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Gong, Ting

McNally, Francis J

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Investigation

Caenorhabditis elegans spermatocytes can segregate achiasmate homologous chromosomes apart at higher than random frequency during meiosis I

Ting Gong, Francis J. McNally*

Department of Molecular and Cellular Biology, University of California, Davis, Davis, CA 95616, USA

*Corresponding author: Department of Molecular and Cellular Biology, University of California, Davis, One Shields Avenue Davis, CA 95616, USA. Email: fjmcnally@ucdavis.edu

Abstract

Chromosome segregation errors during meiosis are the leading cause of aneuploidy. Faithful chromosome segregation during meiosis in most eukaryotes requires a crossover which provides a physical attachment holding homologs together in a "bivalent." Crossovers are critical for homologs to be properly aligned and partitioned in the first meiotic division. Without a crossover, individual homologs (univalents) might segregate randomly, resulting in aneuploid progeny. However, *Caenorhabditis elegans zim-2* mutants, which have crossover defects on chromosome V, have fewer dead embryos than that expected from random segregation. This deviation from random segregation is more pronounced in *zim-2* males than that in females. We found three phenomena that can explain this apparent discrepancy. First, we detected crossovers on chromosome V in both *zim-2(tm574)* oocytes and spermatocytes, suggesting a redundant mechanism to make up for the ZIM-2 loss. Second, after accounting for the background crossover frequency, spermatocytes produced significantly more euploid gametes than what would be expected from random segregation. Lastly, trisomy of chromosome V is viable and fertile. Together, these three phenomena allow *zim-2(tm574)* mutants with reduced crossovers on chromosome V to have more viable progeny. Furthermore, live imaging of meiosis in *spo-11(me44)* oocytes and spermatocytes, which exhibit crossover failure on all 6 chromosomes, showed 12 univalents segregating apart in roughly equal masses in a homology-independent manner, supporting the existence of a mechanism that segregates any 2 chromosomes apart.

Keywords: *Caenorhabditis elegans*, meiosis, chromosome segregation

Introduction

Genetic recombination and random chromosome segregation during sexual reproduction reshuffle genetic variations, giving birth to diverse offspring to adapt to a varying environment. During meiotic prophase, homologs are tethered together as a "bivalent" by sister chromatid cohesion and crossovers between homologs (Fig. 1a) (Miller *et al.*, 2013; Moore and Orr-Weaver, 1997). This bivalent configuration is essential for faithful chromosome segregation. At meiosis I, crossovers between homologs provide physical attachments for the homologs to be properly oriented toward opposite spindle poles and segregate normally. If homologs fail to pair and cohesion between sister chromatids is retained, the 2 univalents might segregate randomly at anaphase I resulting in gametes that have lost or retained both univalents (Fig. 1b) (Buonomo *et al.*, 2000). Alternatively, if cohesion between sister chromatids is lost and they segregate apart at anaphase I, nonsisters would segregate randomly at anaphase II (Nicklas, 1977; LeMaire-Adkins and Hunt, 2000). Either case would result in a high percentage of progeny with monosomy or trisomy. The importance of crossover-based attachments between homologous chromosomes is supported by the high frequency of dead and aneuploid progeny (Dernburg *et al.*, 1998; Blokhina *et al.*, 2019) or a complete lack of functional gametes (Romanienko and Camerini-Otero, 2000) produced by meiotic recombination mutants in a wide range of sexually reproducing organisms, including plants, yeast, worms, and mammals. In addition, aging causes spontaneous loss of inter-homolog attachments and production of aneuploid progeny in both *Caenorhabditis elegans* (Raices *et al.*, 2021) and humans (Wartosch *et al.*, 2021).

Parental aneuploidy and crossover failure are two situations where meiosis must proceed without a crossover. Whereas aneuploidy is often lethal, there are cases of viable and fertile aneuploids, which reveal backup mechanisms to restore euploidy. In XO mice, preferential retention of the univalent X at the first meiotic division is observed in 60% of oocytes rather than the 50% expected from random segregation (LeMaire-Adkins and Hunt, 2000). In trisomy IV or trisomy X in *C. elegans*, the extra univalents are preferentially eliminated during anaphase I of meiosis (Hodgkin *et al.*, 1979; Cortes *et al.*, 2015; Vargas *et al.*, 2017). Keeping the univalent from monosomic parents or getting rid of the extra chromosome from trisomic parents both increase the frequency of euploid progeny.

Meiosis with 2 achiasmate homologs is likely more common than meiosis with a single univalent due to monosomy or trisomy. Some species naturally do not undergo meiotic recombination but still accurately segregate pairs of homologous chromosomes. Recombination does not occur between homologs in oocytes of

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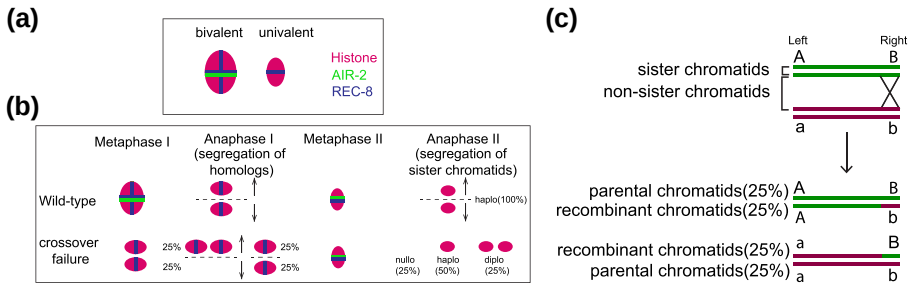

Fig. 1. Crossovers in *zim-2(tm574)* female oocytes and male spermatocytes. a) Diagram of bivalent, univalent, and associated AIR-2 and cohesin subunit REC-8 in *C. elegans*. b) Schematic of sequential cohesion loss and chromosome segregation during meiosis in wild type and in case of crossover failure with random segregation. c) Schematic of a crossover that gives rise to 50% recombinant gametes and 50% parental gametes. d) Percentage of parental gametes and recombinant gametes produced by *zim-2* females crossed with wild-type males or *zim-2* males crossed with *fog-2(–)* females. The *P*-values are shown on the right by 2 × 2 Fisher's exact test.

the silkworm *Bombyx mori*, but homologs remain attached at telomeres after breakdown of the synaptonemal complex to allow accurate segregation (Rosin *et al.*, 2021). Likewise, in the plant species *Luzula elegans*, sister chromatid cohesion is lost during anaphase I of a "reverse meiosis" but homologous chromosomes remain tethered at telomeres at metaphase II to allow accurate segregation during anaphase II (Heckmann *et al.*, 2014). During *Drosophila* male meiosis, homolog pairs are naturally held together by spermatocyte-specific protein complexes instead of crossovers (Thomas *et al.*, 2005; Weber *et al.*, 2020). During *Drosophila* female meiosis, the 4th chromosome naturally does not undergo recombination, but homologs of the 4th chromosome accurately segregate apart (Theurkauf and Hawley, 1992). This female-specific mechanism also functions when recombination is artificially blocked on the X chromosome and is promoted by heterochromatin bridges connecting the 2 homologs (Dernburg *et al.*, 1996; Hughes *et al.*, 2009).

The existence of backup mechanisms to promote accurate segregation of chromosomes lacking normal attachments has also been suggested in humans where loss of cohesion occurs as a spontaneous error. In the "reverse segregation" (RS) error, where sister chromatids segregate prematurely at meiosis I, nonsister chromatids would be expected to segregate randomly at anaphase II (50% euploid). Instead, 78% of the nonsisters remained correctly aligned at the spindle equator and segregated correctly at meiosis II. Similar to achiasmate segregation in *Drosophila* oocytes, chromatin threads between nonsister chromatids were observed in 46% of RS MII eggs (Gruhn *et al.*, 2019). Whether chromatin threads promote faithful chromosome segregation in the absence of normal attachments remains to be determined.

