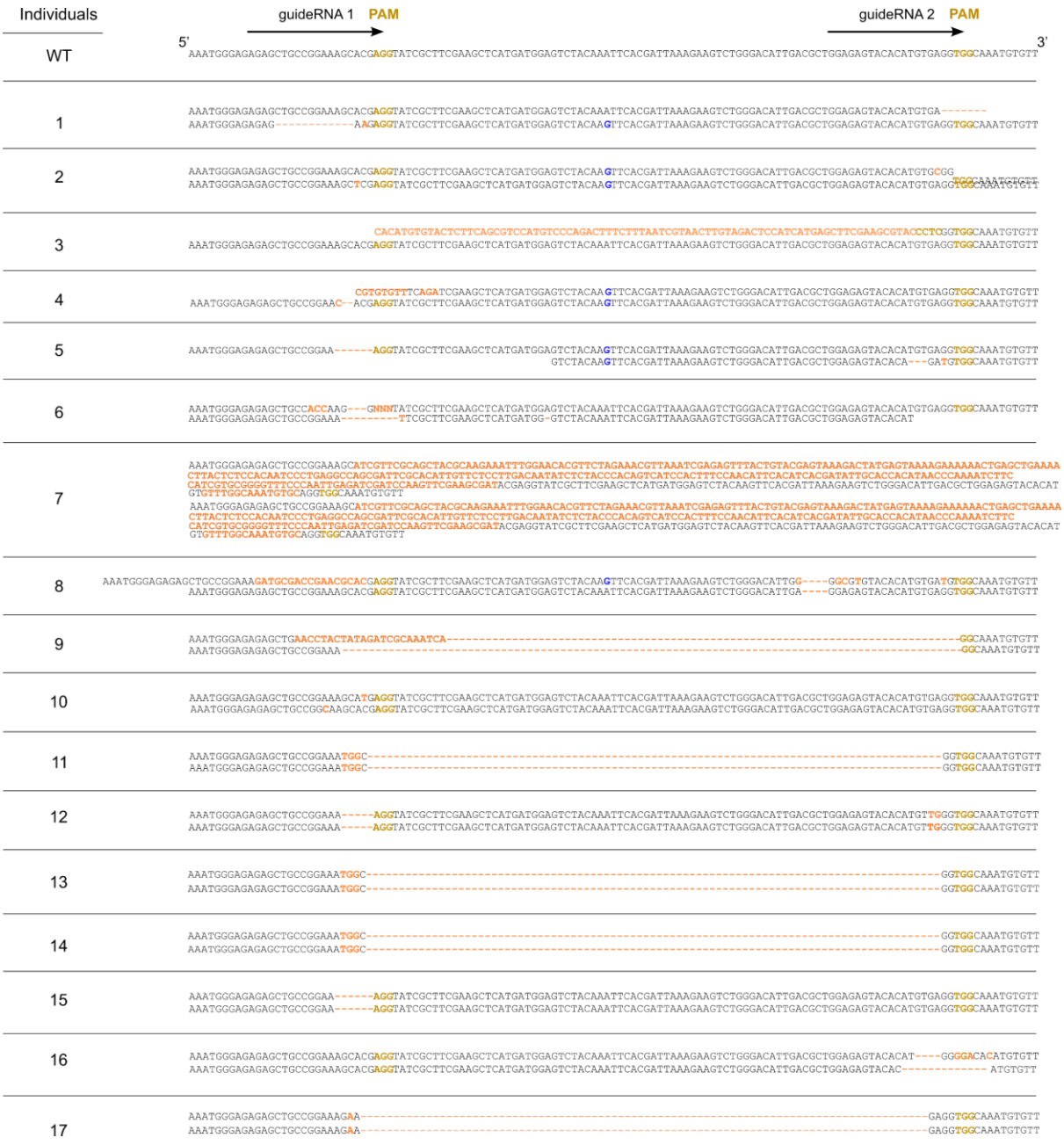


Figure S1: Sequencing alignment of 17 F1 twitchers. For each 17 individuals both chromosomes are represented. Individuals 1-4 are from the same mother (P0). Individuals 5-17 are from the same mother (P0). Individual n°3 present a inversion. PAM sequences are in yellow, mutations in orange and polymorphic site in blue.

References

Adams, S., Pathak, P., Shao, H., Lok, J. B., & Pires-daSilva, A. (2019). Liposome-based transfection enhances RNAi and CRISPR-mediated mutagenesis in non-model nematode systems. *Scientific Reports*, *9*, 483. https://doi.org/10.1038/s41598-018-37036-1

Adli, M. (2018). The CRISPR tool kit for genome editing and beyond. *Nature Communications*, *9*(1), Article 1. https://doi.org/10.1038/s41467-018-04252-2

Bai, Y., He, Y., Shen, C.-Z., Li, K., Li, D.-L., & He, Z.-Q. (2023). CRISPR/Cas9-Mediated genomic knock out of tyrosine hydroxylase and yellow genes in cricket Gryllus bimaculatus. *PLOS ONE*, *18*(4), e0284124. https://doi.org/10.1371/journal.pone.0284124

Bono, J. M., Olesnicky, E. C., & Matzkin, L. M. (2015). Connecting genotypes, phenotypes and fitness: Harnessing the power of CRISPR/Cas9 genome editing. *Molecular Ecology*, *24*(15), 3810–3822. https://doi.org/10.1111/mec.13252

Chen, S.-H., Chao, A., Tsai, C.-L., Sue, S.-C., Lin, C.-Y., Lee, Y.-Z., Hung, Y.-L., Chao, A.-S., Cheng, A.-J., Wang, H.-S., & Wang, T.-H. (2019). Utilization of HEPES for Enhancing Protein Transfection into Mammalian Cells. *Molecular Therapy. Methods & Clinical Development*, *13*, 99–111. https://doi.org/10.1016/j.omtm.2018.12.005

Cho, S. W., Kim, S., Kim, J. M., & Kim, J.-S. (2013). Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. *Nature Biotechnology*, *31*(3), 230–232. https://doi.org/10.1038/nbt.2507

Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P. D., Wu, X., Jiang, W., Marraffini, L. A., & Zhang, F. (2013). Multiplex Genome Engineering Using CRISPR/Cas Systems. *Science (New York, N.Y.)*, *339*(6121), 819–823. https://doi.org/10.1126/science.1231143

Crawford, K., Diaz Quiroz, J. F., Koenig, K. M., Ahuja, N., Albertin, C. B., & Rosenthal, J. J. C. (2020). Highly Efficient Knockout of a Squid Pigmentation Gene. *Current Biology*, *30*(17), 3484-3490.e4. https://doi.org/10.1016/j.cub.2020.06.099

DiCarlo, J. E., Norville, J. E., Mali, P., Rios, X., Aach, J., & Church, G. M. (2013). Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems. *Nucleic Acids Research*, *41*(7), 4336–4343. https://doi.org/10.1093/nar/gkt135

Dickinson, D. J., Ward, J. D., Reiner, D. J., & Goldstein, B. (2013). Engineering the Caenorhabditis elegans Genome Using Cas9-Triggered Homologous Recombination. *Nature Methods*, *10*(10), 1028–1034. https://doi.org/10.1038/nmeth.2641

Ding, Q., Regan, S. N., Xia, Y., Oostrom, L. A., Cowan, C. A., & Musunuru, K. (2013). Enhanced efficiency of human pluripotent stem cell genome editing through replacing TALENs with CRISPRs. *Cell Stem Cell*, *12*(4), 393–394. https://doi.org/10.1016/j.stem.2013.03.006

Dockendorff, T. C., Estrem, B., Reed, J., Simmons, J. R., Zadegan, S. B., Zagoskin, M. V., Terta, V., Villalobos, E., Seaberry, E. M., & Wang, J. (2022). The nematode Oscheius tipulae as a genetic model for programmed DNA elimination. *Current Biology: CB*, *32*(23), 5083-5098.e6. https://doi.org/10.1016/j.cub.2022.10.043

Doudna, J. A., & Charpentier, E. (2014). Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science (New York, N.Y.)*, *346*(6213), 1258096. https://doi.org/10.1126/science.1258096

Evans, T. (2006). Transformation and microinjection. *WormBook*. https://doi.org/10.1895/wormbook.1.108.1

Gao, C. (2018). The future of CRISPR technologies in agriculture. *Nature Reviews Molecular Cell Biology*, *19*(5), Article 5. https://doi.org/10.1038/nrm.2018.2

Good, N. E., Winget, G. D., Winter, W., Connolly, T. N., Izawa, S., & Singh, R. M. M. (1966). Hydrogen Ion Buffers for Biological Research*. *Biochemistry*, *5*(2), 467–477. https://doi.org/10.1021/bi00866a011

Gratz, S. J., Cummings, A. M., Nguyen, J. N., Hamm, D. C., Donohue, L. K., Harrison, M. M., Wildonger, J., & O'Connor-Giles, K. M. (2013). Genome Engineering of Drosophila with the CRISPR RNA-Guided Cas9 Nuclease. *Genetics*, *194*(4), 1029–1035. https://doi.org/10.1534/genetics.113.152710

Grosmaire, M., Launay, C., Siegwald, M., Brugière, T., Estrada-Virrueta, L., Berger, D., Burny, C., Modolo, L., Blaxter, M., Meister, P., Félix, M.-A., Gouyon, P.-H., & Delattre, M. (2019). Males as somatic investment in a parthenogenetic nematode. *Science*, *363*(6432), 1210–1213. https://doi.org/10.1126/science.aau0099

Hellekes, V., Claus, D., Seiler, J., Illner, F., Schiffer, P. H., & Kroiher, M. (2023). CRISPR/Cas9 mediated gene editing in non-model nematode Panagrolaimus sp. PS1159. *Frontiers in Genome Editing*, *5*, 1078359. https://doi.org/10.3389/fgeed.2023.1078359

Hruscha, A., & Schmid, B. (2015). Generation of zebrafish models by CRISPR /Cas9 genome editing. *Methods in Molecular Biology (Clifton, N.J.)*, *1254*, 341–350. https://doi.org/10.1007/978-1-4939-2152-2_24

Jinek, M., East, A., Cheng, A., Lin, S., Ma, E., & Doudna, J. (2013). RNA-programmed genome editing in human cells. *ELife*, *2*, e00471. https://doi.org/10.7554/eLife.00471

Levy, A. D., Yang, J., & Kramer, J. M. (1993). Molecular and genetic analyses of the Caenorhabditis elegans dpy-2 and dpy-10 collagen genes: A variety of molecular alterations affect organismal morphology. *Molecular Biology of the Cell*, *4*(8), 803–817. https://doi.org/10.1091/mbc.4.8.803

Lieber, M. R. (2010). The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annual Review of Biochemistry*, *79*, 181–211. https://doi.org/10.1146/annurev.biochem.052308.093131

Liu, M., Rehman, S., Tang, X., Gu, K., Fan, Q., Chen, D., & Ma, W. (2019). Methodologies for Improving HDR Efficiency. *Frontiers in Genetics*, *9*. https://www.frontiersin.org/articles/10.3389/fgene.2018.00691

Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., Norville, J. E., & Church, G. M. (2013). RNA-Guided Human Genome Engineering via Cas9. *Science (New York, N.Y.)*, *339*(6121), 823–826. https://doi.org/10.1126/science.1232033

Moerman, D. G., & Baillie, D. L. (1979). GENETIC ORGANIZATION IN CAENORHABDITIS ELEGANS: FINE-STRUCTURE ANALYSIS OF THE unc-22 GENE. *Genetics*, *91*(1), 95–103. https://doi.org/10.1093/genetics/91.1.95

Munro, C., Cadis, H., Pagnotta, S., Houliston, E., & Huynh, J.-R. (2023). Conserved meiotic mechanisms in the cnidarian Clytia hemisphaerica revealed by Spo11 knockout. *Science Advances*, *9*(4), eadd2873. https://doi.org/10.1126/sciadv.add2873

Paix, A., Folkmann, A., Rasoloson, D., & Seydoux, G. (2015). High Efficiency, Homology-Directed Genome Editing in Caenorhabditis elegans Using CRISPR-Cas9 Ribonucleoprotein Complexes. *Genetics*, *201*(1), 47–54. https://doi.org/10.1534/genetics.115.179382

Paix, A., Wang, Y., Smith, H. E., Lee, C.-Y. S., Calidas, D., Lu, T., Smith, J., Schmidt, H., Krause, M. W., & Seydoux, G. (2014). Scalable and versatile genome editing using linear DNAs with microhomology to Cas9 Sites in Caenorhabditis elegans. *Genetics*, *198*(4), 1347–1356. https://doi.org/10.1534/genetics.114.170423

Stiernagle, T. (2006). Maintenance of C. elegans. *WormBook*. https://doi.org/10.1895/wormbook.1.101.1

Trible, W., Olivos-Cisneros, L., McKenzie, S. K., Saragosti, J., Chang, N.-C., Matthews, B. J., Oxley, P. R., & Kronauer, D. J. C. (2017). Orco Mutagenesis Causes Loss of Antennal Lobe Glomeruli and Impaired Social Behavior in Ants. *Cell*, *170*(4), 727-735.e10. https://doi.org/10.1016/j.cell.2017.07.001

Wang, H., Yang, H., Shivalila, C. S., Dawlaty, M. M., Cheng, A. W., Zhang, F., & Jaenisch, R. (2013). One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering. *Cell*, *153*(4), 910–918. https://doi.org/10.1016/j.cell.2013.04.025

Zhang, L., Mazo-Vargas, A., & Reed, R. D. (2017). Single master regulatory gene coordinates the evolution and development of butterfly color and iridescence. *Proceedings of the National Academy of Sciences*, *114*(40), 10707–10712. https://doi.org/10.1073/pnas.1709058114

Conclusion

In this chapter, we investigated the impact of the zwitterionic molecule HEPES on the efficiency of CRISPR Cas9. We demonstrated that in *M. belari,* a minimum of 20 mM HEPES is necessary to obtain CRISPR/Cas9-based mutants in the F1 generation. We went on to show that the addition of HEPES to the injection mix facilitates the transport of the CRISPR complex also in *C. elegans*. Indeed, in the presence of 20 mM HEPES, CRISPR/Cas9-based mutants were obtained after microinjection outside the gonad.

