Sexual dimorphic regulation of recombination by the synaptonemal complex in *C. elegans*

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ABSTRACT

In sexually reproducing organisms, germ cells faithfully transmit the genome to the next generation by forming haploid gametes, such as eggs and sperm. Although most meiotic proteins are conserved between eggs and sperm, many aspects of meiosis are sexually dimorphic including the regulation of recombination. The synaptonemal complex (SC), a large ladder-like structure that forms between homologous chromosomes, is essential for regulating meiotic chromosome organization and promoting recombination. To assess whether sex-specific differences in the SC underpin sexually dimorphic aspects of meiosis, we examined Caenorhabditis elegans SC central region proteins (known as SYP proteins) in oogenesis and spermatogenesis and uncovered sex-specific roles for the SYPs in regulating meiotic recombination. We find that SC composition, specifically SYP-2, SYP-3, SYP-5 and SYP-6, is regulated by sex-specific mechanisms throughout meiotic prophase I. During pachytene, both oocytes and spermatocytes differentially regulate the stability of SYP-2 and SYP-3 within an assembled SC. Further, we uncover that the relative amount of SYP-2 and SYP-3 within the SC is independently regulated in both a sex-specific and a recombination-dependent manner. Specifically, we find that SYP-2 regulates the early steps of recombination in both sexes, while SYP-3 controls the timing and positioning of crossover recombination events across the genomic landscape in only oocytes. Finally, we find that SYP-2 and SYP-3 dosage can influence the composition of the other SYPs in the SC via sex-specific mechanisms during pachytene. Taken together, we demonstrate dosage-dependent regulation of individual SC components with sex-specific functions in recombination. These sexual dimorphic features of the SC provide insights into how spermatogenesis and oogenesis adapted similar chromosome structures to differentially regulate and execute recombination.
INTRODUCTION

In most sexually reproducing organisms, meiosis ensures the faithful inheritance of genetic information in each generation through the formation of haploid gametes, such as eggs and sperm. Many aspects of meiosis are sexually dimorphic from the differences in the size of egg and sperm cells to the molecular mechanisms ensuring accurate segregation of the chromosomes (reviewed in Cahoon & Libuda, 2019). However, many meiotic proteins are present in both sexes and how each sex utilizes a highly similar proteome to produce dimorphic phenotypes remains unclear.

Multiple studies in mice and plants indicate that meiotic chromosome structures, such as the synaptonemal complex (SC), are sexually dimorphic (reviewed in Cahoon & Libuda, 2019; Morgan et al., 2017). The SC assembles between homologous chromosomes in early prophase I (called the transition zone in C. elegans or late zygotene in mice, plants, and yeast) and organizes the genome to both facilitate and enable the essential meiotic processes of homolog pairing and recombination (reviewed in Cahoon & Hawley, 2016). This scaffold between the homologs formed by the SC allows for the accurate repair of DNA double strand breaks (DSBs) induced by the topoisomerase-like protein Spo11 (Keeney et al., 1997; Zickler & Kleckner, 1999, 2015). A subset of these DSBs must be repaired as crossover recombination events to allow for the accurate segregation of the homologs at anaphase I.

The SC is both required for the formation of crossovers and influences the frequency of crossovers occurring per homolog pair in multiple organisms (Colaiacovo et al., 2003; Costa et al., 2005; de Vries et al., 2005; Gordon et al., 2021; Hayashi et al., 2010; Higgins et al., 2005; Hillers & Villeneuve, 2003; Hurlock et al., 2020; Jeffress et al., 2007; Libuda et al., 2013; Liu et al., 2021; MacQueen et al., 2002; Page & Hawley, 2001; Smolikov, Eizinger, Hurlburt, et al., 2007; Smolikov et al., 2009; Sym et al., 1993; Woglar & Villeneuve, 2018). Proteins within the SC can influence crossover distribution, DSB repair mechanisms, and the crossover licensing process (Capilla-Perez et al., 2021; Durand et al., 2022; Garcia-Muse et al., 2019; Gordon et al.,
Voelkel-Meiman et al., 2015; Voelkel-Meiman et al., 2022). In *C. elegans*, pro-crossover proteins are recruited to the SC by the central region proteins of the SC (Cahoon et al., 2019; Libuda et al., 2013). Notably, the regulation of recombination is sexually dimorphic in many organisms (Arbeithuber et al., 2015; Bherer et al., 2017; Brick et al., 2018; Choi et al., 2018; Clement & Arndt, 2013; de Boer et al., 2015; Drouaud et al., 2007; Durand et al., 2022; Giraut et al., 2011; Gruhn et al., 2013; Halldorsson et al., 2019; Halliwell & Hoffmann, 2021; Morelli & Cohen, 2005; Pratto et al., 2021; Underwood et al., 2018). The exact mechanism(s) of how the SC regulates crossing over and obtains sex-specific outcomes remains an active area of study in multiple organisms.

Similar to other organisms, many aspects of *C. elegans* meiosis are sexually dimorphic from the timing of egg and sperm development to the regulation of checkpoints and recombination (Cahoon & Libuda, 2021; Checchi et al., 2014; Gartner & Engebrecht, 2022; Gumienny et al., 1999; Jaramillo-Lambert et al., 2007; Jaramillo-Lambert & Engebrecht, 2010; Jaramillo-Lambert et al., 2016; Jaramillo-Lambert et al., 2010; Lamelza & Bhalla, 2012; Li et al., 2020; Rourke & Jaramillo-Lambert, 2022; Saito & Colaiacovo, 2017). These sex-specific differences suggest that other critical recombination regulatory processes, such as the SC, may also have sexually dimorphic features in *C. elegans*. Both sexes in *C. elegans* are assumed to assemble the same proteins into the SC, but this aspect has not been extensively investigated as most of our knowledge about the SC in *C. elegans* focuses on oocytes.

Here we show that the SC is sexually dimorphic in *C. elegans*. Specifically, we demonstrate that the composition of the SC is not uniform during prophase I and instead is regulated in a sex-specific and protein dosage-dependent manner to facilitate specific steps of recombination. We find that a threshold level of SYP-2 in the SC is critical for the establishment and/or stabilization of recombination intermediates, while SYP-3 levels in the SC modulate the timing of crossover designation during pachytene. In addition, we identify sexual dimorphic
regulation of SC composition whereby specific SC proteins independently influence the levels of other proteins within the complex. Taken together, our study reveals novel regulation of recombination whereby the SC composition is dynamically altered throughout pachytene to facilitate sexually dimorphic mechanisms of DNA repair.

RESULTS

SYP-2 and SYP-3 are sexually dimorphic

To understand the relationship between the sexually dimorphic aspects of meiosis and the SC, we used the model system *C. elegans* where oocyte and spermatocyte development can be easily accessed and analyzed at both the same time and developmental stage. Adult male worms undergo spermatogenesis, while adult hermaphrodite worms undergo oogenesis (Figure 1A). The germline for both sexes is organized as a spatial-temporal gradient along the distal-proximal axis, thereby allowing for easy and simultaneous access to all stages of meiotic prophase I (Gartner & Engebrecht, 2022; Hillers et al., 2017). The SC initiates assembly late in the transition zone (leptotene/zygotene) and is fully assembled by pachytene (MacQueen et al., 2002). While many proteins have been identified within the *C. elegans* SC, we focused our analyses here on the central region proteins, which are all called SYP (SYnaPsis protein) and, to date, six SYP proteins (SYP-1 through SYP-6) span the gap between the homologous chromosomes (Figure 1B) (Colaiacovo et al., 2003; Hurlock et al., 2020; MacQueen et al., 2002; Smolikov, Eizinger, Hurlburt, et al., 2007; Smolikov et al., 2009; Zhang et al., 2020).

To assess if SC dynamics differ between spermatocytes and oocytes, we performed fluorescent recovery after photobleaching (FRAP) assays with two SYP proteins endogenously tagged with fluorescent proteins: (1) GFP::SYP-2 from (Gao et al., 2016), and (2) mCherry::SYP-3 that we generated using CRISPR/Cas9 (see Methods) (Figure 1C, Figure 1 – figure supplement 1, Figure 1 – figure supplement 2). Both SYP-2 (213 amino acids) and SYP-3 (224 amino acids) are the smallest SYP proteins in the *C. elegans* SC and have very little
sequence similarity between themselves and to the other SYP proteins (Kursel et al., 2021).

However, structurally SYP-2 and SYP-3 are similar to the other SYP proteins in that all the SYP proteins have predicted coiled-coil protein domains with SYP-2 having one putative coiled-coil domain and SYP-3 having three putative coiled-coil domains (Kursel et al., 2021). We found that both SYP-2 and SYP-3 have sex-specific differences in protein turnover, with SYP-2 dynamics higher in spermatocytes and SYP-3 dynamics higher in oocytes.

For GFP::SYP-2, spermatocytes displayed significantly higher recovery dynamics throughout pachytene compared to oocytes (Figure 1C, Figure 1 – figure supplement 1, Figure 1 – figure supplement 2; P<0.001, Bonferroni-Dunn adjusted, Mann-Whitney). In contrast, mCherry::SYP-3 recovered more quickly in oocytes during early pachytene compared to spermatocytes (early pachytene P=0.0015; Bonferroni-Dunn adjusted, Mann-Whitney). During mid and late pachytene, SYP-3 recovery rates were similar between the sexes. Notably, we observed that the progressive stabilization of spermatocyte SYP-3 was less pronounced and more subtle than that of oocyte SYP-3, suggesting that spermatocytes may not modulate SYP-3 stability via the same mechanisms as oocytes (Figure 1C). Collectively, these data indicate that SYP dynamics are not uniformly regulated throughout prophase and instead exhibit both SYP-specific and sex-specific dynamics.

Overall, both sexes showed the same overall trend of progressive stabilization of SYP-2 and SYP-3 throughout pachytene matching previous observations with the transgene GFP::SYP-3 (Figure 1, Figure 1 – figure supplement 1, Figure 1 – figure supplement 2; see Methods) (Nadarajan et al., 2017; Pattabiraman et al., 2017; Rog et al., 2017). Upon comparing SYP mobilization between the sexes, we found that SYP-2 and SYP-3 displayed differences in mobility when compared to each other within each sex. In oocytes, GFP::SYP-2 turnover was significantly reduced compared to mCherry::SYP-3 turnover (Figure 1C, Figure 1 – figure supplement 1, Figure 1 – figure supplement 2; P<0.01, Bonferroni-Dunn adjusted, Mann-Whitney). Comparatively in spermatocytes, only early pachytene displayed significant
differences in GFP::SYP-2 and mCherry::SYP-3 turnover (Figure 1C; \( P=0.0237 \), Bonferroni-Dunn adjusted, Mann-Whitney). These results demonstrate that SYP-2 and SYP-3 can be independently regulated during pachytene.

To determine whether differential regulation of SYP-2 and SYP-3 might be influenced by chromosome length, we measured SC length during early, mid, and late pachytene in both sexes. Our results found that SC length was similar throughout pachytene in each sex (Figure 1 – figure supplement 3). The slightly shorter SC lengths in late pachytene spermatocytes was likely due to differences in chromosome compaction between the sexes (Rourke & Jaramillo-Lambert, 2022; Samson et al., 2014). These results suggest that independent regulation of SYP-2 and SYP-3 within an assembled SC is not due to changes in chromosome length, but rather by other factors.

**SYP-2 and SYP-3 composition within the SC is sexually dimorphic**

The differences in SYP-2 and SYP-3 mobility between oogenesis and spermatogenesis suggests that the abundance or concentration of each protein within the SC may also be sexually dimorphic. To compare the relative assembled SYP compositions within the SC between sexes during meiotic prophase I, we calculated the mean fluorescence intensity per cubic micrometer of assembled SC for individual nuclei throughout pachytene in oogenesis and spermatogenesis, and then calculated the average SYP intensity among nuclei in a sliding window across the normalized germline length (see Methods). We found that the accumulation of GFP::SYP-2 in the SC is sexually dimorphic (Figure 2A, 2B, Figure 2 – figure supplement 1). Wild type oocytes progressively accumulated SYP-2 up until the early to mid pachytene transition (early pachytene mean intensity 153,292.27 ± SD 13,983.85; early/mid pachytene mean peak intensity 161,878.14 ± SD 13,930.43; mid pachytene mean intensity 148,430.1 ± SD 13,058.02) and then reduce the amount of SYP-2 slightly before maintaining a relatively
constant level of SYP-2 through late pachytene. In contrast to oocytes, wild type spermatocytes progressively loaded SYP-2 into the SC until the onset of late pachytene at which point the amount of SYP-2 began to stabilize (Figure 2A, 2B, Figure 2 – figure supplement 1). Notably, early pachytene spermatocytes had less SYP-2 loaded than oocytes, but late pachytene spermatocytes had near or slightly more amounts of SYP-2 in the SC than oocytes (P<0.001, Bonferroni adjusted, Mann-Whitney). Additionally, slightly past the early/mid pachytene transition of SYP-2 intensity, we noted only oocytes display a few persisting bright nuclei in nearly all germlines examined (8 out of 9 germlines with persisting bright nuclei; Figure 2B). Thus, the incorporation of SYP-2 throughout pachytene differs between sexes.

In contrast to SYP-2, mCherry::SYP-3 progressively accumulated throughout pachytene in both sexes, matching previous observations using a GFP::SYP-3 transgene (Figure 2C, 2D, Figure 2 – figure supplement 1) (Pattabiraman et al., 2017). Therefore, differences in fluorescent tags on SYP-3 do not appear to largely influence the incorporation of SYP-3 into the SC during pachytene (see Methods). In comparison to oocytes, spermatocytes incorporated less SYP-3 throughout pachytene (P<0.001, Bonferroni adjusted, Mann-Whitney), which was also observed in the early regions of pachytene with SYP-2. Thus, spermatocytes have less SYP-3 and SYP-2 in the SC than oocytes, specifically within the early and mid regions of pachytene. Moreover, unlike with SYP-2, we did not observe in either sex any bright SYP-3 nuclei that were not surrounded by other nuclei of similar intensity, thereby suggesting that these bright SYP-2 nuclei may have defects or changes that only trigger a response with SYP-2 levels (Figure 2D, Figure 2 – figure supplement 1). Taken together, these data demonstrate that SYP-2 and SYP-3 are differentially incorporated in the SC both over the course of meiotic prophase I and between sexes.