A nematode-specific family of Zn finger proteins provides a unique opportunity to study the response of the meiotic segregation machinery to crossover failure because loss of each family member results in crossover failure on specific chromosomes in *C. elegans*. There are 2 sexes in *C. elegans*, hermaphrodites and males, determined by the sex chromosome (X) to autosome ratio. Normally, hermaphrodites are XX and males are XO with a univalent at MI. HIM-8 mediates pairing and crossover formation on the X chromosome (Phillips *et al.*, 2005), ZIM-1 mediates pairing of chromosomes II and III, ZIM-2 mediates pairing of chromosome V, and ZIM-3 mediates pairing of chromosomes I and IV (Phillips and Dernburg, 2006) by binding to chromosome-specific DNA sequences (Phillips *et al.*, 2009). Random meiotic segregation after crossover failure on the X in one parent due to loss of HIM-8 should result in 25% of progeny with monosomy (XO), 50% disomy (XX), and 25% trisomy (XXX). An early study first demonstrated that these 3 karyotypes have easily scorable phenotypes, then used these phenotypes to demonstrate sex-specific responses to crossover failure on the X (Hodgkin *et al.*, 1979). *him-8*; XX males produced significantly more euploid haplo-X gametes than the 50% expected from random segregation (Supplementary Fig. 1; Hodgkin *et al.*, 1979) suggesting that 2 unpaired sex chromosomes tend to be segregated apart during spermatogenesis. In contrast, *him-8* XX females produced an excess of nullo-X gametes (Supplementary Fig. 1) (Hodgkin *et al.*, 1979) indicating that univalent chromosomes are preferentially deposited into polar bodies during oocyte meiosis (Cortes *et al.*, 2015).

It is unclear whether these sex-specific responses to crossover failure are restricted to the X chromosome because unambiguous phenotypes have not been assigned to most autosomal aneuploidies in *C. elegans*. However, *zim-1* or *zim-2* females mated with WT males have less inviable progeny than what would be expected from random segregation if trisomies and monosomies of chromosomes II, III, or V are lethal. This deviation from random segregation was more substantial in *zim-1* or *zim-2* males mated with WT females (Jaramillo-Lambert *et al.*, 2010; Fig. 2a, this study), suggesting a stronger sex-specific correction system in males to maximize the number of viable progeny in response to crossover failure.

In addition to accurate meiotic segregation of achiasmate chromosomes, two other phenomena which could contribute to the results of Hodgkin *et al.* (1979) and Jaramillo-Lambert *et al.* (2010) are the potential for viable aneuploidy and mitotic correction. Whereas most aneuploidies in humans are lethal, viable trisomy of chromosome IV (Sigurdson *et al.*, 1984) and X (Hodgkin *et al.*, 1979; Vargas *et al.*, 2017) in *C. elegans* have been reported in previous work.

An additional mechanism that could allow parents with crossover failure to have more viable progeny is correction of meiosis-derived aneuploidy by segregation errors during embryonic mitosis. Mosaicism and aneuploidy are very common in early human embryos, but significantly lower rates of aneuploidy or mosaicism are detected at birth (Nagaoka *et al.*, 2012; Bielanska *et al.*, 2002). The first mitotic divisions of human embryos are extremely error-prone (McCoy *et al.*, 2015), which could result in

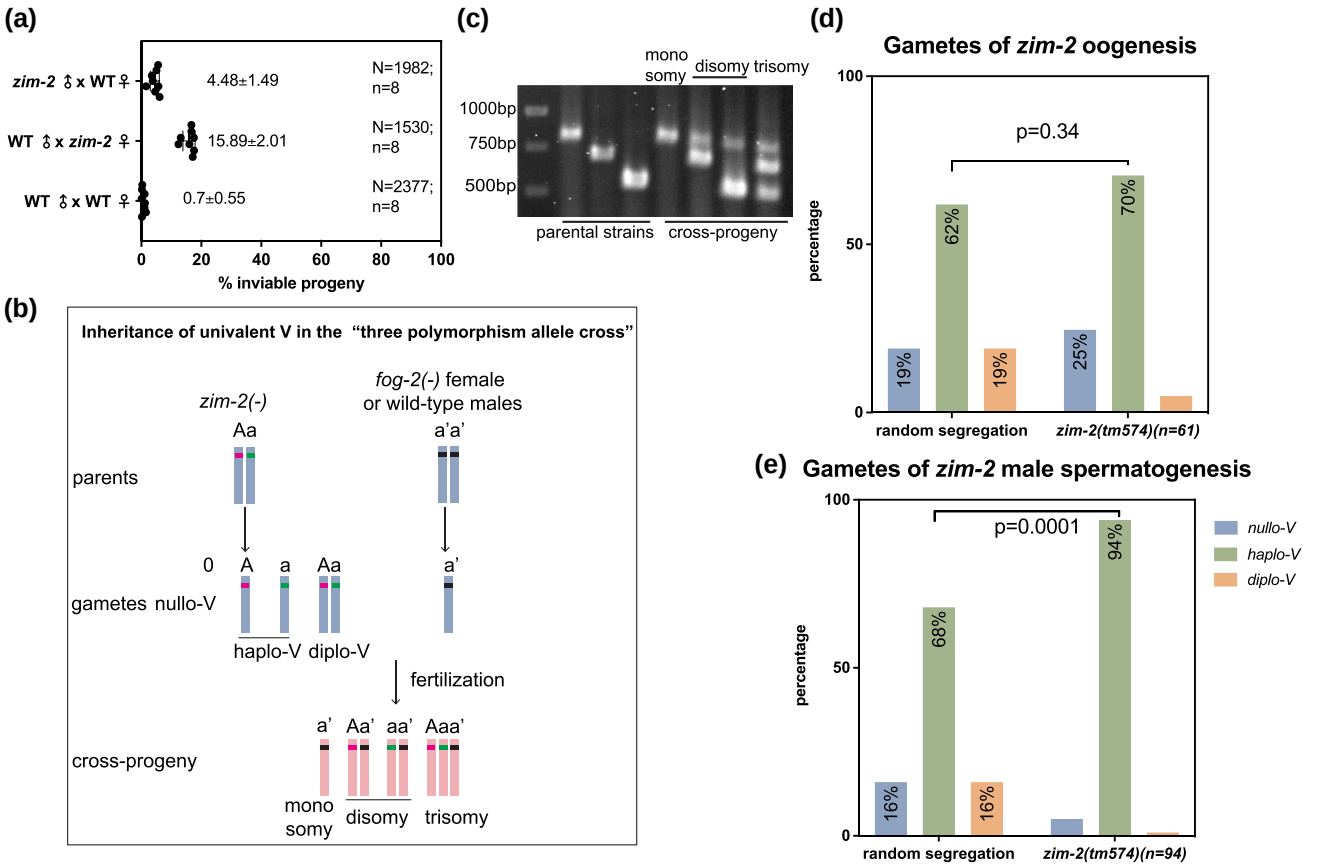

Fig. 2. *Zim-2(tm574)* males produce more euploid progeny than what would be expected from random segregation. a) Percentage of inviable progeny produced by *zim-2(tm574)* males mated with *fog-2(–)* females, *zim-2(tm574)* females mated with WT males, and WT males mated with WT females. Each dot represents progeny from one parent. N: total number of progeny counted; *n*: number of parents. The numbers shown next to the scatter plot are mean ± SD. b) Diagram of the "three-polymorphism allele crosses" to detect nullo-V, haplo-V, and diplo-V gametes by *zim-2(tm574)* female oocytes and males. *Zim-2(tm574)* females or males with 2 different polymorphism alleles were mated/mated with males or *fog-2(–)* females with a third polymorphism allele on chromosome V. Their progeny was subject to PCRs for corresponding polymorphism alleles. Primers amplifying alleles are listed in Supplemental Material. c) Agarose gel showing examples of parental strains with 3 distinguishable polymorphism alleles, and examples of monosomic V progeny (nullo-V gamete, 1 band); disomic V progeny (haplo-V gamete, 2 bands), and trismic V progeny (diplo-V gamete, 3 bands). d) Percentage of nullo-V, haplo-V, and diplo-V gametes from *zim-2(tm574)* female oogenesis and e) *zim-2(tm574)* male spermatogenesis. Random segregation: percentage of 3 types of gametes expected from random segregation after correcting for pre-existing crossover rate. *P*-value by Fisher's exact test (2 × 2, comparing aneuploidy and euploidy).

both mitotic aneuploidy as well as correction of meiotic aneuploidy. In addition, aneuploid cells are progressively eliminated during embryogenesis (Orvieto *et al.*, 2020; Yang *et al.*, 2021). This "mitotic correction" of meiotic aneuploidy likely explains uniparental disomy, the condition of having 2 chromosome copies from 1 parent and none from the other parent, in humans (Nakka *et al.*, 2019).

