ASY1 acts as a dosage-dependent antagonist of telomere-led recombination and mediates crossover interference in Arabidopsis

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During meiosis, interhomolog recombination produces crossovers and noncrossovers to create genetic diversity. Meiotic recombination frequency varies at multiple scales, with high subtelomeric recombination and suppressed centromeric recombination typical in many eukaryotes. During recombination, sister chromatids are tethered as loops to a polymerized chromosome axis, which, in plants, includes the ASY1 HORMA domain protein and REC8-cohesin complexes. Using chromatin immunoprecipitation, we show an ascending telomere-to-centromere gradient of ASY1 enrichment, which correlates strongly with REC8–cohesin ChIP-seq data. We mapped crossovers genome-wide in the absence of ASY1 and observe that telomere-led recombination becomes dominant. Surprisingly, asy1/+ heterozygotes also remodel crossovers toward subtelomeric regions at the expense of the pericentromeres. Telomeric recombination increases in asy1/+ occur in distal regions where ASY1 and REC8 ChIP enrichment are lowest in wild type. In wild type, the majority of crossovers show interference, meaning that they are more widely spaced along the chromosomes than expected by chance. To measure interference, we analyzed double crossover distances, MLH1 foci, and fluorescent pollen tetrads. Interestingly, while crossover interference is normal in asy1/+, it is undetectable in asy1−/− mutants, indicating that ASY1 is required to mediate crossover interference. Together, this is consistent with ASY1 antagonizing telomere-led recombination and promoting spaced crossover formation along the chromosomes via interference. These findings provide insight into the role of the meiotic axis in patterned recombination frequency within plant genomes.

Significance

Meiosis is fundamental to eukaryotic reproduction and shapes patterns of genetic variation. Meiotic recombination is also a vital tool during crop improvement, which allows introgression of wild variation into agriculturalstrains. Despite this, the levels and distributions of crossovers along chromosomes can limit breeding. For example, many crops show highly skewed crossover distributions toward the telomeres. This can lead to the problem of linkage drag when variation within non-recombining regions is selected. Our findings demonstrate how gene dosage of key components of the meiotic chromosome axis can be used to remodel the recombination landscape. Therefore, modifying ASY1 and ASY3 gene dosage in crop species may provide a strategy to change recombination patterns or levels in order to accelerate strain improvement.

Meiosis | crossover | axis | ASY1 | interference

Meiosis is a specialized cell division that increases genetic diversity in populations (1, 2). Meiosis halves the chromosome number to produce haploid gametes via a single round of DNA replication and two rounds of chromosome segregation (1, 3). During prophase I, meiotic homologous chromosomes undergo DNA double-strand breaks (DSBs) that can be repaired using an interhomolog pathway, which may result in crossovers or non–crossovers (1, 3). In plants, meiotic DSBs are formed via a topoisomerase-VI–like complex containing SPO11-1, SPO11-2, and MTOVPVB (4). Meiotic DSBs are resected to form 3′-overhanging single-stranded DNA (ssDNA), which is bound by the RecA homologs RAD51 and DMCI that promote strand invasion of a homolog (1, 3). A set of pro-crossover factors, termed the ZMM pathway, act to protect interhomolog strand invasion events from antirecombination pathways (3). Class I crossover events triggered via the ZMM pathway are more widely spaced along the chromosomes than expected by chance, which is known as interference (5). A minority of crossovers are generated by the Class II repair pathways in wild type, which do not show interference (3).

Homologous chromosomes associate with a specialized axis structure during meiosis, which is conserved across eukaryotes and is required for efficient and accurate interhomolog recombination (6). Following S-phase, replicated sister chromatids are associated via cohesin complexes containing the meiosis-specific kleisin REC8 (7, 8). Immunostaining of REC8 during prophase I reveals a linear axis, to which the chromatin is attached (6). In addition to REC8–cohesin, major components of the plant meiotic chromosome axis include the HORMA domain protein ASY1 and the coiled-coil proteins ASY3 and ASY4 (9–12). In this configuration, coalesged chromatin loops project laterally from the axis, resembling mitotic lampbrush configurations, although with a juxtaposed homolog (6). The tethered-loop axis model proposes that meiotic DSBs are generated on the chromatin loops that become tethered to the axis during interhomolog repair (6, 13). Axis-localized HORMA domain proteins are required during meiosis to promote homolog pairing, DSB repair, and synaptonemal complex (SC) assembly (14–19). However, there are also important differences in the function of meiotic HORMA proteins between species. For example, mouse HORMAD1, budding yeast Hop1, and Caenorhabditis elegans HORMAD1–3, but not Arabidopsis ASY1, are required for meiotic DSB formation (9, 15, 20–22). In late prophase I, the axis is remodeled, which is associated with depletion of HORMA proteins and loading of transverse filament SC proteins, including ZYP1a and ZYP1b (18, 23).
Genome-wide analyses have revealed that meiotic DSB and crossover frequency are highly variable between the telomeres and centromeres of plant chromosomes (24–29). For example, the centromeres and surrounding repetitive sequences (peri- centromeric heterochromatin) are frequently suppressed for meiotic recombination (24–29). High meiotic crossover levels are typically observed in distal subtelomeric regions, which also tend to have higher gene density (24–29). However, the factors and mechanisms that shape the meiotic recombination landscape along chromosomes remain incompletely understood. To investigate the role of the axis during meiosis, we mapped ASY1 enrichment throughout the Arabidopsis genome using chromatin immunoprecipitation sequencing (ChIP-seq). We observe an ascending ASY1 gradient from the telomere to the centromere, which correlates positively with REC8 ChIP-seq enrichment data (30). We mapped crossovers genome-wide in asyl mutants and observe that recombination becomes telomere-led, likely reflecting telomere pairing observed early in prophase I (31). We show that asyl heterozygotes maintain crossover numbers but remodel recombination frequency toward the telomeres at the expense of the pericentromeres. The zone of telomere-led recombination in asyl and asyl/+/corresponds to distal regions of the chromosomes with lowest ASY1 and REC8 ChIP-seq enrichment in wild type. Through analysis of double crossover distances, fluorescent pollen tetrads, and MLH1 foci, we show that crossover interference is normal in asyl+/heterozygotes, but is undetectable in asyl homozygotes. Together, our data show that ASY1 exerts a major effect on the crossover landscape via mediating interference and acting as a gene dosage-dependent antagonist of telomere-led recombination.