Sex-specific recombination dependent regulation of SYP-2 and SYP-3 within the SC
During pachytene, one of the main functions of the SC is to facilitate and regulate recombination. In *C. elegans*, SPO-11 induced DSBs are formed in the context of fully assembled SC in early pachytene, and these breaks get repaired as the nuclei traverse through pachytene (Gartner & Engebrecht, 2022). By the transition to late pachytene, crossover recombination events are designated and marked by the pro-crossover protein COSA-1 (Yokoo et al., 2012). Notably, in *C. elegans* oocytes, the SC is modified in response to recombination and alterations of the SC influences and/or impairs recombination (Almanzar et al., 2023; Cahoon et al., 2019; Colaiacovo et al., 2003; Gao et al., 2016; Gordon et al., 2021; Köhler et al., 2020; Libuda et al., 2013; Nadarajan et al., 2016; Rog et al., 2017; Sato-Carlton et al., 2018; Schild-Prufert et al., 2011). To determine whether the changes we observed in SYP-2 and SYP-3 accumulation during pachytene were caused by recombination, we assessed how the absence of recombination influenced the incorporation of each protein within the SC. To achieve this, we inhibited the formation of crossovers at different stages of recombination using two mutants: (1) *spo-11(me44)* mutants, which cannot form DSBs; and (2) *cosa-1(tm3298)* mutants, which cannot designate DSBs for crossover formation.

The amount of both GFP::SYP-2 and mCherry::SYP-3 loaded into the SC was significantly increased in *spo-11* mutant oocytes, but not spermatocytes (Figure 3A, 3C, Figure 3 – figure supplement 1, Figure 3 – figure supplement 2). The pattern of SYP-2 accumulation was altered in *spo-11* oocytes, in which SYP-2 amounts continued to increase within the SC displaying a 1.27-fold increase in SYP-2 amounts during early pachytene, 1.62-fold increase during mid pachytene, and 1.57-fold increase during late pachytene (Figure 3A). While the overall pattern of SYP-3 accumulation in *spo-11* oocytes remains very similar to wild type with a progressive accumulation throughout pachytene, the total amounts of SYP-3 significantly increase within the SC (Figure 3C). In contrast, *spo-11* spermatocytes displayed mild changes in amounts of SYP-2 and SYP-3 in the SC compared to wild type and the trend of SYP incorporation through pachytene was unaltered (Figure 3A, 3C). Additionally, western blot whole
worm analysis of GFP::SYP-2 protein showed an slight, but not statistically significant, increase in SYP-2 protein levels in only oocytes (Figure 3 – figure supplement 3). Thus, oocytes and spermatocytes differentially regulate the amount of SYP-2 protein in the absence of DSBs. Taken together, these results demonstrate that the incorporation of SYPs is regulated in a recombination-dependent and in a sex-specific manner during prophase I.

This sex-specific regulation of the SYPs in response to DSB formation was also observed in the crossover deficient cosa-1 mutants, albeit to a different degree in comparison to spo-11 mutant oocytes (Figure 3B, 3D, Figure 3 – figure supplement 1, Figure 3 – figure supplement 2). Specifically, cosa-1 oocytes did not increase the levels of SYP-2 and SYP-3 to the same amounts as observed in spo-11 oocytes (Figure 3). In early pachytene, cosa-1 oocytes showed a 0.8-fold decrease in SYP-2 amounts that changed in mid and late pachytene with SYP-2 amounts increasing by 1.16-fold and 1.22-fold over wild type amounts, respectively (Figure 3B). Whereas spo-11 oocytes increased the amount of assembled SYP-2 to a greater degree than cosa-1 oocytes. Thus, SYP-2 and SYP-3 levels in oocytes can be differentially regulated depending on the specific recombination stage that is impeded. Similar to spo-11 mutant spermatocytes, cosa-1 mutant spermatocytes did not largely alter SYP-2 and SYP-3 levels (Figure 3). Even when recombination is hindered, the pattern of SYP-2 and SYP-3 incorporation throughout pachytene is not the same for each sex or between the SYPs. Overall, the SYPs within the SC of oocytes largely respond to alterations in recombination whereas the SC of spermatocytes do not significantly respond to recombination.

Reduced SYP-2 causes altered SC assembly in oocytes

The SYP-specific changes in SC composition and in response to recombination defects raised the possibility that SYP protein dosage may regulate specific steps of recombination. To alter the dosage of each SYP, we used heterozygous null mutants for either syp-2(ok307) or syp-3(ok785) referred to as syp-2/+ or syp-3/+.

In oocytes, reducing the dosage of SYP-1, SYP-2 or...
SYP-3 by 60-70% was sufficient to permit SC assembly and crossover designation (Libuda et al., 2013). It remained unclear, however, whether altering the dosage of the SYPs also influenced chromosome pairing or the timing of SC assembly, which can also influence downstream meiotic processes like recombination (Couteau et al., 2004; Couteau & Zetka, 2005; Goodyer et al., 2008; MacQueen et al., 2005; Martinez-Perez & Villeneuve, 2005; Mlynarczyk-Evans & Villeneuve, 2017; Nabeshima et al., 2004; Zhang et al., 2012).

In syp-2/+ and syp-3/+ mutants, the transition zone (determined by DAPI-stained DNA morphology) was not significantly extended in either sex, indicating that chromosome pairing is not impaired by SYP dosage (Figure 4 – figure supplement 1). Since SYP-1, SYP-2 and SYP-3 are dependent on each other for assembly, we assessed SC assembly using SYP-1 staining in syp-2/+ and syp-3/+ oocytes and spermatocytes (Colaiacovo et al., 2003; MacQueen et al., 2002; Smolikov, Eizinger, Schild-Prufert, et al., 2007). SC assembly and/or the maintenance of full length SC was altered by reducing the dosage of SYP-2 only in oocytes (Figure 4). In early pachytene, syp-2/+ oocytes displayed more discontinuities in the SC along the chromosomes than both wild type and syp-3/+ suggesting that there is a SC assembly or maintenance defect in syp-2/+ (Figure 4A, yellow arrow heads). Notably, these SC defects in early pachytene caused a significant increase in the length of the SC assembly zone in syp-2/+ oocytes, but these mutants did maintain full length SC after this zone (Figure 4C, 4E; P<0.001, Bonferroni P adjusted, Mann-Whitney). In contrast, syp-2/+ and syp-3/+ spermatocytes did not display any significant defects in SC assembly in early pachytene (Figure 4B, 4D). Additionally, syp-2/+ or syp-3/+ mutants in both sexes did not display any changes in SC disassembly or pachytene length, as indicated by DAPI morphology (Figure 4, Figure 4 – figure supplement 1). We also checked SUN-1 S8 phosphorylation in both syp-2/+ and syp-3/+ to assess whether synapsis checkpoints were activated, but there was no significant change in the length of the SUN-1 S8 phosphorylation zone in either mutant or sex (Figure 4 – figure supplement 1) (Woglar et al., 2013). Thus, SC assembly and/or maintenance only in oocytes is sensitive to SYP-2 dosage.
during the early stages of pachytene and this defect is not severe enough to trigger synapsis checkpoint activation.

**SYP-2 and SYP-3 dosage regulate recombination via separate, sex-specific mechanisms**

Since altering SYP dosage permitted assembly of full length SC and did not impair chromosome pairing, we next assessed how reducing the dosage of SYP-2 and SYP-3 influenced recombination. To assess the mechanics of recombination, we used immunofluorescence to quantify and detect specific proteins that mark sites undergoing three different stages of recombination: (1) DSB formation with RAD-51 (Colaiacovo et al., 2003) (note: the subsequent removal of RAD-51 also indicates progression of a DSB down a repair pathway); (2) joint molecule formation with GFP::MSH-5 (Janisiw et al., 2018); and, (3) crossover designation with GFP::COSA-1 in oocytes (Yokoo et al., 2012) and OLLAS::COSA-1 in spermatocytes (Janisiw et al., 2018) (see Methods). For simplicity, here we refer to the tagged versions of GFP::MSH-5 and GFP::COSA-1 or OLLAS::COSA-1 as only the protein name either MSH-5 or COSA-1. The average number of foci of each protein was determined by using a sliding window of 0.01 along the normalized germline length, which was divided into early, mid, and late pachytene (see Methods).

**RAD-51 foci are relatively unaffected by SYP dosage**

Similar to previous studies, we found that the number and timing of RAD-51 foci per nucleus during pachytene is very different between the sexes (Figure 5A, 5B, Figure 5 – figure supplement 1, Figure 5 – figure supplement 2) (Checchi et al., 2014; Jaramillo-Lambert & Engebrecht, 2010). Oocytes initiate DSBs later in the germline than spermatocytes, however, both sexes progressively repair these breaks throughout pachytene (Checchi et al., 2014; Colaiacovo et al., 2003; Jaramillo-Lambert & Engebrecht, 2010; Toraason et al., 2021). Based on the RAD-51 foci counts, altering SYP dosage did not appear to have large effects on DSB
initiation or subsequent progression through a repair pathway in either sex (Figure 5A, 5B, Figure 5 – figure supplement 1, Figure 5 – figure supplement 2). We did note changes in the number of RAD-51 foci specifically in oocytes of syp-2/+ and syp-3/+ during early pachytene (P<0.001; Figure 5A), but these slight alternations did not change the DSB repair dynamics with foci numbers declining at similar rates to wild type during pachytene progression. Additionally, we checked DSB-2 staining in oocytes, which marks the region of the germline where DSBs are induced and found no significant changes in the DSB-2 zone in either mutant (Figure 5 – figure supplement 3). Thus, DSB formation and repair is not largely impacted by altering the dosage of SYP-2 or SYP-3.

SYP dosage influences MSH-5 foci via sex-specific mechanisms

Consistent with the early loading of RAD-51 in spermatocytes, we found that MSH-5 is also loaded earlier in spermatocytes than oocytes during pachytene (Figure 5C, 5D, Figure 5 – figure supplement 1, Figure 5 – figure supplement 2). Wild type spermatocytes reach peak amounts of MSH-5 foci around the transition between early and mid pachytene, whereas oocytes have peak amounts of MSH-5 foci in mid pachytene. Moreover, the peak amount of MSH-5 foci loaded per nucleus is higher in spermatocytes than oocytes (mean MSH-5 oocytes 11.6 foci ± SD 6.0 vs. spermatocytes 15.4 ± SD 7.0). These differences in MSH-5 foci between the sexes contribute to a growing body of work illustrating that the processing of recombination events is sexually dimorphic (Brick et al., 2018; Checchi et al., 2014; Li et al., 2020).

Reducing the dosage of SYP-2 and SYP-3 caused significant changes in the number of MSH-5 foci per nucleus during pachytene (Figure 5C, 5D, Figure 5 – figure supplement 1, Figure 5 – figure supplement 2), particularly in oocytes. syp-2/+ oocytes showed significant reductions in the average number of MSH-5 foci throughout pachytene, indicating that the amount of SYP-2 is crucial to load and/or maintain MSH-5 at a DSB (Figure 5C; P<0.001, Bonferroni adjusted P, Mann-Whitney). syp-3/+ oocytes displayed significant increases in the
average number of MSH-5 foci per nucleus during early and mid pachytene (Figure 5C, P<0.001, Bonferroni adjusted P, Mann-Whitney). However, during mid pachytene, syp-3/+ oocytes rapidly lost MSH-5 foci earlier than wild type. Taken together, our data suggests that SYP-3 dosage regulates the timing of MSH-5 loading and off-loading (or maintenance at a DSB) in oocytes.

In spermatocytes, only reducing the dosage of SYP-2 caused significant changes in MSH-5 foci during pachytene (Figure 5D, Figure 5 – figure supplement 1, Figure 5 – figure supplement 2). syp-2/+ spermatocytes initially form MSH-5 foci to levels similar to wild type in early pachytene. However, during mid and late pachytene syp-2/+ spermatocytes rapidly lose MSH-5 foci, suggesting that SYP dosage is important for the maintenance of MSH-5 at joint molecules (Figure 5D; P<0.001, Bonferroni adjusted P, Mann-Whitney). Notably, for both sexes the dosage of SYP-2 appears to be important for MSH-5 stability, suggesting a conserved role for SYP-2 in both sexes. Additionally, unlike oocytes, SYP-3 dosage does not change MSH-5 foci during pachytene in spermatocytes illustrating a sex-specific role for SYP-3 in regulating MSH-5 at DSB sites during recombination.

SYP dosage alters the timing of COSA-1 foci loading during pachytene

Unlike the sexually dimorphic DNA repair dynamics observed in RAD-51 and MSH-5, both oocytes and spermatocytes load COSA-1 in mid pachytene, and by late pachytene, all 6 COSA-1 foci in oocytes and 5-6 COSA-1 foci in spermatocytes have been established (Figure 5E, 5F, Figure 5 – figure supplement 1, Figure 5 – figure supplement 2) (Cahoon et al., 2023; Yokoo et al., 2012). However, altering the dosage of SYP-2 and SYP-3 cause significant changes in the timing of COSA-1 loading in both oocytes and spermatocytes (Figure 5E, 5F, Figure 5 – figure supplement 1, Figure 5 – figure supplement 2). In both syp-2/+ and syp-3/+ oocytes, the loading of COSA-1 foci was shifted to occur earlier in mid pachytene than wild type (Figure 5E; syp-2/+ P<0.001, syp-3/+ P<0.001, Bonferroni adjusted P, Mann-Whitney). In contrast, syp-3/+
spermatocytes exhibit a delay in the loading of COSA-1 foci during mid pachytene (Figure 5F; $P<0.05$, Bonferroni adjusted $P$, Mann-Whitney). Although $syp-2/+\) spermatocytes also displayed a potential delay in the loading of COSA-1, the difference is not statistically different from wild type (Figure 5F; $P=1$, Bonferroni adjusted $P$, Mann-Whitney). Taken together, dosage of $SYP-2$ and $SYP-3$ regulates the sex-specific timing of crossover designation.