Here, we conducted an investigation of a *C. elegans zim-2* mutant, which presents a unique opportunity to study the response of the meiotic machinery to a specific crossover defect of a specific autosome.

Materials and methods

C. elegans strains

Worms used in this study were maintained under standard laboratory conditions. Strains are listed in Supplementary Table 1.

Three-polymorphism allele crosses

The F2 progeny were obtained as follows:

For polymorphism allele crosses to identify chromosome V copy number of progeny from *zim-2(tm574)*, *zim-2(tm574)* hermaphrodites at the L4 stage were mated to *zim-2(tm574)* males with JU258 polymorphisms on chromosome V to obtain F1 homozygous *zim-2(tm574)* worms with heterozygous polymorphisms (JU258/N2) on chromosome V. Only hermaphrodites with a mating plug and having more than 50% male progeny were considered to have mated. Males for crosses were generated by heat shocking L4 worms at 32° for 5 hr. To obtain F2 progeny from *zim-2(tm574)* male parents, F1 *zim-2(tm574)* males were mated to *fog-2(–)* CB4856 polymorphic hermaphrodites. To obtain F2 progeny from *zim-2(tm574)* female parents, F1 *zim-2(tm574)* females were mated to CB4856 Hawaiian males.

For polymorphism allele crosses to identify chromosome II and III copy number of progeny from *zim-1**, *zim-1(tm1813)* hermaphrodites at the L4 stage were mated to a *zim-1(xoe6)* CB4856 polymorphic males to obtain F1 *zim-1*(tm1813/xoe6)* worms with heterozygous polymorphisms (N2/CB4856). Only hermaphrodites with a mating plug and having 50% male progeny were considered to have mated. To obtain F2 progeny from *zim-1** male parents, F1 *zim-1** males were mated to JU258 polymorphic hermaphrodites. To obtain F2 progeny from *zim-1** female parents, F1 *zim-1** females were mated to JU258 Hawaiian males.

F2 eggs and hatched larvae (all progeny from the parents within 24 hr) from at least 3 individual mothers were subject to PCRs

within 0–24 hr after being laid. Strains, polymorphisms, and primers for PCRs are listed in Supplementary Tables 1 and 2.

For PCRs, single progeny were sorted into PCR tubes and digested in 6 ml of lysis buffer and proteinase K. Lysis buffer was composed of the following: 1-M KCl, 1-M Tris pH 8.3, 1-M MgCl2, 0.45% IGEPAL, 0.45% Tween 20, and sterile water. The tubes were submerged in liquid nitrogen for 10 min and heated in a thermocycler at 60° for 60 min and 95° for 15 min. Each PCR reaction was 20 ul total. The following final concentrations of each reagent were used: 1x Standard Taq Reaction Buffer, 1-mM $MgCl_2$, 300-μM dNTPs, 0.5-μM forward primer, 0.5-μM reverse primer, 1000-ng template DNA (1/6th of a worm/egg), 0.4 unit/20-μl PCR Hot Start Taq DNA Polymerase, and sterile water. Primers are listed in Supplementary Table 2. All PCR reactions followed the standard Hot Start Taq Polymerase protocol with 38 cycles for denaturation, annealing, and extension. When doing PCR on adult animals, 32 cycles were utilized.

Live imaging

zim-2(tm574), *zim-1(tm1813)*, and *zim-3(tm2303)* strains expressing AIR-2::GFP, mCherry::histone H2B, and mKate::PH; *spo-11(me44)/nT1* strains expressing AIR-2::GFP and mCherry::histone; and *spo-11(me44)/nT1* strains expressing GFP::HIS and mKate::TUB were constructed. Worms were anesthetized with tricaine/tetramisole as described (Kirby et al. 1990; McCarter et al. 1999) and gently mounted between a coverslip and a thin 2% agarose pad on a slide. Images in Figs. 3–5 and Supplementary Figs. 3–5 were captured with a Solamere Spinning Disk Confocal equipped with a Yokogawa CSU10, Hamamatsu Orca Flash 4.0 cMOS, and an Olympus 100x/1.35 oil objective. For counting chromosomes in female diakinesis oocytes and male spermatocytes, z-stack images were taken in a 0.5-μm step size to include all chromosomes. For time-lapse movies of male meiosis, 3 z-stacks in a 1-μm step size were captured every 20 s.

Statistical analysis

Chi-square and Fisher's exact tests of 2 × 2 contingency tables were calculated with GraphPad Prism. Fisher's exact test of 2 × 3 contingency tables was calculated with https://www.danielsoper.com/statcalc/calculator.aspx?id = 58.

Results

Crossovers in *zim-2(tm574)* male and female parents

zim-2(tm574) mutants have been reported to be defective in pairing and recombination of chromosome V (Phillips and Dernburg, 2006) but to have a higher than expected frequency of viable progeny (Jaramillo-Lambert *et al.*, 2010). At diakinesis in *C. elegans*, when homolog pairing, recombination, and chromosome remodeling have completed, every pair of homologous chromosomes appears as 1 chromosome body called a "bivalent," consisting of 4 chromatids held together by a crossover and sister chromatid cohesion. In wild-type *C. elegans*, 6 chromosome bodies will be present, with 5 autosomal bivalents (autosomes I–V), and 1 X chromosome bivalent in oocytes (XX) or 1 unpaired X univalent in spermatocytes of males (XO). The majority of *zim-2(tm574)* diakinesis oocytes have 7 chromosome bodies, 5 bivalents, and 2 V univalents. However, a variable number of oocytes with 6 chromosome bodies have been reported, 28% (Phillips and Dernburg, 2006), 38% (Cortes *et al.*, 2015), and 24% (Supplementary Fig. 2a, Supplementary Fig. 2c, this study). This is possibly due to chromosome V crossovers or noncrossover inter-homolog chromosome connections in the absence of ZIM-2. To distinguish between these possibilities, we measured the crossover incidence in *zim-2(tm574)* oocytes by analyzing recombination between polymorphism alleles on the ends of chromosome V. *zim-2(tm574)* females heterozygous for polymorphism alleles on the 2 ends of V were crossed with wild-type males carrying a third set of polymorphism alleles on the 2 ends of chromosome V (Fig. 1c, Supplementary Fig. 3). We found that 14.72% of cross progeny from *zim-2(tm574)* females were recombinant for chromosome V (Fig. 1d). Because there is exactly one crossover per *C. elegans* bivalent, equal numbers of recombinant and nonrecombinant progeny result from normal recombination. Thus *zim-2(tm574)* females have a crossover frequency of 2 × 14.72% or 29.44% for chromosome V. The detected crossovers likely resulted from a redundant mechanism, instead of incomplete knockdown of *zim-2*, as no ZIM-2 was detected by antibody staining in *zim-2(tm574)* gonads (Phillips and Dernburg, 2006). This crossover frequency was also not significantly different than the 28% of *zim-2* oocytes with 6 DAPI-staining bodies observed by Phillips and Dernburg (2006) indicating that 29.44% of *zim-2* oocytes have a crossover rather than a noncrossover connection between homologous copies of chromosome V.