Results

Telomere–Centromere Gradients of ASY1 and REC8 ChIP-Seq Enrichment. To investigate the genome-wide localization of ASY1, we performed ChIP-seq using a polyclonal rabbit α-ASY1 antibody raised against ful-length recombinant protein (12). Immunostaining of anther spreads using the -ASY1 antibody on meiotic- and stage floral buds and obtained two independent biological replicate libraries with 26,488,565 and 39,593,737 mapping read pairs (17.5× and 28.2× genome coverage, respectively; SI Appendix, Table S1). The ChIP-seq replicates are highly correlated at the genome scale (r = 0.91 using 10-kb adjacent windows; SI Appendix, Table S2). To determine the specificity of ASY1 ChIP-seq enrichment, two controls were performed. First, the α-ASY1 antibody was used for ChIP-seq from leaf tissue, where ASY1 is not expressed (12). Second, preimmune serum was used for ChIP-seq from floral tissue. After deduplication, only 0.29% and 0.39% of reads in these libraries mapped to the Arabidopsis genome (SI Appendix, Table S1). In contrast, 90.1% and 93.2% of deduplicated ASY1 ChIP-seq reads were mapped (SI Appendix, Table S1). This demonstrates the low background of reads that map to the Arabidopsis genome obtained from our ChIP protocol, in the absence of the epitope or the α-ASY1 antibody. For further analysis, ASY1 ChIP-seq libraries were normalized using an input chromatin library to generate log2(ChIP/input) enrichment values across the genome (Fig. 1C).

At the genome scale, we observed highest ASY1 ChIP-seq enrichment over the centromeric and pericentromeric regions (Fig. 1C and SI Appendix, Fig. S1). An ascending gradient of ASY1 ChIP-seq enrichment was observed from telomeres to centromeres, with the sharpest increase observed as the centromeres are approached (Fig. 1D). We observed a striking positive correlation between ASY1 and REC8-HA ChIP-seq enrichment (e.g., r = 0.88–0.93 at 10-kb scale; Fig. 1C and SI Appendix, Fig. S1 and Table S2) (30), which is consistent with their highly correlated immunostaining patterns (Fig. 1B). We compared ASY1 ChIP-seq enrichment to DSBs using SPO11-1-oligos as a marker (Fig. 1C) (25). At the chromosome scale, the regions in proximity to the centromere where ASY1 is highest have the lowest DSBs (Fig. 1C). However, when considering the chromosome arms alone, ASY1 and SPO11-1-oligos show a weak positive correlation (r = 0.48 at 10-kb scale; Fig. 1C and D and SI Appendix, Fig. S1). At the fine scale, SPO11-1-oligos are highest within nucleosome-depleted gene promoters and terminators (Fig. 1E) (25). In contrast, ASY1 and REC8 are highest within nucleosome-enriched gene bodies (Fig. 1E) (30). Variation in ASY1 enrichment within genes correlates positively with REC8 and nucleosome occupancy (MNase-seq), but does not correlate with SPO11-1-oligos in gene promoters or terminators (Fig. 1F). Equally, variation between genes in promoter SPO11-1-oligo levels does not correlate with ASY1 or REC8 ChIP-seq enrichment within gene bodies (Fig. 1F).

Telomere-Led Recombination Dominates in asyl Mutants. As we observed a gradient of ASY1 ChIP-seq enrichment between the telomeres and centromeres, we sought to investigate a stronger patterning along chromosomes in asyl mutants. Homozygous asyl mutants have low fertility due to reduced chiasma and a high incidence of univalent chromosomes at metaphase I, which leads to aneuploid gametes (9, 15). Despite this, low numbers of viable progeny can be obtained from asyl homozygotes. Therefore, we crossed asyl+/individuals in Col [asy1-1-4 (15)] and Ws-4 [hereafter Ws; asyl-1-3/+ (32)] backgrounds to generate wild type or asyl ColWs F1 plants. The F1 plants were self-fertilized, and 187 wild type and 169 asyl F2 progeny were generated and used for DNA sequencing. The TIGER pipeline was used to identify crossover locations from the sequencing data (SI Appendix, Table S3) (33).