Next, I used the same strategy to establish CRISPR/Cas9-based homologous repair, in order to insert specific sequences, such as fluorescent tags, into the genome of *M. belari*. I have tried to repair a double strand break using a single strand oligo, containing 50 bp homology regions on each side of the break. I have also tried to insert plasmid by NHEJ using the CRISPaint technique (Schmid-Burgk et al., 2016). I used different strategies to screen for the insertion: insertion of a restriction site for PCR/digestion detection, PCR detection with specific primers in the repair template, systematic Sanger Sequencing of the region, etc. Unfortunately, despite hundreds of injections and PCR, none of the techniques I have tried worked. Generally, in all species tested so far, repair by HR is more difficult to achieve than repair by NHEJ. For this reason, in cell culture lines for instance, elaborated strategies have been established to downregulate the NHEJ pathway to increase the frequency of repair by HR. Another important point is that HR occurs mainly during S phase, whereas NHEJ happens at all times in the cell cycle. The gonad of *M. belari* is extremely small and contains very few proliferative cells compared to those of other nematodes, in particular *C. elegans*. This is probably a major constraint for the establishment of knock-in techniques through HR in this species.

General Discussion

M. belari is an intriguing species at the boundary between sexuality and asexuality. While both sexes are present in the population, females are largely predominant in the population, accounting for 90% of individuals. Interestingly, although some sexual females might be produced in the wild (see Model in Chapter 2), these females are exclusively asexually produced. The population is therefore composed of 90% asexual females and 10% sexual males. The contribution of males to the genome of the species is most likely null since they represent only 10% of the individuals and their genome is diluted by two at each generation into the female genome. Only the Y chromosome represents the male genome.

The contribution of males is clearly not genetic, but their existence remains crucial. Indeed, by providing essential components for the development of the embryo, the sperm cell is necessary to induce both female and male development. Most likely in this species, the transition to strict parthenogenesis has not occurred because the oocytes depend on the sperm for being activated and to find a source of centrosomes. The importance of maintaining both sexes compels females to produce asexual and sexual individuals. Nonetheless, from a genetic point of view, *M. belari* can be qualified as an asexual species.

In *M. belari*, the meiosis that generates future males is canonical and results in the formation of a haploid gamete. In this case, diploidy is restored by the fusion of male and female haploid DNA after fertilization. This type of oocyte is, therefore, called amphimictic. In contrast, the meiosis that generates future females is altered from the canonical state and results in the formation of a diploid or unreduced gamete. Since the males do not contribute any gene to the female progeny, these eggs are named gynogenetic. As discussed in Chapter 1, there are multiple manners to generate unreduced gametes. I have found that in the unreduced oocytes of *M. belari*, meiosis I is abortive. My results further suggest that anaphase B is absent during meiosis I (Anaphase B is prevented). It is still not known whether the chromosomes enter anaphase B and arrest or whether anaphase B is not initiated. However, anaphase B is present in meiosis I of amphimictic and in meiosis II of both amphimictic and gynogenetic oocytes. Therefore, during meiosis I, females are able to switch on and/or off the ability to perform anaphase B. This ability could be under the control of mRNAs, proteins or small RNAs that are preferentially deposited by the mother during oogenesis. Further experiments are needed to

understand how anaphase B is prevented or activated and which factors enable this phenomenon.

M. belari species has a widely heterozygous genome (presented in Co-Segregation in Chapter 2). In unreduced oocytes, the abortion of meiosis I leads to the assortment of non-sister chromatids in the gynogenetic eggs. As Chapter 1 argues, in absence of recombination, the assortment of non-sister chromatids promotes the maintenance of heterozygosity. However, both cytological and genomic evidence (presented in Co-Segregation in Chapter 2) demonstrated that all chromosomes recombine and this, at each generation. Importantly, recombination events are not restricted to the tips of chromosomes. The presence of recombination raised a paradox in *M. belari* that could be resolved by combining cytology and genomics analyses. With this combination, we hypothesised the existence of a new mechanism for maintaining heterozygosity, the Directed Chromatid Assortment. We proved that during the meiosis of gynogenetic oocytes, the two recombinant chromatids of a pair of chromosomes are associated to be either expelled into the pole body or retained in the unreduced gamete. In addition, modeling demonstrated that Directed Chromatid Assortment is rapidly selected in an asexual population where the loss of heterozygosity is detrimental.

Directed Chromatid Assortment is to our knowledge a novel mechanism of chromosome segregation. Regardless of its impact on understanding the emergence and maintenance of asexual species, it reveals how flexible is meiosis and how much remains to be discovered by the study of asexual species. Modeling suggests that this segregation bias appeared at the same time as asexuality and thus contributed to the invasion of asexuality in the population. Indeed, in absence of Directed Chromatid Assortment (DCA), an even with rare events of sexuality, the genome of *M. belari* would rapidly suffer from a loss of heterozygosity, which is most likely detrimental for the species.

The existence of DCA raises many questions on the underlying molecular mechanisms. In order to associate recombinant chromatids, the cell must be able to distinguish between recombinant and non-recombinant chromatids within a given pair of chromosomes. Before recombination, future recombinant chromatids are specifically marked for crossover. Consequently, we hypothesise that during recombination, specific ornamental proteins may be maintained on the DNA of recombinant chromatids which allows their identification. Later, for DCA, a biased orientation of these labelled chromatids must occur. Alternatively, the recombinant chromatids may still be connected and linked, which favours their co-orientation

during the next division. There is still a lot of work to be conducted to demonstrate the mechanism behind the process of Directed Chromatid Assortment.

Our work highlights the power of the Directed Chromatid Assortment mechanism. Thus, given the penetrance of the Directed Chromatid Assortment in parthenogenetic populations, this mechanism may not be limited to the *M. belari* species.

As described in Chapter 1, many asexual species have a heterozygous genome although there is no mechanistic explanation for that. Often, the cytology is missing or unclear. Therefore, many asexuals have been considered non-recombining asexuals because only the absence of recombination offers a simple explanation for the maintenance of heterozygosity in the long term. For some recombining asexual, however, it has been shown, that a displacement of the crossing over at the tip of the chromosome maintains a largely heterozygous genome. We propose DCA could have been selected as an alternative mechanism to avoid LOH in several lineages and is probably not restricted to *M. belari*. Indeed, Directed Chromatid Assortment could also promote heterozygosity in oribatid mites, the bee *Apis mellifera capensis* and the rotifer *Adineta vaga*. In these species, modifications in meiosis may also have caused meiosis abortion and biased chromatid segregation. The combination of these two modifications could be a major event in the emergence of asexuality. Furthermore, sister chromatid assortment associated with Directed Chromatid Assortment could sustain the asexual lineage in the long term.

With regard to the fascinating findings reported in *M. belari*, it is now undeniable that these works could not have been done without the combination of several disciplines such as cytology, genomics and modeling. Therefore, in the last sentence of my manuscript, after four magnificent years of work, I invite all readers of my work to re-evaluate the contribution of Cell Biology to the study of the wonderful world of asexual species evolution.

Bibliography

Abdala, C. S., Baldo, D., Juárez, R. A., & Espinoza, R. E. (2016). The First Parthenogenetic Pleurodont Iguanian: A New All-female Liolaemus (Squamata: Liolaemidae) from Western Argentina. *Copeia*, *104*(2), 487–497. https://doi.org/10.1643/CH-15-381

Adams, M., Foster, R., Hutchinson, M. N., Hutchinson, R. G., & Donnellan, S. C. (2003). The Australian Scincid Lizard Menetia Greyii: A New Instance of Widespread Vertebrate Parthenogenesis. *Evolution*, *57*(11), 2619–2627. https://doi.org/10.1111/j.0014-3820.2003.tb01504.x

Adams, S., Pathak, P., Shao, H., Lok, J. B., & Pires-daSilva, A. (2019). Liposome-based transfection enhances RNAi and CRISPR-mediated mutagenesis in non-model nematode systems. *Scientific Reports*, *9*, 483. https://doi.org/10.1038/s41598-018-37036-1

Albertson, D. G. (1984). Localization of the ribosomal genes in Caenorhabditis elegans chromosomes by in situ hybridization using biotin-labeled probes. *The EMBO Journal*, *3*(6), 1227–1234.

Albertson, D. G., & Thomson, J. N. (1993). Segregation of holocentric chromosomes at meiosis in the nematode,Caenorhabditis elegans. *Chromosome Research*, *1*(1), 15–26. https://doi.org/10.1007/BF00710603

Almanzar, D. E., Gordon, S. G., & Rog, O. (2021). Meiotic sister chromatid exchanges are rare in C. elegans. *Current Biology*, *31*(7), 1499-1507.e3. https://doi.org/10.1016/j.cub.2020.11.018

Archetti, M. (2022). Evidence from automixis with inverted meiosis for the maintenance of sex by loss of complementation. *Journal of Evolutionary Biology*, *35*(1), 40–50. https://doi.org/10.1111/jeb.13975

Ashman, T.-L., Bachtrog, D., Blackmon, H., Goldberg, E. E., Hahn, M. W., Kirkpatrick, M., Kitano, J., Mank, J. E., Mayrose, I., Ming, R., Otto, S. P., Peichel, C. L., Pennell, M. W., Perrin, N., Ross, L., Valenzuela, N., Vamosi, J. C., & The Tree of Sex Consortium. (2014). Tree of Sex: A database of sexual systems. *Scientific Data*, *1*(1), Article 1. https://doi.org/10.1038/sdata.2014.15

Avise, J. (2008). *Clonality: The Genetics, Ecology, and Evolution of Sexual Abstinence in Vertebrate Animals*. Oxford University Press, USA.

Barley, A. J., Reeder, T. W., Nieto-Montes de Oca, A., Cole, C. J., & Thomson, R. C. (2021). A New Diploid Parthenogenetic Whiptail Lizard from Sonora, Mexico, Is the "Missing Link" in the Evolutionary Transition to Polyploidy. *The American Naturalist*, *198*(2), 295–309. https://doi.org/10.1086/715056

Barlow, D. P., & Bartolomei, M. S. (2014). Genomic Imprinting in Mammals. *Cold Spring Harbor Perspectives in Biology*, *6*(2), a018382. https://doi.org/10.1101/cshperspect.a018382

Bartelmez, G. W., & Riddle, O. (1924). On parthenogenetic cleavage and on the rôle of water absorption by the ovum in the formation of the subgerminal cavity in the pigeon's egg: PARTHENOGENESIS AND SUBGERMINAL CAVITY. *American Journal of Anatomy*, *33*(1), 57–66. https://doi.org/10.1002/aja.1000330104

Bast, J., Parker, D. J., Dumas, Z., Jalvingh, K. M., Tran Van, P., Jaron, K. S., Figuet, E., Brandt, A., Galtier, N., & Schwander, T. (2018). Consequences of Asexuality in Natural Populations: Insights from Stick Insects. *Molecular Biology and Evolution*, *35*(7), 1668–1677. https://doi.org/10.1093/molbev/msy058

Baudrimont, A., Penkner, A., Woglar, A., Machacek, T., Wegrostek, C., Gloggnitzer, J., Fridkin, A., Klein, F., Gruenbaum, Y., Pasierbek, P., & Jantsch, V. (2010). Leptotene/Zygotene Chromosome Movement Via the SUN/KASH Protein Bridge in Caenorhabditis elegans. *PLOS Genetics*, *6*(11), e1001219. https://doi.org/10.1371/journal.pgen.1001219

Belar, K. (1923). *Über den Chromosomenzyklus von parthenogenetischen Erdnematoden.* 513–518.

Bell, G. (1982). *The Masterpiece of Nature: The Evolution and Genetics of Sexuality.*

Bergmann, P., Laumann, M., Norton, R. A., & Heethoff, M. (2018). Cytological evidence for automictic thelytoky in parthenogenetic oribatid mites (Acari, Oribatida): Synaptonemal complexes confirm meiosis in Archegozetes longisetosus. *Acarologia*, *58*(2), 342–356. https://doi.org/10.24349/acarologia/20184246

Berling, I. (1932). *Zur Biologie von Nemeritis canescens Grav (Hymen Ophion).* 223–249.

Beukeboom, L. W., & Pijnacker, L. P. (2000). Automictic parthenogenesis in the parasitoid Venturia canescens (Hymenoptera: Ichneumonidae) revisited. *Genome*, *43*(6), 939–944.

Beukeboom, L. W., & Vrijenhoek, R. C. (1998). Evolutionary genetics and ecology of sperm-dependent parthenogenesis. *Journal of Evolutionary Biology*, *11*(6), 755–782. https://doi.org/10.1046/j.1420-9101.1998.11060755.x

Birky Jr, C. W. (1996). Heterozygosity, heteromorphy, and phylogenetic trees in asexual eukaryotes. *Genetics*, *144*(1), 427–437.