The higher frequency of viable progeny from *zim-2(tm574)* males in Jaramillo-Lambert *et al.* (2010) might be due to a higher frequency of background crossovers. To test this, we examined the progeny from *zim-2(tm574)* male parents. *zim-2(tm574)* males heterozygous for polymorphism alleles on the left end and right end of chromosome V were crossed with *fog-2(–)* females (unable to make their own sperm) with a third set of polymorphism alleles on the 2 ends of chromosome V. In addition, 18.68% of progeny from *zim-2(tm574)* male parents were recombinant (Fig. 1d). This is not significantly different from females ($P = 0.6614$, 2 × 2 Fisher's exact test) and does not explain the higher progeny viability of *zim-2(tm574)* males.

zim-2(tm574) males produce more euploid progeny than what would be expected from random segregation

To test whether *zim-2(tm574)* mutants have a higher percentage of euploid progeny than expected from random segregation, we analyzed the copy number of chromosome V among the progeny of *zim-2(tm574)* females carrying heterozygous polymorphism alleles on chromosome V mated with wild-type males with a third polymorphism allele on chromosome V. Euploid progeny will inherit only one chromosome V from the *zim-2(tm574)* female parent while trisomic progeny will inherit both maternal copies and monosomic progeny will inherit neither maternal copy of chromosome V (Fig. 2b and c). In addition, 30% of progeny were aneuploid (Fig. 2d), slightly less than what would be expected from random segregation (38%, after correcting for the crossover rate in females). In contrast, *zim-2(tm574)* males mated with *fog-2(–)* females had notably fewer aneuploid progeny (6%) than what would be expected from random segregation (32%, after correcting for the crossover rate in males) (Fig. 2e). This suggested that 2 univalent Vs in males were distributed to spermatids equally instead of randomly. Segregation of 2 achiasmate homologous chromosomes apart at higher than random frequency has been previously referred as distributive segregation (Zhang and Hawley, 1990).

To test the generality of the apparent distributive segregation of chromosome V univalents in *zim-2(tm574)* males, we performed a similar analysis of *zim-1(tm1813/xoe6)* worms which exhibit crossover failures on chromosomes II and III (Phillips and Dernburg, 2006, alleles used are described in Materials and Methods). We observed 8 chromosome bodies in 100% of *zim-1*

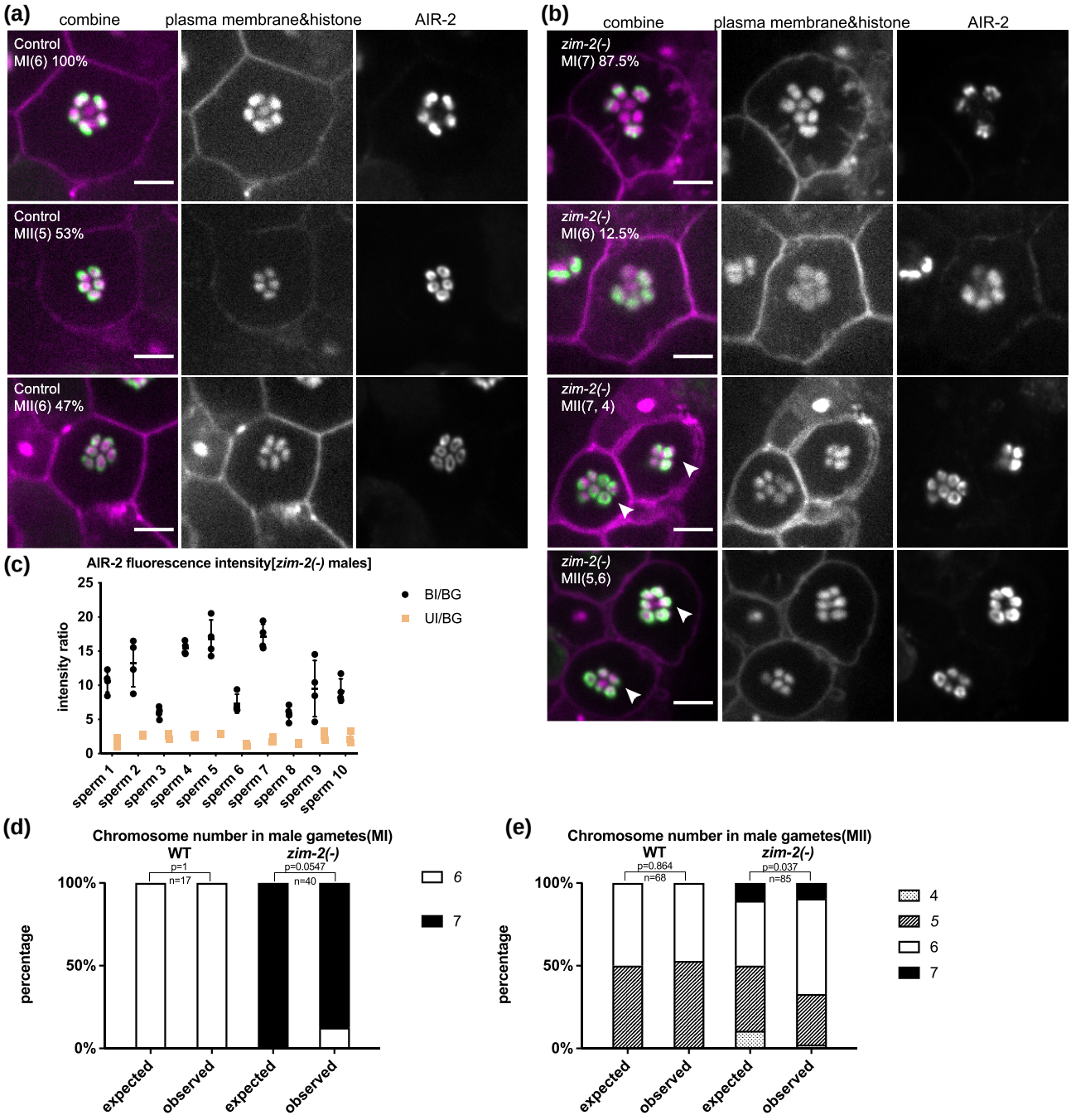

Fig. 3. *zim-2(tm574)* spermatocytes gave rise to more gametes with normal number of chromosomes. a) Representative images of live wild-type spermatocytes with 6 chromosome bodies at metaphase I and 5 or 6 chromosome bodies at metaphase II. AIR-2 in green; histone and plasma membrane in magenta. Scale bar: 2 μm. b) Representative images of live *zim-2(tm574)* spermatocytes at metaphase I (with 7 or 6 chromosome bodies) and II (with 7, 4, 5, or 6 chromosome bodies). c) AIR-2::GFP fluorescence intensity ratio of 4 bivalent to background (BI/BG, black) and 3 univalent to background (UI/BG, yellow) in 10 individual *zim-2(tm574)* spermatocytes. d) Percentage of metaphase I spermatocytes with 6 or 7 chromosome bodies in control and *zim-2* males. e) Percentage of metaphase II spermatocytes with 4, 5, 6, or 7 chromosome bodies in control and *zim-2* males. Expected: expected from random segregation at metaphase II after correcting for existing chromosome alignment at metaphase I. 3.5% with 4 chromosome bodies, 32.5% with 5, 53.5% with 6, 10.5% comparing to 10.7%, 39.3%, 39.3%, and 10.7% expected from random segregation, respectively.

oocytes indicating a very low background crossover frequency on II and III (Supplementary Fig. 2b and c). *zim-1* males had more progeny that was euploid for chromosome II or III than what would be expected from random segregation and more euploid progeny than that of *zim-1* hermaphrodites (Supplementary Fig. 2e). Among *zim-3(tm2303)* diakinesis oocytes, 13.95% had 7 chromosome bodies and 78.05% had 8 chromosome bodies (Supplementary Fig. 2b and c). The polymorphism assay to detect euploid progeny from *zim-3* mutant was not conducted due to unavailability of *zim-3* mutant carrying distinguishable polymorphisms. Interestingly, univalents loaded less Aurora B kinase AIR-2 than bivalents in *zim-1*, *zim-2*, and *zim-3* mutants (Supplementary Fig. 2d), consistent with a previous study (Muscat *et al.*, 2015).

