Natural variation and dosage of the HEI10 meiotic E3 ligase control Arabidopsis crossover recombination

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Recent studies have shown that natural variation of crossover recombination modifiers can have significant effects on genetic diversity and adaptation. To further investigate this, we used natural Arabidopsis thaliana accessions to identify major crossover quantitative trait loci (rQTLs) that explain 56.9% of crossover variation in Col×Ler F2 populations. We mapped rQTL1 to semidominant polymorphisms in HEI10, which encodes a conserved ubiquitin E3 ligase that regulates crossovers. Null hei10 mutants are haploinsufficient, and, using genome-wide mapping and immunocytology, we showed that transformation of additional HEI10 copies is sufficient to more than double euchromatic crossovers. However, heterochromatic centromeres remained recombination-suppressed. The strongest HEI10-mediated crossover increases occur in subtelomeric euchromatin, which is reminiscent of sex differences in Arabidopsis recombination. Our work reveals that HEI10 naturally limits Arabidopsis crossovers and has the potential to influence the response to selection.

Keywords: meiosis; recombination; modifier; HEI10; ZMM; Arabidopsis

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The majority of eukaryotes reproduces via the meiotic cell division, where a diploid cell replicates DNA once and segregates chromosomes twice to produce tetrads of haploid gametes (Barton and Charlesworth 1998). Genetic diversity is generated between gametes due to independent chromosome segregation in addition to recombination between homologous chromosomes during meiotic prophase I (Barton and Charlesworth 1998). Despite the importance of crossovers for balanced chromosome segregation during meiosis and fertility, extensive genetic variation in recombination frequency is observed within and between species (Sanchez-Moran et al. 2002; Coop and Przeworski 2006; Fledel-Alon et al. 2011; Hinch et al. 2011; Sandor et al. 2012; Bauer et al. 2013; Rodgers-Melnick et al. 2015; Ziolkowski et al. 2015). Importantly, natural variation that modifies crossover frequency has the potential to widely influence genetic adaptation and the response to selection (Hill and Robertson 1966; Feldman et al. 1996; Barton and Charlesworth 1998).

Genetic polymorphisms that modify crossover frequency can be classified as cis- or trans-acting, according to whether they control recombination on the same chromosome or throughout the genome, respectively (Coop and Przeworski 2006; Yandeau-Nelson et al. 2006; Baudat and de Massy 2007; Ziolkowski et al. 2015). Examples of human trans modifier loci include the RNF212 meiotic E3 ligase gene, which controls crossover levels (Kong et al. 2008, Fledel-Alon et al. 2011), and the PRDM9 zinc finger SET domain gene, which specifies recombination hot spot locations (Fledel-Alon et al. 2011; Hinch et al. 2011). Polymorphisms are also known to exert local cis effects, where heterozygous polymorphisms can inhibit crossover repair of interhomolog strand invasion events.

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Multiple nonredundant pathways that include crossover repair of strand invasion events is promoted by pathway that includes MUS81 (Mercier et al. 2015). Non-Arabidopsis Holliday junctions, and promote crossover resolution. Therefore, we sought to use high-throughput fluorescent reporter systems to measure recombination and identify trans-acting crossover modifier loci that vary between A. thaliana accessions.

Meiotic recombination initiates via formation of DNA double-strand breaks (DSBs) by SPO11 transestersases (Mercier et al. 2015). DSBs are resected to generate ssDNA, which is bound by the RecA-related recombinases RAD51 and DMC1 (Mercier et al. 2015). The resulting nucleoprotein filaments can then invade a replicated sister chromatid or a homologous DNA duplex to form a displacement loop (Mercier et al. 2015). Immunostaining of Arabidopsis meiocytes for DMC1, RAD51, or the DSB-associated histone variant yH2A.X has revealed ~100–200 foci distributed along the paired homologous chromosomes at the leptotene stage (Girard et al. 2015; Yin et al. 2015). Approximately 10 of these initiating meiotic DSBs mature into crossovers per Arabidopsis meiosis (Girault et al. 2011; Salomé et al. 2012; Rowan et al. 2015; Choi et al. 2016).