By late pachytene, the final number of COSA-1 foci in oocytes (6 foci) and spermatocytes (5 or more foci) was not changed from the required one crossover per chromosome, thereby suggesting that SYP dosage does not largely influence the ability of each sex to designate the crossovers on all chromosomes (Figure 5E, 5F, Figure 5 – figure supplement 1, Figure 5 – figure supplement 2). Further, in oocytes, we observed 6 DAPI staining bodies at diakinesis for both $syp-2/+\) (30/30 oocytes with 6 DAPI bodies) and $syp-3/+\) (30/30 oocytes with 6 DAPI bodies) indicating that the 6 COSA-1 foci per nucleus at late pachytene mature into 6 crossovers that link the homologous chromosomes together at diakinesis.

**SYP-dosage regulates crossover landscape**

Since we found that manipulating the dosage of SYP-2 and SYP-3 altered multiple steps of recombination and previous studies indicate that the SC can regulate crossover numbers (Colaiacovo et al., 2003; Gordon et al., 2021; Hayashi et al., 2010; Köhler et al., 2020; Libuda et al., 2013; MacQueen et al., 2002; Smolikov, Eizinger, Schild-Prufert, et al., 2007), we wanted to determine whether SYP-2 and SYP-3 dosage influences the recombination landscape by changing where crossovers are positioned along the length of the chromosome. To assess the recombination landscape, we used established single nucleotide polymorphism (SNP) recombination mapping between two *C. elegans* isolates (Bristol and Hawaiian) to identify crossovers (see Methods) (Bazan & Hillers, 2011). For oocytes, we mapped recombination on both Chromosome II and the X Chromosome. Since male worms have an unpaired X
chromosome and do not form crossovers on this sex chromosome, only recombination on Chromosome II was mapped in spermatocytes.

On Chromosome II, both syp-2/+ and syp-3/+ only altered the crossover landscape in oocytes (Figure 6, Table 1). In spermatocytes, both syp-2/+ and syp-3/+ displayed no significant changes in crossover frequencies across all of Chromosome II. In contrast, both syp-2/+ and syp-3/+ oocytes displayed significant changes in the crossing over distribution on Chromosome II (Figure 6; syp2/+ P=0.0343, syp3/+ P=0.0333, Chi-Squared). Specifically, both mutants increased crossing over in the first interval (A to B) on the left side of Chromosome II by ~10cM each (Figure 6; wild type 14cM, syp-2/+ 24cM, syp-3/+ 25cM; syp2/+ P=0.0449, syp3/+ P=0.0170, Fisher’s Exact). Intriguingly, this first interval is also where the pairing center is located on Chromosome II. Thus, one possible explanation for the elevated crossing over in the interval is that by reducing the SYP dosage, crossovers are now more often formed where the SC is first assembled at the pairing center (Hayashi et al., 2010; Rog & Dernburg, 2015).

On the X Chromosome, syp-2/+ oocytes displayed a significant decrease in crossover frequency along the entire chromosome (Figure 6 – figure supplement 1; Figure 6 – figure supplement 2; wild type 51cM, syp-2/+ 32cM; P=0.0343, Chi-Squared). This decrease was not observed in syp-3/+ oocytes, which showed no significant changes in crossover frequency to wild type for X Chromosome recombination (Figure 6 – figure supplement 1; Figure 6 – figure supplement 2; wild type 51cM, syp-3/+ 45cM; P=0.5849, Chi-Squared). The large decrease in crossing over in syp-2/+ suggests that these mutants should have a significant amount of X Chromosome nondisjunction or missegregation. In C. elegans, mutants with frequent X Chromosome nondisjunction produce a high incidence of male (Him) phenotype as male worms are hemizygous for the X Chromosome. Notably, syp-2/+ did not display an elevation in the frequency of male progeny nor did they have an increase in dead eggs (Figure 6 – figure supplement 1; wild type 0.4% dead eggs and 0.2% Him, syp-2/+ 0.4% dead eggs and 0.2% Him). Since the SNP recombination mapping experiment is performed in a hybrid
Bristol/Hawaiian background, we also checked the hybrid background for male progeny and dead eggs. Due to known meiotic drive elements between these strains, the Bristol/Hawaiian hybrids produce more dead eggs (Seidel et al., 2011; Seidel et al., 2008). However, syp-2/+ Bristol/Hawaiian hybrids did not display a higher incidence of dead eggs or more male progeny than the wild type Bristol/Hawaiian hybrid (Figure 6 – figure supplement 1; wild type 19.4% dead eggs and 0.2% Him; syp-2/+ 23.3% dead eggs and 0.1% Him). Thus, it remains unclear as to why reducing SYP-2 causes a significant decrease in recombination without a corresponding increase in X chromosome nondisjunction. One possible explanation is that syp-2/+ have a chromosome distortion event occurring in the later stages of meiosis that is removing recombinant X chromosomes. Future studies are needed to understand what is happening to the recombinant X chromosomes in syp-2/+.

In comparison to wild type, the broods from syp-3/+ hermaphrodites exhibited both an increased lethality (more dead eggs; Figure 6 – figure supplement 1, wild type Bristol 0.4% and hybrid 19.4% dead eggs; syp-3/+ Bristol 1.2% and hybrid 27.0% dead eggs) as well as an higher incidence of male progeny than wild type (Figure 6 – figure supplement 1, wild type Bristol 0.2% and hybrid 0.2% Him; syp-3/+ Bristol 0.7% and hybrid 1.6% Him, Bristol p<0.0001, Hybrid p<0.0001, Chi-Squared). In addition, syp-3/+ also produced a higher rate of progeny with dumpy and/or uncoordinated mutant phenotypes than both wild type and syp-2/+ (Figure 6 – figure supplement 1, Bristol 0.06% mutant progeny, syp-2/+ Bristol 0.06% mutant progeny, syp-3/+ Bristol 0.47% mutant progeny; wild type Bristol p=0.0207, syp-2/+ Bristol p=0.0388, Fisher’s Exact). SYP proteins have been implicated in regulating DSB repair pathway choice by preventing access to error prone and sister chromatid repair (Lascarez-Lagunas et al., 2022; Lemmens et al., 2013; Macaisne et al., 2018; Rosu et al., 2011; Smolikov, Eizinger, Hurlburt, et al., 2007; Yin & Smolikove, 2013). Additionally, SYP-3 directly interacts with the pro-crossover protein BRC-1 to promote crossover DSB repair (Janisiw et al., 2018). Thus, the mutant phenotypes observed in progeny from syp-3/+ hermaphrodites are likely due to error prone
repair using nonhomologous end joining, which is used by \textit{syp}-3 nulls to repair persisting DSBs (Smolikov, Eizinger, Schild-Prufert, et al., 2007). These results suggest that SYP-3 amounts are important for suppressing error prone repair. Notably, in contrast to oocytes, spermatocyte fertility was unaffected in \textit{syp}-2+/ or \textit{syp}-3+/ (Figure 6 – figure supplement 1). Thus, reducing the dosage of SYP-2 or SYP-3 has sexually dimorphic consequences on fertility.

\textit{SYP-2 and SYP-3 dosage influences the SC composition during pachytene}

The SYP dosage-dependent regulation of recombination (Figures 4, 5), different patterns of SYP protein incorporation throughout pachytene (Figure 2), and independent regulation of SYP loading into the SC (Figures 2, 3) suggest that SYP accumulation in the SC may be dynamically altered during pachytene to regulate the steps of recombination. Thus, reducing the gene dosage of each SYP may influence how the SYPs are accumulating within the SC, which could influence the regulation of recombination. To determine how \textit{syp} gene dosage influences the incorporation of each SYP during pachytene, we used heterozygous null mutants of each \textit{syp} gene with a wild type fluorescently tagged GFP::SYP-2 or mCherry::SYP-3: \textit{syp-2(ok307)/gfp::syp-2} (referred to as \textit{syp}-2+) or \textit{syp-3(ok785)/mCherry::syp-3} (referred to as \textit{syp}-3+). Indeed, we found that the dosage of \textit{syp}-2 and \textit{syp}-3 influenced both SYP-2 and SYP-3 accumulation during pachytene in both sexes (Figure 7, Figure 7 – figure supplement 1).

Altering the \textit{syp}-2 gene dosage resulted in both sexes to initially have a ~0.7-fold decrease in SYP-2 amounts within the SC, however, SYP-2 levels progressively increased throughout pachytene until wild type amounts were reached or pachytene ended (Figure 7A). Western blot analysis revealed that GFP::SYP-2 proteins levels were not significantly increased in \textit{syp}-2+ oocytes and spermatocytes (Figure 3 – figure supplement 3). Overall, these results suggest that both sexes attempt to compensate for the haploinsufficiency of losing a \textit{syp}-2 gene by loading as much SYP-2 into the SC as possible during pachytene.
In contrast, SYP-3 accumulation in syp-2/+ mutants displayed sex-specific responses (Figure 7B). In oocytes, reducing the syp-2 gene dosage resulted in a slight decrease in SYP-3 levels in early pachytene that becomes more pronounced in late pachytene, which we noted was the same window when SYP-2 protein levels reached wild type amounts in syp-2/+ mutants (Figure 7B; P<0.001, Bonferroni adjusted, Mann-Whitney). In spermatocytes, reducing the syp-2 gene dosage resulted in an increase in SYP-3 amount in early pachytene that are indistinguishable from wild type by late pachytene (Figure 7B). Thus, while altering syp-2 gene dosage caused both sexes to respond similarly with reduced loading of SYP-2 into assembled SC, SYP-3 levels within assembled SC were inversely affected via sex-specific mechanisms.

We found that altering syp-3 gene dosage resulted in the same overall changes to SYP-2 and SYP-3 amounts in each sex as altering syp-2 gene dosage, albeit the degree in which each SYP changed was different (Figure 7, Figure 7 – figure supplement 1). Specifically, reducing syp-3 gene dosage caused an initial reduction of SYP-2 levels in both sexes that was less than the reduction in syp-2/+ (Figure 7A, 7C; early pachytene syp-2/+ oocytes 0.72-fold change vs. syp-3/+ oocytes 0.87-fold change; early pachytene syp-2/+ spermatocytes 0.71-fold change vs. syp-3/+ spermatocytes 0.89-fold change). However, similar to syp-2/+, both sexes progressively increase SYP-2 amounts in syp-3/+ until wild type levels were reached or pachytene ended (Figure 7A, 7C). Additionally, western blot analysis of GFP::SYP-2 protein levels showed that both sexes only slightly increase SYP-2 protein levels (Figure 3 – figure supplement 3). Thus, both sexes respond to alterations in syp gene dosages with similar trends of SYP-2 accumulation within the SC.

In oocytes, reducing syp-3 gene dosage caused a stronger decrease in overall SYP-3 protein amounts compared to syp-2/+ (Figure 7B, 7D, early pachytene syp-2/+ 0.97-fold change vs syp-3/+ 0.91-fold change, mid pachytene 0.99-fold change vs syp-3/+ 0.90-fold change, late pachytene 0.91-fold change vs. syp-3/+ 0.85-fold change). In contrast, syp-3/+ spermatocytes displayed an increase in SYP-3 levels that was similar to the increase in SYP-3 observed in
syp-2/+ spermatocytes (Figure 7B, 7D). Thus, the dosage of each SYP gene influences the accumulation of SYP-2 and SYP-3 within the assembled SC similarly for each sex. Oocytes respond to reduced syp gene dosage by decreasing both SYP-2 and SYP-3 within the SC, while spermatocytes respond to reduced syp gene dosage by decreasing SYP-2 and increasing SYP-3 in the SC. Overall, these differences are likely influencing and/or responding to the changes we observed in recombination for both syp-2/+ and syp-3/+ (Figure 5) and suggest that the dosage of each SYP throughout pachytene regulates recombination in a sex-specific manner (see Discussion).

SYP dosage differentially influences SYP-5 and SYP-6 composition within the SC

The ability of both SYP-2 and SYP-3 to affect the incorporation of each other within the SC suggested that the dosage of each SYP may influence and/or regulate the incorporation of other SYP proteins in the SC. Since the inability to functionally tag SYP-1 and SYP-4 with fluorescent proteins precluded analysis of these two proteins, we assessed how the composition of SYP-5 and SYP-6 within the SC were influenced by syp-2 and syp-3 gene dosage in both oocytes and spermatocytes. Using endogenously CRISPR tagged lines containing either SYP-5::GFP or SYP-6::GFP (Zhang et al., 2020) combined with heterozygous null mutants of syp-2 (syp-2/+) or syp-3 (syp-3/+), we found that syp-2 and syp-3 gene dosage can influence SYP-5 and SYP-6 incorporation in the SC via sex-specific mechanisms (Figure 8, Figure 8 – figure supplement 1).

Sexually dimorphic SYP-5 accumulation is differentially influenced by syp-2 and syp-3 gene dosage

Similar to the SYP-2 and SYP-3, the composition of SYP-5 within the SC is also sexually dimorphic. In wild type oocytes, SYP-5::GFP accumulation in the SC progressively increased throughout pachytene, matching previous observations of SYP-5 localization (Figure 8A) (Hurlock et al., 2020; Zhang et al., 2020). While wild type spermatocytes also displayed a
progressive increase in SYP-5::GFP through pachytene, the total amount of SYP-5::GFP was significantly less than oocytes (Figure 8A, p<0.001, Bonferroni adjusted, Mann-Whitney).

Notably, the sex-specific difference in wild type SYP-5 amounts was especially apparent in the late pachytene region where oocytes have 1.8-fold more SYP-5 within the SC than spermatocytes. Intriguingly, the accumulation pattern of wild type SYP-3 and SYP-5 were very similar - progressively increasing in protein levels loaded throughout pachytene and with spermatocytes loading overall less protein (Figure 2, 8).

