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Meiotic crossover patterning in the absence of ATR: Loss of interference and assurance but not the centromere effect

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ABSTRACT Meiotic crossovers must be properly patterned to ensure accurate disjunction of homologous chromosomes during meiosis I. Disruption of the spatial distribution of crossovers can lead to nondisjunction, aneuploidy, gamete dysfunction, miscarriage, or birth defects. One of the earliest identified genes involved proper crossover patterning is *mei-41*, which encodes the *Drosophila* ortholog of the checkpoint kinase ATR. Although analysis of hypomorphic mutants suggested the existence of crossover patterning defects, it has not been possible to assess these in null mutants because these mutants exhibit maternal-effect embryonic lethality. To overcome this lethality, we expressed wild-type Mei-41 only after the completion of meiotic recombination, allowing embryos to survive. We find that crossovers are decreased more severely in null mutants, to about one third of wild-type levels. Crossover interference, a patterning phenomenon that ensures that crossovers are widely spaced along a chromosome, is eliminated in these mutants. Similarly, crossover assurance, which describes the distribution of crossovers among chromosomes, is lost. Despite the loss of interference and assurance, a third important patterning phenomenon – the centromere effect – remains intact. We propose a model in which the centromere effect is established prior to and independently of interference and assurance.

KEYWORDS meiotic recombination, interference, centromere effect, Drosophila, ATR kinase

Introduction

Meiotic crossovers are subject to numerous mechanisms of spatial control to ensure genetic diversity and proper disjunction of homologous chromosomes (Wang *et al.* 2015). Crossover assurance is the phenomenon in which there is at least one crossover per bivalent, generating the "obligate chiasma" that ensures disjunction (Owen 1949). Crossover interference is the inhibition of crossover formation within intervals flanking sites of crossover precursors (Sturtevant 1913; Berchowitz and Copenhaver 2010). Together with crossover homeostasis, which buffers crossover formation from increases or decreases in potential crossover precursors (Martini *et al.* 2006), assurance and interference demarcate the minimum and maximum number of crossovers possible per meiosis. Modeling suggests that crossover assurance, interference, and homeostasis are the result of a single patterning process with varying degrees of plasticity depending on the meiotic environment (Wang *et al.* 2015). However, less is known regarding the centromere effect, a phenomenon wherein crossover formation is suppressed within pericentromic euchromatic regions (Beadle 1932; Mather 1939).

Perturbation of crossover control can be viewed in the context of the two-pathway paradigm, wherein crossovers created within the 'Class I' pathway use canonical meiotic proteins that result in crossover patterning characteristic of that species (Kohl and Sekelsky 2013). Alternatively, in some cases mutants that lack one or more of these meiosis-specific proteins default back to a more mitotic-like 'Class II' pathway. This switch from Class I to Class II is often associated with a significant reduction in crossover formation and abnormal crossover patterning that results in gamete aneuploidy (Argueso *et al.* 2004; Lu *et al.* 2008; Hatkevich *et al.* 2017). Therefore, teasing apart the relationship between pathway usage and crossover control phenomena may

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help elucidate evolutionarily conserved mechanisms resulting in proper crossover placement.

The *Drosophila mei-41* gene, originally identified in a screen for meiotic mutants in 1972, encodes the ortholog of the DNA damage checkpoint kinase ATR (Baker and Carpenter 1972; Hari *et al.* 1995). Progeny from *mei-41*^{1} females exhibited altered crossover distribution, suggesting a critical role for the protein in meiotic crossover patterning (Baker and Carpenter 1972). However, meiotic recombination has not been assayed in the complete absence of Mei-41 because null mutants exhibit maternal-effect embryonic lethality due to DNA replication checkpoint failure: Embryos that lack maternal Mei-41 fail to slow rapid nuclear cycles leading up to the midblastula transition, do not cellularize, and eventually degenerate (Sibon *et al.* 1999). Thus, alleles used in previous studies of meiotic recombination are either hypomorphic or separation-of-function (Laurençon *et al.* 2003).

To overcome the embryonic requirement for maternal Mei-41, we expressed Mei-41 under control of a promoter that turns on during oogenesis after recombination has been completed, generating a fertile *mei-41* null mutant. Crossover and nondisjunction phenotypes are more severe in the mei 41 null mutant. With regard to crossover patterning, crossover interference and assurance are completely lost in *mei-41* null mutants, but the centromere effect remains intact. Intriguingly, progeny from mothers lacking both Mei-41 and the presumed *Drosophila* Class I Holliday junction resolvase Mei-9 have phenotypes nearly identical to those of *mei-41* single mutants, indicating a switch to the Class II pathway following the establishment of the centromere effect. We conclude that the centromere effect is established prior to, or separate from, the essential role of Mei-41 in the Class I crossover pathway and that the centromere effect is achieved independently of interference and assurance.

Materials and Methods

Drosophila stocks

Flies were maintained at 25° on standard medium. To overcome the maternal-effect embryonic lethality of *mei-41*^{29D} null mutation (Sibon *et al.* 1999; Laurençon *et al.* 2003), wild-type genomic *mei-41* was cloned into the pPattB, UASp::w vector (courtesy of Steve Rogers) via In-Fusion HD (Takara Bio USA, Inc., Mountain View, CA) and transformed into XL10-Gold ultracompetent cells (Agilent Technologies, Inc., Santa Clara, CA). This construct was injected via phiC31 integrase-mediated transgenesis into the *X* chromosome landing site 2A (BestGene Inc., Chino Hills, CA). Integrants were crossed into a *P{matα4::GAL4-VP16}* background. *w mei-41*^{29D}*/y P{UASp::mei-41} w mei-4129D; matα4::GAL4-VP16/+* was used in all *mei-41* null assays.