BLACKMAN, R. L. (1987). Reproduction, cytogenetics and development. *Aphids: Their Biology, Natural Enemies and Control*, *2*, 163–195.

Blanc, C., Saclier, N., Faou, E. L., Marie-Orleach, L., Wenger, E., Diblasi, C., Glemin, S., Galtier, N., & Delattre, M. (2023). *Co-segregation of recombinant chromatids maintains genome-wide heterozygosity in an asexual nematode* (p. 2023.03.17.533182). bioRxiv. https://doi.org/10.1101/2023.03.17.533182

Bogart, J. P., Bartoszek, J., Noble, D. W. A., & Bi, K. (2009). Sex in unisexual salamanders: Discovery of a new sperm donor with ancient affinities. *Heredity*, *103*(6), Article 6. https://doi.org/10.1038/hdy.2009.83

Bogart, J. P., Bi, K., Fu, J., Noble, D. W. A., & Niedzwiecki, J. (2007). Unisexual salamanders (genus Ambystoma) present a new reproductive mode for eukaryotes. *Genome*, *50*(2), 119–136. https://doi.org/10.1139/g06-152

Bohr, T., Nelson, C. R., Giacopazzi, S., Lamelza, P., & Bhalla, N. (2018). Shugoshin Is Essential for Meiotic Prophase Checkpoints in C. elegans. *Current Biology*, *28*(20), 3199-3211.e3. https://doi.org/10.1016/j.cub.2018.08.026

Booth, W., Million, L., Reynolds, R. G., Burghardt, G. M., Vargo, E. L., Schal, C., Tzika, A. C., & Schuett, G. W. (2011). Consecutive virgin births in the new world boid snake, the Colombian rainbow Boa, Epicrates maurus. *The Journal of Heredity*, *102*(6), 759–763. https://doi.org/10.1093/jhered/esr080

Booth, W., Smith, C. F., Eskridge, P. H., Hoss, S. K., Mendelson, J. R., & Schuett, G. W. (2012). Facultative parthenogenesis discovered in wild vertebrates. *Biology Letters*, *8*(6), 983–985. https://doi.org/10.1098/rsbl.2012.0666

Braig, H., Normark, B. B., Turner, B., & Stouthamer, R. (2002). Microorganism-induced parthenogenesis. *Reproductive Biology of Invertebrates. Vol XI.*, 1–62.

Brandt, A., Tran Van, P., Bluhm, C., Anselmetti, Y., Dumas, Z., Figuet, E., François, C. M., Galtier, N., Heimburger, B., Jaron, K. S., Labédan, M., Maraun, M., Parker, D. J., Robinson-Rechavi, M., Schaefer, I., Simion, P., Scheu, S., Schwander, T., & Bast, J. (2021). Haplotype divergence supports long-term asexuality in the oribatid mite Oppiella nova. *Proceedings of the National Academy of Sciences*, *118*(38), e2101485118. https://doi.org/10.1073/pnas.2101485118

Brenner, S. (1988). *The nematode Caenorhabditis elegans.* 1091–1105.

Broday, L. (2017). The SUMO system in Caenorhabditis elegans development. *International Journal of Developmental Biology*, *61*(3-4–5), Article 3-4–5. https://doi.org/10.1387/ijdb.160388LB

Buchwitz, B. J., Ahmad, K., Moore, L. L., Roth, M. B., & Henikoff, S. (1999). A histone-H3-like protein in C. elegans. *Nature*, *401*(6753), 547–548.

Bullini, L. (1994). Origin and evolution of animal hybrid species. *Trends in Ecology & Evolution*, *9*(11), 422–426. https://doi.org/10.1016/0169-5347(94)90124-4

Bulteau, R., & Francesconi, M. (2022). Real age prediction from the transcriptome with RAPToR. *Nature Methods*, *19*(8), Article 8. https://doi.org/10.1038/s41592-022-01540-0

Cahoon, C. K., Helm, J. M., & Libuda, D. E. (2019). Synaptonemal Complex Central Region Proteins Promote Localization of Pro-crossover Factors to Recombination Events During Caenorhabditis elegans Meiosis. *Genetics*, *213*(2), 395–409. https://doi.org/10.1534/genetics.119.302625

Card, D. C., Vonk, F. J., Smalbrugge, S., Casewell, N. R., Wüster, W., Castoe, T. A., Schuett, G. W., & Booth, W. (2021). Genome-wide data implicate terminal fusion automixis in king cobra facultative parthenogenesis. *Scientific Reports*, *11*(1), Article 1. https://doi.org/10.1038/s41598-021-86373-1

Carmena, M., Riparbelli, M. G., Minestrini, G., Tavares, Á. M., Adams, R., Callaini, G., & Glover, D. M. (1998). Drosophila polo kinase is required for cytokinesis. *The Journal of Cell Biology*, *143*(3), 659–671.

Carmena, M., Wheelock, M., Funabiki, H., & Earnshaw, W. C. (2012). The chromosomal passenger complex (CPC): From easy rider to the godfather of mitosis. *Nature Reviews Molecular Cell Biology*, *13*(12), 789–803.

Carrier, J. C., Musick, J. A., & Heithaus, M. R. (2012). *Biology of Sharks and Their Relatives*. CRC Press.

Castellano-Pozo, M., Pacheco, S., Sioutas, G., Jaso-Tamame, A. L., Dore, M. H., Karimi, M. M., & Martinez-Perez, E. (2020). Surveillance of cohesin-supported chromosome structure controls meiotic progression. *Nature Communications*, *11*(1), 4345. https://doi.org/10.1038/s41467-020-18219-9

Castellano-Pozo, M., Sioutas, G., Barroso, C., Lopez-Jimenez, P., Jaso-Tamame, A. L., Crawley, O., Shao, N., Page, J., & Martinez-Perez, E. (2022). *The kleisin subunit controls the function of meiotic cohesins by determining the mode of DNA binding and differential regulation by SCC-2 and WAPL-1* [Preprint]. Cell Biology. https://doi.org/10.1101/2022.10.12.511771

Cavin-Meza, G., Kwan, M. M., & Wignall, S. M. (2022). Multiple motors cooperate to establish and maintain acentrosomal spindle bipolarity in C. elegans oocyte meiosis. *ELife*, *11*, e72872. https://doi.org/10.7554/eLife.72872

Cavin-Meza, G., Mullen, T. J., Czajkowski, E. R., Wolff, I. D., Divekar, N. S., Finkle, J. D., & Wignall, S. M. (2022). ZYG-9ch-TOG promotes the stability of acentrosomal poles via regulation of spindle microtubules in C. elegans oocyte meiosis. *PLOS Genetics*, *18*(11), e1010489. https://doi.org/10.1371/journal.pgen.1010489

Chapman, D. D., Firchau, B., & Shivji, M. S. (2008). Parthenogenesis in a large-bodied requiem shark, the blacktip Carcharhinus limbatus. *Journal of Fish Biology*, *73*(6), 1473–1477. https://doi.org/10.1111/j.1095-8649.2008.02018.x

Chapman, D. D., Shivji, M. S., Louis, E., Sommer, J., Fletcher, H., & Prodöhl, P. A. (2007). Virgin birth in a hammerhead shark. *Biology Letters*, *3*(4), 425–427. https://doi.org/10.1098/rsbl.2007.0189

Chavez, D. R., Snow, A. K., Smith, J. R., & Stanfield, G. M. (2018). Soma-germ line interactions and a role for muscle in the regulation of C. elegans sperm motility. *Development (Cambridge, England)*, *145*(24), dev167734. https://doi.org/10.1242/dev.167734

Cheeseman, I. M., Chappie, J. S., Wilson-Kubalek, E. M., & Desai, A. (2006). The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. *Cell*, *127*(5), 983–997.

Chuang, C.-H., Schlientz, A. J., Yang, J., & Bowerman, B. (2020). Microtubule assembly and pole coalescence: Early steps in Caenorhabditis elegans oocyte meiosis I spindle assembly. *Biology Open*, *9*(6), bio052308. https://doi.org/10.1242/bio.052308

Colaiácovo, M. P., MacQueen, A. J., Martinez-Perez, E., McDonald, K., Adamo, A., La Volpe, A., & Villeneuve, A. M. (2003). Synaptonemal Complex Assembly in C. elegans Is Dispensable for Loading Strand-Exchange Proteins but Critical for Proper Completion of Recombination. *Developmental Cell*, *5*(3), 463–474. https://doi.org/10.1016/S1534-5807(03)00232-6

Cole-Clark, M. P., Barton, D. A., Allsopp, M. H., Beekman, M., Gloag, R. S., Wossler, T. C., Ronai, I., Smith, N., Reid, R. J., & Oldroyd, B. P. (2017). Cytogenetic basis of thelytoky in Apis mellifera capensis. *Apidologie*, *48*(5), 623–634. https://doi.org/10.1007/s13592-017-0505-7

Connolly, A. A., Osterberg, V., Christensen, S., Price, M., Lu, C., Chicas-Cruz, K., Lockery, S., Mains, P. E., & Bowerman, B. (2014). Caenorhabditis elegans oocyte meiotic spindle pole assembly requires microtubule severing and the calponin homology domain protein ASPM-1. *Molecular Biology of the Cell*, *25*(8), 1298–1311.

Connolly, A. A., Sugioka, K., Chuang, C.-H., Lowry, J. B., & Bowerman, B. (2015). KLP-7 acts through the Ndc80 complex to limit pole number in C. elegans oocyte meiotic spindle assembly. *Journal of Cell Biology*, *210*(6), 917–932.

Corsi, A. K., Wightman, B., & Chalfie, M. (2015). A Transparent Window into Biology: A Primer on Caenorhabditis elegans. *Genetics*, *200*(2), 387–407. https://doi.org/10.1534/genetics.115.176099

Crawley, O., Barroso, C., Testori, S., Ferrandiz, N., Silva, N., Castellano-Pozo, M., Jaso-Tamame, A. L., & Martinez-Perez, E. (2016). Cohesin-interacting protein WAPL-1 regulates meiotic chromosome structure and cohesion by antagonizing specific cohesin complexes. *ELife*, *5*, e10851. https://doi.org/10.7554/eLife.10851

Cubides-Cubillos, S. D., Patané, J. S. L., Pereira da Silva, K. M., Almeida-Santos, S. M., Polydoro, D. S., Galassi, G. G., Travaglia Cardoso, S. R., & Silva, M. J. de J. (2020). Evidence of facultative parthenogenesis in three Neotropical pitviper species of the Bothrops atrox group. *PeerJ*, *8*, e10097. https://doi.org/10.7717/peerj.10097

Dai, J., Sultan, S., Taylor, S. S., & Higgins, J. M. (2005). The kinase haspin is required for mitotic histone H3 Thr 3 phosphorylation and normal metaphase chromosome alignment. *Genes & Development*, *19*(4), 472–488.

Danlasky, B. M., Panzica, M. T., McNally, K. P., Vargas, E., Bailey, C., Li, W., Gong, T., Fishman, E. S., Jiang, X., & McNally, F. J. (2020). Evidence for anaphase pulling forces during C. elegans meiosis. *The Journal of Cell Biology*, *219*(12), e202005179. https://doi.org/10.1083/jcb.202005179

Davis, G. K. (2012). Cyclical Parthenogenesis and Viviparity in Aphids as Evolutionary Novelties. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, *318*(6), 448–459. https://doi.org/10.1002/jez.b.22441

Davis-Roca, A. C., Divekar, N. S., Ng, R. K., & Wignall, S. M. (2018). Dynamic SUMO remodeling drives a series of critical events during the meiotic divisions in Caenorhabditis elegans. *PLOS Genetics*, *14*(9), e1007626. https://doi.org/10.1371/journal.pgen.1007626

Davis-Roca, A. C., Muscat, C. C., & Wignall, S. M. (2017). Caenorhabditis elegans oocytes detect meiotic errors in the absence of canonical end-on kinetochore attachments. *Journal of Cell Biology*, *216*(5), 1243–1253. https://doi.org/10.1083/jcb.201608042

de Carvalho, C. E., Zaaijer, S., Smolikov, S., Gu, Y., Schumacher, J. M., & Colaiácovo, M. P. (2008). LAB-1 antagonizes the Aurora B kinase in C. elegans. *Genes & Development*, *22*(20), 2869–2885. https://doi.org/10.1101/gad.1691208

de Meeûs, T., Prugnolle, F., & Agnew, P. (2007). Asexual reproduction: Genetics and evolutionary aspects. *Cellular and Molecular Life Sciences*, *64*(11), 1355–1372. https://doi.org/10.1007/s00018-007-6515-2

Dedryver, C.-A., Le Gallic, J.-F., Mahéo, F., Simon, J.-C., & Dedryver, F. (2013). The genetics of obligate parthenogenesis in an aphid species and its consequences for the maintenance of alternative reproductive modes. *Heredity*, *110*(1), Article 1. https://doi.org/10.1038/hdy.2012.57

Dedukh, D., Altmanová, M., Klíma, J., & Kratochvíl, L. (2022). Premeiotic endoreplication is essential for obligate parthenogenesis in geckos. *Development*, *149*(7), dev200345. https://doi.org/10.1242/dev.200345

Dedukh, D., da Cruz, I., Kneitz, S., Marta, A., Ormanns, J., Tichopád, T., Lu, Y., Alsheimer, M., Janko, K., & Schartl, M. (2022). Achiasmatic meiosis in the unisexual Amazon molly, Poecilia formosa. *Chromosome Research*, *30*(4), 443–457. https://doi.org/10.1007/s10577-022-09708-2

Dedukh, D., Majtánová, Z., Marta, A., Pšenička, M., Kotusz, J., Klíma, J., Juchno, D., Boron, A., & Janko, K. (2020). Parthenogenesis as a Solution to Hybrid Sterility: The Mechanistic Basis of Meiotic Distortions in Clonal and Sterile Hybrids. *Genetics*, *215*(4), 975–987. https://doi.org/10.1534/genetics.119.302988

Delattre, M., & Gönczy, P. (2004). The arithmetic of centrosome biogenesis. *Journal of Cell Science*, *117*(9), 1619–1630.