Univalent V tends to be evenly distributed in *zim-2(tm574)* male spermatocytes

To examine whether the high incidence of euploid progeny from *zim-2(tm574)* males originates from meiosis (mitotic correction can also increase euploidy), we took images of spermatocytes at metaphase I and metaphase II. If distributive segregation occurs during anaphase I, then 2 univalents in metaphase I spermatocytes would segregate away from each other, resulting in more spermatocytes having a normal number of chromosomes at metaphase II than what would be expected from random segregation. Wild-type metaphase I spermatocytes had 6 chromosome bodies, of which 1 is the univalent X. If the sister chromatids of the univalent X remain intact at anaphase I, 50% of metaphase II spermatocytes will inherit the X and have 6 chromosome bodies, 1 X, and 5 autosomal chromosomes. In addition, 50% of metaphase II spermatocytes that do not inherit the X chromosome will have 5 autosomal chromosome bodies. If sister chromatids of the X split at anaphase I, then metaphase II spermatocytes will all have 6 chromosome bodies. Among wild-type metaphase II spermatocytes ($n = 68$), 53% ($n = 36$) had 5 chromosome bodies and 47% ($n = 32$) had 6 chromosome bodies (Fig. 3a and d), indicating the X univalent remains intact at anaphase I, consistent with previous studies (Shakes *et al.*, 2009).

12.5% ($n = 5$) of *zim-2(tm574)* metaphase I spermatocytes ($n = 40$ analyzed) had 6 chromosome bodies (Fig. 3b and d), indicating a chiasma between chromosome V homologs in those spermatocytes, and consistent with the crossovers detected by polymorphism analysis. This 12.5% of spermatocytes should behave like wild-type and at metaphase II should have 50% 5 autosomal bodies and 50% 6 chromosome bodies (5 autosomes and 1 X). In addition, 87.5% ($n = 35$) of *zim-2* metaphase I spermatocytes had 7 chromosome bodies, comprised of 4 bivalents, 1 univalent X, and 2 univalent Vs (Fig. 3b and d). If 2 univalent Vs segregate randomly, 75% of their metaphase II spermatocytes will have 5 or 6 chromosome bodies. Therefore, 78% of total metaphase II spermatocytes will have 5 or 6 chromosome bodies (12.5%∗1 +87.5%∗0.75∗). In addition, 85% ($n = 72$) of metaphase II spermatocytes ($n = 85$ analyzed) had 5 or 6 chromosome bodies, significantly more than what would be expected from random segregation (78%, Fig. 3b and e, $P = 0.037$ by Fisher's exact test 2×2 after correcting for 12.5% nuclei with normal chromosome number). In conclusion, chromosome counting of the images of meiotic metaphase I and II in *zim-2(tm574)* males suggests 2 univalent Vs tend to be more equally distributed to the metaphase II spermatocytes than which would result from random segregation.

Faithful chromosome segregation relies on sequential cohesion release between homologs at anaphase I and sister chromatids at anaphase II as a result of phosphorylation of the cohesin subunit REC-8, which depends on the spatial and temporal activity of Aurora B kinase AIR-2 in *C. elegans* (Ferrandiz *et al.*, 2018; De Carvalho *et al.*, 2008; Tzur *et al.*, 2012). We found that, like the univalent X in wild-type, V univalents in *zim-2(tm574)* metaphase I spermatocytes, in which 7 chromosome bodies were shown, loaded very little AIR-2 compared with bivalents (Fig. 3c). This suggests sister chromatids of univalents might remain intact at anaphase I due to insufficient AIR-2 loading. Moreover, AIR-2 appeared on all metaphase II chromosomes in both wild-type and *zim-2* mutants (Fig. 3a and b), suggesting that sister chromatids likely segregate at anaphase II.

Time-lapse imaging of univalent V segregation in *zim-2(tm574)* males

To track univalent behavior more directly, we filmed meiotic chromosome segregation in wild-type and *zim-2(tm574)* males labeled with AIR-2::GFP, mCherry::histone, and mKate::PH (plasma membrane) (Fig. 4a and b, Supplementary Fig. 4). In *C. elegans* wild-type XO males, the univalent X frequently lags at meiosis I and sister chromatids of the X chromosome separate at meiosis II (Albertson and Thomson, 1993; Shakes *et al.*, 2009). In all wild-type meiosis I spermatocytes ($n = 21$), when segregation of autosomal homologs completed, the single X univalent still lagged at the midzone and the intensity of AIR-2::GFP on the lagging X was much lower than on metaphase bivalents. Meanwhile, separating autosomes had AIR-2 attached to their side at anaphase I (Fig. 4a). This is similar to what has been reported in a previous study by immunohistochemistry (Shakes *et al.*, 2009) and is very different from oocytes, in which AIR-2 dissociates from chromosomes at anaphase I and relocates to the midzone (Rogers *et al.*, 2002). Why the localization and dynamics of AIR-2 in males differ from female meiosis and whether this underlies different segregation mechanisms between sexes are not clear. Localization of AIR-2 in the middle of the univalent X indicated sister chromatids were oriented toward opposite spindle poles. If sister chromatids segregate precociously at anaphase I, the resulting single sister chromatids would be likely to lag at anaphase II. No lagging chromosomes were observed in anaphase II spermatocytes in both wild-type ($n = 25$, Fig. 4c) and *zim-2(tm574)* males ($n = 20$, Fig. 4c), suggesting univalents did not split at anaphase I.

Three out of 24 meiosis I spermatocytes in *zim-2(tm574)* males recapitulated wild-type male meiosis, in having a single lagging chromosome at anaphase I (Supplementary Fig. 4), consistent with 12.5% metaphase I spermatocytes having crossovers on V. In the remaining 21 spermatocytes, more than 1 univalent was observed lagging in the midzone when all other autosomes had segregated (Fig. 4b and c). In the spermatocytes ($n = 20$) where 3 univalents (1 X and 2 V univalents) were present, the intensity of AIR-2::GFP on the lagging univalents was much lower than that on metaphase bivalents. In 1 spermatocyte, all 3 univalents moved to the same pole at anaphase I (4 aneuploid-V embryos will be made after fertilization) whereas in the other 19 spermatocytes, the 3 univalent segregated in a "two and one" manner to the opposite spindle pole during late anaphase (Fig. 4b). It is not possible to distinguish the X univalent from the 2 V univalents in these time-lapse sequences. However, the polymorphism assays demonstrated that *zim-2* males produce only 6% aneuploid-V gametes (4.8 out of 80 spermatids will be aneuploid). We therefore inferred that the other 19 spermatocytes with a "two and one" segregation pattern produced 76 euploid-V spermatids, suggesting that the 3 univalents segregating in the "two and one" manner were 1 X and 1 V univalents segregating away from the other V univalent. This is significantly higher than expected from random segregation (Fig. 4d). In conclusion, live imaging of *zim-2(tm574)* male meiosis suggests sister chromatids of univalents did not split at anaphase I and that 2 univalent Vs tend to segregate apart to opposite poles instead of segregating together to the same pole at anaphase I.

Segregation of univalents in *spo-11(me44)* oocytes and spermatocytes

To eliminate the uncertainty generated by the univalent X in identifying autosomal univalents in *zim-2* males, we utilized a *spo-11*