Double mutant stock creation used the above transgenic rescue in conjunction with appropriate null alleles. The *mei-41; mei-P22* double mutant genotype was *y w mei-41*^{29D}*/y* PUASp::mei-41 *w mei-41*^{29D}*; mei-P22*^{103} *st/mei-P22*^{103} *Blm*^{D2} *Sb P{matα4::GAL4-VP16}*. The *mei-9 mei-41* double mutant genotype was *y mei-9*^{a} *mei-41*^{29D}*/y P{UASp::mei-41} w mei-9*^{a} *mei-41*^{29D}*; P{matα4::GAL4-VP16}/+*. The *mei-41 Blm* double mutant genotype was *w mei-41*^{29D}*/y P{UASp::mei-41} w mei-41*^{29D}*; st Blm*^{D2} *ry*^{531} *P{matα4::GAL4-VP16}/Blm*^{N1} *ry*^{606} *Sb P{UASp::Blm}*.

Hatch rates

To test *P{UASp::mei-41}* rescue efficiency, 60 virgin females of appropriate genotypes were crossed to 20 isogenized Oregon-Rm males (courtesy of Scott Hawley). Adults were mated in grape-juice agar cages containing yeast paste for two days prior to collection. Embryos were collected on grape-juice agar plates for five hours and scored for hatching 48 hours later.

Meiotic assays

Meiotic crossovers were quantified by crossing *net dpp*$^{d\text{-}ho}$ *dp b pr cn / +* virgin females of the appropriate mutant background to *net dpp*$^{d\text{-}ho}$ *dp b pr cn* males. All six markers were scored in progeny from each genotype, with the exception *mei-41; mei-P22*. In that case, 731 *XX* females were scored for all six markers and 1023 *XXY* females and *XY* males were scored for *net – b*; eye color markers *pr* and *cn* were excluded because of the presence of a *w* mutation in the mothers. These data were pooled for a final *n* of 1754. Crossover density was calculated using *Drosophila melanogaster* reference genome release 6.12 with transposable elements excluded, as described in Hatkevich *et al.* (Hatkevich *et al.* 2017). Complete progeny counts are given in Supplemental Table S1.

Interference was assayed by crossing *dp wg*$^{Sp\text{-}1}$ *b / +* virgin *mei-41* null and wild-type females to *net dpp*$^{d\text{-}ho}$ *dp b pr cn* males. Complete progeny counts are given in Supplemental Table S2.

X nondisjunction was scored by crossing virgin mutant females of the appropriate genotypes to *y sc cv v g f / Dp(1;Y)B*^{S} males. Exceptional progeny for *X* nondisjunction events originate from diplo-*X* and nullo-*X* ova, resulting in Bar-eyed females (*XXY*) and wild-type-eyed males (*XO*), respectively. Counts of scored exceptional progeny were multiplied by two to account for *X* nondisjunction progeny that do not survive to adulthood (*XXX* and *YO*).

mei-41; Blm *double mutant morphology analysis*

mei-41; Blm double mutant females reach adulthood at less than expected Mendelian ratios relative to sibling classes, scored over a five day period. Double mutants and wild-type virgins were mated to isogenized *w*^{1118} males for two days in vials containing yeast paste, followed by ovary dissection in PBS buffer. Female morphology and ovary defects were photographed using the EOS Rebel T3i (Canon U.S.A., Inc., Long Island, NY) with an MM-SLR Adapter (Martin Microscope Company, Easley, SC).

Data Availability

The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article. *Drosophila* stocks are available upon request.

Results

Post-germarium expression of mei-41 *rescues embryonic lethality and creates a meiotic recombination null*

Drosophila females homozygous for null mutations in *mei-41* produce no viable progeny due to a requirement for maternally-deposited Mei-41 at the midblastula transition, resulting in cleavage-stage arrest (Sibon *et al.* 1999). *Blm* null mutants also exhibit maternal-effect embryonic lethality (McVey *et al.* 2007). To study meiotic recombination in *Blm* null mutants, Kohl *et al.* (Kohl *et al.* 2012) expressed wild-type Blm under indirect control of the alpha tubulin 67C (matα) promoter via the Gal4-*UASp* system. This promoter does not express until the early vitellarium (Sanghavi *et al.* 2013), by which time recombination should be compete. In support of this conclusion, crossover assays on surviving progeny of females with null mutations in *Blm* give similar results to those from embryos rescued by expressing

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UASp::Blm with the *matα4::GAL4-VP16* driver (McVey *et al.* 2007; Kohl *et al.* 2012; Hatkevich *et al.* 2017).