Delmotte, F., Leterme, N., Bonhomme, J., Rispe, C., & Simon, J.-C. (2001). Multiple routes to asexuality in an aphid species. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *268*(1483), 2291–2299.

Dernburg, A. F., McDonald, K., Moulder, G., Barstead, R., Dresser, M., & Villeneuve, A. M. (1998). Meiotic Recombination in C. elegans Initiates by a Conserved Mechanism and Is Dispensable for Homologous Chromosome Synapsis. *Cell*, *94*(3), 387–398. https://doi.org/10.1016/S0092-8674(00)81481-6

Divekar, N. S., Davis-Roca, A. C., Zhang, L., Dernburg, A. F., & Wignall, S. M. (2021). A degron-based strategy reveals new insights into Aurora B function in C. elegans. *PLOS Genetics*, *17*(5), e1009567. https://doi.org/10.1371/journal.pgen.1009567

Dorée, M., & Hunt, T. (2002). From Cdc2 to Cdk1: When did the cell cycle kinase join its cyclin partner? *Journal of Cell Science*, *115*(Pt 12), 2461–2464. https://doi.org/10.1242/jcs.115.12.2461

Dudgeon, C. L., Blower, D. C., Broderick, D., Giles, J. L., Holmes, B. J., Kashiwagi, T., Krück, N. C., Morgan, J. a. T., Tillett, B. J., & Ovenden, J. R. (2012). A review of the application of molecular genetics for fisheries management and conservation of sharks and rays. *Journal of Fish Biology*, *80*(5), 1789–1843. https://doi.org/10.1111/j.1095-8649.2012.03265.x

Dumont, J., & Desai, A. (2012). Acentrosomal spindle assembly and chromosome segregation during oocyte meiosis. *Trends in Cell Biology*, *22*(5), 241–249. https://doi.org/10.1016/j.tcb.2012.02.007

Dumont, J., Oegema, K., & Desai, A. (2010). A kinetochore-independent mechanism drives anaphase chromosome separation during acentrosomal meiosis. *Nature Cell Biology*, *12*(9), 894–901.

Elinson, R. P., Bogart, J. P., Licht, L. E., & Lowcock, L. A. (1992). Gynogenetic mechanisms in polyploid hybrid salamanders. *Journal of Experimental Zoology*, *264*(1), 93–99. https://doi.org/10.1002/jez.1402640114

Engebrecht, J., Hirsch, J., & Roeder, G. S. (1990). Meiotic gene conversion and crossing over: Their relationship to each other and to chromosome synapsis and segregation. *Cell*, *62*(5), 927–937.

Engelstädter, J. (2008). Constraints on the evolution of asexual reproduction. *BioEssays*, *30*(11-12), 1138–1150.

Eweis, D. S., Delattre, M., & Plastino, J. (2022). Asymmetry is defined during meiosis in the oocyte of the parthenogenetic nematode Diploscapter pachys. *Developmental Biology*, *483*, 13–21. https://doi.org/10.1016/j.ydbio.2021.12.013

Feldheim, K. A., Chapman, D. D., Sweet, D., Fitzpatrick, S., Prodöhl, P. A., Shivji, M. S., & Snowden, B. (2010). Shark Virgin Birth Produces Multiple, Viable Offspring. *Journal of Heredity*, *101*(3), 374–377. https://doi.org/10.1093/jhered/esp129

Feldheim, K. A., Clews, A., Henningsen, A., Todorov, L., McDermott, C., Meyers, M., Bradley, J., Pulver, A., Anderson, E., & Marshall, A. (2017). Multiple births by a captive swellshark Cephaloscyllium ventriosum via facultative parthenogenesis. *Journal of Fish Biology*, *90*(3), 1047–1053. https://doi.org/10.1111/jfb.13202

Feldheim, K. A., Dubach, J., & Watson, L. (2022). Parthenogenesis in an elasmobranch in the presence of conspecific males. *Journal of Fish Biology*, 1–3. https://doi.org/10.1111/jfb.15268

Félix, M.-A., & Sternberg, P. W. (1996). Symmetry breakage in the development of one-armed gonads in nematodes. *Development*, *122*(7), 2129–2142. https://doi.org/10.1242/dev.122.7.2129

Ferrandiz, N., Barroso, C., Telecan, O., Shao, N., Kim, H.-M., Testori, S., Faull, P., Cutillas, P., Snijders, A. P., & Colaiácovo, M. P. (2018). Spatiotemporal regulation of Aurora B recruitment ensures release of cohesion during C. elegans oocyte meiosis. *Nature Communications*, *9*(1), 834.

Fournier, D., Hellemans, S., Hanus, R., & Roisin, Y. (2016). Facultative asexual reproduction and genetic diversity of populations in the humivorous termite Cavitermes tuberosus. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1832), 20160196. https://doi.org/10.1098/rspb.2016.0196

Fradin, H., Kiontke, K., Zegar, C., Gutwein, M., Lucas, J., Kovtun, M., Corcoran, D. L., Baugh, L. R., Fitch, D. H. A., Piano, F., & Gunsalus, K. C. (2017). Genome Architecture and Evolution of a Unichromosomal Asexual Nematode. *Current Biology: CB*, *27*(19), 2928-2939.e6. https://doi.org/10.1016/j.cub.2017.08.038

Freitas, S. N., Harris, D. J., Sillero, N., Arakelyan, M., Butlin, R. K., & Carretero, M. A. (2019). The role of hybridisation in the origin and evolutionary persistence of vertebrate parthenogens: A case study of Darevskia lizards. *Heredity*, *123*(6), Article 6. https://doi.org/10.1038/s41437-019-0256-5

Galis, F., & van Alphen, J. J. M. (2020). Parthenogenesis and developmental constraints. *Evolution & Development*, *22*(1–2), 205–217. https://doi.org/10.1111/ede.12324

Gassmann, R., Essex, A., Hu, J.-S., Maddox, P. S., Motegi, F., Sugimoto, A., O'Rourke, S. M., Bowerman, B., McLeod, I., & Yates, J. R. (2008). A new mechanism controlling kinetochore–microtubule interactions revealed by comparison of two dynein-targeting components: SPDL-1 and the Rod/Zwilch/Zw10 complex. *Genes & Development*, *22*(17), 2385–2399.

Geiger, W. (1998). Population dynamics, life histories and reproductive modes. *Sex and Parthenogenesis. Evolutionary Ecology of Reproductive Modes in Non-Marine Ostracods. Backhuys, Leiden*, *215*, 228.

Ghiselin, M. T. (1974). *The economy of nature and the evolution of sex.*

Gillies, C. B. (1975). Synaptonemal Complex and Chromosome Structure. *Annual Review of Genetics*, *9*(1), 91–109. https://doi.org/10.1146/annurev.ge.09.120175.000515

Gillies, C. B., & Moens, P. B. (1984). The synaptonemal complex in higher plants. *Critical Reviews in Plant Sciences*, *2*(2), 81–116. https://doi.org/10.1080/07352688409382191

Giraud, T., Refrégier, G., Le Gac, M., de Vienne, D. M., & Hood, M. E. (2008). Speciation in fungi. *Fungal Genetics and Biology*, *45*(6), 791–802.

Glemin, S., & Galtier, N. (2012). Genome evolution in outcrossing versus selfing versus asexual species. *Evolutionary Genomics: Statistical and Computational Methods, Volume 1*, 311–335.

Gokhman, V. E., & Kuznetsova, V. G. (2018). Parthenogenesis in Hexapoda: Holometabolous insects. *Journal of Zoological Systematics and Evolutionary Research*, *56*(1), 23–34. https://doi.org/10.1111/jzs.12183

Goldstein, B., & Hird, S. N. (1996). Specification of the anteroposterior axis in Caenorhabditis elegans. *Development*, *122*(5), 1467–1474.

González, D. P., Lamb, H. V., Partida, D., Wilson, Z. T., Harrison, M.-C., Prieto, J. A., Moresco, J. J., Diedrich, J. K., Yates, J. R., & Olson, S. K. (2018). CBD-1 organizes two independent complexes required for eggshell vitelline layer formation and egg activation in C. elegans. *Developmental Biology*, *442*(2), 288–300. https://doi.org/10.1016/j.ydbio.2018.08.005

Grill, S. W., Gönczy, P., Stelzer, E. H., & Hyman, A. A. (2001). Polarity controls forces governing asymmetric spindle positioning in the Caenorhabditis elegans embryo. *Nature*, *409*(6820), 630–633. https://doi.org/10.1038/35054572

Grismer, J. L., Bauer, A. M., Grismer, L. L., Thirakhupt, K., Aowphol, A., Oaks, J. R., Wood, P. L., Jr, Onn, C. K., Thy, N., Cota, M., & Jackman, T. (2014). Multiple origins of parthenogenesis, and a revised species phylogeny for the Southeast Asian butterfly lizards, Leiolepis. *Biological Journal of the Linnean Society*, *113*(4), 1080–1093. https://doi.org/10.1111/bij.12367

Groot, T. V. M., Bruins, E., & Breeuwer, J. a. J. (2003). Molecular genetic evidence for parthenogenesis in the Burmese python, Python molurus bivittatus. *Heredity*, *90*(2), Article 2. https://doi.org/10.1038/sj.hdy.6800210

Grosmaire, M., Launay, C., Siegwald, M., Brugière, T., Estrada-Virrueta, L., Berger, D., Burny, C., Modolo, L., Blaxter, M., Meister, P., Félix, M.-A., Gouyon, P.-H., & Delattre, M. (2019). Males as somatic investment in a parthenogenetic nematode. *Science*, *363*(6432), 1210–1213. https://doi.org/10.1126/science.aau0099

Gueth-Hallonet, C., Antony, C., Aghion, J., Santa-Maria, A., Lajoie-Mazenc, I., Wright, M., & Maro, B. (1993). Gamma-Tubulin is present in acentriolar MTOCs during early mouse development. *Journal of Cell Science*, *105*(1), 157–166.

Haber, J. E. (1999). DNA recombination: The replication connection. *Trends in Biochemical Sciences*, *24*(7), 271–275.

Hagstrom, K. A., Holmes, V. F., Cozzarelli, N. R., & Meyer, B. J. (2002). *C. elegans* condensin promotes mitotic chromosome architecture, centromere organization, and sister chromatid segregation during mitosis and meiosis. *Genes & Development*, *16*(6), 729–742. https://doi.org/10.1101/gad.968302

Hales, D. F., Wilson, A. C., Sloane, M. A., Simon, J.-C., Legallic, J.-F., & Sunnucks, P. (2002). Lack of detectable genetic recombination on the X chromosome during the parthenogenetic production of female and male aphids. *Genetics Research*, *79*(3), 203–209.

Harmon, T. S., Kamerman, T. Y., Corwin, A. L., & Sellas, A. B. (2016). Consecutive parthenogenetic births in a spotted eagle ray Aetobatus narinari. *Journal of Fish Biology*, *88*(2), 741–745. https://doi.org/10.1111/jfb.12819

Harper, N. C., Rillo, R., Jover-Gil, S., Assaf, Z. J., Bhalla, N., & Dernburg, A. F. (2011). Pairing Centers Recruit a Polo-like Kinase to Orchestrate Meiotic Chromosome Dynamics in C. elegans. *Developmental Cell*, *21*(5), 934–947. https://doi.org/10.1016/j.devcel.2011.09.001

Hattersley, N., Cheerambathur, D., Moyle, M., Stefanutti, M., Richardson, A., Lee, K.-Y., Dumont, J., Oegema, K., & Desai, A. (2016). A Nucleoporin Docks Protein Phosphatase 1 to Direct Meiotic Chromosome Segregation and Nuclear Assembly. *Developmental Cell*, *38*(5), 463–477. https://doi.org/10.1016/j.devcel.2016.08.006

Hebert, P. D. (1978). The population bilogy of daphnia (crustacea, daphnidae). *Biological Reviews*, *53*(3), 387–426.

Hebert, P. D., Ward, R. D., & Weider, L. J. (1988). Clonal-diversity patterns and breeding-system variation in Daphnia pulex, an asexual-sexual complex. *Evolution*, *42*(1), 147–159.

Heckmann, S., Jankowska, M., Schubert, V., Kumke, K., Ma, W., & Houben, A. (2014). Alternative meiotic chromatid segregation in the holocentric plant Luzula elegans. *Nature Communications*, *5*(1), Article 1. https://doi.org/10.1038/ncomms5979

Hillers, K. J., Jantsch, V., Martinez-Perez, E., & Yanowitz, J. L. (2018). Meiosis. *WormBook: The Online Review of C. Elegans Biology [Internet]*.