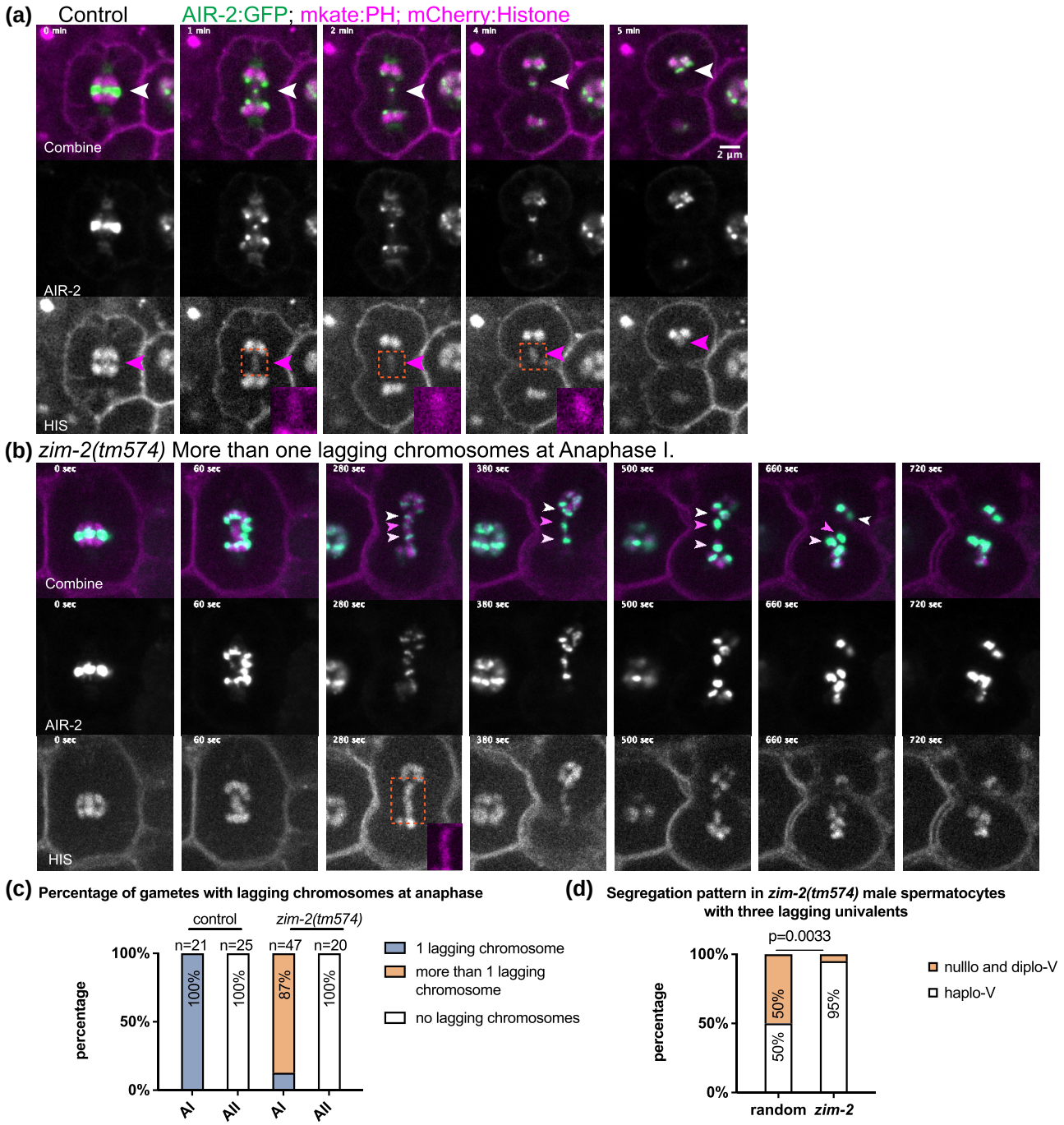

Fig. 4. Univalent V in *zim-2(tm574)* males tends to segregate apart at meiosis i. a) Time-lapse images of male meiosis I spermatocytes labeled with AIR-2::GFP, mKate::PH, and mCherry::histone in wild-type and b) in *zim-2(tm574)* chromosomes, with more than one lagging chromosome (87.5%). The arrowheads point to lagging chromosomes. The zoomed insets from the midzone highlighted by dashed boxes at anaphase were shown on the right bottom. c) Percentage of gametes with lagging chromosomes in wild-type and *zim-2(tm574)* at anaphase I and anaphase II. d) Percentage of *zim-2(tm574)* spermatocytes having 3 univalents segregated in "nullo- and diplo-V" or "haplo-V" pattern.

mutant in which oocytes and spermatocytes both enter meiosis I with all univalents. SPO-11 is required for inducing double-strand breaks that initiate meiotic DNA recombination. Deletion of SPO-11 prevents the formation of chiasmata and crossovers, so that 12 univalents are observed in diakinesis oocytes (Dernburg *et al.*, 1998) (13/13 *spo-11* oocytes, this study).

We utilized time-lapse imaging to monitor meiosis I in *spo-11* spermatocytes (Fig. 5a) and *spo-11* oocytes (Fig. 5b). We evaluated chromosome segregation by measuring the fluorescence intensity ratio of the 2 separating chromosome masses at anaphase I. This ratio was very close to 1 in control spermatocytes ($n = 12$) and oocytes ($n = 12$), indicating chromosomes are equally partitioned (Fig. 5c, Supplementary Fig. 5). Univalents also segregated roughly in half at anaphase I in most *spo-11* spermatocytes and oocytes. A ratio of separating chromosome masses within 1.5 was observed in 79% of *spo-11* spermatocytes ($n = 24$) and all *spo-11* oocytes ($n = 12$) (Fig. 5c). This suggested a mechanism that moves roughly equal masses of chromatin to opposite poles during anaphase I. It is not clear why the ratio in *spo-11* spermatocytes had a bigger variation than that in *spo-11* oocytes. However, homozygous