Meiotic interhomolog strand invasion intermediates can follow alternative repair fates, including crossover or noncrossover, which differ in exchange of flanking markers (Mercier et al. 2015). The majority of Arabidopsis crossovers is dependent on the conserved ZMM pathway (named after the budding yeast genes Zip1, Zip2, Zip3, Zip4, Msh4, Msh5, and Mer3), which includes SHOC1, HEI10, ZIP4, MSH4, MSH5, MER3, PTD, MLH1, and MLH3 (Lynn et al. 2007; Mercier et al. 2015). Crossovers generated by the ZMM pathway show interference, where double crossovers are spaced farther apart than expected at random, which is detectable over the scale of meiotic chromosomes in Arabidopsis (Lynn et al. 2007; Mercier et al. 2015). The ZMM pathway is thought to stabilize interhomolog recombination intermediates, including double Holliday junctions, and promote crossover resolution (Lynn et al. 2007; Gray and Cohen 2016). A minority of Arabidopsis crossovers is generated by a noninterfering pathway that includes MUS81 (Mercier et al. 2015). Noncrossover repair of strand invasion events is promoted by multiple nonredundant pathways that include FANCONI ANEMIA COMPLEMENTATION GROUP M (FANCM), MHF1, MHF2, FIDGETIN-LIKE1 (FIGL1), RECQ4A, RECQ4B, TOPOISOMERASE3a (TOP3a), and MSH2 (Mercier et al. 2015). The combined action of these anti-crossover pathways results in repair of ~90% of initiating meiotic DSBs as noncrossovers.

In this study, we identified an Arabidopsis natural genetic variation that acts in trans to control meiotic crossover frequency. Although A. thaliana is predominantly self-fertilizing, clear evidence for outcrossing exists. For example, Arabidopsis linkage disequilibrium decays rapidly over kilobase distances, and strong historical crossover hot spots are detectable (Kim et al. 2007; Horton et al. 2012; Choi et al. 2013). Genotyping of natural Arabidopsis populations has also revealed standing heterozygosity and evidence for local outcrossing between subpopulations (Bomblies et al. 2010). Therefore, recombination-modifying polymorphisms have had the opportunity to exert an effect on the genetic history of this species. Here we identify natural genetic polymorphisms in the HEI10 meiotic E3 ligase gene that associate with quantitative variation in crossover frequency between Arabidopsis accessions. We further show that HEI10 is highly dosage-sensitive and that transformation of additional HEI10 copies is sufficient to more than double crossover recombination throughout euchromatin. Together, this demonstrates that HEI10 is a limiting factor for interference-sensitive crossover formation in Arabidopsis.

Results

Detecting recombination modifier loci using Col/Ler chromosome substitution lines (CSLs)

Genetic segregation of linked, hemizygous T-DNAs expressing different colors of fluorescent proteins in pollen or seed [fluorescent-tagged lines (FTLs)] can be used to measure Arabidopsis crossover frequency (Fig. 1; Emmanu-el et al. 2006; Berchowitz and Copenhaver 2008; Yin et al. 2013; Ziolkowski et al. 2015). We previously analyzed crossovers in an F2 population derived from crosses between the Col-420 subtelomeric FTL and Catania-1 (Ct) parents, which did not identify significant trans-acting recombination modifier loci (Ziolkowski et al. 2015). To further screen for natural crossover modifiers, we generated a Col-420×Landsberg erecta (Ler) F2 population, which showed higher mean recombination than Col-420×Ct [20.2 cM vs. 15.0 cM] (Fig. 1A; Supplemental Tables S1, S2) and significantly greater variation in crossover frequency between individuals [Brown-Forsythe test, $P = 2.91 \times 10^{-14}$]. This is consistent with the presence of trans modifier loci (Fig. 1A; Supplemental Tables S1, S2).