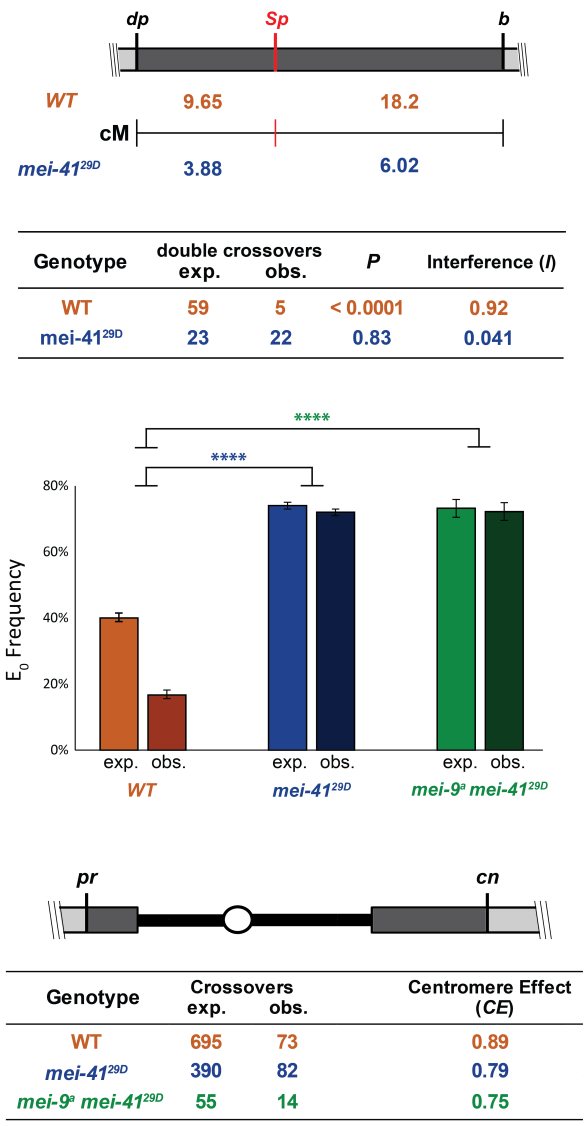
We used the same system to overcome the maternal-effect lethality in embryos from *mei-41*^{29D} homozygous null females (see Materials and Methods). To quantify the extent of maternal *P{UASp::mei-41}* rescue, we compared hatch rates of embryos from wild-type, *mei-41*^{29D}, and *P{UASp::mei-41} mei-41*^{29D} with and without *P{matα4::GAL4-VP16}* (Table 1). Embryos from females homozygous for *mei-41*^{29D} with or without *P}UASp::mei-41}* but lacking *P{matα4::GAL4-VP16}* did not survive to hatching, whereas embryos from females with both components of the Gal4-*UASp* rescue system had a hatch rate of 52.8%. It is possible that the rescue is complete and that the remaining embryonic lethality is due to aneuploidy resulting from high nondisjunction in *mei-41* mutants. Larvae that did hatch survived to adulthood, allowing for analysis of the crossover patterning landscape in a *mei-41* null mutant. For simplicity, flies carrying this transgene system are denoted below as *mei-41*^{29D} or *mei-41* null mutants.

Crossover reduction in mei-41 *null mutants*

Drosophila mei-41 was initially characterized as a meiotic mutant by Baker and Carpenter in 1972 (Baker and Carpenter 1972). Hypomorphic *mei-41* alleles resulted in an overall 46% decrease relative to wild-type controls, measured across adjacent intervals spanning the entirety of *2L* and proximal *2R*, about 20% of the euchromatic genome. Progeny from *mei-41*^{29D} mothers had a significantly more severe phenotype, with a 67% reduction in crossovers summed across this region (Figure 1A). Given the many functions of Mei-41 in mitotically proliferating cells, we wanted to determine whether the remaining crossovers were meiotic or occurred within the pre-meiotic germline. Mei-P22 is the binding partner of Mei-W68, the Drosophila Spo11 ortholog, and is required to generate meiotic DSBs (Liu *et al.* 2002; Robert *et al.* 2016). In the absence of Mei-P22, resulting crossovers must be mitotic in origin and occur prior to meiotic recombination. *Drosophila* males hemizygous for *mei-41*^{29D} do not display mitotic recombination in the pre-meiotic germline (LaRocque *et al.* 2007). Likewise, meiotic crossovers were completely abolished in *mei-41*^{29D}; *mei-P22*^{103} double mutants (n = 1754). One vial had two female progeny that appeared to be either a double crossover in the adjacent *b – pr* and *pr – cn* regions, gene conversion of the pr mutation, or reversion of this mutation (an insertion of a *412* transposable element). Since these were in the same vial they likely represent a single pre-meiotic event. We conclude that the crossovers observed in the *mei-41* null mutant females are meiotic in origin.

Crossover interference and crossover assurance are lost in mei-41 *null mutants*

Meiotic crossover control includes a phenomenon known as interference, which is a decreased likelihood of having two crossovers close to each other within the same chromosome arm (Sturtevant 1913; Berchowitz and Copenhaver 2010). While the strength of crossover interference differs between organisms, complete interference in *Drosophila* extends out to about 10 cM (Weinstein 1958). Baker and Carpenter (Baker and Carpenter 1972) reported that interference is reduced in *mei-41* hypomorphic mutants. We determined the extent of this reduction in null mutants by analyzing two adjacent intervals on *2L* (Figure 2A). Single and double crossovers were scored and interference (I) was calculated using the method of Stevens (Stevens 1936). In Stevens' definition, I = 1 indicates complete positive inter-