As expected, a significant decrease in crossovers per F2 was observed in asyl (mean = 4.6) compared to wild type [mean = 7.9; Mann–Whitney–Wilcoxon (MWW) test P = 4.37 × 10−3; Fig. 2A and SI Appendix, Table S3]. However, the number of crossovers observed per asyl F2 individual was higher than predicted from bivalent counts per meiosis in asyl-3 [mean = 1.5 (32)] and asyl-4 [mean = 1.9 (9); Fig. 2A and SI Appendix, Table S3]. This may reflect generation of viable F2 plants selecting for gametes with at least one crossover per chromosome in order to balance segregation at metaphase I. Alternatively, as chiasma measurements are made from male meiosis, whereas F2 crossover data reflect both male and female meiosis, this could indicate sex differences in crossover reduction in asyl. A further possibility is that closely spaced crossovers may be counted as single chiasma in asyl, causing an underestimation of recombination. In wild type, crossover number per chromosome is positively correlated with physical length (r = 0.98, P = 3.4 × 10−15), whereas no significant correlation exists in asyl (Fig. 2B). Exceptionally, chromosome 2 shows a crossover frequency close to wild type in asyl, with a striking increase on the short, nucleolar organizing region (NOR)-bearing arm (Fig. 2 B and D and SI Appendix, Table S3). This is consistent with chiasma and fluorescent in situ hybridization (FISH) analysis in asyl mutants in the Ws accession, where the short arm of chromosome 2 also showed high chiasma frequency (34). Interestingly, in ColWs F1 hybrids, NOR2 rDNA gene clusters are transcriptionally silenced, whereas NOR4 on chromosome 4 are expressed (35). Nucleolar silencing is known to involve formation of heterochromatin at the transcriptionally repressed NOR (36). Hence, heterochromatin formation at NOR2 could contribute to closer alignment of homologs and thereby promote crossover formation on chromosome 2 in asyl.

When recombination was analyzed along scaled telomere–centromere axes, we observed a strong bias of asyl crossover formation toward the subtelomeric regions (Fig. 2 C–E). Analysis
Fig. 1. The landscape of ASY1 ChIP-seq enrichment throughout the Arabidopsis genome. (A) Meiotic cells in early prophase I or adjacent somatic cells immunostained for ASY1 (green) and stained for chromatin (DAPI, blue). (B) Male meiocytes in early prophase I immunostained for ASY1 (green) and REC8-HA (red) and stained for chromatin (DAPI, blue). (Inset) Correlation plot of ASY1 and REC8-HA signal intensity (n = 10 cells). (Scale bars, 10 μM.) (C) ASY1 (blue) and REC8-HA (pink) ChIP-seq enrichment [log2(ChIP/input)] and SPO11-1-oligos [log2(SPO11-1-oligos/gDNA), red] along the Arabidopsis genome in adjacent 10-kb windows, smoothed using a moving average (25, 30). Vertical solid and dotted lines indicate the telomeres and centromeres, respectively. The pericentromere boundaries are indicated by pale blue ticks on the x axis. (D) Data as in C, but analyzing proportionally scaled chromosome arms from telomeres to centromeres. (E) Data as in C, but showing mean coverage profiles for ASY1 (blue), REC8-HA (pink), nucleosomes [log2(MNase-seq/gDNA), green], and SPO11-1-oligos (red) over proportionally scaled windows between gene transcriptional start (TSS) and termination (TTS) sites and 2-kb flanking regions. The same number of randomly positioned windows of the same widths were analyzed as a control. (F) Data as in C, but analyzed as heat maps within genes and 2-kb flanking regions. Genes were ranked by ASY1 levels in gene bodies (TSS–TTS; Upper) or by promoter SPO11-1-oligos (Lower). Shading is equal to defined quantiles of coverage values mapped linearly to a vector of six colors.
of chiasmata in asy1, asy3, and asy4 axis mutants has shown a high incidence of rod bivalent configurations (Fig. 2F) (9, 10, 34), which may reflect distal crossover locations. To investigate recombination in relation to telomere position, we assigned each crossover a distance to its nearest telomere and plotted events on a common axis (Fig. 2G and SI Appendix, Fig. S2). The crossover
counts observed were analyzed in windows relative to the telomere in wild type and asy1 and used to perform χ² tests, with correction for multiple testing (SI Appendix, Table S4). We observed that windows in the first megabase of chromosomes show significantly greater crossovers in asy1 (Fig. 2G and SI Appendix, Figs. S2 and S3 and Table S4), which we term the telomere-lead zone (TLZ). Interestingly, the TLZ corresponds to distal regions that have relatively low ASY1 and REC8 CHIP-seq enrichment in wild type (Fig. 2G). At the fine scale, we observed that asy1 crossovers show a preference to form in nucleosome-depleted, AT-rich regions both with higher-than-average SPO11-1-oligos, which were similar to a preference to form in nucleosome-depleted, AT-rich regions between wild type (mean = 7.54) and asy1-4/+ (mean = 7.92) populations (MWW test, P = 0.059; Fig. 3A and SI Appendix, Table S3). A positive correlation exists between number of crossovers per chromosome and physical chromosome length in both asy1+/+ (r = 0.97 P = 6.17 x 10⁻⁷) and wild type (r = 0.97 P = 4.83 x 10⁻⁷) ColxLer (Fig. 3B). Hence, global crossover numbers are maintained in asy1+/+ heterozygotes, relative to wild type. At the chromosome scale, despite crossover numbers being maintained, we observed that the asy1+/+ recombination landscape was remodelled (Fig. 3 C and D). Specifically, crossovers increased in the distal subtelomeric regions in asy1+/+ compared to wild type at the expense of the pericentromeric regions (Fig. 3 C and D). The centromeres remained crossover-suppressed in both wild type and asy1+/+ populations (Fig. 3 C and D). We repeated analysis of crossover positions relative to the nearest telomere and compared crossover counts between wild type and asy1+/+ using χ² tests (Fig. 3E and SI Appendix, Table S4). This identified the first megabase within the subtelomeric regions as showing significantly elevated crossovers in asy1+/+ compared to wild type (Fig. 3E and SI Appendix, Fig. S3 and Table S4), which overlaps with the TLZ observed in asy1 (Fig. 2G). As noted, the TLZ contains regions of relatively low ASY1 and REC8 CHIP-seq enrichment in wild type, which may explain the sensitivity of crossovers in these regions to reduced ASY1 gene dosage (Fig. 3E). These findings demonstrate that asy1+/+ heterozygotes maintain crossover numbers, but show remodeling of recombination toward distal regions.