Examining SYP-5 accumulation in both syp-2/+ and syp-3/+ showed more similarities between SYP-5 and SYP-3 accumulation in oocytes. In syp-2/+ and syp-3/+ oocytes, SYP-5::GFP accumulation significantly decreased within the SC throughout pachytene, which was the same response SYP-3 levels had to altered syp-2 and syp-3 gene dosage (Figure 8A, 7B, 7D). In spermatocytes, syp-2/+ did not influence SYP-5::GFP amounts, but syp-3/+ did significantly decrease SYP-5::GFP accumulation through pachytene (Figure 8A). This spermatocyte SYP-5 result contrasts with spermatocyte SYP-3 where reduced syp-2 and syp-3 gene dosage caused increased SYP-3 levels in both cases (Figure 7B, 7D). Thus, in oocytes, reducing the dosage of either syp-2 or syp-3 causes a reduction in both SYP-5 and SYP-3 levels within the SC during pachytene, thereby suggesting that SYP-5 and SYP-3 may be regulated similarly with the oocyte SC. Whereas in spermatocytes, there may be differential regulation of SYP-5 and SYP-3 which potentially indicates different roles for these proteins during spermatocyte meiosis (see Discussion).

**Sexually dimorphic localization of SYP-6**

SYP-6 is the paralog of SYP-5 and has some functional redundancy with SYP-5 in SC assembly and synapsis in oocytes (Hurlock et al., 2020; Zhang et al., 2020). While both SYP-5 and SYP-6 are assembled into the SC within the same region of the germline, SYP-5 and SYP-6 are disassembled at different times (Hurlock et al., 2020; Zhang et al., 2020). In oocytes, SYP-5 is
disassembled with the other SYPs in diplotene; whereas SYP-6 is disassembled in mid pachytene and is largely absent from late pachytene nuclei (Hurlock et al., 2020; Zhang et al., 2020). Since SYP-6 localization in spermatocytes was unknown, we used immunofluorescence staining of SYP-1 and SYP-6::GFP to determine if spermatocytes displayed the same early disassembly of SYP-6 (see Methods). Surprisingly, unlike oocytes, SYP-6 persists in spermatocytes through late pachytene and disassembling in diplotene with SYP-1 with all the other SYPs (Figure 8B, 8C). SYP-5 localization was identical in each sex throughout pachytene, where it co-localized with SYP-1 (Figure 8 – figure supplement 2) (Hurlock et al., 2020; Zhang et al., 2020). Thus, the retention of SYP-6 in late pachytene SC indicates that composition of late pachytene SC is sexually dimorphic.

Since reducing syp-2 and syp-3 gene dosage led to changes in SYP-5 levels within the SC, we assessed whether similar changes in SYP-6 levels occurred in syp-2/+ and syp-3/+ heterozygotes. Similar to (Zhang et al., 2020), we also noticed in oocytes that SYP-6 has a different incorporation pattern than SYP-5 (Figure 8D, Figure 8 – figure supplement 1). Since SYP-6 disassembles prior to the end of late pachytene in oocytes, we measured the intensity of SYP-6::GFP per nucleus from the start of pachytene until the end of the GFP::SYP-6 signal (Figure 8D, see Methods). In wild type oocytes, SYP-6::GFP remained at a fairly constant amount before it is disassembled in mid pachytene. This SYP-6 result contrasts with SYP-5, which progressively increases throughout pachytene (Figure 8A). Further, unlike SYP-5, the syp-2 and syp-3 gene dosage does not appear to have a strong effect on altering the amount of SYP-6::GFP in oocytes (Figure 8D). Thus, SYP-5 and SYP-6 within oocyte SC are differentially regulated, thereby suggesting that these proteins might have some non-redundant roles during meiosis (Zhang et al., 2020).

Given that SYP-6 is retained to the end of pachytene in spermatocytes, we assessed the incorporation of SYP-6 throughout pachytene in spermatocytes (Figure 8E, Figure 8 – figure supplement 1, see Methods). In early pachytene, both wild type oocytes and spermatocytes
display similar amounts of SYP-6::GFP (Figure 8D, 8E). By mid pachytene, while oocytes are

disassembling SYP-6, spermatocytes only display a slight reduction in SYP-6::GFP levels from

mid/late pachytene to the end of pachytene (Figure 8D, 8E). Unlike oocyte SC, altering the
dosage of SYP-2 influences SYP-6::GFP incorporation within the spermatocyte SC. Specifically,
syp-2/+ spermatocytes display a significant increase in the incorporation of SYP-6::GFP throughout pachytene (Figure 8E, p<0.001, Bonferroni adjusted, Mann-Whitney). In contrast,
syp-3/+ spermatocytes largely did not alter the incorporation of SYP-6::GFP during pachytene
progression (Figure 8E).

Taken together, the regulation of SYP-2, SYP-3, SYP-5, and SYP-6 are different in both
spermatocytes and oocytes suggesting sex-specific roles for each protein during meiosis.

Further, altering SYP-2 and SYP-3 dosage appears to cause global, sexually dimorphic
changes within the SC that is likely a response to and/or consequence of the defects in specific
steps of recombination.

DISCUSSION

Sexually dimorphic regulation of recombination by the SC

One of the roles for the SC during meiosis is regulating recombination to promote the
establishment of a crossover on each homolog pair. Our data here demonstrate that the SC in
C. elegans regulates recombination via sexually dimorphic mechanisms. We show that the
central region proteins SYP-2 and SYP-3 have sex-specific differences in protein turnover rates
within the SC that may influence the sexually dimorphic composition of the SC. Specifically,
SYP-2, SYP-3, SYP-5 and SYP-6 are incorporated into the SC at different levels via sex-
specific mechanisms. Moreover, we find that SYP protein levels in the SC are dependent on
recombination such that SYP protein dosage impacts the regulation of recombination. Our data
support a model where SYP-2 and SYP-3 levels within the SC regulate the proper timing and execution of specific steps of recombination (Figure 9).

Our data suggest that the amount of SYP-2 within the SC of oocytes promotes the formation and/or maintenance of joint molecules (Figure 9A). *syp-2/+* oocytes incorporate less SYP-2 in the SC during meiotic stages when MSH-5 stabilized joint molecules are formed (Figures 5, 7) and exhibit a severe reduction in MSH-5 foci (Figure 5). This relationship between the SC and joint molecules is SYP-2 specific, as a reduction in SYP-3 levels within an assembled SC did not result in reduced MSH-5 foci (Figures 5, 7). We therefore suggest that a specific threshold of SYP-2 in the SC ensures the formation and/or stabilization of joint molecules.

Similar to oocytes, spermatocyte SYP-2 dosage is required for the maintenance of joint molecules marked by MSH-5 (Figure 9B). Unlike oocytes, reducing SYP-2 levels in spermatocytes did not alter the assembly of the SC (Figure 4) and is likely why MSH-5 foci are able to initially form near wild type levels in early pachytene (Figure 5). However, the rapid loss of MSH-5 in spermatocytes during mid pachytene suggests that these joint molecules are either being rapidly resolved and designated for crossover recombination or destabilized, resulting in MSH-5 removal. We favor a model where the rapid loss of MSH-5 foci is underpinned by destabilization of joint molecules, as the crossover designation marker COSA-1 is not prematurely loaded when SYP-2 dosage is reduced. This function of SYP-2 in the maintenance of joint molecules appears conserved between the sexes, as *syp-2/+* spermatocytes initially form wild type levels of MSH-5 foci that are rapidly lost (Figure 5). However, the specific threshold of SYP-2 required in spermatocytes to establish and/or stabilize joint molecules is significantly reduced compared to oocytes (Figures 2, 5, 7). This discrepancy between the sexes suggests that there are mechanistic differences in how joint molecules are regulated between spermatocytes and oocytes. Thus, our work here adds to a growing body of work illustrating that spermatocytes process DSBs into crossovers differently than oocytes (Brick et
We further propose that SYP-3 dosage in oocytes regulates the timing of recombination steps (Figure 9A). SYP-3 accumulates within the SC throughout pachytene as recombination intermediates are successively processed (Figure 2). Notably, MSH-5 marked joint molecules and COSA-1 marked crossover designation appear prematurely when the levels of SYP-3 in the SC are reduced (Figures 5, 7). This acceleration in crossover designation coincides with a disproportionate formation of crossovers near the pairing center side of Chromosome II (Figure 6). This change in crossover designation and positioning was also present in syp-2/+. However, we assert that this phenotype is likely underpinned by SYP-3 levels, as SYP-3 incorporation is also reduced in syp-2/+ (Figure 7). Thus, the timing of recombination events in the germline appears to be sensitive to the amounts of SYP-3. We suggest that SYP-3 incorporation is dynamically regulated in response to meiotic stresses to ensure that recombination is completed by the end of pachytene, which is spatially and temporally limited by the length of the gonad.

In spermatocytes, SYP-3 also regulates the timing of recombination events during pachytene. However, spermatocytes SYP-3 incorporation is minimally impacted by syp-3/+ heterozygosity (Figure 9B). We suggest that the relatively normal levels of SYP-3 in the syp-3/+ spermatocyte SC explains the absence of crossover positioning defects (Figure 7). In fact, SYP-3 levels are subtly elevated in early pachytene in syp-3/+ spermatocytes and coincides with a delay in crossover designation (Figures 5, 7). As spermatogenesis and oogenesis operate on very different timescales (Jaramillo-Lambert et al., 2007) and the amount of SYP-3 in the SC of spermatocytes and oocytes differs, we raise the possibility that titrating the level of SYP-3 incorporation may function to regulate the timing of recombination events between the sexes.

**Spermatocytes regulate the SC differently than oocytes**
Sex comparative studies are critical to understand the differences in egg and sperm development. Here we demonstrate that the SC in *C. elegans* is sexually dimorphic. Intriguingly, the sex-specific differences in SYP-2 and SYP-3 dynamics within the SC suggest a difference in protein regulation between the sexes (Figure 1). The progressive stabilization of SC during pachytene in oocytes has been linked with phosphorylation of specific SYP proteins (Nadarajan et al., 2017; Pattabiraman et al., 2017). Additionally, during oogenesis, many SYP proteins are known to be post-translationally modified, including SYP-2, in response to recombination and many of these modifications are critical for DSB formation and repair as well as the timing of SC assembly and disassembly (Garcia-Muse et al., 2019; Lascarez-Lagunas et al., 2022; Nadarajan et al., 2017; Nadarajan et al., 2016; Sato-Carlton et al., 2018). Since spermatocytes also displayed the same progressive stabilization of the SYP-2 and SYP-3 during pachytene as oocytes, it is possible that the same post-translational modifications of SYP proteins may also have similar functions in spermatocytes.

We found that SYP-2 was more dynamic in spermatocytes even at the “stabilized” state in late pachytene (Figure 1). Thus, spermatocytes may not stabilize the SC to the same degree as oocytes. One reason for this could be to allow DSBs in late pachytene to be repaired with the homolog. In oocytes, the stabilization of the SC in late pachytene is thought to prevent the formation of more crossovers with the homolog. Spermatocytes have been shown to have differences in DNA repair, the number of crossovers, and the checkpoints that monitor repair events, and these differences may require a more dynamic SC (Checchi et al., 2014; Gabdank & Fire, 2014; Jaramillo-Lambert et al., 2007; Jaramillo-Lambert & Engebrecth, 2010; Jaramillo-Lambert et al., 2010; Li et al., 2020). Future studies are needed in spermatocytes to examine these relationships between SC dynamics, post translational modification of the SC, and DNA repair outcomes.

The amount of SYP-2, SYP-3 and SYP-5 required to properly execute the same steps of recombination is significantly different between oocytes and spermatocytes (Figure 8, 9).
Oocytes require more of both SYP-2, SYP-3, and SYP-5 during early and mid pachytene than spermatocytes (Figures 2, 8). One possibility for this difference is due to male worms having a hemizygous X chromosome, which does not normally assemble the SC (Jaramillo-Lambert & Engebrecht, 2010). This lack of SC on one chromosome should potentially reduce the amount of SC proteins we get from our analysis. If this were the case, then we would expect the SYP proteins to be reduced in spermatocytes along the entire length of pachytene. Instead, we observe SYP-2 levels in spermatocytes reach those of oocytes during late pachytene and SYP-6 levels are nearly identical between the sexes in early pachytene regions (Figure 2, 9). Further, spermatocytes have increased SYP-2 turnover dynamics in late pachytene compared to oocyte SYP-2 (Figure 1), which may help facilitate the repair of DSBs in late pachytene since spermatocytes do not undergo checkpoint-mediated apoptosis (Jaramillo-Lambert et al., 2010). Thus, having a more dynamic SYP-2 may aid DSB repair when errors occur late in pachytene.

Our observation that reduced SYP-3 dosage triggers an increase in SYP-3 composition within the SC suggests that spermatocytes may be able to compensate for the loss of a functional copy of syp-3. Given that spermatocytes differ in the dynamics of protein turnover within the assembled SC, one way spermatocytes could alter SYP-3 levels is to alter the SYP-3 turnover rates within the SC. We demonstrate that spermatocytes do indeed have altered protein dynamics in the SC compared to oocytes (Figure 1). Therefore, sex-specific differences in SYP-2 and SYP-3 dynamics may regulate the composition of the SC during pachytene.

Independent regulation of the SYPs

One common feature amongst SC proteins in nearly all SC-containing organisms is that most, if not all, of the central region proteins are dependent upon each other for assembly of the SC (reviewed in Cahoon & Hawley, 2016). In C. elegans, the SYP proteins are also dependent on each other for protein stability. Specifically, the result where depletion of one SYP leads to the degradation of the other SYPs, has led to the assumption that the SYPs are all completely
interdependent for assembly, stability, and function (Colaiacovo et al., 2003). Here we show that fluctuations in both SYP-2 and SYP-3 levels can not only differentially influence the amount of each other within the SC and other SYPs (Figures 7, 8), but also each SYP protein can be regulated independently (Figures 2, 3, 7, 8). Notably, we show that these differences in the proportion of SYP-2 and SYP-3 within the SC is directly involved in regulating specific steps of recombination. Additionally, some of the SYPs display similar accumulation patterns and respond similarly to changes in SYP protein levels during pachytene, such as SYP-5 and SYP-3 in oocytes (Figures 7, 8). Thus, we hypothesize that each SYP in the SC maintains both SYP-dependent functions where they function together as a group (e.g. assembling the SC) and SYP-independent functions where they can individually or in smaller groups to influence other aspects of meiosis (e.g. regulating specific steps of recombination).