Genotype	double crossovers exp.	double crossovers obs.	*P*	Interference (*I*)
WT	59	5	< 0.0001	0.92
mei-41^{29D}	23	22	0.83	0.041

Genotype	Crossovers exp.	Crossovers obs.	Centromere Effect (*CE*)
WT	695	73	0.89
mei-41^{29D}	390	82	0.79
mei-9^{a} mei-41^{29D}	55	14	0.75

Figure 1 Crossover reduction in *mei-41* null mutants. (A) Crossover distribution on *2L* in *mei-41*^{29D} mutants compared to wild-type. Marker location indicated at top based on genome assembly position (Mb) and excludes the centromere and unassembled peri-centromeric satellite sequences (black circle). Crossover density (solid line) scored between each marker for wild-type flies (n = 4222) and *mei-41* mutants (n = 7801). Mean density (dashed line) shows overall crossover reduction across all intervals. Statistical significance determined via chi-square tests on crossovers observed versus crossovers expected if crossovers are a reflection of mean density (ns, $p > 0.05$; **, $p < 0.01$; ****, $p < 0.0001$ after Bonferroni correction; † $p < 0.0001$ after G test of goodness of fit of overall *mei-41*^{29D} crossover distribution compared to wild-type). Wild-type data are from Hatkevich et al. (Hatkevich *et al.* 2017) (used with permission). (B) Genetic length (cM) for *2L* in wild-type and *mei-41*^{29D}. Marker location indicated at bottom based on genetic length. Numbers represent cM for each interval. For the full dataset, refer to Supplemental Table 1.

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Table 1 Hatch rates for embryos from *mei-41* mutants.

Maternal Genotype	Hatched (%)	Total (n)
wild type	73.1 [a]	2035
mei-41^{29D}	0	527
P{UASp::mei-41} mei-41^{29D}	0	837
P{UASp::mei-41} mei-41^{29D}*; P{matα4::GAL4VP16} / +*	52.8 [b]	1187

[a] This number is lower than expected for wild type. The cause of this is unknown.
[b] The apparent lack of complete rescue may largely be the result of a high frequency of aneuploidy resulting from the absence of mei-41 during meiotic recombination.

ference and $I = 0$ indicates no interference. Among progeny of wild-type females ($n = 3325$), there are significantly fewer double crossovers observed (5) than expected (59; $p < 0.0001$), demonstrating strong interference between these intervals ($I = 0.915$; Figure 2A). Compared to wild-type, *mei-41*^{29D} mutants ($n = 9740$) show a significant reduction in interference ($p < 0.0001$; Figure 2A), with no significant difference between expected (23) and observed (22) ($p = 0.83$; $I = 0.041$; Figure 2A). Based on this, we conclude that interference is completely lost in *mei-41* null mutants.

Meiotic crossover reduction in *Drosophila* leads to an increase in nondisjunction events, often due to a failure to form at least one crossover (known as the obligate chiasma) between homologs (Hawley 1988; Koehler *et al.* 1996). These observations imply the existence of a mechanistic phenomenon known as crossover assurance, where bivalents must achieve a minimum number of crossovers to create the meiotic spindle tension required for stable homolog orientation at metaphase I (McKim *et al.* 1993). *X* chromosome nondisjunction occurs at a frequency of less than 0.1% in wild-type *Drosophila*, and original hypomorphic alleles of *mei-41* cause a significant increase, to 9-10% (Baker and Carpenter 1972). We crossed *mei-41*^{29D} females to males carrying a dominant *Y*-linked B^S mutation and scored progeny. NDJ was significantly increased relative to the rate in hypomorphic mutants (13.6%; p = 0.0018) (Supplementary Table 3).

Based on the loss of interference, the severe reduction in crossovers per meiosis, and the significant increase in *X* nondisjunction seen in *mei-41* null mutants, we hypothesized that crossover assurance would be severely reduced, if not completely lost. If the reduction in crossovers on *2L* is representative of the entire genome, then *mei-41* null mutants average less than two crossovers per meiosis. It is therefore not possible to have full assurance, which would require a minimum of three (one per major chromosome) or five (one per major chromosome arm) crossovers. Nonetheless, assurance could manifest as the two crossovers being on different chromosomes more often than expected by chance. This would predict a decrease in double crossovers; since we observed no such decrease (Figure 2A) it suggests that crossover assurance is indeed lost.

We also assessed assurance by comparing the observed and expected frequency of meioses in which there were no crossovers (E_0) on *2L*. For *mei-41*^{29D} mutants the expected E_0 frequency (0.740, based on Poisson distribution) is similar to the observed E_0 frequency (0.720, based on the equations of Weinstein (Weinstein 1936)). This differs significantly compared to wild-type females ($p < 0.0001$) (Figure 2B). Together, the significant increase in *X* chromosome nondisjunction, the loss of interference,