Crossovers Are Sensitive to asy1 and asy3 Gene Dosage, but Not REC8. To further investigate changes to crossover frequency associated with meiotic axis gene dosage, we used fluorescent tagged lines (FTLs) (38, 39). FTL intervals are defined by T-DNA insertions that express different colors of fluorescent protein (green, red, or blue) in pollen (LAT52 promoter) or seed (NpaI promoter) (38, 39). When an individual is hemizygous for linked T-DNAs, patterns of fluorescence in pollen or seed can be used to quantify crossover frequency within the intervals flanked by the T-DNAs (40, 41). We crossed the subtelomeric FTL interval 420 on chromosome 3 to asy1+/+, asy3+/+, and rec8+/+, using two independent alleles in each case (Fig. 3F and SI Appendix, Table S5). We observed that all asy1+/+ and asy3+/+ heterozygotes showed significantly increased 420 crossover frequency compared to wild type (t test P value range = 2.39 x 10⁻⁷ to 2.00 x 10⁻⁹; Fig. 3G and SI Appendix, Table S5). In contrast, rec8+/+ heterozygotes showed no significant difference (t test P = 0.26; Fig. 3G and SI Appendix, Table S5). As 420 is located distally, we observed a relatively high genetic distance in asy1 and asy3 (∼10 cM; Fig. 3F and SI Appendix, Table S3) despite these backgrounds having reduced crossovers genome-wide (Fig. 2) (9, 11). It is not possible to measure FTLs in rec8 homozygotes, as they are completely sterile (7, 42, 43).

Due to the remodeling of crossovers along the chromosomes observed in asy1+/+ (Fig. 3C), we also measured recombination using the CEN3 FTL, which spans the DNA methylated centromere and pericentromere of chromosome 3 (Fig. 3F and SI Appendix, Table S6). CEN3 showed a significant decrease in crossover frequency in asy1+/+ and asy3+/+ heterozygotes compared to wild type (t test P = 4.60 x 10⁻⁷ and 2.05 x 10⁻⁶; Fig. 3G and SI Appendix, Table S6). No significant difference in CEN3 crossover frequency was observed between rec8+/+ and wild type (t test P = 0.11; Fig. 3G and SI Appendix, Table S6). In asy1 and asy3 homozygotes, CEN3 crossovers were virtually eliminated compared to wild type (t test P = 5.85 x 10⁻¹¹ and 1.40 x 10⁻⁸; Fig. 3G and SI Appendix, Table S6), consistent with telomere-led recombination dominating in these backgrounds. These experiments demonstrate remodeling of the crossover landscape toward the telomeres via reduced gene dosage of ASY1 and ASY3, but not REC8.

Cytogenetic Analysis of Meiosis in asy1+/+ and asy3+/+ Heterozygotes. We next analyzed meiotic progression of asy1+/+ and asy3+/+ heterozygotes using chromosome spreads of pollen mother cells and DAPI staining of chromatin (Fig. 4A). Chromosomes paired normally at pachytene in the asy1+/+ and asy3+/+ heterozygotes, and heterochromatic regions of dense DAPI staining were visible during prophase I (Fig. 4A). Five bivalents were detected at diakinesis, and no missegregation of chromosomes was observed at anaphase I or meiosis II in asy1+/+ and asy3+/+ (Fig. 4A). Consistently, no significant decrease in asy1+/+ or asy3+/+ seed count or pollen viability was observed (SI Appendix, Tables S7 and S8). To further assess chromatin organization, we immunostained male meiocytes for ASY1 and the heterochromatic histone modification H3K9me2 in wild type, asy1+/+, asy1, asy3+/+, and asy3 (Fig. 4B) (26, 44). H3K9me2 staining on chromosomes was observed in all genotypes, consistent with normal heterochromatin formation (Fig. 4B). ASY1 was undetectable in asy1, and showed an altered punctate staining pattern in asy3, as reported (Fig. 4B) (9).