In worms and yeast, the SC appears to grow in width throughout pachytene suggesting that the composition of proteins within the complex is highly dynamic (Pattabiraman et al., 2017; Voelkel-Meiman et al., 2012). Here we found that the incorporation of SYP-2 and SYP-3 are not proportional during pachytene (Figure 1). Further, our results suggest that recombination influences the pattern of SYP accumulation within the SC and that each of the SYPs are independently regulated within the SC (Figure 3). The ability to adjust the amount of SYP-2, SYP-3, SYP-5, and SYP-6 within the SC demonstrates flexibility in the requirements of SYPs to assemble the complex. SYP-2 is positioned in the very center of the SC and may be slightly more external on the complex to SYP-3, SYP-5, and SYP-6 (Köhler et al., 2020), which would facilitate regulation of SYP-2 levels without compromising the whole complex. Also, the proteins within this very central region of the SC play a critical role in recombination (Gordon et al., 2021; Voelkel-Meiman et al., 2019; Voelkel-Meiman et al., 2015; Voelkel-Meiman et al., 2022). Thus, these proteins in the center of the SC, such as SYP-2, may not have as strong of structural roles in the complex as those positioned broadly in the central region, such as SYP-3.
The sex-specific differences with SYP-6 localization and the sexually dimorphic response of SYP-5 and SYP-6 to SYP-2 and SYP-3 levels suggest that SYP-5 and SYP-6 have some individual sex-specific roles during meiosis. Null mutants of syp-5 and syp-6 in oocytes also indicate that there are shared and distinct functions between these proteins (Hurlock et al., 2020; Zhang et al., 2020). syp-5 mutants have stronger defects in fertility and crossing over than syp-6 mutants, thereby suggesting that SYP-5 may play a more significant role in oocyte recombination than that of SYP-6 (Hurlock et al., 2020; Zhang et al., 2020). However, the sex-specific retention of SYP-6 localization into late pachytene of spermatocytes suggests that SYP-6 has a spermatocyte-specific role in late pachytene (Figure 8). Since spermatocytes do not undergo checkpoint-mediated apoptosis (Jaramillo-Lambert et al., 2010), retaining SYP-6 late in pachytene may facilitate repair of any DSBs still present into late pachytene. Future studies examining the role of SYP-6 in spermatocyte recombination may reveal that the fertility defects in syp-6 mutants are caused by defective sperm rather than oocytes.

Not only are the patterns of SYP-2 and SYP-3 accumulation in the SC different, but the dosage of each SYP protein can influence the amount of the other SYP proteins. Regardless of which SYP dosage was altered, the same change in SYP-2 and SYP-3 accumulation occurred individually for each sex. In oocytes, both SYP-2, SYP-3, and SYP-5 amounts decreased in response to reduced SYP-2 or SYP-3 (Figure 7). Whereas in spermatocytes reduced SYP-2 or SYP-3 decreased SYP-2 amounts, increased SYP-3 amounts, and did not change SYP-5 amounts (Figure 7, 8). The exception to this was SYP-6, which increased upon reduced SYP-2 dosage and did not change significantly in response to altered SYP-3 dosage (Figure 8). One explanation for these sex-specific differences is that spermatocytes require a different stoichiometry of proteins in the SC compared to oocytes. In mice, the SC in oocytes is narrower than the SC between spermatocytes due to structural differences in the organization of proteins within the central element and the chromosome axis (Agostinho et al., 2018). While C. elegans does not have a defined central element based on electron micrographs of the SC, SYP-2 is
located in the very center of the SC where the central element proteins are located in other organisms (Cahoon & Hawley, 2016). Future studies to determine the stoichiometric ratios of chromosome axis proteins may reveal that spermatocytes assemble an SC that is structurally different from oocytes.

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METHODS

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**C. elegans strains, genetics, CRISPR, and culture conditions.** All strains were generated from the N2 background and were maintained and crossed at 20°C under standard conditions on nematode growth media (NGM) with lawns of *Escherichia coli* (*E. coli*). InVivo Biosystems tagged the C-terminus of SYP-3 with a piRNA optimized mCherry using CRISPR/Cas9. The CRISPR homology-directed repair template was constructed containing at least 500 base pairs of homology on either side of the insertion site at the SYP-3 locus. A small region of DNA was recoded section at the sgRNA site to avoid Cas9 cutting the template and mCherry was attached to SYP-3 with a glycine serine linker (GGSGGGGS). These repair constructs were synthesized into plasmids and injected into *unc-119(ed3)* mutant worms with two sgRNAs. Successful CRISPR/Cas9 integrations were screened using a *loxP* flanked *unc-119* rescue transgene, which was inserted into an intron of *syp-3* and removed following successful PCR.
confirmation of the integration by injecting Cre recombinase (Dickinson et al., 2013). All sequences and screening primers for the CRISPR/Cas9 tagging of SYP-3 are in Supplemental file 1. CRISPR/Cas9 worm lines were backcrossed to N2 worms three times and loss of unc-119(ed3) mutation was confirmed by PCR before processing with any strain construction.

The following strains were used in this study:

N2: Bristol wild type strain.
CB4856: Hawaiian wild type strain.
DLW114: unc-18(knu969[unc-18::AID*]) X. reSi7 [rgef-1p::TIR1::F2A::mTagBFP2::AID*::NLS::tbb-2 3'UTR] I.
DLW118: unc-18(knu969[unc-18::AID*]) X. reSi7 [rgef-1p::TIR1::F2A::mTagBFP2::NLS::AID*:tbb-2 3'UTR] I. GFP::syp-2 V.
DLW119: syp-3(knu999[mCherry::syp-3]) II.
DLW128: unc-18(knu969[unc-18::AID*]) X. reSi7 [rgef-1p::TIR1::F2A::mTagBFP2::NLS::AID*:tbb-2 3'UTR] syp-3(knu999[mCherry::syp-3]) I.
DLW160: unc-18(knu969[unc-18::AID*]) X. reSi7 [rgef-1p::TIR1::F2A::mTagBFP2::AID*::NLS::tbb-2 3'UTR] I. GFP::syp-2 V.
DLW163: unc-18(knu969[unc-18::AID*]) X. reSi7 [rgef-1p::TIR1::F2A::mTagBFP2::AID*::NLS::tbb-2 3'UTR]. spo-11(me44)/nT1 [qls51] IV. GFP::syp-2/nT1 [qls51] V.
DLW188: syp-2(ok307)/tmC16 [unc-60(tmls1210)] V.
DLW190: syp-3(ok785)/ tmC18 [dpy-5(tmls1200)] I.
DLW192: unc-18(knu969[unc-18::AID*]) X. reSi7 [rgef-1p::TIR1::F2A::mTagBFP2::AID*::NLS::tbb-2 3'UTR] syp-3(knu999[mCherry::syp-3]) I. GFP::syp-2 V.
DLW193: unc-18(knu969[unc-18::AID*]) X. reSi7 [rgef-1p::TIR1::F2A::mTagBFP2::AID*::NLS::tbb-2 3'UTR] syp-3(knu999[mCherry::syp-3]) I. GFP::syp-2 V.
DLW195: mels8[unc-119(+)] pie-1promoter::gfp::cosa-1 II. syp-2(ok307)/tmC16 [unc-60(tmls1210)] V.
DLW196: mels8[unc-119(+)] pie-1promoter::gfp::cosa-1 II. syp-3(ok785)/ tmC18 [dpy-5(tmls1200)] I.
DLW197: mels8[unc-119(+)] pie-1promoter::gfp::cosa-1 II. syp-2(ok307)/tmC16 [unc-60(tmls1210)] V.
DLW198: mels8[unc-119(+)] pie-1promoter::gfp::cosa-1 II. syp-3(ok785)/ tmC18 [dpy-5(tmls1200)] I.
DLW208: msh-5[ddr22(GFP::msh-5)] IV; syp-2(ok307)/tmC16 [unc-60(tmls1210)] V.
DLW209: syp-3(ok785)/ tmC18 [dpy-5(tmls1200)] I; msh-5[ddr22(GFP::msh-5)] IV.
DLW211: cosa-1[ddr12(OLLAS::cosa-1)] III; syp-2(ok307)/tmC16 [unc-60(tmls1210)] V.
Microscopy. Worms were mounted for all live imaging studies using our auxin-inducible conditional paralysis method, which is described in (Cahoon & Libuda, 2021). Briefly, young adult worms (18-24hrs post L4) from a parental generation that was grown on NGM with either 1mM auxin (for oocyte studies) or 10mM auxin (for spermatocyte studies) were picked into 1µL drop of live imaging media (M9 media with 25mM serotonin (Sigma-Aldrich, cat no. H7752, (Rog & Dernburg, 2015)), 0.08% tricaine (Ethyl 3-aminobenzoate methanesulfonate; Sigma-Aldrich, cat. no. E10521-50G), 0.008% tetramisole hydrochloride (Sigma-Aldrich, cat. no. T1512-10G) and either 1mM or 10mM auxin (Naphthaleneacetic Acid (K-NAA), PhytoTechnology Laboratories, cat no. N610) (Martinez et al., 2020)) on a 22x40mm (no. 1.5) coverslip. (note: we found that poly-lysine treating the coverslips was not necessary for immobilization of the worms in most cases as long as the liquid under the agarose pad is minimal.) 7-9% agarose pads (Invitrogen, cat no. 16500500) were gently placed over the top of the worms and excess liquid was wicked away using Whatman paper. A microscope slide was adhered to the agarose pad worm coverslip sandwich using a ring of Vaseline around the pad. Worms were then imaged.
using the setting described below. For both SC intensity and photobleaching studies, worms
were imaged immediately following mounting and worms were only kept mounted for a max of 1
hour even though worms can survive being mounted for 2-3hrs (Cahoon & Libuda, 2021).

We did note that previous studies displayed higher fractions of SYP-3 recovery, which is
likely caused by our studies not upshifting the worms to 25ºC overnight (Nadarajan et al., 2017;
Pattabiraman et al., 2017; Rog et al., 2017). Shifting the worms to 25ºC is known to cause
significant elevations in meiotic gene expression and is used to enhance expression of
fluorescently tagged meiotic proteins (Pattabiraman et al., 2017; Song et al., 2010; Yokoo et al.,
2012). Notably, we found that in recombination deficient mutants, such as cosa-1, the elevated
levels of GFP::SYP-2 protein in the SC with the elevation in protein expression that comes at
25ºC caused large aggregates to form in mid/late pachytene that would persist into diakinesis
(Figure 3 – figure supplement 4). When the worms were only grown at 20ºC without any 25ºC
upshift, these aggregates did not form. Thus, none of the worms for the studies in this paper
were placed at 25ºC overnight. Nevertheless, the trends appear the same for progressive
stabilization with both GFP::SYP-3 and mCherry::SYP-3 (Figure 1, Figure 1 – figure supplement
2) suggesting that mCherry and GFP fluorescent tags of SYP-3 do not appear to illicit
differential dynamics (Nadarajan et al., 2017; Pattabiraman et al., 2017; Rog et al., 2017).

All live imaging studies of SYP-2::GFP, mCherry::SYP-3, SYP-5::GFP, and SYP-6::GFP
were imaged on a Nikon CSU SoRa Spinning Disk Microscope with a 60x water lens/N.A. 1.2
using a Z-step size of 0.3µm. For SC intensity quantifications, the laser power, and exposure
times were kept consistent for all genotypes. All GFP::SYP-2, SYP-5::GFP, and SYP-6::GFP
were imaging used the 488 laser at 16% power and 500msec exposure time. All mCherry::SYP-
3 imaging used the 561 laser at 25% power and 700msec exposure time. Additionally, only the
bottom half of the germline closest to the coverslip was imaged and germlines were not imaged
if the position of the mounted worm caused the gut to cover germline or moved parts of the
ermline deeper into the worm.
The fluorescent recovery after photobleaching (FRAP) studies were performed as described in (Pattabiraman et al., 2017) with minor changes. Briefly, a Z-stack was taken prior to photobleaching to obtain a pre-bleach image. Then, a region of interest defined by the point tool in Elements was photobleached. A timelapse was started immediately post-photobleaching with images captured every 5 minutes for 35 minutes to monitor the fluorescence recovery. Pilot experiments showed that the 35-minute timepoint displayed the highest recovery fluorescence observed before the signal plateaued. So, to minimize photobleaching and phototoxicity effects, we concluded the recovery timelapses at 35 minutes. For photobleaching small regions GFP::SYP-2 or mCherry::SYP-3, a 405 laser was used with 1-5% laser power and 10-30msec exposure depending on the germline location of the nucleus and the tagged protein with GFP::SYP-2 requiring less laser power to photobleach than mCherry::SYP-3. Previous studies showed that immobilized worms without serotonin have significantly diminished or absent chromosome motion, and the loss of this motion does not impair SC recovery dynamics (Pattabiraman et al., 2017; Rog & Dernburg, 2015; Rog et al., 2017). Since the addition of chromosome motion makes the FRAP recovery analysis very challenging, we did not include serotonin to minimize the motion of the chromosomes and to allow for better tracking of the photobleached SC region within each nucleus during the recovery timelapse.

Immunofluorescence slides of fixed gonad were imaged on a GE DeltaVision microscope with a 63x/N.A. 1.42 lens and 1.5x optivar at 1024x1024 pixel dimensions. Images were acquired using 0.2µm Z-step size and deconvolved with softWoRx deconvolution software.