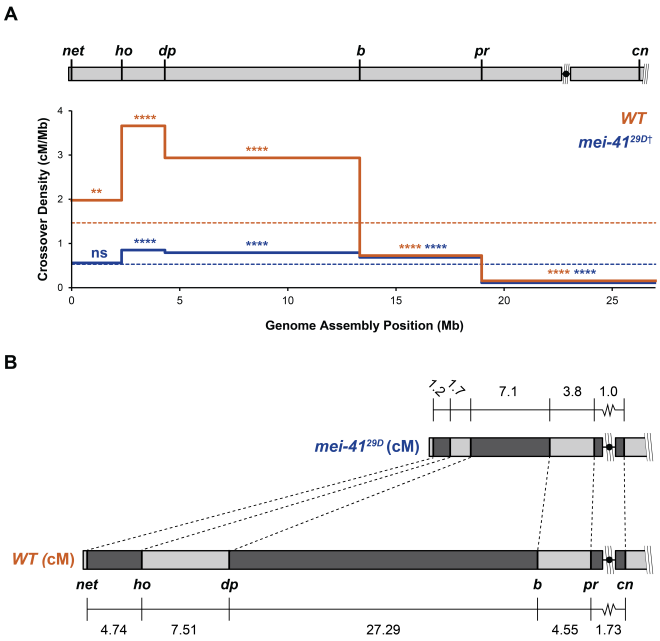

Figure 2 Distal meiotic crossovers are reduced in *mei-41 null mutants.* (A) Crossover distribution on *2L* in *mei-41*^{29D} mutants compared to wild-type. Marker location indicated at top based on genome assembly position (Mb) and excludes the centromere and unassembled peri-centromeric satellite sequences (black circle). Crossover density (solid line) scored between each marker for wild-type flies ($n = 4222$) and *mei-41* null mutants ($n = 7801$). Mean density (dashed line) shows overall crossover reduction across all intervals. Statistical significance determined via chi-square tests on crossovers observed versus crossovers expected if crossovers are a reflection of mean density (ns, $p > 0.05$; **, $p < 0.01$; ****, $p < 0.0001$ after Bonferroni correction; † $p < 0.0001$ after G test of goodness of fit of overall *mei-41*^{29D} crossover distribution compared to wild-type). Wild-type data are from Hatkevich *et al.* (Hatkevich *et al.* 2017) (used with permission). (B) Genetic length (cM) for *2L* in wild-type and *mei-41*^{29D}. Marker location indicated at bottom based on genetic length. Numbers represent cM for each interval. For the full dataset, refer to Supplemental Table 1.

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and the E_0 frequency indicate that crossover assurance is lost in *mei-41*^{29D} mutants.

The centromere effect is retained in mei-41 *mutants*

A striking observation from both *mei-41*^{1} and *mei-41*^{29D} mutants is that despite significant reduction in medial and distal crossovers, a proportional reduction in the number of crossovers within the proximal regions is not seen. As in hypomorphic mutants, crossover reduction in *mei-41* null mutants was more severe within the three distal intervals (>70% reduction in each) compared to the two proximal intervals (15% for b to pr and 29% for pr to cn, which spans the pericentric heterochromatin) (Figure 1B). This suggests that the centromere effect on recombination remains intact despite severe reduction or complete loss of crossover interference and assurance. To evaluate this hypothesis in *mei-41* null mutants, we calculated *CE*, a measure of the centromere effect that is similar to *I* (Hatkevich *et al.* 2017), in the centromere-spanning *pr* - *cn* interval. As in wild-type females (*CE* = 0.89; (Hatkevich *et al.* 2017), in *mei-41*^{29D} mutants there was a significant difference between expected and observed crossovers in this region ($p < 0.0001$), yielding a *CE* value of 0.79 (Figure 2C).

Crossovers in mei-41 *mutants are not dependent upon the Class I meiotic resolvase Mei 9*

The change in crossover distribution seen in *mei-41* null mutants differs from patterning seen in Blm mutants, which encodes a helicase required for proper meiotic patterning and recombination through the Class I pathway (Hatkevich *et al.* 2017). Loss of Blm results in loss of interference, assurance, and the centromere effect. These crossovers are generated in the Class II pathway as they do not use the Class I resolvase Mei 9, suggesting that Blm helicase is required for shuttling meiotic DSBs into the Class I pathway early in the repair pathway, and that there is no patterning in the Class II pathway.

As *mei-41*^{29D} null mutants lose crossover assurance and interference but retain the centromere effect, we hypothesized that loss of Mei-41 shifts meiotic recombination into the Class II pathway later than loss of Blm, after establishment of the centromere effect. To determine whether crossovers generated in *mei-41* null mutants rely upon the Class I resolvase, we generated *mei-9*^{a} *mei-41*^{29D} double mutants and analyzed crossover patterning on *2L* as described above. Similar to *mei-41*^{29D} single mutants, double mutants displayed a 66% reduction in crossovers and exhibited a similar same distribution. (Figure 3A & 3B). Consistent with this, nondisjunction frequency in the *mei-9 mei-41* double mutant (15.9%, $p = 0.3424$) was not significantly different to that of the *mei-41* single mutant (Supplementary Table 2) and crossover assurance was lost ($p = 0.488$; Figure 22A). Most importantly, the centromere effect in double mutants (*CE* = 0.75) was similar to that of *mei-41* single mutants, with a significant difference between expected and observed crossovers ($p < 0.0001$; Figure 2C). We conclude that crossovers generated in *mei-41* mutants do not require the Class I Mei-9 resolvase, suggesting that loss of Mei-41 shifts meiotic recombination into the Class II pathway after or separate from the establishment of the centromere effect.

mei-41; Blm *double mutants display partial synthetic lethality and low brood size*

To further tease apart the establishment of crossover control mechanisms in the context of the two-pathway paradigm, we