To cytologically analyze class I crossovers, we immunostained diakinesis-stage male meiocytes using α-MLH1 antibodies and stained chromatin with DAPI (Fig. 4C and SI Appendix, Figs. S5 and S9). We did not observe significant differences in total MLH1 foci between asy1+/+ and wild type (MWW test P = 0.128), but a small yet significant decrease occurred in asy3+/+ (MWW test P = 2.26 x 10⁻⁷; Fig. 4 C and D and SI Appendix, Table S9). We quantified MLH1 foci overlapping pericentromeric heterochromatin, defined by DAPI-dense regions, and observed a significant decrease in asy1+/+ and asy3+/+ compared to wild type (MWW test P = 4.53 x 10⁻⁷ and P = 8.94 x 10⁻⁸; Fig. 4 C and D and SI Appendix, Fig. S5 and Table S9). This is further consistent with distalization of crossovers away from centromere-proximal regions in asy1+/+ and asy3+/+ heterozygotes.

To investigate the effect of asy1+/+ heterozygosity on axis loading of ASY1, we quantified α-ASY1 immunostained signal intensity during early prophase I and observed a 21% reduction in asy1+/+ compared to wild type (MWW test P = 0.019; Fig. 4 D and E and SI Appendix, Table S10). As ASY3 is required for polymerization of ASY1 during meiosis (Fig. 4B) (9), we also quantified α-ASY1 signal intensity in asy3+/+ and observed a 25% reduction compared to wild type (MWW test P = 1.6 x 10⁻³; Fig. 4 D and E and SI Appendix, Table S10). We immunostained pachytene-stage cells for the synaptonemal complex (SC) transverse filament ZYP1 (45). Continuous ZYP1 signal was observed along chromosomes in asy1+/+ and asy3+/+ compared to wild type,
Fig. 3. Distal increases in crossover frequency in asy1/+ and asy3/+ axis heterozygotes. (A) Histograms of crossovers per F2 individual for wild type and asy1/+. Red dashed lines indicate mean values. (B) Crossovers per chromosome per F2 for wild type (blue) and asy1/+ (red) plotted against chromosome length in megabases. (C) Crossover frequency in wild type (blue) and asy1/+ (red) and ASY1 ChIP-seq enrichment [log2(ChIP/input), black] analyzed along proportionally scaled chromosome arms, oriented from telomeres to centromeres. ColxLer SNP density is shown by green shading. (D) Crossover frequency (crossovers/150 kb per F2) plotted along the Arabidopsis genome for wild type (blue) and asy1/+ (red), with ColxLer SNP density (green) shaded. Telomere and centromere positions are indicated by vertical solid and dotted lines, respectively. (E) Crossovers analyzed relative to the closest telomere in wild type (blue) and asy1/+ (red). The lower plot shows ASY1 (green) and REC8-HA (pink) ChIP-seq enrichment [log2(ChIP/input)] analyzed over the same regions. (F) DNA methylation (CG, CHG, CHH) in wild type (Col) is plotted along chromosome 3, and the positions of the 420 and CEN3 FTL intervals are indicated. (G) Crossover frequency (in cM) within the 420 and CEN3 FTL intervals in the indicated genotypes. Black dots represent replicate measurements, and red dots represent mean values. To assess significant differences, t tests were performed (n.s., not significant; ***P < 0.001).
and no significant differences in SC length were observed (MWW tests $P = 0.74$ and $P = 0.27$; Fig. 4 D and E and SI Appendix, Table S11). Hence, although we detect a reduction in ASY1 loading in $asy1^+\text{ and } asy3^+$ heterozygotes, full pairing and synapsis occurs in these backgrounds.

**Crossover Interference Is Maintained in $asy1^+$ but Is Absent in $asy1$.**

We next investigated crossover interference in $asy1^+$ and $asy1$ using analysis of double crossover (DCO) events. As we sequenced F$_2$ individuals, which derive from two independent meioses, in some cases, there is uncertainty about whether an observed DCO occurs in cis or trans (SI Appendix, Fig. S6) (46). Importantly, only cis DCOs, which occurred in the same meiosis, are relevant for measurement of crossover interference (46). In our F$_2$ data, a subset of cis DCOs can be identified from Col–Het–Col, Ler–Het–Ler, or Ws–Het–Ws genotype blocks (SI Appendix, Fig. S6) (46, 47). Therefore, we filtered for DCOs following this genotype pattern, which resulted in 118, 86, 98, and 73 DCOs in the Col x Ler wild type and $asy1^+$ and Col x Ws wild type and $asy1$ populations, respectively (SI Appendix, Table S12). For each population, a matched set of randomly positioned DCOs of the same widths was generated as a control comparison. We analyzed recq4a recq4b crossover data in the same way (37), where the interference-insensitive Class II crossover repair pathway is greatly increased (37, 48). In $asy1$, the majority of the remaining crossovers have been shown to be dependent on the Class I pathway (9, 15).