**Immunohistochemistry.** Immunofluorescence was performed as described in (Cahoon & Libuda, 2021; Libuda et al., 2013). Briefly, gonads were dissected in egg buffer with 0.1% Tween20 on to VWR Superfrost Plus slides from 18-24 hour post L4 worms. Dissected gonads were fixed in 5% paraformaldehyde for 5 minutes, flash frozen in liquid nitrogen, and fixed for 1 minute in 100% methanol at -20°C. Slides were washed three times in PBS+0.1% Tween20
(PBST) for 5 minutes each and incubated in block (0.7% bovine serum albumin in PBST) for 1 hour. Primary antibodies (chicken anti-RAD-51, 1:1500 (Kurhanewicz et al., 2020; Toraason et al., 2021); rabbit anti-SYP-1, 1:1000 (gift from Silva Lab); rabbit anti-DSB-2 (Rosu et al., 2013); 1:5000; rabbit anti-OLLAS 1:1000 (Genscript, A01658); guinea pig anti-SUN-1 S8P, 1:700 (Woglar et al., 2013)) were added and incubated overnight in a humid chamber with a parafilm cover. Slides were then washed three times in PBST for 10 minutes each and incubated with secondary antibodies (goat anti-rabbit AlexaFluor488, ThermoFisher, cat. no. A11034; goat anti-chicken AlexaFluor488, ThermoFisher, cat. no. A11039; goat anti-rabbit AlexaFluor555, ThermoFisher, cat. no. A21428; GFP booster, Chromotek, gb2AF488-50; goat anti-guinea pig AlexFluor488, ThermoFisher, cat. no. A11073) at 1:200 dilution for 2 hours in a humid chamber with a parafilm cover. Slides were washed two times in PBST then incubated with 2µg/mL DAPI for 15-20 minutes in a humid chamber. Prior to mounting slides were washed once more in PBST for 10 minutes and mounted using Vectashield with a 22x22mm coverslip (no. 1.5). Slides were sealed with nail polish and stored at 4ºC until imaged. GFP::MSH-5 slides were imaged within 24-48hrs of mounting due to significant signal loss in the GFP::MSH-5 staining if the slide were stored longer.

Image Analysis and Quantification.

FRAP quantification. The quantification of fluorescence recovery of GFP::SYP-2 and mCherry::SYP-3 was determined using FIJI. All photobleaching movies were first stabilized using the FIJI plugin “StackRegJ” (https://research.stowers.org/imagejplugins/) to reduce the nuclear and worm motion in the germline. Photobleached nuclei were cropped to exclude as much extra z volume outside the size of the nucleus as possible. Then, these nuclei were sum intensity z-projected and the fluorescence intensity of the photobleached region was monitored using the rectangle tool. A small box was drawn on the segment of SC that will be photobleached and through each frame of the timelapse the fluorescence intensity was
recorded to obtain pre-bleach, bleach, and post-bleach fluorescence intensity values for a total
of 35 minutes. Similar to (Pattabiraman et al., 2017), we also excluded any nucleus that rotated
or shifted in such a way that the photobleached SC segment could not be tracked between
frames of the timelapse.

Nuclei in early pachytene were defined by being within the first 5-6 rows of pachytene
and nuclei in late pachytene were defined by being within the last 5-6 rows of pachytene. Mid
pachytene nuclei were selected by being located within the middle region of pachytene. At each
of these regions, the fluorescent intensity of three background regions of interest were
determined per germline and averaged together to give the average background intensity. The
average background intensity was subtracted from the fluorescence intensity of the
photobleached SC segment. Additionally, the FRAP data from each SC segment was
normalized such that the segment intensity pre-photobleach was 1 and the intensity immediately
post-photobleached was 0. This allows us to determine the fraction of fluorescence intensity of
each SYP protein that recovered following 35 minutes post-photobleaching. For oocytes, 3-5
germlines were used for GFP::SYP-2 and mCherry::SYP-3 analysis. For spermatocytes 3-6,
germlines were used for GFP::SYP-2 and mCherry::SYP-3 analysis. For both sexes, 8-11 nuclei
were analyzed in each region of pachytene and the specific n values in each region are reported
in the figure legend. All images have been sum intensity projected and slightly adjusted for
brightness and contrast. Additionally, any brightness and contrast adjustments made to oocyte
images was also applied to spermatocyte images.

SC intensity quantification. The quantification of GFP::SYP-2, mCherry::SYP-3, SYP-5::GFP
and SYP-6::GFP was performed using Imaris (Oxford Instruments) in combination with our
whole gonad analysis, described in (Toraason et al., 2021). The assembled, chromatin-
associated SYP signal in each nucleus was surfaced in Imaris to obtain the sum intensity and
volume of the assembled. The start of pachytene was defined by the first row that did not
contain more the 1-2 nuclei of transition zone nuclei (nuclei with DNA in a polarized or
“crescent” shape morphology) and full length SC. We assessed nuclei shape using both the
nuclear fluorescent haze produced by unassembled fluorescently tagged SYPs and the
assembled chromatin-associated SC. The end of pachytene was defined by the last row that
contained all pachytene nuclei with the occasional single diplotene nucleus. The pachytene
region was then equally divided into three zones based on the length of this region within the
germline to generate early pachytene, mid pachytene, and late pachytene. These criteria were
used for establishing the early pachytene, mid pachytene, and late pachytene in both
hermaphrodite and male germlines. Nuclei were excluded from the analysis if they were not in a
single layer on the bottom half of the imaged germline rachis due to an intensity decrease
caused by higher amounts of light scatter from being deeper in germline.

Our whole gonad analysis was used to align GFP::SYP-2, mCherry::SYP-3, SYP-5::GFP
and SYP-6::GFP surfaced nuclei along the germline length (Toraason et al., 2021). Each
nucleus was then normalized by their volume to determine the normalized sum intensity of each
nucleus during pachytene. The length of pachytene was also normalized per germline from 0
(early pachytene) to 1 (late pachytene). Since SYP-6 disassembles in oocytes prior to the end
of pachytene, SYP-6::GFP was normalized to the SYP-6::GFP distance, which was measured
from early pachytene (0) to the end of the SYP-6::GFP signal (1) for each germline. To calculate
the average and standard deviation of the normalized SYP intensity of each nucleus during
pachytene, we binned the data using a sliding window of 0.01. 7-12 germlines were analyzed for
all genotypes and both sexes. During the course of this study, we discovered that from all
images from May 2022 up to September 2022 needed to be corrected for a 15% drop in the
power of the 561nm laser. This correction was applied to the sum intensity of mCherry::SYP-3
for all genotypes imaged during this time period, which included spo-11 oocytes and
spermatocytes, syp-2/+ oocytes and spermatocytes, cosa-1 spermatocytes, syp-3/+ oocytes
and spermatocytes, and wild type oocytes and spermatocytes. The number of nuclei analyzed

within early, mid and late pachytene in each genotype is reported in as figure supplement for
Figures 2, 3, 7, and 8. All images have been sum intensity projected and slightly adjusted for
brightness and contrast with the same settings between mutants and sexes.

SC length quantification. SC length measurements were determined on deconvolved
DeltaVision images in Imaris using the filament tracer tool. Each chromosome within a nucleus
was traced following the SYP-1 signal. If all six chromosomes could not be traced, then that
nucleus was excluded from the analysis. Nuclei in early pachytene were defined by being within
the first 5-6 rows of pachytene and nuclei in late pachytene were defined by being within the last
5-6 rows of pachytene. Mid pachytene nuclei were selected by being located within the middle
region of pachytene. For spermatocyte nuclei with six SC tracks, the smallest trace length was
removed from the analysis because we inferred it to be the hemizygous X chromosome
inappropriately assembled the SC (Jaramillo-Lambert & Engebrecht, 2010). For oocytes, 10
nuclei in early pachytene were traced, 10 nuclei in mid pachytene were traced, and 12 nuclei in
late pachytene were traced. For spermatocytes, 11 nuclei in early pachytene were traced, 12
nuclei in mid pachytene were traced, and 13 nuclei in late pachytene were traced.

Germline measurement quantifications of transition zone, DSB-2 staining, SUN-1 S8P, and
SYP-1 assembly. Germline measurement quantification were performed using Imaris in
combination with our whole gonad analysis protocol, described in (Toraason et al., 2021).
Imaged gonads were stitched together using the FIJI (NIH) plugin Stitcher (Preibisch et al.,
2009) and using the measurement tool in Imaris. The positions of points along the germline
were recorded marking specific regions indicating the start of the germline at the pre-meiotic tip,
start of the transition zone, start of DSB-2 staining, start of SUN-1 S8P, start of SYP-1
assembly, end of transition zone, end of DSB-2 zone, end of SUN-1 S8P, end of SYP-1
assembly, end of SYP-1 zone, end of pachytene, last nuclei with SUN-1 S8P, and end of
straggler DSB-2 nuclei. DAPI morphology was used to determine the start and end of the transition and pachytene. From these recorded point positions, we calculated the length of: (1) the transition zone, (2) SYP-1 assembly zone, (3) full length SYP-1 zone, (4) SUN-1 S8P zone, (5) last nucleus with SUN-1 S8P zone, (6) DSB-2 zone, (7) DSB-2 straggler nuclei zone, and (8) pachytene. The start of the transition zone and pachytene was defined by the first row that did not contain more the 1-2 nuclei of either pre-meiotic nuclei (compact nuclei) or transition zone nuclei (nuclei with DNA in a polarized or “crescent” shape morphology), respectively. The end of pachytene was defined by the last row that contained all pachytene nuclei with the occasional single diplotene nucleus. The start of DSB-2 and SUN-1 S8P was determined by the position where the staining of each antibody began in a majority of the nuclei within a row, and the end of DSB-2 and SUN-1 S8P staining was determined by the position where the staining of each antibody was largely absent from a majority of the nuclei. The end of the DSB-2 straggler nuclei and SUN-1 S8P last nucleus was defined by the last nucleus in the germline with bright DSB-2 staining or SUN-1 S8P staining, respectively. The start of the SYP-1 assembly zone was defined by the germline position where small linear fragments of SYP-1 were observed, and the end of the SYP-1 assembly zone was defined by the germline position where all the nuclei in a row had full length SYP-1 with only 1-2 discontinuities. The end of the SYP-1 zone was determined by region where SYP-1 began to disassemble at the end of pachytene. The germline length was normalized per germline where 0 was the start of the germline at the pre-meiotic tip and 1 was the end of late pachytene. The number of germlines analyzed in each experiment is reported in the figure legends. All images have been max intensity projected and slightly adjusted for brightness and contrast.

**RAD-51, MSH-5, COSA-1 quantification.** Imaged gonads were stitched together using the FIJI (NIH) plugin Stitcher (Preibisch et al., 2009) and analyzed in Imaris as described in (Toraason et al., 2021) with minor changes. Each gonad from the start of pachytene through the end of
pachytene was analyzed for RAD-51, MSH-5 or COSA-1 foci per nucleus, which was determined by DAPI morphology. The start of pachytene was defined by the first row that did not contain more the 1-2 nuclei of transition zone nuclei (nuclei with DNA in a polarized or “crescent” shape morphology). The end of pachytene was defined by the last row that contained all pachytene nuclei with the occasional single diplotene nucleus. The pachytene region was then equally divided into three zones based the length of this region within the germline to generate early pachytene, mid pachytene, and late pachytene. The length of pachytene was also normalized per germline from 0 (early pachytene) to 1 (late pachytene). These criteria were used for establishing the early pachytene, mid pachytene, and late pachytene in both hermaphrodite and male germlines. For RAD-51, MSH-5 and COSA-1 foci per nucleus, sliding window averages and standard error of the mean (SEM) were calculated using a 0.01 bin size. 7-12 germlines were analyzed for all genotypes and both sexes. The number of nuclei analyzed within early, mid and late pachytene in each genotype is reported in the figure legends for each plot in Figure 6. All images have been max intensity projected and slightly adjusted for brightness and contrast.

**Western Blot Analysis.** For both male and hermaphrodite 100 adult worms were picked and washed once with M9 before being boiled for 5-10 minutes in sample buffer with occasional vortexing. For all western blots, samples were run on SDS-PAGE and wet transferred to low fluorescence PVDF membranes (ThermoFisher cat. no. 22860). Membranes were blocked in 5% milk in TBS+0.1% Tween20 (TBST) for 1 hour at room temperature. Primary antibodies (anti-SYP-2 rabbit 1:1000 (gift from Yumi Kim), anti-Tubulin mouse 1:1000 (Abcam cat. no. ab7291)) were incubated and agitated on an orbital shaker overnight at 4°C in 5% milk. Blots were washed three times for 10 minutes with TBST and LiCor secondary antibodies (IRDye 680 Donkey anti-Mouse (LiCor cat. no. 926-68072), IRDye 800CW Donkey anti-Rabbit (LiCor cat. no. 926-80072), and IRDye 800CW donkey anti-goat (LiCor cat. no. 926-80073)).
no. 926-32213)) were incubated at 1:1000 in TBST at room temperature for 1 hour. Blots were washed twice for 10 minutes each and imaged using LiCor Odyssey Fc.

Since male worms only have one germline, all hermaphrodite samples were diluted to adjust for the two germlines present in hermaphrodite worms. To determine the correct dilution factor, we performed a dilution series of the hermaphrodite sample and measured the sum intensity of the SYP-2 bands in each dilution using FIJI (Figure 3 – figure supplement 3). Then, we subtracted the background intensity from each SYP-2 measurement and normalized each dilution by the average intensity of SYP-2 from two undiluted male sample (Figure 3 – figure supplement 3). From the normalized dilution curve, we determined that a 40% dilution of the hermaphrodite sample equated to similar amounts of SYP-2 in the undiluted male samples. This 40% dilution was applied to all hermaphrodite samples in Figure 3 – figure supplement 3. To quantify the amount of SYP-2 in each sample, we used FIJI to measure the sum intensity of the SYP-2 and loading control alpha-tubulin bands that were both subtracted both from a background intensity. Then, the background subtracted SYP-2 intensity was normalized by the background subtracted loading control alpha-tubulin intensity to generate a normalized SYP-2 intensity (Figure 3 – figure supplement 3).

**Fertility assay.** To assess hermaphrodite fertility, L4 hermaphrodite worms were placed onto new NGM plates and were transferred every 24 hours for a total of 2 days. To assess male fertility, single L4 male worms were mated to single L4 *fog-2* obligate females and transferred every 24 hours for a total of 2 days before permanently removing the parental worms. After 3 days from removing the parental worms, each plate was scored for dead eggs. Then, the following day (4 days post removing parental worms) each plate was scored for living hermaphrodite and male progeny (male progeny was not scored in male fertility assays). Additionally, any progeny with mutant Unc or Dpy phenotypes were also scored for 5 worms in each genotype. 7-10 worms were assayed for fertility for each genotype.
SNP recombination mapping. SNP recombination mapping of Chromosome II and X was performed as described in (Bazan & Hillers, 2011) with minor changes. syp-2(ok307) and syp-3(ok785) were generated in Bristol (N2) backgrounds and we PCR confirmed that both mutant strains carried all Bristol SNPs for both chromosomes assayed prior to mapping recombination.