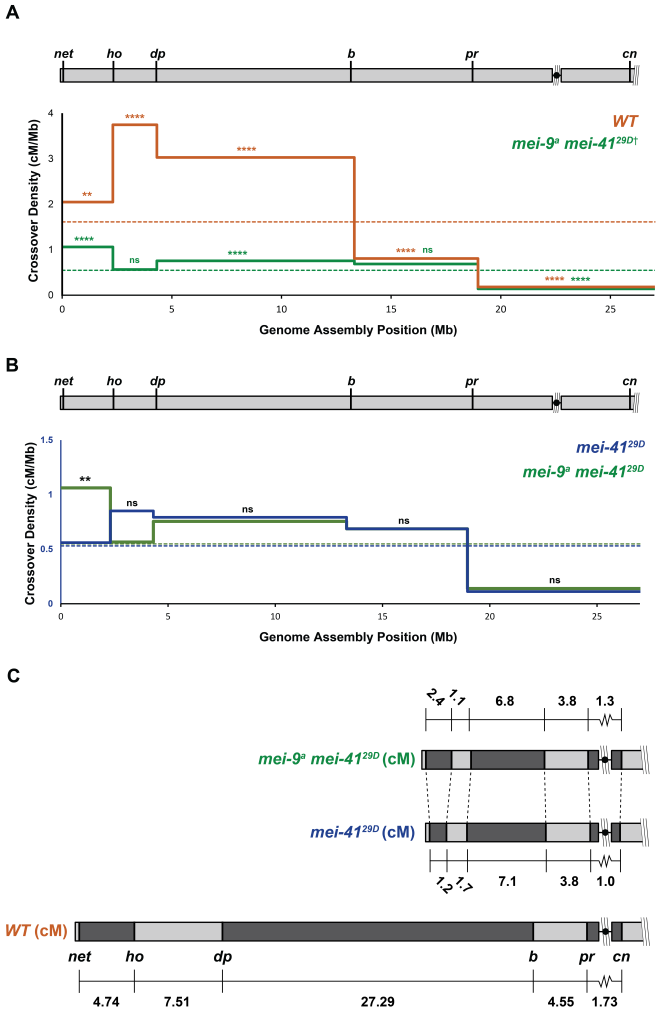

Figure 3 Crossover patterning in *mei-9*^{a} *mei-41*^{29D} double mutant similar to *mei-41*^{29D} single mutant. (A) Crossover distribution on *2L* in *mei-9*^{a} *mei-41*^{29D} compared to previous wild-type data (Hatkevich *et al.* 2017), used with permission). For schematic explanation and statistical calculations refer to Figure 1A (*mei-9*^{a} *mei-41*^{29D} $n = 1059$). (B) Comparison of crossover distribution between single and double mutant. Statistical significance determined for each interval via chi-square tests between expected crossovers (*mei-41*^{29D}) and observed crossovers (*mei-9*^{a} *mei-41*^{29D}) (ns, $p > 0.05$; ** $p < 0.01$). Only the most distal region (*net* to *ho*) is significantly different between single and double mutants, but it is unclear whether this is biologically meaningful or merely a consequence of low sample size or genetic background effects. (C) Genetic length (cM) comparison for *2L* in *mei-41*^{29D} versus *mei-9*^{a} *mei-41*^{29D} double mutants. Wild-type genetic length included below for marker reference. Numbers represent cM for each interval. For the full dataset, refer to Supplemental Table 1.

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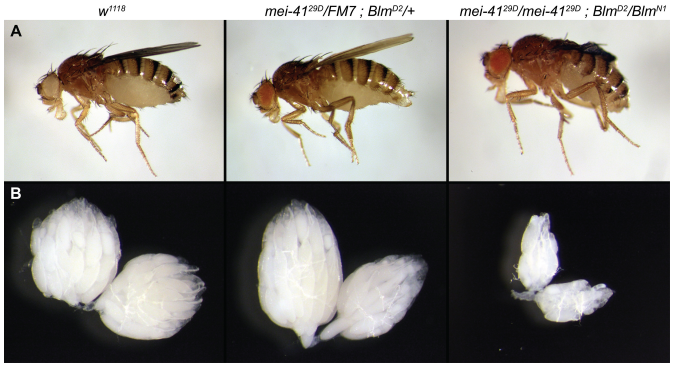

Figure 4 *mei-41^{29D}*; *Blm* double mutants display morphological abnormalities and underdeveloped ovaries at six days post-eclosion. (A) Morphological differences between control, heterozygous, and homozygous female adults (genotypes listed at top). Of adult who survived to six days post-eclosion, control and heterozygous females display wild-type morphology, while double mutant females show abnormal tergites. (B) Ovaries from control and heterozygous appear wild-type, while ovaries from double mutant females are severely undeveloped, resulting in low brood size.

hypothesized that Blm acts earlier in the recombination pathway than Mei-41, since there is a complete loss of crossover patterning in *Blm* mutants compared to loss of only interference and assurance in *mei-41* mutants. Therefore, *mei-41*; *Blm* double mutants should have a phenotype like that of *Blm* single mutants with respect to crossover patterning. However, while both *mei-41* and *Blm* single mutants are fully viable, we recovered fewer *mei-41*; *Blm* double mutant females than expected, suggesting partial synthetic lethality. Of the few obtained, females exhibited underdeveloped abdomens and abnormal tergites (Figure 4A). Most lacked midgut and hindgut structures, displayed ovary epithelial sheaths that contained under-developed ovaries or lacked ovaries entirely, and died within 1-2 days of eclosion. Survivors retained underdeveloped ovaries at six days post-eclosion (Figure 4B), corresponding to a severe reduction in brood size and makinge crossover and nondisjunction analyses impractical. These synthetic phenotypes (reduced viability and developmental defects) are likely the result of a combination of mitotic defects seen in *mei-41* and *Blm* single mutants, which include elevated spontaneous apoptosis (LaRocque *et al.* 2007; Trowbridge *et al.* 2007).