Hiraki, H., Kagoshima, H., Kraus, C., Schiffer, P. H., Ueta, Y., Kroiher, M., Schierenberg, E., & Kohara, Y. (2017). Genome analysis of Diploscapter coronatus: Insights into molecular peculiarities of a nematode with parthenogenetic reproduction. *BMC Genomics*, *18*(1), 478. https://doi.org/10.1186/s12864-017-3860-x

Hiruta, C., Nishida, C., & Tochinai, S. (2010). Abortive meiosis in the oogenesis of parthenogenetic Daphnia pulex. *Chromosome Research*, *18*(7), 833–840. https://doi.org/10.1007/s10577-010-9159-2

Hiruta, C., & Tochinai, S. (2012). Spindle Assembly and Spatial Distribution of γ-tubulin during Abortive Meiosis and Cleavage Division in the Parthenogenetic Water Flea Daphnia pulex. *Zoological Science*, *29*(11), 733–737. https://doi.org/10.2108/zsj.29.733

Hollis, J. A., Glover, M. L., Schlientz, A. J., Cahoon, C. K., Bowerman, B., Wignall, S. M., & Libuda, D. E. (2020). Excess crossovers impede faithful meiotic chromosome segregation in C. elegans. *PLoS Genetics*, *16*(9), e1009001.

Hsu, J.-Y., Sun, Z.-W., Li, X., Reuben, M., Tatchell, K., Bishop, D. K., Grushcow, J. M., Brame, C. J., Caldwell, J. A., & Hunt, D. F. (2000). Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. *Cell*, *102*(3), 279–291.

Hsu, W. S. (1956a). Oogenesis in Habrotrocha tridens (Milne). *The Biological Bulletin*, *111*(3), 364–374.

Hsu, W. S. (1956b). Oogenesis in the Bdelloidea rotifer Philodina roseola Ehrenberg. *Cellule*, *57*, 283–296.

Hubbs, C. L., & Hubbs, L. C. (1932). Apparent Parthenogenesis in Nature, in a Form of Fish of Hybrid Origin. *Science*, *76*(1983), 628–630. https://doi.org/10.1126/science.76.1983.628

Hubstenberger, A., Courel, M., Bénard, M., Souquere, S., Ernoult-Lange, M., Chouaib, R., Yi, Z., Morlot, J.-B., Munier, A., Fradet, M., Daunesse, M., Bertrand, E., Pierron, G., Mozziconacci, J., Kress, M., & Weil, D. (2017). P-Body Purification Reveals the Condensation of Repressed mRNA Regulons. *Molecular Cell*, *68*(1), 144-157.e5. https://doi.org/10.1016/j.molcel.2017.09.003

Huelgas-Morales, G., & Greenstein, D. (2018). Control of oocyte meiotic maturation in C. elegans. *Seminars in Cell & Developmental Biology*, *84*, 90–99.

Hung, C., & Jenkins, W. R. (1969). Oogenesis and Embryology of Two Plant-parasitic Nematodes, Pratylenchus penetrans and P. zeae. *Journal of Nematology*, *1*(4), 352–356.

Itono, M., Morishima, K., Fujimoto, T., Bando, E., Yamaha, E., & Arai, K. (2006). Premeiotic endomitosis produces diploid eggs in the natural clone loach, Misgurnus anguillicaudatus (Teleostei: Cobitidae). *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, *305A*(6), 513–523. https://doi.org/10.1002/jez.a.283

Jaquiéry, J., Stoeckel, S., Larose, C., Nouhaud, P., Rispe, C., Mieuzet, L., Bonhomme, J., Mahéo, F., Legeai, F., & Gauthier, J.-P. (2014). Genetic control of contagious asexuality in the pea aphid. *PLoS Genetics*, *10*(12), e1004838.

Jaron, K. S., Bast, J., Nowell, R. W., Ranallo-Benavidez, T. R., Robinson-Rechavi, M., & Schwander, T. (2021). Genomic features of parthenogenetic animals. *Journal of Heredity*, *112*(1), 19–33.

Jessus, C., Munro, C., & Houliston, E. (2020). Managing the oocyte meiotic arrest—Lessons from frogs and jellyfish. *Cells*, *9*(5), 1150.

Joachim, B. L., & Schlupp, I. (2012). Mating preferences of Amazon mollies (Poecilia formosa) in multi-host populations. *Behaviour*, *149*(2), 233–249. https://doi.org/10.1163/156853912X636302

Jordan, M. A., Perrine-Ripplinger, N., & Carter, E. T. (2015). An Independent Observation of Facultative Parthenogenesis in the Copperhead (Agkistrodon contortrix). *Journal of Herpetology*, *49*(1), 118–121. https://doi.org/10.1670/14-017

Kaitna, S., Pasierbek, P., Jantsch, M., Loidl, J., & Glotzer, M. (2002). The aurora B kinase AIR-2 regulates kinetochores during mitosis and is required for separation of homologous chromosomes during meiosis. *Current Biology*, *12*(10), 798–812.

Khan, Z., Khan, M. S., Bawazeer, S., Bawazeer, N., Suleman, Irfan, M., Rauf, A., Su, X.-H., & Xing, L.-X. (2022). A comprehensive review on the documented characteristics of four *Reticulitermes* termites (Rhinotermitidae, Blattodea) of China. *Brazilian Journal of Biology*, *84*. https://doi.org/10.1590/1519-6984.256354

Kharche, S. D., & Jha, B. K. (2016). Parthenogenesis. In G. Steinhoff (Ed.), *Regenerative Medicine—From Protocol to Patient: 2. Stem Cell Science and Technology* (pp. 425–448). Springer International Publishing. https://doi.org/10.1007/978-3-319-27610-6_16

Kinney, M. E., Wack, R. F., Grahn, R. A., & Lyons, L. (2013). Parthenogenesis in a Brazilian Rainbow Boa (Epicrates cenchria cenchria). *Zoo Biology*, *32*(2), 172–176. https://doi.org/10.1002/zoo.21050

Kitagawa, R. (2009). Key players in chromosome segregation in Caenorhabditis elegans. *Frontiers in Bioscience : A Journal and Virtual Library*, *14*, 1529–1557.

Köhler, S., Wojcik, M., Xu, K., & Dernburg, A. F. (2017). Superresolution microscopy reveals the three-dimensional organization of meiotic chromosome axes in intact *Caenorhabditis elegans* tissue. *Proceedings of the National Academy of Sciences*, *114*(24), E4734–E4743. https://doi.org/10.1073/pnas.1702312114

Komatsu, N., Kawakami, Y., Banzai, A., Ooi, H. K., & Uchida, A. (2015). Species clarification of Ogasawara cockroaches which inhabit Japan. *Tropical Biomedicine*, *32*(1), 98–108.

Laband, K., Le Borgne, R., Edwards, F., Stefanutti, M., Canman, J. C., Verbavatz, J.-M., & Dumont, J. (2017). Chromosome segregation occurs by microtubule pushing in oocytes. *Nature Communications*, *8*(1), Article 1. https://doi.org/10.1038/s41467-017-01539-8

Labella, S., Woglar, A., Jantsch, V., & Zetka, M. (2011). Polo Kinases Establish Links between Meiotic Chromosomes and Cytoskeletal Forces Essential for Homolog Pairing. *Developmental Cell*, *21*(5), 948–958. https://doi.org/10.1016/j.devcel.2011.07.011

Lahl, V., Sadler, B., & Schierenberg, E. (2006). Egg development in parthenogenetic nematodes: Variations in meiosis and axis formation. *The International Journal of Developmental Biology*, *50*(4), 393–398. https://doi.org/10.1387/ijdb.052030vl

Lamelza, P., Young, J. M., Noble, L. M., Caro, L., Isakharov, A., Palanisamy, M., Rockman, M. V., Malik, H. S., & Ailion, M. (2019). Hybridization promotes asexual reproduction in Caenorhabditis nematodes. *PLOS Genetics*, *15*(12), e1008520. https://doi.org/10.1371/journal.pgen.1008520

Lampert, K. P., Lamatsch, D. K., Fischer, P., Epplen, J. T., Nanda, I., Schmid, M., & Schartl, M. (2007). Automictic Reproduction in Interspecific Hybrids of Poeciliid Fish. *Current Biology*, *17*(22), 1948–1953. https://doi.org/10.1016/j.cub.2007.09.064

Lampert, K. P., & Schartl, M. (2010). A little bit is better than nothing: The incomplete parthenogenesis of salamanders, frogs and fish. *BMC Biology*, *8*(1), Article 1. https://doi.org/10.1186/1741-7007-8-78

Láscarez-Lagunas, L. I., Nadarajan, S., Martinez-Garcia, M., Quinn, J. N., Todisco, E., Thakkar, T., Berson, E., Eaford, D., Crawley, O., & Montoya, A. (2022). ATM/ATR kinases link the synaptonemal complex and DNA double-strand break repair pathway choice. *Current Biology*, *32*(21), 4719-4726. e4.

Laumann, M., Bergmann, P., & Heethoff, M. (2008). Some remarks on the cytogenetics of oribatid mites. *SOIL ORGANISMS*, *80*(2), Article 2.

Launay, C., Félix, M.-A., Dieng, J., & Delattre, M. (2020). Diversification and hybrid incompatibility in auto-pseudogamous species of Mesorhabditis nematodes. *BMC Evolutionary Biology*, *20*(1), Article 1. https://doi.org/10.1186/s12862-020-01665-w

Lehtonen, J., Schmidt, D. J., Heubel, K., & Kokko, H. (2013). Evolutionary and ecological implications of sexual parasitism. *Trends in Ecology & Evolution*, *28*(5), 297–306.

Lenormand, T., Engelstädter, J., Johnston, S. E., Wijnker, E., & Haag, C. R. (2016). Evolutionary mysteries in meiosis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1706), 20160001.

Leonard, J. L. (2013). Williams' Paradox and the Role of Phenotypic Plasticity in Sexual Systems. *Integrative and Comparative Biology*, *53*(4), 671–688. https://doi.org/10.1093/icb/ict088

Li, Y., & Sasaki, H. (2011). Genomic imprinting in mammals: Its life cycle, molecular mechanisms and reprogramming. *Cell Research*, *21*(3), Article 3. https://doi.org/10.1038/cr.2011.15

Libuda, D. E., Uzawa, S., Meyer, B. J., & Villeneuve, A. M. (2013). Meiotic chromosome structures constrain and respond to designation of crossover sites. *Nature*, *502*(7473), 703–706.

Lindquist, E. E. (1984). Current theories on the evolution of major groups of Acari and on their relationships with other groups of Arachnida, with consequent implications for their classification. *Acarology*, 28–62.

Liu, Q. L., Thomas, V. P., & Williamson, V. M. (2007). Meiotic Parthenogenesis in a Root-Knot Nematode Results in Rapid Genomic Homozygosity. *Genetics*, *176*(3), 1483–1490. https://doi.org/10.1534/genetics.107.071134

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego, Calif.)*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), Article 12. https://doi.org/10.1186/s13059-014-0550-8

Lutes, A. A., Neaves, W. B., Baumann, D. P., Wiegräbe, W., & Baumann, P. (2010). Sister Chromosome Pairing Maintains Heterozygosity in Parthenogenetic Lizards. *Nature*, *464*(7286), 283–286. https://doi.org/10.1038/nature08818

Lynch, M., Ye, Z., Urban, L., Maruki, T., & Wei, W. (2022). The Linkage-Disequilibrium and Recombinational Landscape in Daphnia pulex. *Genome Biology and Evolution*, *14*(11), evac145. https://doi.org/10.1093/gbe/evac145

MacQueen, A. J., Phillips, C. M., Bhalla, N., Weiser, P., Villeneuve, A. M., & Dernburg, A. F. (2005). Chromosome Sites Play Dual Roles to Establish Homologous Synapsis during Meiosis in C. elegans. *Cell*, *123*(6), 1037–1050. https://doi.org/10.1016/j.cell.2005.09.034

Maddox, P. S., Oegema, K., Desai, A., & Cheeseman, I. M. (2004). "Holo"er than thou: Chromosome segregation and kinetochore function in C. elegans. *Chromosome Research*, *12*(6), 641–653. https://doi.org/10.1023/B:CHRO.0000036588.42225.2f

Malone, C. J., Misner, L., Le Bot, N., Tsai, M.-C., Campbell, J. M., Ahringer, J., & White, J. G. (2003). The C. elegans Hook Protein, ZYG-12, Mediates the Essential Attachment between the Centrosome and Nucleus. *Cell*, *115*(7), 825–836. https://doi.org/10.1016/S0092-8674(03)00985-1

Manandhar, G., Schatten, H., & Sutovsky, P. (2005). Centrosome reduction during gametogenesis and its significance. *Biology of Reproduction*, *72*(1), 2–13. https://doi.org/10.1095/biolreprod.104.031245

Mandrioli, M., & Manicardi, G. C. (2020). Holocentric chromosomes. *PLOS Genetics*, *16*(7), e1008918. https://doi.org/10.1371/journal.pgen.1008918

Marais, M. (2012). *The cytogenetics of some South African root-knot nematodes (Heteroderidae: Nematoda)*.