To generate Bristol/Hawaiian hybrids for mapping recombination, we crossed Bristol, syp-2(ok307) (DLW188), and syp-3(ok785) (DLW190) hermaphrodites to Hawaiian (CB4856) males. 8-10 Bristol/Hawaiian hybrid L4 hermaphrodites were picked off the cross plates for oocyte recombination mapping and 10-15 Bristol/Hawaiian hybrid L4 males were picked off the cross plates for spermatocyte recombination. For oocyte recombination mapping, hybrid L4 hermaphrodites of each genotype were crossed to Bristol males and male progeny were picked into 96-well plates for lysis and recombination PCR screening. For spermatocyte recombination mapping, hybrid L4 males of each genotype were crossed to Bristol hermaphrodites and male progeny were picked into 96-well plates for lysis and recombination PCR screening.

We used previously designed PCR primers and restriction digests to map six Bristol and Hawaiian SNPs on both Chromosomes II and X (Bazan & Hillers, 2011). However, we were unable to get the primers to work for SNP E on Chromosome II, so we redesigned new primers for this SNP that worked with the existing restriction digest for SNP identification at this genomic location. All SNP positions, PCR primers, restriction digests, and band sizes of the products for the Bristol or Hawaiian SNPs can be found in Supplemental file 2. The recombination frequency or map length in centiMorgans (cM) was calculated by taking the total number of crossovers identified in each interval divided by the total chromosomes scored multiplied by 100.

Statistics. Statistically analysis of the FRAP data, SC length, SNP recombination mapping and fertility assays was done using Prism. Mann-Whitney U tests adjusted for multiple comparisons using the Bonferroni-Dunn method was performed on the FRAP data. Kruskal-Wallis tests were
performed on the SC lengths with corrections for multiple comparisons. Chi-squared tests were performed on the entire SNP recombination mapping distribution. Pairwise comparisons between recombination intervals were performed using Fisher’s Exact test. Chi-squared and Fisher’s Exact tests were performed on the fertility assays. Mann-Whitney U tests adjusted for multiple comparisons using the Bonferroni-Dunn method were performed on the SC intensity, quantification of RAD-51, MSH-5 and COSA-1, transition zone length, pachytene length, end of SYP-1 assembly zone, SUN-1 S8P zone, end of DSB-2 zone, and end of DSB-2 straggler zone using R. Each test used is indicated in the Results section next to the reported p-value and all n values are reported in the figure legends.

Data Availability. All strains developed as part of this study will be available at the CGC or are available upon request. All data generated or analyzed during this study are included in the manuscript and supporting files. Source Data have been provided for the numerical values plotted in each figure: Figure 1 source data (Figure 1 source data.xlsx), Figure 1 – figure supplement 3 source data (Figure 1 – figure supplement 3 source data.xlsx), Figure 2 source data (Figure 2 source data.xlsx), Figure 2 – figure supplement 2 source data (Figure 2 – figure supplement 2 source data.zip), Figure 3 source data (Figure 3 source data.xlsx), Figure 4 source data (Figure 4 source data.xlsx), Figure 4 – figure supplement 1 source data (Figure 4 – figure supplement 1 source data.zip), Figure 5 source data 1 (Figure 5 source data.xlsx), Figure 5 – figure supplement 3 source data (Figure 5 – figure supplement 3.xlsx), Figure 6 source data 1 (Table 1), Figure 6 – figure supplement 1 source data (Figure 6 – figure supplement 2), Figure 7 source data (Figure 7 source data.xlsx), and Figure 8 source data (Figure 8 source data.xlsx).

**FIGURE LEGENDS**

**Figure 1: SYP-2 and SYP-3 dynamics are sexually dimorphic.** (A) Diagrams of hermaphrodite (top, red) and male (bottom, blue) germlines with developing oocytes and spermatocytes, respectively. The stages of the germlines are labeled starting at the pre-meiotic
tip (PMT) and ending at the meiotic divisions. Nuclei proliferate at the distal end of the germline
(pre-meiotic tip) and physically move proximally as they proceed into the stages of meiosis: the
transition zone (leptotene/zygotene), pachytene, diplotene, and diakinesis (in spermatocytes
diplotene/diakinesis is termed the condensation zone). At the end of prophase I, oocyte nuclei
arrest at diakinesis until they are fertilized, but spermatocytes rapidly complete the meiosis I and
meiosis II divisions to generate mature sperm. (B) Diagram of the synaptonemal complex
showing the positions of SYP-1 (gray), SYP-2 (yellow), SYP-3 (green), SYP-5 (blue-purple), and
SYP-6 (red-purple) within the central region of the complex. (C) Quantification of the fraction of
fluorescence recovered 35 minutes after photobleaching a small region of either GFP::SYP-2
(left) or mCherry::SYP-3 (right) in both oocytes (red) and spermatocytes (blue) (a.u. = arbitrary
units). All statistics are multiple comparisons Bonferroni-Dunn adjusted Mann Whitney U test
and unless the p value is indicated the asterisk indicates number of significant digits from
p=0.05 (n.s. = not significant). Oocyte data with GFP::SYP-2 is from 10 nuclei (early), 9 nuclei
(mid), and 9 nuclei (late), and with mCherry::SYP-3 is from 11 nuclei (early), 9 nuclei (mid), and
9 nuclei (late). Spermatocyte data with GFP::SYP-2 is from 9 nuclei (early), 9 nuclei (mid), and
10 nuclei (late), and with mCherry::SYP-3 is from 8 nuclei (early), 9 nuclei (mid), and 10 nuclei
(late).

Figure 1 – source data. The source data for Figure 1C are provided. [Figure 1 source
data.xlsx]. The normalized fluorescence recovery for the FRAP data for each nucleus analyzed
with GFP::SYP-2 and mCherry::SYP-3 at early, mid, and late pachytene in both sexes.

Figure 1 – figure supplement 1. Montages of FRAP from oocytes and spermatocytes.
Representative image montages of GFP::SYP-2 and mCherry::SYP-3 FRAP from oocytes (A)
and spermatocytes (B) in early and late pachytene. Yellow box indicates the bleached region of
each nucleus.

Figure 1 – figure supplement 2. FRAP recovery curves from oocytes and spermatocytes.
Normalized recovery curves from FRAP analysis of nuclei with GFP::SYP-2 (A) or
mCherry::SYP-3 (B) from oocytes (left plot) and spermatocytes (right plot) (see Methods).
Nuclei were analyzed at early pachytene (pink triangles), mid pachytene (grey squares), and
late pachytene (blue circles). Error bars indicate standard deviation.

Figure 1 – figure supplement 3. SC lengths are not different between the sexes in early
and mid pachytene. Quantification of the measurement of SYP-1 length on each chromosome
between oocytes (red) and spermatocytes (blue) throughout pachytene. Only late pachytene
display significant differences in SC lengths (Mann-Whitney U test; n.s. = not significant).

Figure 1 – figure supplement 3 source data. The source data for Figure 1 – figure
supplement 3 are provided. [Figure 1 – figure supplement 3 source data.xlsx]. The SYP-1
length measurements for each chromosome in wild type oocytes and spermatocytes.

Figure 2: Accumulation of SYP-2 and SYP-3 in the SC are sexually dimorphic. (A,C)
Quantification of the mean intensity of GFP::SYP-2 (A) or mCherry::SYP-3 (C) per nucleus
normalized by the volume of each nucleus (see Methods) throughout pachytene for wild type
(WT) oocytes (red, pale red band is the standard deviation) and spermatocytes (blue, pale blue
band is the standard deviation). P values on the plot are comparisons between oocytes and
spermatocytes for each region of pachytene using Mann Whitney U tests. (B,D) Represented
images of GFP::SYP-2 (B) or mCherry::SYP-3 (D) in wild type (WT) hermaphrodite (top,
oocytes) and male (bottom, spermatocytes) germlines. Germlines are oriented with the start of
pachytene on the left and meiotic progression continues to the right. Yellow boxes identify the
regions enlarged in each image below to show representatives of early, mid and late pachytene.

Arrowheads indicate GFP::SYP-2 bright nuclei in mid pachytene. The intensity adjustments are
the same for both GFP::SYP-2 germlines and mCherry::SYP-3 germlines, respectively. n values
for number of germlines and nuclei can be found in Figure 2 – figure supplement 1.

**Figure 2 – source data.** The source data for Figure 2A, 2C are provided. [Figure 2 source
data.xlsx]. Raw sum intensity and normalized sum intensity per nucleus for GFP::SYP-2,
mCherry::SYP-3 in both sexes and wild type genotypes.

**Figure 2 – figure supplement 1.** SC intensity n values for nuclei and germlines scored.

**Figure 3: Recombination influences the incorporation of SYP-2 and SYP-3 in the SC
differently in each sex.** (A,B) Quantification of the mean intensity of GFP::SYP-2 per nucleus
normalized by the volume of each nucleus (see Methods) throughout pachytene for spo-11 (A)
and cosa-1 (B). (C,D) Quantification of the mean intensity of mCherry::SYP-3 per nucleus
normalized by the volume of each nucleus (see Methods) throughout pachytene for spo-11 (C)
and cosa-1 (D). Oocytes are shown in red with the standard deviation shown as a pale red band
and spermatocytes are shown in blue with the standard deviation shown as a pale blue band.
The mean intensity of GFP::SYP-2 (A,B) or mCherry::SYP-3 (C,D) for wild type (WT) oocytes
(black) and wild type (WT) spermatocytes (gray) are shown as dashed lines. Heat maps below
each pachytene region show the Bonferroni adjusted P values from Mann-Whitney U tests with
dark gray indicating p<0.001, light gray indicating p<0.05, and white indicating not significant
(n.s.). The self-comparison between spermatocyte spo-11 or cosa-1 mutants was not
determined (n.a.). n values for number of germlines and nuclei can be found in Figure 3 – figure
supplement 2.

**Figure 3 – source data.** The source data for Figure 3 are provided. [Figure 3 source
data.xlsx]. Raw sum intensity and normalized sum intensity per nucleus for GFP::SYP-2 and
mCherry::SYP-3 in both sexes and all genotypes analyzed in Figure 3.

**Figure 3 – figure supplement 1.** Representative images of spo-11 and cosa-1 germlines
with GFP::SYP-2 and mCherry::SYP-3 in oocytes and spermatocytes. Scale bar represents
25 µm.

**Figure 3 – figure supplement 2.** SC intensity n values for nuclei and germlines scored.

**Figure 3 – figure supplement 3.** Western Blot Analysis of SYP-2 protein levels in oocytes
and spermatocytes. (A) Dilution series blot with adult hermaphrodite samples to determine the
level of dilution necessary to match the oocyte GFP::SYP-2 amounts to the spermatocyte
GFP::SYP-2 amounts (see Methods). The loading percent above each well are the percent of
hermaphrodite sample and diluted with sample buffer prior to loading. 100% is undiluted sample
for both hermaphrodite (oocytes) and male (spermatocytes) samples. To the right of the blot is
the quantification of the GFP::SYP-2 band normalized to the averaged undiluted spermatocyte
SYP-2 amounts. From the plot, we determined that a 40% dilution of the hermaphrodite
samples was necessary to be equivalent to an undiluted spermatocyte sample from male
worms. (B) Blot of GFP::SYP-2 levels in wild type (WT) and mutant oocytes and spermatocytes.
To the right of the blot is quantification of the GFP::SYP-2 band normalized to tubulin loading
control. Three independent western blots were quantified with 100 worm samples loaded per
genotype and the error bars represent the standard deviation.
Figure 3 – figure supplement 3 source data. The source data for Figure 3 – figure supplement 3 are provided. [Figure 3 – figure supplement source data.zip]. Original western blot images, raw SYP-2 band intensity measurements, and normalized SYP-2 amounts in both sexes and all genotypes analyzed in this manuscript.

Figure 3 – figure supplement 4. GFP::SYP-2 aggregates in cosa-1 at 25°C during mid and late pachytene. Representative hermaphrodite germline images of GFP::SYP-2 in cosa-1 mutants from worms kept at 20°C and worms upshifted to 25°C for 18-24hrs prior to imaging. White arrows indicate the direction oocyte nuclei are moving within the germline and the stages of prophase are labeled.

Figure 4: Oocyte SYP-1 assembly is uniquely sensitive to SYP-2 dosage. (A,B) Representative images early pachytene stained for SYP-1 in oocytes (A) and spermatocytes (B) from wild type (WT), syp-2/+, and syp-3/+. Yellow dashed box shows the nucleus that is enlarged below each merge image. Yellow arrowheads indicate regions where the SYP-1 signal is broken/not continuous. Scale bar represents 5µm (C, D) Measurement of the relative length of the SYP-1 assembly zone in oocytes (C) and spermatocytes (D) from the pre-meiotic tip (PMT) to the end of pachytene in wild type (WT, gray), syp-2/+ (pink), and syp-3/+ (purple). Dashed lines represent the SC assembly zone and solid lines represent fully assembled SC. (E) Quantification of the end of the SYP1 assembly zone in wild type (WT, gray), syp-2/+ (pink), and syp-3/+ (purple) in oocytes (left) and spermatocytes (right). All statistics are Bonferroni adjusted P values from Mann-Whitney U tests (n.s. = not significant). Oocyte data is from 8 wild type germlines, 10 syp-2/+ germlines, and 9 syp-3/+ germlines. Spermatocyte data is from 9 wild type germlines, 8 syp-2/+ germlines, and 10 syp-3/+ germlines.

Figure 4 – source data. The source data for Figure 4C, 4D, 4E are provided. [Figure 4, source data.xlsx]. Distance data from germlines where the length of SYP-1 assembly was determined.