To identify trans recombination modifier loci, we first used Col/Ler CSLS (Fig. 1B; Wijnker et al. 2012). For example, CSL LCCCC denotes Ler [L] and Col [C] genotypes for each of the five chromosomes (Fig. 1B). Fourteen CSLS were crossed to Col-J2f and Col-420 FTLs (crossover reporters located on chromosomes 2 and 3, respectively), and replicate F1 measurements were collected (Fig. 1B–F; Supplemental Tables S3–S5). We observed that all F1 genotypes that were chromosome 1 Col/Ler heterozygous showed significantly reduced crossovers compared with control Col-420×LCCCC F1 plants, with weaker effects detected from the other chromosomes [Fig. 1E,F; Supplemental Tables S3–S5]. This reveals the presence of a semidominant trans-acting recombination modifier on chromosome 1.
Genetic mapping of Arabidopsis recombination quantitative trait loci (rQTLs)

We observed previously that juxtaposition of homozygous and heterozygous regions can influence recombination in cis at the megabase scale (Ziolkowski et al. 2015). To eliminate cis effects and specifically map trans recombination modifiers, we generated an F2 population from a Col-420 × LCCC cross. In this population, chromosome 3 is Col/Col homozygous, which is where the 420 FTL interval is located, and therefore cis effects were excluded. We identified two major trans rQTLs on chromosomes 1 and 4, with logarithm of the [base 10] odds ratio (LOD) scores of 40.2 and 53.5, which explain 23.3% and 33.6% of the variance in recombination, respectively (F-test, $P < 2 \times 10^{-16}$) (Fig. 2A; Supplemental Table S6). rQTL1Ler genotypes associate with low recombination, with heterozygotes showing intermediate crossover frequency [Fig. 2B], consistent with the semidominant effects observed for chromosome 1 in the CSL F1 experiments [Fig. 1E,F; Supplemental Tables S3, S4]. In contrast, rQTL4Ler genotypes associate with high recombination and behaves recessively, explaining why it was not detected in the CSL experiments [Fig. 2C].

To investigate the influence of rQTL1 and rQTL4 on meiotic recombination elsewhere in the genome, we performed cytogenetic analysis in Col and Ler in addition to recombinants with low [rQTL1Ler rQTL4Col] or high [rQTL1Col rQTL4Ler] 420 crossovers (Supplemental Fig. S1A–E; Supplemental Tables S7–S9). MLH1 foci occurring along the meiotic synaptonemal complex (visualized by ZYP1 immunostaining) serve as a measure of total interfering crossovers per nucleus [Lambing et al. 2015]. We observed significantly more MLH1 foci in rQTL1Col rQTL4Ler lines compared with the other genotypes (Mann-Whitney-Wilcoxon test, $P = 0.0396$) (Supplemental Fig. S1A,C; Supplemental Table S7). We confirmed the same trend via analysis of chiasmata at metaphase I (Mann-Whitney-Wilcoxon test, $P = 2.20 \times 10^{-5}$) (Supplemental Fig. S1B,D; Supplemental Table S8; Sanchez-Moran et al. 2002). These analyses confirm that Col and Ler polymorphisms underlying rQTL1 and rQTL4 influence crossovers not only in the 420 interval but throughout the chromosomes.

Genetic variation in the HEI10 meiotic E3 ligase gene underlies rQTL1

We sought to identify rQTL1 using an F2 population derived from a Col-420 × LCCC cross, which again revealed...
We selected an F2 individual that was Col/Ler heterozygous spanning rQTL1 (19.54–21.24 Mb), which was then self-fertilized to generate a large F3 population (n = 3072) (Fig. 2E; Supplemental Table S11). Genotyping identified 307 F3 plants with crossovers within the rQTL1 region, which were then measured for 420 crossover frequency and genotyped for 15 additional markers (Fig. 2E; Supplemental Table S11). This narrowed the credible rQTL1 interval to a 34-kb region containing 14 genes (Fig. 2E,F). The most strongly associated marker pair (LOD = 51.02) defined a 4.3-kb interval containing two genes: MRD1 (At1g53480) and HEI10 (At1g53490) (Fig. 2F). As HEI10 belongs to a conserved gene family, which encodes RING domain SUMO/ubiquitin E3 ligases that promote crossovers in diverse eukaryotes, this was the strongest candidate gene for rQTL1 [Supplemental Figs. S2, S3j; Bhalla et al. 2008; Kong et al. 2008; Fledel-Alon et al. 2011; Chelysheva et al. 2013; De Muyt et al. 2014; Qiao et al. 2014; Johnston et al. 2016; Rao et al. 2017]. HEI10 family proteins possess N-terminal RING domains, central coiled-coil domains, and C-terminal regions of unknown function [Supplemental Figs. S2, S3b; Gray and Cohen 2016].