Marescalchi, O., Zauli, C., & Scali, V. (2002). Centrosome dynamics and inheritance in related sexual and parthenogenetic Bacillus (Insecta Phasmatodea). *Molecular Reproduction and Development*, *63*(1), 89–95. https://doi.org/10.1002/mrd.10177

Mark Welch, D., & Meselson, M. (2000). Evidence for the evolution of bdelloid rotifers without sexual reproduction or genetic exchange. *Science (New York, N.Y.)*, *288*(5469), 1211–1215. https://doi.org/10.1126/science.288.5469.1211

Martens, K., Rossetti, G., & Horne, D. J. (2003). How ancient are ancient asexuals? *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1516), 723–729.
Martens, K., Schön, I., Meisch, C., & Horne, D. J. (2008). Global diversity of ostracods (Ostracoda, Crustacea) in freshwater. *Hydrobiologia*, *595*, 185–193.
Martinez-Perez, E., Schvarzstein, M., Barroso, C., Lightfoot, J., Dernburg, A. F., & Villeneuve, A. M. (2008). Crossovers trigger a remodeling of meiotic chromosome axis composition that is linked to two-step loss of sister chromatid cohesion. *Genes & Development*, *22*(20), 2886–2901. https://doi.org/10.1101/gad.1694108
Martinez-Perez, E., & Villeneuve, A. M. (2005). HTP-1-dependent constraints coordinate homolog pairing and synapsis and promote chiasma formation during C. elegans meiosis. *Genes & Development*, *19*(22), 2727–2743. https://doi.org/10.1101/gad.1338505
Mather, K. (1938). Crossing-Over. *Biological Reviews*, *13*(3), 252–292. https://doi.org/10.1111/j.1469-185X.1938.tb00516.x
Maton, G., Edwards, F., Lacroix, B., Stefanutti, M., Laband, K., Lieury, T., Kim, T., Espeut, J., Canman, J. C., & Dumont, J. (2015). Kinetochore components are required for central spindle assembly. *Nature Cell Biology*, *17*(5), 697–705.
Maynard Smith, J. (1971). *The origin and maintenance of sex Pp. 163–175 in Williams G. C., ed. Group selection.*
Maynard Smith, J. (1978). *The evolution of sex.*
Maynard Smith, J. (1986). Evolution: Contemplating life without sex. *Nature*, *324*, 300–301.
McKim, K. S., Green-Marroquin, B. L., Sekelsky, J. J., Chin, G., Steinberg, C., Khodosh, R., & Hawley, R. S. (1998). Meiotic Synapsis in the Absence of Recombination. *Science*, *279*(5352), 876–878. https://doi.org/10.1126/science.279.5352.876
McNally, K. P., Panzica, M. T., Kim, T., Cortes, D. B., & McNally, F. J. (2016). A novel chromosome segregation mechanism during female meiosis. *Molecular Biology of the Cell*, *27*(16), 2576–2589.
Mehlmann, L. M. (2005). Stops and starts in mammalian oocytes: Recent advances in understanding the regulation of meiotic arrest and oocyte maturation. *Reproduction*, *130*(6), 791–799.
Merlin, J., Goldstein, A., & Goldstein, P. (2003). Three-Dimensional Ultrastructural Karyotype Analysis from the Meiotic Parthenogenetic Nematode Heterodera betulae. *Journal of Nematology*, *35*(2), 228–231.
Mestdagh, P., Van Vlierberghe, P., De Weer, A., Muth, D., Westermann, F., Speleman, F., & Vandesompele, J. (2009). A novel and universal method for microRNA RT-qPCR data normalization. *Genome Biology*, *10*(6), Article 6. https://doi.org/10.1186/gb-2009-10-6-r64
Mikeladze-Dvali, T., von Tobel, L., Strnad, P., Knott, G., Leonhardt, H., Schermelleh, L., & Gönczy, P. (2012). Analysis of centriole elimination during C. elegans oogenesis. *Development*, *139*(9), 1670–1679.
Miller, K. L., Rico, S. C., Muletz-Wolz, C. R., Campana, M. G., McInerney, N., Augustine, L., Frere, C., Peters, A. M., & Fleischer, R. C. (2019). Parthenogenesis in a captive Asian water dragon (Physignathus cocincinus) identified with novel microsatellites. *PLOS ONE*, *14*(6), e0217489. https://doi.org/10.1371/journal.pone.0217489
Mira, A. (1998). Why is meiosis arrested? *Journal of Theoretical Biology*, *194*(2), 275–287.
Mogie, M. (1992). *The evolution of asexual reproduction in plants*. Springer.
Monen, J., Maddox, P. S., Hyndman, F., Oegema, K., & Desai, A. (2005). Differential role of CENP-A in the segregation of holocentric C. elegans chromosomes during meiosis and mitosis. *Nature Cell Biology*, *7*(12), Article 12. https://doi.org/10.1038/ncb1331
Moreira, M. O., Fonseca, C., & Rojas, D. (2021). Parthenogenesis is self-destructive for scaled reptiles. *Biology Letters*, *17*(5), rsbl.2021.0006, 20210006. https://doi.org/10.1098/rsbl.2021.0006
Morgan, C., Knight, E., & Bomblies, K. (2022). The meiotic cohesin subunit REC8 contributes to multigenic adaptive evolution of autopolyploid meiosis in Arabidopsis arenosa. *PLoS Genetics*, *18*(7), e1010304.
Mullen, T. J., & Wignall, S. M. (2017). Interplay between microtubule bundling and sorting factors ensures acentriolar spindle stability during C. elegans oocyte meiosis. *PLoS Genetics*, *13*(9), e1006986.
Muller, H. J. (1932). Some genetic aspects of sex. *The American Naturalist*, *66*(703), 118–138.
Muller, H. J. (1964). The relation of recombination to mutational advance. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *1*(1), 2–9.
Muscat, C. C., Torre-Santiago, K. M., Tran, M. V., Powers, J. A., & Wignall, S. M. (2015). Kinetochore-independent chromosome segregation driven by lateral microtubule bundles. *ELife*, *4*, e06462. https://doi.org/10.7554/eLife.06462
Nadarajan, S., Altendorfer, E., Saito, T. T., Martinez-Garcia, M., & Colaiácovo, M. P. (2021). HIM-17 regulates the position of recombination events and GSP-1/2 localization to establish short arm identity on bivalents in meiosis. *Proceedings of the National Academy of Sciences*, *118*(17), e2016363118.
Nahaboo, W., Zouak, M., Askjaer, P., & Delattre, M. (2015). Chromatids segregate without centrosomes during Caenorhabditis elegans mitosis in a Ran- and CLASP-dependent manner. *Molecular Biology of the Cell*, *26*(11), 2020–2029. https://doi.org/10.1091/mbc.E14-12-1577
Neiman, M., Sharbel, T. F., & Schwander, T. (2014). Genetic causes of transitions from sexual reproduction to asexuality in plants and animals. *Journal of Evolutionary Biology*, *27*(7), 1346–1359.
Nigon, V. M. (1949). *Les modalites de la reproduction et le determinisme due sexe chez quelques nematodes libres.* 1–132.
Nigon, V. M., & Félix, M.-A. (2018). History of research on C. elegans and other free-living nematodes as model organisms. In *WormBook: The Online Review of C. elegans Biology [Internet]*. WormBook. https://www.ncbi.nlm.nih.gov/books/NBK453431/
Nishiyama, T., Tachibana, K., & Kishimoto, T. (2010). Cytostatic Arrest: Post-Ovulation Arrest until Fertilization in Metazoan Oocytes. *Oogenesis: The Universal Process*, 357–384.
Normark, B. B. (2014). Modes of reproduction. In *Evolution of insect mating systems* (Oxford: Oxford Univ Press, pp. p1-19).

Normark, B. B., & Kirkendall, L. R. (2009). Chapter 192—Parthenogenesis in Insects and Mites. In V. H. Resh & R. T. Cardé (Eds.), *Encyclopedia of Insects (Second Edition)* (pp. 753–757). Academic Press. https://doi.org/10.1016/B978-0-12-374144-8.00201-0

Oegema, K., & Hyman, A. A. (2006). *Cell division. WormBook*.

Ohkura, H. (2015). Meiosis: An Overview of Key Differences from Mitosis. *Cold Spring Harbor Perspectives in Biology*, *7*(5), a015859. https://doi.org/10.1101/cshperspect.a015859

Oldroyd, B. P., Yagound, B., Allsopp, M. H., Holmes, M. J., Buchmann, G., Zayed, A., & Beekman, M. (2021). Adaptive, caste-specific changes to recombination rates in a thelytokous honeybee population. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1952), 20210729. https://doi.org/10.1098/rspb.2021.0729

Olsen, M. W. (1965). Twelve year summary of selection for parthenogenesis in Beltsville Small White turkeys. *British Poultry Science*, *6*(1), 1–6. https://doi.org/10.1080/00071666508415546

Olsen, M. W., Wilson, S. P., & Marks, H. L. (1968). Genetic Control of Parthenogenesis in Chickens. *Journal of Heredity*, *59*(1), 41–42. https://doi.org/10.1093/oxfordjournals.jhered.a107639

Olsen, W. W., & Marsden, S. J. (1954). Natural parthenogenesis in turkey eggs. *Science (New York, N.Y.)*, *120*(3118), 545–546. https://doi.org/10.1126/science.120.3118.545

Olson, S. K., Greenan, G., Desai, A., Müller-Reichert, T., & Oegema, K. (2012). Hierarchical assembly of the eggshell and permeability barrier in C. elegans. *Journal of Cell Biology*, *198*(4), 731–748. https://doi.org/10.1083/jcb.201206008

Omilian, A. R., Cristescu, M. E. A., Dudycha, J. L., & Lynch, M. (2006). Ameiotic recombination in asexual lineages of Daphnia. *Proceedings of the National Academy of Sciences*, *103*(49), 18638–18643. https://doi.org/10.1073/pnas.0606435103

Oxley, P. R., Ji, L., Fetter-Pruneda, I., McKenzie, S. K., Li, C., Hu, H., Zhang, G., & Kronauer, D. J. C. (2014). The genome of the clonal raider ant Cerapachys biroi. *Current Biology: CB*, *24*(4), 451–458. https://doi.org/10.1016/j.cub.2014.01.018

Palmer, S. C., & Norton, R. A. (1992). Genetic diversity in thelytokous oribatid mites (Acari; Acariformes: Desmonomata). *Biochemical Systematics and Ecology*, *20*(3), 219–231. https://doi.org/10.1016/0305-1978(92)90056-J

Parker, H. M., & McDaniel, C. D. (2009). Parthenogenesis in unfertilized eggs of Coturnix chinensis, the Chinese painted quail, and the effect of egg clutch position on embryonic development1. *Poultry Science*, *88*(4), 784–790. https://doi.org/10.3382/ps.2008-00368

Pasierbek, P., Jantsch, M., Melcher, M., Schleiffer, A., Schweizer, D., & Loidl, J. (2001). A Caenorhabditis elegans cohesion protein with functions in meiotic chromosome pairing and disjunction. *Genes & Development*, *15*(11), 1349–1360. https://doi.org/10.1101/gad.192701

Pelisch, F., Bel Borja, L., Jaffray, E. G., & Hay, R. T. (2019). Sumoylation regulates protein dynamics during meiotic chromosome segregation in C. elegans oocytes. *Journal of Cell Science*, *132*(14), jcs232330. https://doi.org/10.1242/jcs.232330

Pellegrino, K. C. M., Rodrigues, M. T., & Yonenaga-Yassuda, Y. (2003). Triploid Karyotype of Leposoma percarinatum (Squamata, Gymnophthalmidae). *Journal of Herpetology*, *37*(1), 197–199. https://doi.org/10.1670/0022-1511(2003)037[0197:TKOLPS]2.0.CO;2

Pfaffl, M. W., Tichopad, A., Prgomet, C., & Neuvians, T. P. (2004). Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper – Excel-based tool using pair-wise correlations. *Biotechnology Letters*, *26*(6), 509–515. https://doi.org/10.1023/B:BILE.0000019559.84305.47

Phillips, C. M., & Dernburg, A. F. (2006). A family of zinc-finger proteins is required for chromosome-specific pairing and synapsis during meiosis in C. elegans. *Developmental Cell*, *11*(6), 817–829. https://doi.org/10.1016/j.devcel.2006.09.020

Phillips, C. M., Wong, C., Bhalla, N., Carlton, P. M., Weiser, P., Meneely, P. M., & Dernburg, A. F. (2005). HIM-8 Binds to the X Chromosome Pairing Center and Mediates Chromosome-Specific Meiotic Synapsis. *Cell*, *123*(6), 1051–1063. https://doi.org/10.1016/j.cell.2005.09.035

Picelli, S., Faridani, O. R., Björklund, Å. K., Winberg, G., Sagasser, S., & Sandberg, R. (2014). Full-length RNA-seq from single cells using Smart-seq2. *Nature Protocols*, *9*(1), Article 1. https://doi.org/10.1038/nprot.2014.006

Pintard, L., & Bowerman, B. (2019). Mitotic Cell Division in Caenorhabditis elegans. *Genetics*, *211*, 35–73. https://doi.org/10.1534/genetics.118.301367

Poinar, G. O., & Ricci, C. (1992). Bdelloid rotifers in Dominican amber: Evidence for parthenogenetic continuity. *Experientia*, *48*(4), 408–410. https://doi.org/10.1007/BF01923444

Portnoy, D. S., & Heist, E. J. (2012). Molecular markers: Progress and prospects for understanding reproductive ecology in elasmobranchs. *Journal of Fish Biology*, *80*(5), 1120–1140. https://doi.org/10.1111/j.1095-8649.2011.03206.x