Consistent with the action of crossover interference, distances between observed DCOs were significantly greater than random
in both wild type ColxLer (MWW P = 5.67 × 10−5) and ColxWs (MWW test P = 3.79 × 10−2; Fig. 5 A and B and SI Appendix, Table S12). The *asy1/+* DCOs were also more widely spaced than random (MWW P = 4.94 × 10−3), which is consistent with normal crossover interference in this background (Fig. 5 A and B and SI Appendix, Table S12). In contrast, the spacing of DCOs in *asy1* was not significantly different from random (MWW P = 0.852; Fig. 5 A and B and SI Appendix, Table S12), showing an absence of detectable crossover interference. Using the same analysis method, DCOs observed in recq4a recq4b were not significantly different from random (P = 0.187), as expected due to greatly elevated noninterfering crossover repair (37, 48).

To independently measure crossover interference in wild type, *asy1/+*, and *asy1*, we used the distally located three-color FTL interval *I3bc*, which overlaps the 420 FTL interval on chromosome 3 (Fig. 5C, Table 1, and SI Appendix, Fig. S7). We measured crossover frequency between the *I3bc* T-DNAs in a qrt1 background, where the four sister haploid cells produced from each male meiosis remain physically attached, allowing tetrad pollen analysis (Table 1) (38). To estimate crossover interference, we calculated *I3b* crossover frequency with and without a crossover in the adjacent *I3c* interval (Table 1). These measurements are used to calculate an interference ratio, where values closer to 0 indicate stronger interference and values close to 1 indicate an absence of interference (38).

In wild type, the more distal interval *I3b* shows higher crossover frequency than *I3c* and an interference ratio of 0.34 (Fig. 5D and Table 1). In *asy1/+*, a significant increase in *I3b* crossover frequency occurred compared to wild type (Perkins *P* = 5.20 × 10−9), whereas *I3c* was not significantly changed (Fig. 5D and Table 1). Consistent with our previous observations, no significant difference was observed in the interference ratio between wild type and *asy1/+* (Perkins *P* = 0.997; Fig. 5D and Table 1). In *asy1*, both genetic intervals showed a significant reduction in crossover frequency compared to wild type (*I3b* Perkins *P* = 3.95 × 10−49 and *I3c* Perkins *P* = 1.06 × 10−41), although the more distal *I3b* interval maintains a higher level of crossovers than *I3c* (Fig. 5D and Table 1). In contrast to *asy1/+*, the *asy1* homozygotes showed an interference ratio of 1.24 that was significantly different than wild type (Perkins *P* = 7.20 × 10−10; Fig. 5D and Table 1), further consistent with an absence of crossover interference.

Finally, to investigate Class I crossovers in wild type and *asy1*, we immunostained MLH1 at diakinesis stage and stained DNA with DAPI (Fig. 5E and SI Appendix, Tables S9 and S13). In wild type (Col), no univalent chromosomes are observed, and, on average, 10.4 MLH1 foci occurred on the bivalents (Fig. 5E and SI Appendix, Tables S9 and S13). In *asy1*, we observed a higher incidence of univalent chromosomes per cell (mean = 5.6) compared to bivalents (mean = 2.6; Fig. 5E and SI Appendix, Table S13). Interestingly, we observed MLH1 foci on both univalents (mean = 5.3) and bivalents (mean = 5) in *asy1* (Fig. 5E and SI Appendix, Table S13). The presence of MLH1 foci on *asy1* bivalents is consistent with crossover formation via the Class I pathway. MLH1 foci have been reported on univalent chromosomes in *dmc1* and in haploid meiosis and may represent sites of interister repair (49). In order to estimate crossover interference, we measured MLH1 interfoci distances on bivalents in wild type and *asy1* (SI Appendix, Table S14). We observed that MLH1 foci were significantly closer in *asy1* compared to wild type (MWW test *P* = 2.19 × 10−2; SI Appendix, Table S14), which is further consistent with a loss of crossover interference. Therefore, our combined analysis of DCO spacing from sequencing data, fluorescent pollen tetrads, and MLH1 foci show that crossover interference is absent in *asy1*.

**Discussion**

Our data inform a model for how ASY1 and the meiotic axis pattern crossover frequency along plant chromosomes (Fig. 6). Previous work has shown that *asy1* mutants undergo normal telomere clustering, formation of meiotic DSB foci during early leptotene, and polymerization of an axial structure marked by REC8 and ASY3 (Fig. 6d) (9, 15, 31). However, DMC1 foci dynamics are altered in *asy1*, resulting in a failure of interhomolog recombination and depletion of crossovers (9, 15). Using high-resolution mapping of crossovers via sequencing F2 plants, we show that recombination becomes largely restricted to a telomere-led zone (TLZ) in *asy1* homozygotes (Fig. 6b). We propose that the proximities of telomeres during early prophase in *asy1* is responsible for telomere-led recombination (Fig. 6d) (31). Telomere-led recombination is active in wild type, but ASY1 antagonizes this activity to promote crossover formation in interstitial and centromere-proximal chromosome regions (Fig. 6b).

Using ChiP-seq, we observe a gradient of ASY1 enrichment from the telomeres to the centromeres, which is paralleled by REC8 cohesin enrichment (30). We propose that differential ASY1 enrichment represents a mechanism to distribute recombination more evenly along the chromosome arms. However, as heterochromatin increases in proximity to the centromere, this causes suppression of meiotic DSBs and crossovers, despite high levels of ASY1 and REC8 (25, 26, 50).