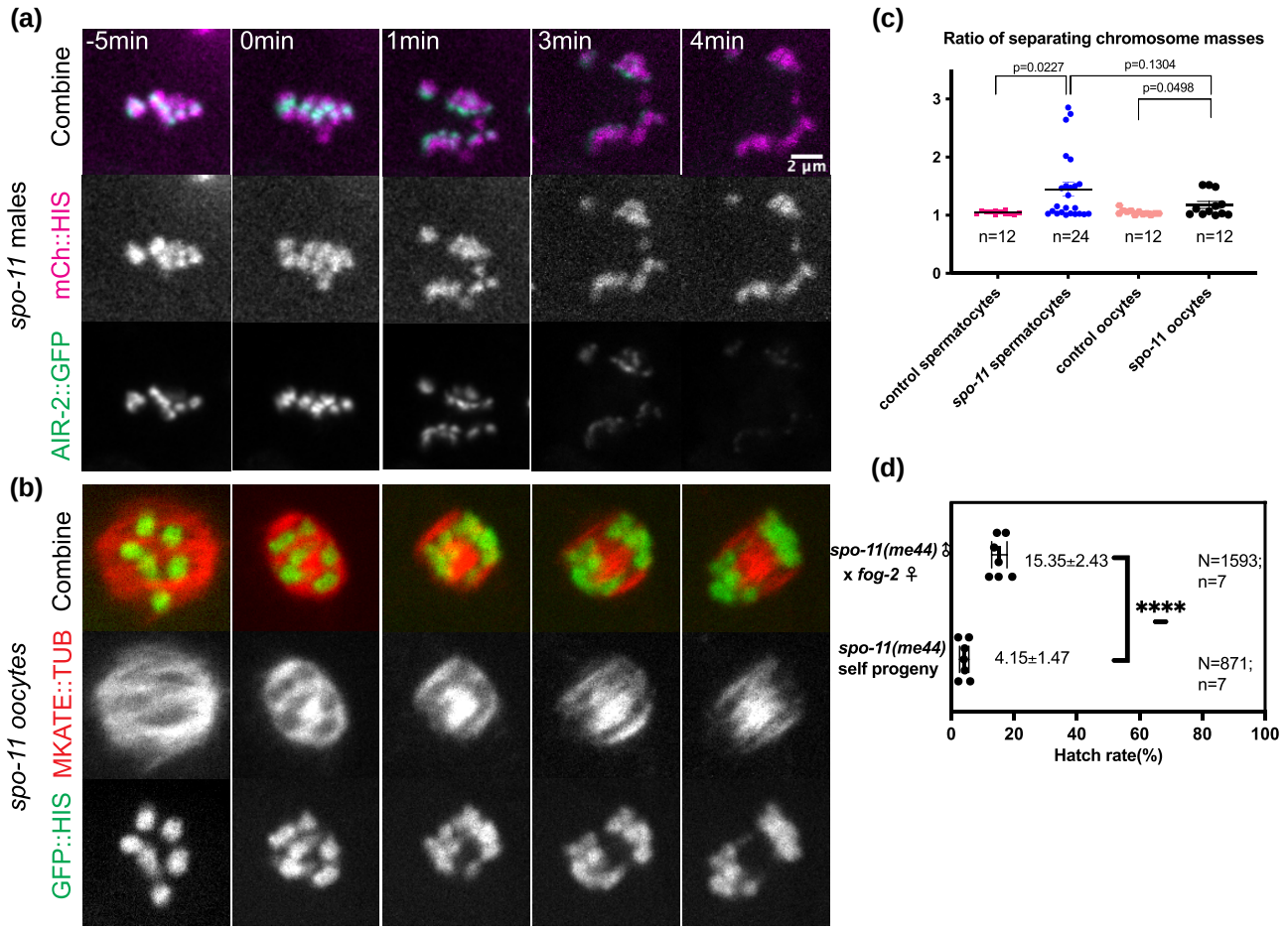

Fig. 5. A total of 12 univalents in *spo-11(me44)* segregated into 2 roughly equal masses at anaphase I. a) Single focal plane time-lapse images of *spo-11(me44)* male meiosis I spermatocytes labeled with AIR-2::GFP; mCherry::histone. A total of 7 AIR-2-labeled chromosomes are visible at metaphase in a single plane compared with 3 in control single planes (Supplementary Fig. 5). Chromosomes aligned at meiotic metaphase I plate and segregated roughly in half (12/16). Scale bar: 2 μm. b) Single focal plane time-lapse images of *spo-11(me44)* meiosis I oocytes labeled with GFP::HIS; mKate::TUB. A total of 6 chromosomes are visible at metaphase in a single plane compared with 3 in control single planes (Supplementary Fig. 5). c) Fluorescence intensity ratio of separating chromosome masses in control male spermatocytes, *spo-11(me44)* male spermatocytes, control oocytes, and *spo-11(me44)* oocytes. Each dot represents the ratio of 2 separating chromosome masses in 1 cell by measuring the fluorescence intensity of maximum projection of separating masses from z-stack images of oocytes/spermatocytes at anaphase I. *n*, number of cells analyzed. d) The hatch rate of progeny from *spo-11(me44)* males crossed with *fog-2* females and *spo-11(me44)* hermaphrodites. *N*, total number of progeny counted; *n*, number of parents. $P < 0.0001$, unpaired *t* test.

spo-11(me44) hermaphrodites are viable but produce more than 90% dead self-progeny (Stamper *et al.*, 2013). *Fog-2(–)* hermaphrodites crossed with *spo-11(me44)* males produce 84.65% dead eggs (Fig. 5d), significantly less than that of self-progeny from *spo-11(me44)* hermaphrodites ($P < 0.0001$, unpaired *t* test). This indicates that a high degree of aneuploidy is generated in both oocytes and spermatocytes, consistent with a previous study (Severson *et al.*, 2009). The equal distribution of mCherry::histone-labeled chromatin in the majority of *spo-11(me44)* meiotic cells but high progeny lethality suggests that distributive segregation might separate any 2 univalents apart, instead of partitioning homologs specifically. This would result in gametes with a relatively normal number of chromosomes but high chromosomal aneuploidy (e.g. 1 gamete having 2 copies of chromosome I, II, and III, but no chromosome IV, V, and X).

Trisomy I, II, and V are viable and fertile while monosomy is lethal

Our polymorphism-based karyotyping indicated that *zim-1* and *zim-2* females do not have significantly more euploid progeny than expected from random segregation, but progeny viability was higher than expected if all aneuploidies are lethal. It might be that viable trisomy and monosomy could contribute to a higher-than-expected hatch rate. There are 6 possible trisomies and monosomies for *C. elegans*. Viable trisomy X and trisomy IV have been reported in previous studies (Sigurdson *et al.*, 1984; Hodgkin *et al.*, 1979; Vargas *et al.*, 2017). The phenotypes of trisomy and monosomy of other chromosomes have not yet been reported. We examined the viability and fertility of trisomic, disomic, and monosomic female progeny from *zim-1* females crossed with WT males by a "three-polymorphism allele cross." *zim-1* hermaphrodites that were heterozygous for PCR polymorphism alleles on chromosome II and III were crossed with wild-type males homozygous for a third polymorphism allele. The F1 progeny (could be trisomic, disomic, or monosomic) was singled into individual plates, allowed to develop, and then scored as dead embryo, hatched larvae, or adults (Fig. 6a and b). If F1s hatched, DIC images of F1s were obtained to score for phenotypic characteristics (Fig. 6a), and hatch rates of F2 self-progeny were obtained after allowing F1's to lay eggs for 24 hr (Fig. 6c). Then F1s were subjected to the PCR analysis to determine the copy number of chromosomes II and III. Among all the trisomy II that we detected, the majority (83%)

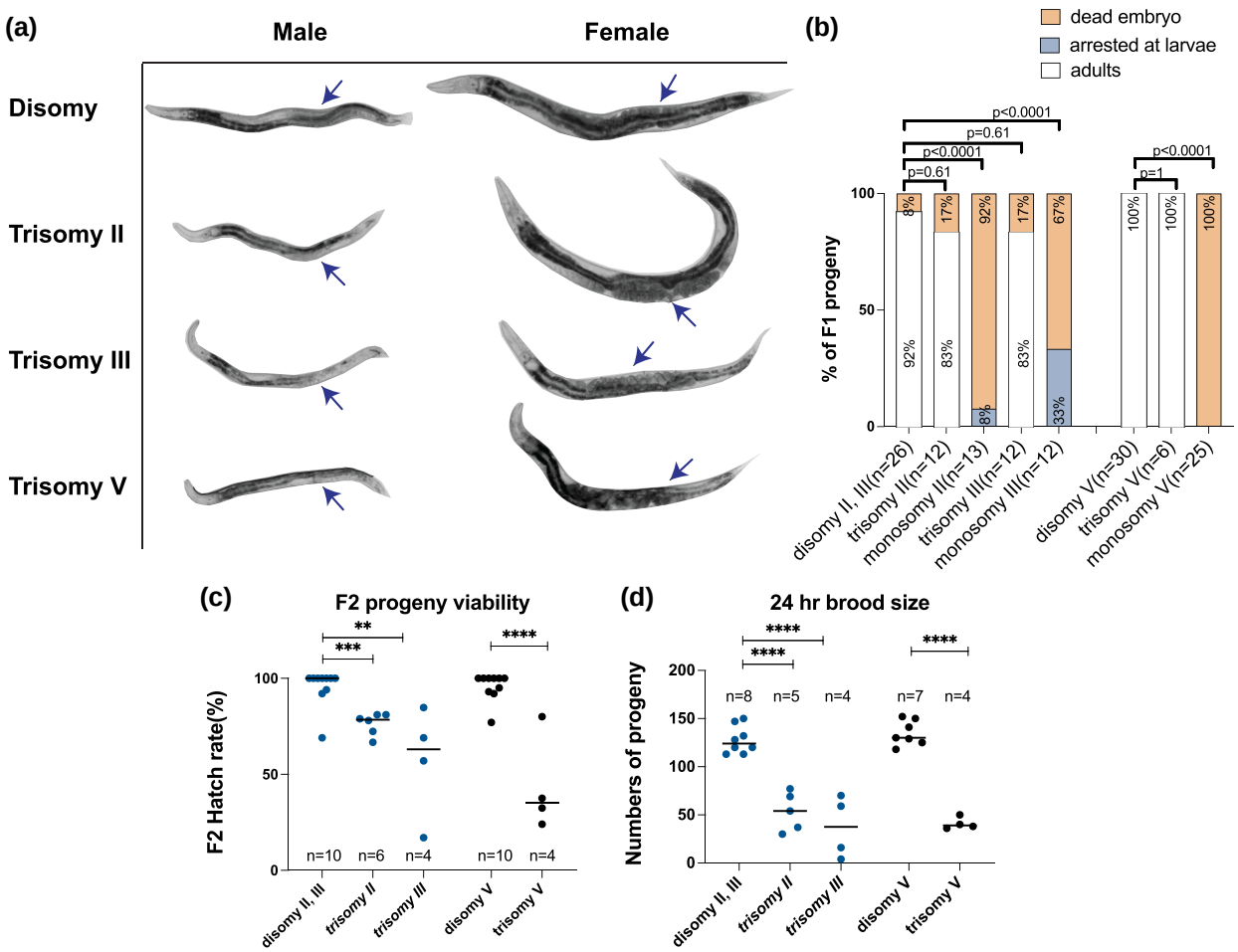

Fig. 6. Most trisomies are viable with reduced fertility whereas monosomies are lethal. a) Differential interference contrast (DIC) images of disomic/trisomic II, III, and V adults. The arrows point to gonads. b) Percentage of dead embryos, arrested larvae, and adults among disomic, trisomic, and monosomic F1 offspring from "*zim-1* female x wild-type male polymorphism II and III cross" (left) and from "*zim-2* female x wild-type male polymorphism V cross" (right) are presented. c) The F2 hatch rate and d) 24-hr brood size of disomy vs trisomy for chromosomes II, III, and V. For b, c, and d, disomies are F1 siblings from the same cross used to generate the trisomies. For a, b, and d, all worms are *zim-1*/+ or *zim-2*/+, and these mutations are recessive. For c, 25% of the F2 progeny should be homozygous *zim-1* or *zim-2*.