Portnoy, D. S., Hollenbeck, C. M., Johnston, J. S., Casman, H. M., & Gold, J. R. (2014). Parthenogenesis in a whitetip reef shark *Triaenodon obesus* involves a reduction in ploidy: PARTHENOGENESIS AND PLOIDY REDUCTION IN *T. OBESUS*. *Journal of Fish Biology*, *85*(2), 502–508. https://doi.org/10.1111/jfb.12415

Rabeling, C., & Kronauer, D. J. C. (2013). Thelytokous parthenogenesis in eusocial Hymenoptera. *Annual Review of Entomology*, *58*, 273–292. https://doi.org/10.1146/annurev-ento-120811-153710

Rabeling, C., Lino-Neto, J., Cappellari, S. C., Dos-Santos, I. A., Mueller, U. G., & Bacci, M. (2009). Thelytokous Parthenogenesis in the Fungus-Gardening Ant Mycocepurus smithii (Hymenoptera: Formicidae). *PLoS ONE*, *4*(8), e6781. https://doi.org/10.1371/journal.pone.0006781

Ramesh, M. A., Malik, S.-B., & Logsdon, J. M. (2005). A Phylogenomic Inventory of Meiotic Genes: Evidence for Sex in Giardia and an Early Eukaryotic Origin of Meiosis. *Current Biology*, *15*(2), 185–191. https://doi.org/10.1016/j.cub.2005.01.003

Reeder, T. W., Cole, C. J., & Dessauer, H. C. (2002). Phylogenetic Relationships of Whiptail Lizards of the Genus Cnemidophorus (Squamata: Teiidae): A Test of Monophyly, Reevaluation of Karyotypic Evolution, and Review of

Hybrid Origins. *American Museum Novitates*, *2002*(3365), 1–61. https://doi.org/10.1206/0003-0082(2002)365<0001:PROWLO>2.0.CO;2

Rey, O., Loiseau, A., Facon, B., Foucaud, J., Orivel, J., Cornuet, J.-M., Robert, S., Dobigny, G., Delabie, J. H. C., Mariano, C. D. S. F., & Estoup, A. (2011). Meiotic recombination dramatically decreased in thelytokous queens of the little fire ant and their sexually produced workers. *Molecular Biology and Evolution*, *28*(9), 2591–2601. https://doi.org/10.1093/molbev/msr082

Rillo-Bohn, R., Adilardi, R., Mitros, T., Avşaroğlu, B., Stevens, L., Köhler, S., Bayes, J., Wang, C., Lin, S., Baskevitch, K. A., Rokhsar, D. S., & Dernburg, A. F. (2021). Analysis of meiosis in Pristionchus pacificus reveals plasticity in homolog pairing and synapsis in the nematode lineage. *ELife*, *10*, e70990. https://doi.org/10.7554/eLife.70990

Riparbelli, M. G., Gottardo, M., & Callaini, G. (2017). Parthenogenesis in insects: The centriole renaissance. *Oocytes: Maternal Information and Functions*, 435–479.

Riparbelli, M. G., Stouthamer, R., Dallai, R., & Callaini, G. (1998). Microtubule Organization during the Early Development of the Parthenogenetic Egg of the HymenopteranMuscidifurax uniraptor. *Developmental Biology*, *195*(2), 89–99. https://doi.org/10.1006/dbio.1997.8841

Robinson, D. P., Baverstock, W., Al-Jaru, A., Hyland, K., & Khazanehdari, K. A. (2011). Annually recurring parthenogenesis in a zebra shark Stegostoma fasciatum. *Journal of Fish Biology*, *79*(5), 1376–1382. https://doi.org/10.1111/j.1095-8649.2011.03110.x

Robinson, F. E., Hardin, R. T., & Robblee, A. R. (1990). Reproductive senescence in domestic fowl: Effects on egg production, sequence length and inter-sequence pause length. *British Poultry Science*, *31*(4), 871–879. https://doi.org/10.1080/00071669008417318

Robinson, F. E., Hardin, R. T., Robinson, N. A., & Williams, B. J. (1991). The Influence of Egg Sequence Position on Fertility, Embryo Viability, and Embryo Weight in Broiler Breeders. *Poultry Science*, *70*(4), 760–765. https://doi.org/10.3382/ps.0700760

Rogers, E., Bishop, J. D., Waddle, J. A., Schumacher, J. M., & Lin, R. (2002). The aurora kinase AIR-2 functions in the release of chromosome cohesion in Caenorhabditis elegans meiosis. *The Journal of Cell Biology*, *157*(2), 219–229. https://doi.org/10.1083/jcb.200110045

Roughgarden, J. (1991). The Evolution of Sex. *The American Naturalist*, *138*(4), 934–953. https://doi.org/10.1086/285261

Rubin, T., Macaisne, N., & Huynh, J.-R. (2020). Mixing and Matching Chromosomes during Female Meiosis. *Cells*, *9*(3), Article 3. https://doi.org/10.3390/cells9030696

Ryder, O. A., Thomas, S., Judson, J. M., Romanov, M. N., Dandekar, S., Papp, J. C., Sidak-Loftis, L. C., Walker, K., Stalis, I. H., Mace, M., Steiner, C. C., & Chemnick, L. G. (2021). Facultative Parthenogenesis in California Condors. *Journal of Heredity*, *112*(7), 569–574. https://doi.org/10.1093/jhered/esab052

Sakuno, T., & Hiraoka, Y. (2022). Rec8 cohesin: A structural platform for shaping the meiotic chromosomes. *Genes*, *13*(2), 200.

Salt, S. (1976). *The host of Nemeritis canescens: A problem in the host specificit of insect parasitoids.* 63–67.

Sanford, C., & Perry, M. D. (2001). Asymmetrically distributed oligonucleotide repeats in the Caenorhabditis elegans genome sequence that map to regions important for meiotic chromosome segregation. *Nucleic Acids Research*, *29*(14), 2920–2926. https://doi.org/10.1093/nar/29.14.2920

Sato, A., Isaac, B., Phillips, C. M., Rillo, R., Carlton, P. M., Wynne, D. J., Kasad, R. A., & Dernburg, A. F. (2009). Cytoskeletal Forces Span the Nuclear Envelope to Coordinate Meiotic Chromosome Pairing and Synapsis. *Cell*, *139*(5), 907–919. https://doi.org/10.1016/j.cell.2009.10.039

Saunders, A. M., Powers, J., Strome, S., & Saxton, W. M. (2007). Kinesin-5 acts as a brake in anaphase spindle elongation. *Current Biology : CB*, *17*(12), R453–R454. https://doi.org/10.1016/j.cub.2007.05.001

Schaefer, I., Domes, K., Heethoff, M., Schneider, K., Schön, I., Norton, R. A., Scheu, S., & Maraun, M. (2006). No evidence for the 'Meselson effect' in parthenogenetic oribatid mites (Oribatida, Acari). *Journal of Evolutionary Biology*, *19*(1), 184–193. https://doi.org/10.1111/j.1420-9101.2005.00975.x

Schmid-Burgk, J. L., Höning, K., Ebert, T. S., & Hornung, V. (2016). CRISPaint allows modular base-specific gene tagging using a ligase-4-dependent mechanism. Nat Commun. *Nature*, *7*, 12338.

Schneider, M. V., Beukeboom, L. W., Driessen, G., Lapchin, L., Bernstein, C., & Van Alphen, J. J. (2002). Geographical distribution and genetic relatedness of sympatrical thelytokous and arrhenotokous populations of the parasitoid Venturia canescens (Hymenoptera). *Journal of Evolutionary Biology*, *15*(2), 191–200.

Schut, E., Hemmings, N., & Birkhead, T. R. (2008). Parthenogenesis in a passerine bird, the Zebra Finch Taeniopygia guttata. *Ibis*, *150*(1), 197–199. https://doi.org/10.1111/j.1474-919X.2007.00755.x

Schvarzstein, M., Wignall, S. M., & Villeneuve, A. M. (2010). Coordinating cohesion, co-orientation, and congression during meiosis: Lessons from holocentric chromosomes. *Genes & Development*, *24*(3), 219–228. https://doi.org/10.1101/gad.1863610

Segbert, C., Barkus, R., Powers, J., Strome, S., Saxton, W. M., & Bossinger, O. (2003). KLP-18, a Klp2 kinesin, is required for assembly of acentrosomal meiotic spindles in Caenorhabditis elegans. *Molecular Biology of the Cell*, *14*(11), 4458–4469.

Segers, H. (2007). Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution</p>. *Zootaxa*, *1564*(1), Article 1. https://doi.org/10.11646/zootaxa.1564.1.1

Severson, A. F., Ling, L., van Zuylen, V., & Meyer, B. J. (2009). The axial element protein HTP-3 promotes cohesin loading and meiotic axis assembly in C. elegans to implement the meiotic program of chromosome segregation. *Genes & Development*, *23*(15), 1763–1778. https://doi.org/10.1101/gad.1808809

Severson, A. F., & Meyer, B. J. (2014). Divergent kleisin subunits of cohesin specify mechanisms to tether and release meiotic chromosomes. *ELife*, *3*, e03467. https://doi.org/10.7554/eLife.03467

Simion, P., Narayan, J., Houtain, A., Derzelle, A., Baudry, L., Nicolas, E., Arora, R., Cariou, M., Cruaud, C., Gaudray, F. R., Gilbert, C., Guiglielmoni, N., Hespeels, B., Kozlowski, D. K. L., Labadie, K., Limasset, A., Llirós, M., Marbouty, M., Terwagne, M., … Van Doninck, K. (2021). Chromosome-level genome assembly reveals homologous chromosomes and recombination in asexual rotifer Adineta vaga. *Science Advances*, *7*(41), eabg4216. https://doi.org/10.1126/sciadv.abg4216

Simon, J.-C., Delmotte, F., Rispe, C., & Crease, T. (2003). Phylogenetic relationships between parthenogens and their sexual relatives: The possible routes to parthenogenesis in animals. *Biological Journal of the Linnean Society*, *79*(1), 151–163.

Sinclair, E. A., Pramuk, J. B., Bezy, R. L., Crandall, K. A., & Sites Jr., J. W. (2010). Dna Evidence for Nonhybrid Origins of Parthenogenesis in Natural Populations of Vertebrates. *Evolution*, *64*(5), 1346–1357. https://doi.org/10.1111/j.1558-5646.2009.00893.x

Siomos, M. F., Badrinath, A., Pasierbek, P., Livingstone, D., White, J., Glotzer, M., & Nasmyth, K. (2001). Separase is required for chromosome segregation during meiosis I in Caenorhabditis elegans. *Current Biology*, *11*(23), 1825–1835.

Sköld, H. N., Obst, M., Sköld, M., & Åkesson, B. (2009). Stem cells in asexual reproduction of marine invertebrates. *Stem Cells in Marine Organisms*, 105–137.

Smith, J. M. (1980). Selection for recombination in a polygenic model. *Genetics Research*, *35*(3), 269–277. https://doi.org/10.1017/S0016672300014130

Smith, J. R., & Stanfield, G. M. (2011). TRY-5 Is a Sperm-Activating Protease in Caenorhabditis elegans Seminal Fluid. *PLOS Genetics*, *7*(11), e1002375. https://doi.org/10.1371/journal.pgen.1002375

Smolikov, S., Eizinger, A., Schild-Prufert, K., Hurlburt, A., McDonald, K., Engebrecht, J., Villeneuve, A. M., & Colaiácovo, M. P. (2007). SYP-3 restricts synaptonemal complex assembly to bridge paired chromosome axes during meiosis in Caenorhabditis elegans. *Genetics*, *176*(4), 2015–2025.

Smolikov, S., Schild-Prüfert, K., & Colaiacovo, M. P. (2009). A yeast two-hybrid screen for SYP-3 interactors identifies SYP-4, a component required for synaptonemal complex assembly and chiasma formation in Caenorhabditis elegans meiosis. *PLoS Genetics*, *5*(10), e1000669.

Speicher, B. R. (1937). Oogenesis in a thelytokous wasp, Nemeritis canescens (Grav.). *Journal of Morphology*, *61*(3), 453–471.

Speicher, B., Speicher, K., & Roberts, F. (1965). *Genetic segregation in the unisexual wasp Devorgilla.* 1035–1041.

Speijer, D., Lukeš, J., & Eliáš, M. (2015). Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. *Proceedings of the National Academy of Sciences*, *112*(29), 8827–8834. https://doi.org/10.1073/pnas.1501725112

Srayko, M., Buster, D. W., Bazirgan, O. A., McNally, F. J., & Mains, P. E. (2000). MEI-1/MEI-2 katanin-like microtubule severing activity is required for Caenorhabditis elegans meiosis. *Genes & Development*, *14*(9), 1072–1084.

Srinivasan, D. G., Fenton, B., Jaubert-Possamai, S., & Jaouannet, M. (2010). Analysis of meiosis and cell cycle genes of the facultatively asexual pea aphid, Acyrthosiphon pisum (Hemiptera: Aphididae). *Insect Molecular Biology*, *19*, 229–239.

Stanfield, G. M., & Villeneuve, A. M. (2006). Regulation of Sperm Activation by SWM-1 Is Required for Reproductive Success of C. elegans Males. *Current Biology*, *16*(3), 252–263. https://doi.org/10.1016/j.cub.2005.12.041

Stelzer, C.-P., Schmidt, J., Wiedlroither, A., & Riss, S. (2010). Loss of sexual reproduction and dwarfing in a small metazoan. *PLoS One*, *5*(9), e12854.