We show that plants heterozygous for *asy1/+* and *asy3/+* mutations undergo remodeling of the crossover landscape, with a shift toward the distal subtelomeres, at the expense of interstitial and pericentromeric regions. Interestingly, the distal regions that undergo crossover increases in *asy1/+* overlap the TLZ observed in *asy1* and have relatively low levels of ASY1 and REC8 ChiP-seq enrichment in wild type.

Using meiotic immunocytochemistry, we quantified a ~21% reduction in *asy1* loading on chromatin in *asy1/+*. This could indicate a threshold effect over which ASY1 antagonizes telomere-led recombination and promotes crossovers in the chromosome arms, toward the centromere. In *asy1/+* heterozygotes, the distal regions would drop below this putative threshold and the strength of telomere-led recombination would increase. As interference remains operational in *asy1/+*, this would lead to a relative loss of crossovers in the interstitial and pericentromeric regions (Fig. 6b).

Alternatively, this may reflect a non-linear effect of decreased ASY1 expression on recombination along the chromosomes. It is notable that genetic variation in axis components, including *ASY1* and *ASY3*, has been strongly associated with adaptation to tetraploidy in *Arabidopsis arenosa*, which may include distalization of crossovers (51, 52).

Our results show that gene dosage of *ASY1* and *ASY3* may contribute to these effects, in addition to the influence from specific variations on the chromosomes.

Crossover interference is mediated by topoisomerase II and the axis protein Red1 in budding yeast (53), while the SC component SYP-1 has been implicated in *Caenorhabditis elegans* (54, 55). Using analysis of double crossover distances, MLH1 foci, and fluorescent pollen tetrads, we show that *asy1* is required for detectable crossover interference in *Arabidopsis*. Interestingly, crossovers in axis mutants are largely dependent on the Class I interfering repair pathway (9, 15). For example, chiasma are eliminated in *asy3 msh4* double mutants, and we show that MLH1 foci form on *asy1* bivalents (9, 15). Therefore, despite the Class I pathway mediating the majority of crossover formation in *asy1*, interference signaling between recombination sites is inactive. Crossover interference has been proposed to occur via mechanical stress acting across paired homologous chromosomes, which is transmitted along the axis and relieved at crossover designated sites (13, 53). In this respect, *ASY1* may mediate crossover interference via transmission of mechanical stress, when chromatins loops connected to the axis undergo cycles of expansion and contraction during early prophase I (13). In the absence of ASY1, the mechanical properties of the axis may be altered, meaning force can no longer be transmitted and crossover interference is not detected. Alternatively, ASY1 may control sensitivity of interhomolog repair sites to the interference signal or mediate transmission of a biochemical signal along the chromosome axis (56).

Our work indicates that axis HORMA domain proteins can play a critical role in mediating crossover interference along chromosomes during meiosis.
Fig. 5. Crossover interference is maintained in asy1/+ but is absent in asy1. (A) Histograms showing the distribution of observed double crossover distances (DCOs, red) in megabases in wild type, recq4a recq4b (37), and asy1/+ Col×Ler F2 individuals or wild type and asy1 Col×Ws F2 individuals. Alongside are identical histograms showing the distribution of matched randomly generated distances (blue). Mann–Whitney–Wilcoxon (MWW) tests were performed to assess significant differences between observed DCOs and random, with $P$ values indicated. (B) Diagrams showing spacing of identified DCOs along the proportional physical length of chromosomes (as percentages). DCOs are connected via arcs and color-coded proportional to the distance between them (red, greatest; blue, smallest). (C) DNA methylation (CG, CHG, CHH) in wild type plotted along chromosome 3 with the positions of the I3bc T-DNAs indicated by vertical lines and colored triangles. (D) Crossover frequency (in cM) within I3b, contingent on crossover in the adjacent interval I3c, in wild type, asy1/+ and asy1. Interference ratios calculated from the I3bc data are plotted for the same genotypes. (E) Representative images of pollen mother cells immunostained for MLH1 (red) at diakinesis stage in wild type and asy1. Chromatin was stained with DAPI (blue). White and orange arrows in the merged images indicate MLH1 foci located on bivalents or univalents, respectively. (Scale bars, 10 μM.)
Table 1. Polen tetrad analysis of crossover frequency and interference within the I3bc FTL intervals in wild type (Col), asy1-4/+, and asy1-4

| Tetrad class | Wild type (Col) | asy1-4/+ | asy1-4 |
|--------------|----------------|---------|--------|
| A – NCO      | 2,338          | 2,092   | 913    |
| B – SCO 13c  | 328            | 317     | 46     |
| C – SCO 13b  | 1,313          | 1,574   | 113    |
| D – SCO 13b and SCO 13c | 12 | 16 | 6    |
| E – SCO 13b and SCO 13c | 8 | 17 | 1    |
| F – SCO 13b and SCO 13c | 13 | 10 | 0    |
| G – SCO 13b and SCO 13c | 14 | 13 | 1    |
| H – DCO 13c  | 3              | 2       | 2      |
| I – DCO 13b  | 5              | 8       | 5      |
| J – DCO 13c and SCO 13b | 0 | 0 | 1    |
| K – SCO 13c and DCO 13b | 0 | 0 | 0    |
| L – DCO 13c and DCO 13b | 0 | 0 | 0    |
| Total        | 4,034          | 4,049   | 1,089  |