hatched and developed into adults with gonads, and 17% did not hatch, the viability of which is not significantly different from their disomic peers (Fig. 6b). Whereas for monosomy II, only 8% hatched and those all arrested at earlier larvae stages. Similarly, trisomy III had similar viability as disomy III: 83% trisomy III reached adulthood, and all monosomy III either did not hatch or were arrested before growing into adults (Fig. 6a and b). Similar crosses with *zim-2* worms revealed that trisomy V progeny are viable whereas monosomy V progeny did not hatch (Fig. 6a and b).

In terms of phenotypic variations, monosomies II and III were extremely sick, small, and usually had motility issues. Trisomy II or III had variable length and width compared to disomy. Interestingly, trisomy II, III, or V hermaphrodites had significantly smaller brood sizes, and their progeny had lower hatch rates (Fig. 6c and d), suggesting that trisomy II or III was viable but had reduced fertility. Our results indicate that survival of trisomy II or trisomy III worms contributes to the high viability of progeny from *zim-1* parents.

Discussion

Our results indicate that spermatocytes in male *C. elegans* can compensate for spontaneous crossover failures by segregating achiasmate univalents apart at anaphase I. In addition, trisomic progeny from a male or female parent is viable, and fertile and previous studies have demonstrated that oocytes of trisomic worms can preferentially eliminate the extra chromosome during meiosis (Cortes *et al.*, 2015; Vargas *et al.*, 2017). Together these mechanisms constitute a robust system for ensuring that a high percentage of healthy euploid progeny can be produced after spontaneous crossover failures.

The different response of male spermatocytes and oocytes to achiasmate univalents could be due to the presence of a third univalent (the X univalent) in males or to some other difference between oocyte and spermatocyte meiosis. Analysis of hermaphrodite spermatogenesis in a *zim-2* mutant could distinguish between these possibilities because there is a bivalent X present. However, our polymorphism approach cannot be applied to hermaphrodite spermatogenesis. The "two and one" segregation pattern in most spermatocytes could be mechanistically related to the "skew" phenomenon. *C. elegans* males that have an asymmetric bivalent, with one homolog larger than the other, preferentially segregate the larger homolog away from the univalent X (Wang *et al.*, 2010; Le *et al.*, 2017). It is possible that a crossover-independent mechanism equalizes the mass of chromatin moving toward each spindle pole and the univalent X affects this equalization only in XO males.

In *Drosophila* (Thomas *et al.*, 2005; Weber *et al.*, 2020), *Bombyx* (Rosin *et al.*, 2021), *Luzula elegans* (Heckmann *et al.*, 2014), and humans (Gruhn *et al.*, 2019), preferential segregation apart of homologs without a crossover is associated with a noncrossover tether between homologs. In *Arabidopsis*, crossover-defective mutants that assemble synaptonemal complex (SC) on univalents are able to segregate homologs apart, whereas mutants that fail to assemble (SC) segregate homologs randomly (Pradillo *et al.*, 2007). Because an aberrant SC is a candidate for a tether that might promote segregation of achiasmate homologs, it is pertinent to note that homologous chromosomes in *C. elegans spo-11* mutants pair and form a synaptonemal complex with transverse elements (Dernburg *et al.*, 1998). However, these inter-homolog attachments are no longer detected in diakinesis oocytes. The univalents in *zim-1*, *zim-2*, and *zim-3* mutant oocytes load SC lateral element proteins, but do not pair or assemble SC transverse element proteins (Phillips and Dernburg, 2006). The naturally unpaired X of XO *C. elegans* males has been reported to form transient inter-sister pseudosynapsis that is part of a mechanism to prevent damage to and prevent checkpoint activation by the male-specific unpaired X (Checchi *et al.*, 2014; Jaramillo-Lambert and Engebrecht, 2010). This male-specific pseudosynapsis mechanism might generate male-specific tethers between univalent autosomes in *zim-1* and *zim-2* mutants. We did not observe such tethers in *C. elegans zim-2* mutants, but they might be revealed with different fluorescent probes or imaging methods.

Alternatively, the sex-specific behavior of achiasmate homolog pairs might be due to one of the several differences between oocyte and spermatocyte meiotic spindles. Unlike acentrosomal female meiotic spindles, male meiotic spindles assemble with centrosomes and robust astral microtubule arrays (Fabig *et al.*, 2020). Plus ends of astral microtubules can interact with cortical dynein to generate pulling forces that contribute to anaphase spindle elongation. In addition, dynein/dynactin is not required for anaphase in oocytes (Laband *et al.*, 2017; Danlasky *et al.*, 2020) but is essential for anaphase in spermatocytes (Barbosa *et al.*, 2021). Anaphase is described as two distinct processes, anaphase A in which chromosomes move closer to spindle poles and anaphase B in which spindle poles move apart. In *C. elegans* oocytes, anaphase A associated with kinetochore-dependent pulling forces (Danlasky *et al.*, 2020) occurs before kinetochore-independent anaphase B (Dumont *et al.*, 2010; McNally *et al.*, 2016) that is associated with outward pushing from the spindle midzone (Laband *et al.*, 2017). In *C. elegans* spermatocytes, anaphases A and B occur simultaneously (Fabig *et al.*, 2020). In *C. elegans* spermatocytes, microtubules run continuously from spindle poles to chromosomes (Fabig *et al.*, 2020) and make end-on attachments to the outside face of chromosomes (Wignall and Villeneuve, 2009; Fabig *et al.*, 2020). End-on microtubule attachments to the outside face of chromosomes are a hallmark of kinetochore-dependent pulling forces generated by microtubule depolymerization coupled with NDC80 complexes (Joglekar *et al.*, 2010). However, microtubules do not shorten during *C. elegans* spermatocyte anaphase A which is instead associated with changes in the shapes of the spindle and chromosomes (Fabig *et al.*, 2020). In contrast, *C. elegans* oocyte microtubules form discontinuous, overlapping arrays of short microtubules (Laband *et al.*, 2017; Redemann *et al.*, 2018) that form only side-on contacts with chromosomes during metaphase (Wignall and Villeneuve, 2009; Redemann *et al.*, 2018) or end-on contacts with the inside face of chromosomes during anaphase (Laband *et al.*, 2017; Redemann *et al.*, 2018). Univalent X chromosomes present during anaphase lag behind the main chromosome masses and are stretched, indicative of pulling forces, in both oocytes (Danlasky *et al.*, 2020) and spermatocytes (Fabig *et al.*, 2020). In *C. elegans* oocytes, midzone microtubules can push chromosomes apart (Laband *et al.*, 2017) and, in the absence of outer kinetochore function, can push intact bivalents apart in a homology-independent manner (Dumont *et al.*, 2010; Danlasky *et al.*, 2020). This midzone pushing mechanism might explain how univalents are separated into equal masses in *spo-11* oocytes. However, it has been reported that the midzone is composed of very few microtubules in *C. elegans* spermatocytes (Fabig *et al.*, 2020), making it unclear if this pushing mechanism is present in spermatocytes.

Data availability

Strains are available upon request. All polymorphisms and PCR primers used are listed in Supplementary Table 2. Representative images are presented within the article, and complete imaging data sets are available upon request.

Supplemental material available at GENETICS online.

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Conflicts of interest

None declared.

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