Discussion

We have demonstrated that the Gal4-*UASp* rescue successfully overcomes maternal-effect embryonic lethality of *mei-41* mutants, allowing us to perform meiotic crossover patterning analysis in *mei-41* null mutants. The crossover reduction in null mutants is more severe than that of the previously reported for hypomophic mutant (Baker and Carpenter 1972). Importantly, we found that crossover interference and assurance are abolished when Mei-41 is absent, yet the centromere effect remains largely intact. Removing the presumptive Class I meiotic resolvase (Mei-9) in a *mei-41* null background resulted in phenotypes similar to those of the *mei-41* single mutant, suggesting that meiosis in *mei-41* null mutants relies on alternative endonucleases to resolve recombination intermediates into crossovers. These crossovers might therefore be defined as being made through the Class II pathway (Kohl and Sekelsky 2013).

Crossovers also appear to be made through the Class II pathway in Drosophila Blm mutants (Hatkevich *et al.* 2017). In these mutants, crossover distribution is more-or-less random along and between chromosomes, perhaps reflecting DSB distribution. Notably, interference, assurance, and the centromere effect are all severely decreased or lost entirely. In contrast, in *mei-41* mutants we find that interference and assurance are lost but a strong centromere effect is retained. If crossover interference and crossover assurance result from a single patterning process (Wang *et al.* 2015), it is not surprising to see loss of both. Retention of a centromere effect, however, suggests a mechanism that is separate from these other patterning phenomena.

The molecular function of Mei-41 that impacts crossover patterning is unknown. One parsimonious interpretation of our results is that the centromere effect is established prior to crossover interference and assurance Figure 5). Blm is required before any of these processes occurs, so loss of Blm results in loss of all three. Mei-41, however, is required later, after the centromere effects has been established but before interference and assurance are achieved. In mice, ATR localizes to unsynapsed chromosome axes (Keegan *et al.* 1996). Immunolocalization of Mei-41 has not been reported, but in Drosophila synapsis is not dependent on DSBs (McKim *et al.* 1998), and both synapsis and DSB formation appear to be normal in mei-41 mutants (Carpenter 1979; Joyce *et al.* 2011). The budding yeast ortholog of ATR, Mec1, is required to ensure inter-homolog bias during meiotic recombination (Grushcow *et al.* 1999). Use of the sister chromatid could disrupt the Class I pathway and reduce the number of interhomolog crossovers, resulting in part or all of the observed decrease. In mitotic DSB repair, mei-41 mutants have no observable defects in the early steps of SDSA, such as resection, strand invasion, and synthesis, but required for subsequent annealing and ligation (LaRocque *et al.* 2007). The molecular function of Mei-41 in mitotic DSB repair has not been elucidated, but it has been hypothesized that Mei-41 activates Marcal1, which then catalyzes annealing of complementary sequences (Holsclaw and Sekelsky 2017). It is possible that Mei-41 activates a protein that catalyzes 2nd-end capture during meiosis, and that loss of this activity prevents recombination from proceeding through the Class I crossover pathway. Assays for sister chromatid exchange and analyses of heteroduplex DNA may shed additional light on the mechanistic role(s) of Mei-41 during meiosis.

Perhaps the most interesting outcome of this work is that the centromere effect is established separately from crossover interference and crossover assurance. The beam-film model postulates that the establishment of crossover interference relies on relief of axial mechanical stress outwards from sites of crossover designation, with crossover homeostasis buffering this spreading inhibitory region based on crossover precursor density (Kleckner *et al.* 2004; Zhang *et al.* 2014). Crossover assurance is therefore observable as the passive byproduct of proper crossover-designation resolution, suggesting that interference, assurance, and homeostasis result from a single patterning process (Zhang *et al.* 2014; Wang *et al.* 2015). We suggest that the centromere effect is achieved through an independent process. Whole-genome sequencing reveals that noncrossover gene events are as common in proximal regions as in other regions, suggesting that DSBs are made throughout the euchromatin (Comeron *et al.* 2012; Miller *et al.* 2016). This implies that the centromere effect is achieved by directing repair of proximal DSBs preferentially into noncrossover pathways or preventing them from entering the crossover pathway. Analysis of recom-