Genetic distance

| I3b cm (P value) | 17.23 | 20.72 | 7.02 |
|------------------|-------|-------|------|
| I3c cm (P value) | 4.87  | 4.75  | 3.35 |
| I3b cm without adjacent | 18.37 | 22.07 | 6.94 |
| I3c CO           | 6.22  | 7.74  | 8.26 |
| Interference ratio | 0.34  | 0.34  | 1.24 |
| P value          | 0.997 | 7.20  | 6×10⁻⁶ |

Materials and Methods

Plant Materials. Arabidopsis plants were grown under long day conditions (16 h light/8 h dark) at 20 °C. The following mutant alleles in the Col-0 background were used: rec8-7 (Salk_019119) (9), rec8-3 (SAIL_087_108) (49), asy1-1 (Salk_144182) (15), asy1-4 (Salk_046272) (23), asy1-3 (Salk_143676) (9), and asy1-2 (SAIL_423_H01) (9). The asy1-3 allele is in the Wassilewskija (WS) background (30).

ChIP-Seq Data Analysis. Deduplicated paired-end ASY1, RE8-HA, H3K9me2, H3K4me1, H3K4me2, H3K4me3, H3K27me1, and H3K27me3 ChIP-seq reads, paired-end MNase-seq reads, and single-end SP011-1-oligo, H2A.Z, and H2A.W reads (25, 30, 58, 59) were aligned to the TAIR10 reference genome using Bowtie2 (version 2.2.9) (60) with the following settings: --very-sensitive -p 4 -k 10. For paired end reads, the Bowtie2 options --no-discordant and --no-mixed were also applied. Prior to alignment, single-end SP011-1-oligo reads were processed as described (25). Up to 10 valid alignments were reported for each read or read pair. Aligned reads with more than two mismatches were discarded using the SAM tool with the following command: "samtools view -S -H -R -b -o temp.bam -@ 4 input.bam | samtools sort -o temp.sorted.bam -@ 4 temp.bam | samtools idxattr -f -t temp.sorted.bam | samtools index -@ 4 temp.sorted.bam".

Cytological Analysis of Meiosis. Fixation of Arabidopsis inflorescences and chromosome spreads of pollen mother cells (PMCs) were performed as described (67). Immunostaining of ASY1, ZYP1, and MLH1 were performed on beaker acid chromosome spreads using fixed inflorescences. After chromosome spreading, the slides were incubated in boiling 10-mM Tris-sodium citrate, pH 7.0, for 45 s, followed by incubation in 1× PBS with 0.1% Triton X-100 (PBST) for 5 min. Primary antibodies were diluted in a solution of 1% BSA diluted in PBST that was added onto the slides, followed by incubation for 20 h at 4 °C for ASY1 and ZYP1 immunostaining or 40 h at 4 °C for MLH1 immunostaining. The
slides were washed in PBST three times for 5 min each at room temperature. Following this, a solution of secondary antibodies diluted in PBST was added, and the slides were incubated for 30 min at 37 ºC. The slides were washed in PBST three times for 5 min each at room temperature, and a solution of DAPI/Vectashield was added and a coverslip added to the slide before imaging. The following antibodies were used for immunostaining: α-ASY1 (rat, 1/1500 dilution) (11), α-ZYP1 (rabbit, 1/500 dilution) (45), and α-MLH1 (rabbit, 1/200 dilution) (68). Immunostaining of ASY1 and H3K9me2 was performed using fresh floral buds. Inflorescences were dissected on damp filter paper under a stereo microscope, and six buds at floral stages 8 to 9 (69) were isolated and transfused to 5 µL of enzyme digestion solution (0.4% cytochalase, 1.5% surose, 1% polyvinylpyrrolidone) on a microscope slide. The buds were dissected to recover the anthers, while the rest of the bud tissue was discarded. The slide was then incubated in a moist box at 37 ºC for 1 min, and the anthers were gently opened with a brass rod to release the meiocytes. A total of 5 µL of enzyme digestion solution was added, and the slide was incubated in a moist box at 37 ºC for 2 min. After this, 10 µL of 1% Liposol was added, and the solution was gently mixed with a needle for 1 min before adding 20 µL of 4% paraformaldehyde. The slides were then left to dry for 4 h. Incubation of slides with antibodies for immunostaining of proteins was performed as described earlier. The following antibodies were used: α-ASY1 (rabbit, 1/500 dilution) and H3K9me2 (mouse, 1/100 diluted; Abcam ab1220).

Microscopy was conducted using a DeltaVision Personal DV microscope (Applied Precision/GE Healthcare) equipped with a CCD CoolSNAP HQ2 camera (Photometrics). Image capture was performed using SoftWoRx software version 5.5 (Applied Precision/GE Healthcare). To analyze colocalization of ASY1 and REC8 immunostaining signal on meiotic cells, the contour of chromatin (stained with DAPI) was marked and signal intensity was quantified for every pixel within the marked area using the package coloc2 from Fiji. Data Availability Statement. All data are publicly available. ASY1 ChiP-seq library data have been deposited in the ArrayExpress database at EMBL-EBI (http://www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-8705 (70). Sequencing data for wild type and asy1 Col/WS GBS libraries have been deposited under ArrayExpress accession E-MTAB-8715 (71), and data for asy1/+ ColxLer GBS libraries has been deposited under ArrayExpress accession E-MTAB-8725 (72).

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