Figure 4 – figure supplement 1: Transition zone length and SUN-1 phosphorylation zone is unaltered by SYP dosage. (A) Measurement of the pre-meiotic zone length (dashed line), transition zone length (thin solid line), and pachytene length (thick solid line) in WT (gray), syp-2/+ (pink), and syp-3/+ (purple) based on DAPI morphology. All germline lengths have been normalized from the pre-meiotic tip (PMT) to the end of pachytene in both oocytes and spermatocytes. Each line represents an individual germline. Overall, neither syp-2/+ nor syp-3/+ displayed altered transition zone length. (B,C) Violin plots of the transition zone (TZ) length and pachytene length in WT (gray), syp-2/+ (pink), and syp-3/+ (purple) in oocytes (B) and spermatocytes (C). There are no statistical differences in transition zone length or pachytene length (Mann-Whitney U test). Oocyte data is from 18 wild type germlines, 19 syp-2/+ germlines, and 18 syp-3/+ germlines. Spermatocyte data is from 9 wild type germlines, 8 syp-2/+ germlines, and 10 syp-3/+ germlines. (D) Measurement of SUN-1 S8 phosphorylation (S8P) zone length in wild type (gray), syp-2/+ (pink), and syp-3/+ (purple) oocytes and spermatocytes. Thin solid line indicates the distance from PMT to start of SUN-1 S8P; a thicker solid line indicates the distance from start to end of most nuclei with SUN-1 S8P staining; and the dashed line indicates the distance to the final nucleus in the germline with SUN-1 S8P staining (see Methods). Each line represents an individual germline. Overall, neither syp-2/+ nor syp-3/+ displayed altered the germline region with SUN-1 S8P.

Figure 4 – figure supplement 1 source data. The source data for Figure 4 – figure supplement 1 are provided. [Figure 4 – figure supplement 1 source data.zip]. Distance data for the length of meiosis stages and SUN-1 S8P.
Figure 5: Sex-specific regulation of recombination by SYP-2 and SYP-3 dosage. (A,B) Quantification of the average number of RAD-51 foci per nucleus throughout pachytene in wild type (WT, gray), syp-2/+ (pink), and syp-3/+ (purple) from oocytes (A) and spermatocytes (B). (C,D) Quantification of the average number of MSH-5 foci per nucleus throughout pachytene in wild type (WT, gray), syp-2/+ (pink), and syp-3/+ (purple) from oocytes (C) and spermatocytes (D). (E,F) Quantification of the average number of COSA-1 foci per nucleus throughout pachytene in wild type (WT, gray), syp-2/+ (pink), and syp-3/+ (purple) from oocytes (E) and spermatocytes (F). Heat maps below each pachytene region show the Bonferroni adjusted P values from Mann-Whitney U tests with dark gray indicating p<0.001, light gray indicating p<0.05, and white indicating not significant (n.s.). The self-comparison between syp-2/+ was not determined (n.a.). n values for number of germlines and nuclei can be found in Figure 5 – figure supplement 1.

Figure 5 – source data. The source data for Figure 5 are provided. [Figure 5 source data.xlsx]. RAD-51, MSH-5, COSA-1 foci per nucleus counts for wild type, syp-2/+ (syp2het) and syp-3/+ (syp3het).

Figure 5 – figure supplement 1. RAD-51, MSH-5, and COSA-1 n values for nuclei and germlines scored.

Figure 5 – figure supplement 2. Representative images of the quantification in Figure 5. Scale bar is 5µm.

Figure 5 – figure supplement 3. DSB-2 zone is unaltered in oocytes when SYP dosage is reduced. (A) Measurement of the length of DSB-2 staining (solid line) and the final DSB-2 stained straggler nucleus (dashed line) in WT (gray), syp-2/+ (pink), and syp-3/+ (purple). All germline lengths have been normalized from the pre-meiotic tip (PMT) to the end of pachytene in oocytes. Each line represents an individual germline. Overall, the zone of DSB-2 staining is largely unaltered in syp-2/+ and syp-3+. (B) Violin plots of the ending of the DSB-2 zone (left) and the ending of the DSB-2 straggler nuclei (right) in WT (gray), syp-2/+ (pink), and syp-3+ (purple). There are no statistical differences in the end of the DSB-2 zone or the end of the DSB-2 straggler nuclei (Mann-Whitney U test). Data is from 10 wild type germlines, 9 syp-2/+ germlines, and 9 syp-3/+ germlines.

Figure 5 – figure supplement 3 source data. The source data for Figure 5 – figure supplement 3 are provided. [Figure 5 – figure supplement 3 source data.xlsx]. Distance data from germlines where the length of DSB-2 zone was determined.

Figure 6: SYP dosage influences the crossover landscape in only oocytes. Recombination SNP mapping of Chromosome II in WT, syp-2+, and syp-3+ from oocytes (left) and spermatocytes (right) (see Methods for details). A diagram of the 15.3Mb Chromosome II shows the megabase location of each SNP assayed (A-E) and the colored boxed between each SNP show the intervals where crossovers were assessed. The map length (cM) is indicated in each crossover interval. The asterisks next to the map lengths indicate significance based on Fisher’s Exact tests compared between the mutants and wild type (syp2/+ P=0.0449; syp3/+ P=0.0170). The asterisks outside the bars indicate significance based on Chi-Squared tests between mutants and wild type (syp2/+ P=0.0343; syp3/+ P=0.0333). The worm counts for these plots can be found in Table 1.

Figure 6 – source data. The source data for Figure 6 are provided in Table 1.
Figure 6 – figure supplement 1. X Chromosome may have some chromosome distortion in syp-2/+ oocytes. (A) Recombination SNP mapping of Chromosome X in wild type, syp-2/+, and syp-3/+ oocytes. A diagram of the 17.7Mb Chromosome X shows the megabase location of each SNP assayed (A-E) and the colored boxed between each SNP show the intervals where crossovers were assessed. The map length (cM) is indicated in each crossover interval. syp-2/+ displays a significant reduction in map length on Chromosome X (P=0.0100, Chi-squared), while syp-3/+ displays no significant changes to wild type. The worm counts for each interval are in Figure 6 – figure supplement 2. (B) Fertility, male progeny and mutant progeny counts for wild type, syp-2/+, and syp-3/+ with either all N2 (Bristol) chromosomes or N2/CB4856 (Bristol/Hawaiian). Mutant progeny were scored by displaying dumpy and/or uncoordinated mutant phenotypes. The first n from the left is for the number of worms scored with live hermaphrodite progeny, male progeny, and dead eggs. The second n from the left is for the number of worms scored having mutant progeny.

Figure 6 – figure supplement 1 source data. The source data for Figure 6 – figure supplement 1 are provided in Figure 6 – figure supplement 2.

Figure 6 – figure supplement 2. Worm counts for Chromosome X SNP mapping recombination.

Figure 7: syp-2 and syp-3 gene dosage influence the amount of each SYP loaded within the SC via sex-specific mechanisms. (A,B) Quantification of the mean intensity of GFP::SYP-2 (A) and mCherry::SYP-3 (B) per nucleus normalized by the volume of each nucleus (see Methods) throughout pachytene for syp-2/+ (left plot) and syp-3/+ (right plot). Mutants are in solid lines with oocytes in red and spermatocytes in blue. Wild type is in dashed lines with oocytes in black and spermatocytes in grey. The pale shading around each line is the standard deviation. (C,D) Representative images of wild type germlines stained with SYP-6::GFP (green) and SYP-1 (magenta) in germlines with oocytes (B) and spermatocytes (C). The white dashed boxed shows the region enlarged in the image on the right. Scale bar represents 10 µm. (D) Quantification of the mean intensity of oocyte SYP-
6::GFP per nucleus normalized by the volume of each nucleus throughout pachytene for syp-2+/ (bright red) and syp-3+/ (dark red). Since SYP-6 disassembles prior to the end of pachytene, the germline length is normalized to the germline region with SYP-6::GFP signal starting at the beginning of pachytene (Pachytene start) to the end of the SYP-6::GFP signal (SYP-6::GFP end) (see Methods). The broken x-axis indicates the unknown distance to the end of pachytene (Pachytene end). Mutants are in solid lines, wild type oocytes is in a dashed line and the pale shading around each line represents the standard deviation. (E) Quantification of the mean intensity of spermatocyte SYP-6::GFP per nucleus normalized by the volume of each nucleus (see Methods) throughout pachytene for syp-2+/ (bright blue) and syp-3+/ (dark blue). Mutants are in solid lines, wild type spermatocytes is in a dashed line and the pale shading around each line represents the standard deviation. Heat maps below plots in panels (A), (D), and (E) show the Bonferroni adjusted P values from Mann-Whitney U tests with dark gray indicating p<0.001, light gray indicating p<0.05, and white indicating not significant (n.s.). The self-comparison between spermatocyte syp-2+/ or syp-3+/ mutants was not determined (n.a.). n values for number of germlines and nuclei can be found in Figure 8 – figure supplement 1.

Figure 8 – source data. The source data for Figure 8 are provided. [Figure 8 source data.xlsx]. Raw sum intensity and normalized sum intensity per nucleus for SYP-5::GFP and SYP-6::GFP in both sexes and all genotypes in Figure 8.

Figure 8 – figure supplement 1. SC intensity n values for nuclei and germlines scored.

Figure 8 – figure supplement 2. Representative images of wild type germlines stained with SYP-5::GFP and SYP-1 in oocytes and spermatocytes. Scale bar represents 10 µm.

Figure 9: SYP dosage influences the sexually dimorphic regulation of recombination. (A) In oocytes, SYP-2 amounts with the SC are critical for the proper formation and/or maintenance of joint molecules stabilized by MSH-5. Reducing the amount of SYP-2 dosage causes decreases in the amount of SYP-2 in early pachytene and shifts the peak amounts of SYP-2 toward mid/late pachytene. This alteration to SYP-2 composition within the SC causes severe reduction in MSH-5. SYP-3 amounts in the SC are important for the proper timing of recombination. When the dosage of SYP-3 is reduced, SYP-3 composition within the SC is reduced throughout pachytene causing faster resolution of jointed molecules and faster designation of crossovers during pachytene. This ultimately causes changes in the recombination landscape where crossovers are more often position near the pairing center. (B) In spermatocytes, SYP-2 amounts are important for the maintenance of MSH-5 stabilized joint molecules. When SYP-2 dosage is reduced, the amount of SYP-2 in the SC is reduced and MSH-5 foci are rapidly lost either because they are resolved quickly or the stability of the joint molecules is compromised. SYP-3 amounts in spermatocytes also influence the timing of recombination but when SYP-3 dosage is reduced SYP-3 amounts are increased rather than decreased. Thus, elevated SYP-3 levels in spermatocytes cause a delay in crossover designation in spermatocytes.

Supplemental file 1. CRISPR/Cas9 primer sequences.

Supplemental file 2. SNP recombination mapping primer sequences.
### Table 1: Chromosome II SNP mapping recombination

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A. Hermaphrodite germline (oocytes)

- Pre-meiotic Transition Zone
- Pre-meiotic tip (PMT)
- Pachytene
- Diplotene
- Oocyte development
- Diakinesis

B. Male germline (spermatocytes)

- Pre-meiotic Transition Zone
- Pre-meiotic tip (PMT)
- Pachytene
- Condensation Zone
- Meiotic divisions/spermatocyte development

C. Graph showing fluorescence recovery 35min post-bleach (a.u.)

- GFP::SYP-2
  - WT oocytes
  - WT spermatocytes

- mCherry::SYP-3
  - Early Pachytene
  - Mid Pachytene
  - Late Pachytene
  - N.S.

Statistical significance:
- oocytes p<0.001; spermatocytes n.s.
- oocytes p<0.001; spermatocytes p<0.01
A. Wild Type oocytes

**GFP::SYP-2**

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**mCherry::SYP-3**

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B. Wild Type spermatocytes

**GFP::SYP-2**

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A

**GFP::SYP-2 oocytes**

normalized intensity (au)

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**GFP::SYP-2 spermatocytes**

normalized intensity (au)

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B

**mCherry::SYP-3 oocytes**

normalized intensity (au)

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**mCherry::SYP-3 spermatocytes**

normalized intensity (au)

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WT oocytes
WT spermatocytes

n.s.
n.s.
p<0.0001

Early Pachytene
Mid Pachytene
Late Pachytene

SC Length (µm)
**Figure A**

- **Y-axis**: Average normalized intensity (a.u.)
- **X-axis**: Normalized germline length
- **Graphs**: Two graphs side by side showing the normalized intensity of GFP::SYP-2 and mCherry::SYP-3 across different stages of pachytene.
- **Stages**: Early, Mid, Late Pachytene
- **Significance**: p<0.001 for all stages

**Figure B**

- **Images**: WT oocytes and WT spermatocytes at early, mid, and late pachytene stages.
- **Cellular Probes**: GFP::SYP-2 and mCherry::SYP-3
- **Scale**: 25µm

**Figure C**

- **Graphs**: Similar to Figure A but with a focus on normalized germline length.

**Figure D**

- **Images**: Similar to Figure B but with a focus on normalized germline length.
A. GFP::SYP-2

- **Oocytes**
  - WT
  - spo-11
  - cosa-1

- **Spermatocytes**

B. mCherry::SYP-3

- **Oocytes**
  - WT
  - spo-11
  - cosa-1

- **Spermatocytes**
Early Pachytene | Mid Pachytene | Late Pachytene

Diplotene

GFP::SYP-2

cosa-1
20°C

25µm

Diakinesis

GFP::SYP-2

cosa-1
25°C

25µm

Diakinesis | Diplotene
Early Pachytene  Mid Pachytene  Late Pachytene

Diplotene

Diakinesis

Early Pachytene  Mid Pachytene  Late Pachytene

Diakinesis  Diplotene
A

WT

syp-2/+ 

syp-3/+ 

Normalized germline length

PMT Pachytene end

B

End of DSB-2

End of DSB-2 straggler

WT

syp-2/+ 

syp-3/+ 

Normalized germline length

n.s. n.s. n.s.

n.s. n.s. n.s.
A

Wild Type
- 16 13 10 12

syp-2/+ - 8 8 6 12

syp-3/+ - 11 13 11 10

Chromosome X recombination (cM)

B

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♀ N2/N2 ♀ fog-2
♂ syp-2/N2 ♀ fog-2
♂ syp-3/N2 ♀ fog-2

Live progeny -- Dead eggs --
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