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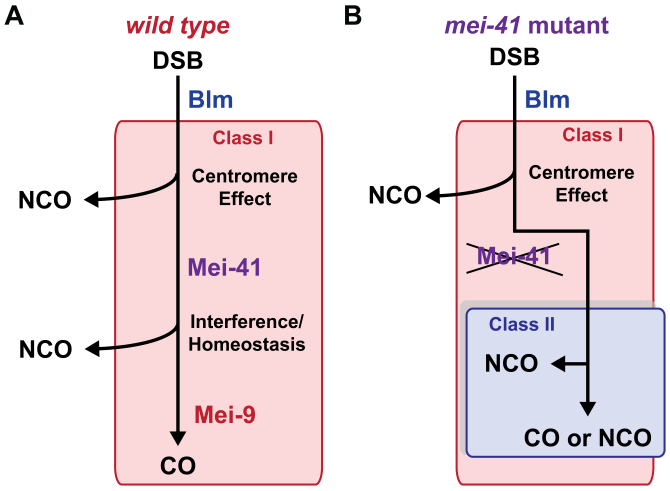

Figure 5 Model for Mei-41 function relative to crossover patterning. (A) Crossovers (CO) in wild-type flies are produced by the Class I pathway (red box). Blm helicase activity is required for entry into the Class I pathway, and therefore for all crossover patterning. The centromere effect is established prior to Mei-41 function, resulting in most proximal DSBs being repaired as noncrossovers (NCO). Mei 41 functions prior to establishment of crossover interference and homeostasis. (B) In the absence of Mei-41, DSBs enter the Class I pathway and the centromere effects is enforced; however, the absence of Mei-41 blocks further progression in this pathway. Instead, repair is completed through the Class II pathway. This may involve formation of noncrossovers through SDSA or dHJ dissolution, or it may involve unbiased resolution of dHJs by an unknown mitotic resolvase to produce either crossover or noncrossover products. The crossovers that are produced are not patterned, except for the centromere effect.

bination in triploid females suggests that the centromere effect might be sensitive to the number of centromeres (Redfield 1932; Sturtevant 1951; Hartmann and Sekelsky 2017), but there is really nothing else known about mechanism. Additional experiments to understand the function of Mei-41 in meiotic recombination may also provide insights into the mechanism of the centromere effect.

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Supplemental Table S1. Progeny counts from crossover distribution experiments.

	Progeny		Maternal Genotype		
			WT	*mei-41*^{29D}	*mei-9*^{a} *mei-41*^{29D}
Parental	+	+ + + + + +	2376	3698	496
	net-cn	*net ho dp b pr cn*		2969	408
SCO	1a (*net*$^{+}$-*ho*)	+ *ho dp b pr cn*	176	16	7
	1b (*net-ho*$^{+}$)	*net* + + + + +		71	15
	2a (*ho*$^{+}$-*dp*)	+ + *dp b pr cn*	290	45	1
	2b (*ho-dp*$^{+}$)	*net ho* + + + +		74	4
	3a (*dp*$^{+}$-*b*)	+ + + *b pr cn*	1099	274	25
	3b (*dp-b*$^{+}$)	*net ho dp* + + +		256	44
	4a (*b*$^{+}$-*pr*)	+ + + + *pr cn*	154	117	14
	4b (*b-pr*$^{+}$)	*net ho dp b* + +		169	25
	5a (*pr*$^{+}$-*cn*)	+ + + + + *cn*	39	44	5
	5b (*pr-cn*$^{+}$)	*net ho dp b pr* +		27	6
DCO	1a-2b	+ *ho* + + + +	1	1	1
	1b-2a	*net* + *dp b pr cn*		2	2
	1a-3b	+ *ho dp* + + +	11	0	0
	1b-3a	*net* + + *b pr cn*		6	0
	1a-4b	+ *ho dp b* + +	10	0	0
	1b-4a	*net* + + + *pr cn*		4	0
	1a-5b	+ *ho dp b pr* +	2	0	0
	1b-5a	*net* + + + + *cn*		1	0
	2a-3b	+ + *dp* + + +	6	1	0
	2b-3a	*net ho* + *b pr cn*		6	2
	2a-4b	+ + *dp b* + +	7	1	0
	2b-4a	*net ho* + + *pr cn*		1	1
	2a-5b	+ + *dp b pr* +	13	0	0
	2b-5a	*net ho* + + + *cn*		2	0
	3a-4b	+ + + *b* + +	19	7	0
	3b-4a	*net ho dp* + *pr cn*		1	0
	3a-5b	+ + + *b pr* +	17	3	0
	3b-5a	*net ho dp* + + *cn*		3	1
	4a-5b	+ + + + *pr* +	2	2	1
	4b-5a	*net ho dp b* + *cn*		0	0
TCO	1b-2a-4b	*net* + *dp b* + +	0	0	1
n			4222	7801	1059

SCO = single crossover; DCO = double crossover; TCO = triple crossover

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Supplementary Table S2. Progeny counts from interference experiment

Progeny Class	Progeny Genotype	Maternal Genotype	
		wild type	*mei-41*^{29D}
Parental	+ + +	1510	5200
	dp Sp b	1897	3686
Single Crossover	+ *Sp b*	172	195
	dp + +	144	164
	+ + *b*	344	272
	dp Sp +	258	297
Double Crossover	+ *Sp* +	4	17
	dp + *b*	1	5
Total *n*		3330	9836

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Supplemental Table 3. X nondisjunction rates for *mei-41* mutants.

Maternal Genotype	Normal Progeny	*XO* NDJ Males	*XXY* NDJ Females	*X* NDJ (%)
mei-41^{1}	815	24	15	8.7
mei-41^{29D}	3791	144	155	13.6
mei-9^{a} *mei-41*^{29D}	499	27	20	15.9

Refer to Materials and Methods for details regarding progeny classes.