Stöck, M., Dedukh, D., Reifová, R., Lamatsch, D. K., Starostová, Z., & Janko, K. (2021). Sex chromosomes in meiotic, hemiclonal, clonal and polyploid hybrid vertebrates: Along the 'extended speciation continuum'. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *376*(1833), 20200103. https://doi.org/10.1098/rstb.2020.0103

STOUTHAMRE, R. (1997). Wolbachia-induced parthenogenesis. *Influential Passengers, Inherited Microorganisms and Arthropod Reproduction.*, 102–124.

Streit, A. (2008). Reproduction in Strongyloides (Nematoda): A life between sex and parthenogenesis. *Parasitology*, *135*(3), 285–294. https://doi.org/10.1017/S003118200700399X

Suomalainen, E. (1950). Parthenogenesis in Animals**Received for publication March 11, 1948. In M. Demerec (Ed.), *Advances in Genetics* (Vol. 3, pp. 193–253). Academic Press. https://doi.org/10.1016/S0065-2660(08)60086-3

Suomalainen, E., Saura, A., & Lokki, J. (1987). *Cytology and evolution in parthenogenesis*. CRC Press.

Szollosi, D., Calarco, P., & Donahue, R. P. (1972). Absence of centrioles in the first and second meiotic spindles of mouse oocytes. *Journal of Cell Science*, *11*(2), 521–541.

Taberly, G. (1958). [The cytology of parthenogenesis in Platynothrus peltifer (Koch) (Acarien, Oribate)]. *Comptes Rendus Hebdomadaires Des Seances De l'Academie Des Sciences*, *247*(19), 1655–1657.

Taberly, G. (1960). *La régulation chromosomique chez Trhypochthonius tectorum (Berlese) espèce parthénogénétique d'Oribate (Acarien) un nouvel exemple de mixocinèse*.

Tanaka, M., & Daimon, T. (2019). First molecular genetic evidence for automictic parthenogenesis in cockroaches. *Insect Science*, *26*(4), 649–655. https://doi.org/10.1111/1744-7917.12572

Tang, C. Q., Obertegger, U., Fontaneto, D., & Barraclough, T. G. (2014). Sexual species are separated by larger genetic gaps than asexual species in rotifers. *Evolution*, *68*(10), 2901–2916.

Terwagne, M., Nicolas, E., Hespeels, B., Herter, L., Virgo, J., Demazy, C., Heuskin, A.-C., Hallet, B., & Van Doninck, K. (2022). DNA repair during nonreductional meiosis in the asexual rotifer Adineta vaga. *Science Advances*, *8*(48), eadc8829. https://doi.org/10.1126/sciadv.adc8829

Tétart, J. (1977). *Les garnitures chromosomiques des Ostracodes d'eau douce*.

Theurkauf, W. E., & Hawley, R. S. (1992). Meiotic spindle assembly in Drosophila females: Behavior of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. *The Journal of Cell Biology*, *116*(5), 1167–1180.

Triantaphyllou, A. C. (1963). Polyploidy and parthenogenesis in the root-knot nematode Meloidogyne arenaria. *Journal of Morphology*, *113*(3), 489–499. https://doi.org/10.1002/jmor.1051130309
Triantaphyllou, A. C. (1966). Polyploidy and reproductive patterns in the root-knot nematode Meloidogyne hapla. *Journal of Morphology*, *118*(3), 403–413. https://doi.org/10.1002/jmor.1051180308
Triantaphyllou, A. C. (1969). Gametogenesis and the Chromosomes of Two Root-knot Nematodes, Meloidogyne graminicola and M. naasi. *Journal of Nematology*, *1*(1), 62–71.
Triantaphyllou, A. C. (1970). Oogenesis and Reproduction of the Birch Cyst Nematode, Heterodera betulae. *Journal of Nematology*, *2*(4), 399–403.
Triantaphyllou, A. C. (1981). Oogenesis and the Chromosomes of the Parthenogenic Root-knot Nematode Meloidogyne incognita. *Journal of Nematology*, *13*(2), 95–104.
Triantaphyllou, A. C. (1987). Cytogenetic Status of Meloidogyne (Hypsoperine) spartinae in Relation to Other Meloidogyne Species. *Journal of Nematology*, *19*(1), 1–7.
Triantaphyllou, A. C., & Moncol, D. J. (1977). Cytology, Reproduction, and Sex Determination of Strongyloides ransomi and S. papillosus. *The Journal of Parasitology*, *63*(6), 961–973. https://doi.org/10.2307/3279827
Tsanov, N., Samacoits, A., Chouaib, R., Traboulsi, A.-M., Gostan, T., Weber, C., Zimmer, C., Zibara, K., Walter, T., Peter, M., Bertrand, E., & Mueller, F. (2016). SmiFISH and FISH-quant – a flexible single RNA detection approach with super-resolution capability. *Nucleic Acids Research*, *44*(22), e165. https://doi.org/10.1093/nar/gkw784
Tucker, A. E., Ackerman, M. S., Eads, B. D., Xu, S., & Lynch, M. (2013). Population-genomic insights into the evolutionary origin and fate of obligately asexual Daphnia pulex. *Proceedings of the National Academy of Sciences*, *110*(39), 15740–15745.
Tzur, Y. B., Egydio de Carvalho, C., Nadarajan, S., Van Bostelen, I., Gu, Y., Chu, D. S., Cheeseman, I. M., & Colaiácovo, M. P. (2012). *LAB-1 targets PP1 and restricts Aurora B kinase upon entrance into meiosis to promote sister chromatid cohesion*.
Van Beneden Edouard. (1883). *Recherches sur la maturation de l'oeuf, la fécondation, et la division cellulaire / par Edouard Van Beneden,...* Librairie Clemm G. Masson.
Van Der Beek, J., Los, J., & Pijnacker, L. (1998). Cytology of parthenogenesis of five Meloidogyne species. *Fundam. Appl. Nemawl., 21 (4),393-399*.
Van Doninck, K., Schön, I., Martens, K., & Goddeeris, B. (2003). The life-cycle of the asexual ostracod Darwinula stevensoni (Brady & Robertson, 1870)(Crustacea, Ostracoda) in a temporate pond. *Hydrobiologia*, *500*, 331–340.
Van Valen, L. (1973). *A new evolutionary law*. 1–13.
Van Wilgenburg, E., Driessen, G., & Beukeboom, L. W. (2006). Single locus complementary sex determination in Hymenoptera: An" unintelligent" design? *Frontiers in Zoology*, *3*(1), 1–15.
Vershinina, A. O., & Kuznetsova, V. G. (2016). Parthenogenesis in Hexapoda: Entognatha and non-holometabolous insects. *Journal of Zoological Systematics and Evolutionary Research*, *54*(4), 257–268. https://doi.org/10.1111/jzs.12141
Von Stetina, J. R., & Orr-Weaver, T. L. (2011). Developmental control of oocyte maturation and egg activation in metazoan models. *Cold Spring Harbor Perspectives in Biology*, *3*(10), a005553.
Wangler, M. F., & Bellen, H. J. (2017). In vivo animal modeling: Drosophila. In *Basic Science Methods for Clinical Researchers* (pp. 211–234). Elsevier.
Warren, W. C., García-Pérez, R., Xu, S., Lampert, K. P., Chalopin, D., Stöck, M., Loewe, L., Lu, Y., Kuderna, L., Minx, P., Montague, M. J., Tomlinson, C., Hillier, L. W., Murphy, D. N., Wang, J., Wang, Z., Garcia, C. M., Thomas, G. C. W., Volff, J.-N., … Schartl, M. (2018). Clonal polymorphism and high heterozygosity in the celibate genome of the Amazon molly. *Nature Ecology & Evolution*, *2*(4), Article 4. https://doi.org/10.1038/s41559-018-0473-y
Wignall, S. M., & Villeneuve, A. M. (2009). Lateral microtubule bundles promote chromosome alignment during acentrosomal oocyte meiosis. *Nature Cell Biology*, *11*(7), Article 7. https://doi.org/10.1038/ncb1891
Williams, G. C. (1971). *Introduction Pp. 1-15 in Williams G.C.* 1–15.
Williams, G. C. (1975). *Sex and Evolution. (MPB-8), Volume 8*. Princeton University Press.
Woglar, A., & Jantsch, V. (2014). Chromosome movement in meiosis I prophase of Caenorhabditis elegans. *Chromosoma*, *123*(1), 15–24. https://doi.org/10.1007/s00412-013-0436-7
Woglar, A., Yamaya, K., Roelens, B., Boettiger, A., Köhler, S., & Villeneuve, A. M. (2020). Quantitative cytogenetics reveals molecular stoichiometry and longitudinal organization of meiotic chromosome axes and loops. *PLOS Biology*, *18*(8), e3000817. https://doi.org/10.1371/journal.pbio.3000817
Wolff, I. D., Hollis, J. A., & Wignall, S. M. (2022). Acentrosomal spindle assembly and maintenance in *Caenorhabditis elegans* oocytes requires a kinesin-12 nonmotor microtubule interaction domain. *Molecular Biology of the Cell*, *33*(8), ar71. https://doi.org/10.1091/mbc.E22-05-0153
Wynn, A. H., Cole, C. J., & Gardner, A. L. (1987). *Apparent triploidy in the unisexual brahminy blind snake, Ramphotyphlops braminus. American Museum novitates ; no. 2868*. https://digitallibrary.amnh.org/handle/2246/5203
Wynne, D. J., Rog, O., Carlton, P. M., & Dernburg, A. F. (2012). Dynein-dependent processive chromosome motions promote homologous pairing in C. elegans meiosis. *Journal of Cell Biology*, *196*(1), 47–64. https://doi.org/10.1083/jcb.201106022
Xu, S., Spitze, K., Ackerman, M. S., Ye, Z., Bright, L., Keith, N., Jackson, C. E., Shaw, J. R., & Lynch, M. (2015). Hybridization and the origin of contagious asexuality in Daphnia pulex. *Molecular Biology and Evolution*, *32*(12), 3215–3225.
Yamashita, M., Jiang, J., Onozato, H., Nakanishi, T., & Nagahama, Y. (1993). A Tripolar Spindle Formed at Meiosis I Assures the Retention of the Original Ploidy in the Gynogenetic Triploid Crucian Carp, Ginbuna Carassius auratus langsdorfii. *Development, Growth & Differentiation*, *35*(6), 631–636. https://doi.org/10.1111/j.1440-169X.1993.00631.x

Yang, H., McNally, K., & McNally, F. J. (2003). MEI-1/katanin is required for translocation of the meiosis I spindle to the oocyte cortex in C. elegans☆. *Developmental Biology*, *260*(1), 245–259.

Yang, K.-T., Li, S.-K., Chang, C.-C., Tang, C.-J. C., Lin, Y.-N., Lee, S.-C., & Tang, T. K. (2010). Aurora-C kinase deficiency causes cytokinesis failure in meiosis I and production of large polyploid oocytes in mice. *Molecular Biology of the Cell*, *21*(14), 2371–2383.

Yu, C.-H., Redemann, S., Wu, H.-Y., Kiewisz, R., Yoo, T. Y., Conway, W., Farhadifar, R., Müller-Reichert, T., & Needleman, D. (2019). Central-spindle microtubules are strongly coupled to chromosomes during both anaphase A and anaphase B. *Molecular Biology of the Cell*, *30*(19), 2503–2514. https://doi.org/10.1091/mbc.E19-01-0074

Yu, Z., Kim, Y., & Dernburg, A. F. (2016). Meiotic recombination and the crossover assurance checkpoint in Caenorhabditis elegans. *Seminars in Cell & Developmental Biology*, *54*, 106–116. https://doi.org/10.1016/j.semcdb.2016.03.014

Zetka, M. C., Kawasaki, I., Strome, S., & Müller, F. (1999). Synapsis and chiasma formation in Caenorhabditis elegans require HIM-3, a meiotic chromosome core component that functions in chromosome segregation. *Genes & Development*, *13*(17), 2258–2270.

Abstract

Sexuality is the most ancestral and common reproductive system but several forms of reproduction without sex exist. This requires modifications of female meiosis, for the production of unreduced gametes. The genomic consequences of asexuality have been well studied but much less is known about the cellular and molecular changes at the origin of new types of meiosis in asexuals.

In my thesis, I first explored how asexual females are produced in the nematode species *Mesorhabditis belari*. I discovered that during meiosis, homologous chromosomes undergo crossing-overs. However, meiosis I is abortive due to a default in anaphase B. Meiosis II next proceeds normally giving rise to diploid oocytes with an assortment of non-sister chromatids. The theory predicts that species undergoing such modified meiosis with recombination should have a widely homozygous genome. However, our collaborators demonstrated that the genome of *M. belari* is widely heterozygous, which raised a paradox. Using a combination of cytology and genomics we uncovered this species undergoes a new type of meiosis, which we named Directed Chromatid Assortment (DCA), whereby the two recombinant chromatids of a given pair of chromosomes co-segregate during the meiotic division. We demonstrated that DCA allows the maintenance of genome-wide heterozygosity in an asexual, despite recombination.

In parallel, I searched for genes involved in oocyte determination, using molecular and cytological tools. In doing so, I developed the CRISPR Cas9 technique in *M. belari*. Finally, I wrote a review which summarizes my readings and thoughts on the importance of cytological approaches for the study of asexuality.