To further investigate HEI10 polymorphisms associated with rQTL1, we sequenced the Ler accession used in our experiments and identified a single nonsynonymous [R264G] substitution and three synonymous intragenic variants relative to the Col reference sequence (Fig. 3A; Supplemental Table S12), which was consistent with 1001 Genomes project data from the closely related Ler-1 and La-0 accessions [Alonso-Blanco et al. 2016]. The HEI10 promoter, which overlaps the antisense gene MRD1, is also polymorphic, with 26 single-nucleotide polymorphisms (SNPs) or indels upstream of the start codon (Fig. 3A; Supplemental Table S12). We generated additional F2 populations derived from Col-420×Bur-0 or Col-420×Cvi-0 crosses and again observed significant association between the HEI10 region and crossover frequency (Bur-0 LOD = 8.30, 95% significance threshold LOD = 2.82; Cvi-0 LOD = 31.74, 95% significance threshold LOD = 2.97) (Fig. 3B,C; Supplemental Table S13). We sequenced HEI10Bur and HEI10Cvi and observed 43 and 30 polymorphisms, respectively, relative to HEI10Col (Fig. 3A; Supplemental Table S12). Twelve polymorphisms are shared between HEI10Ler, HEI10Cvi, and HEI10Ct (Fig. 3A; Supplemental Table S12).
HEI10Bur but absent from HEI10Ct, including the R264 substitution, which we consider as candidates for rQTL1 causal variants [Fig. 3A; Supplemental Table S12]. HEI10 transcript and protein levels measured by quantitative RT–PCR (qRT–PCR) and immunocytogenetic analysis, respectively, did not show significant differences between Col, Ler, and Col/Ler F1 [Supplemental Fig. S1E; Supplemental Tables S14–S16], consistent with the causal rQTL1 polymorphism influencing HEI10 protein function or expression timing rather than expression level. The nonsynonymous R264G polymorphism occurs in the HEI10 C-terminal region (Fig. 3E; Supplemental Figs. S2, S3), which, by analogy with other RING E3 ligases, may play a role in substrate recognition (Deshaies and Joazeiro 2009). We queried 1001 Genomes project data for the frequency and geographic distribution of the HEI10 R264G variants (Supplemental Fig. S4; Alonso-Blanco et al. 2016). Both alleles are globally distributed, with the majority of accessions (959 out of 1008) showing the Ler G264 genotype, and the Col R264 reference allele present in the remaining 123 (11.4%) [Supplemental Fig. S4]. As other Brassicaceae HEI10 orthologs show glycine at position 264, this is consistent with the R264 variant observed in Col-0 and Ct-1 being more recently derived within A. thaliana [Fig. 3E].

Arabidopsis crossover frequency is sensitive to HEI10 dosage
As a genetic test of rQTL1 allelism with HEI10, we crossed recombinant 420 lines with the null hei10-2 allele, which was isolated in the Col background and shows substantially reduced crossovers and fertility when homozygous (Chelysheva et al. 2012). Four independent recombinant lines were used for crosses, two of which were rQTL1Col homozygous, and two that were rQTL1Ler homozygous [Supplemental Table S17]. All lines analyzed were rQTL4Ler homozygous. F1 individuals that were hei10-2 heterozygous showed significantly reduced recombination compared with wild-type crosses [Fig. 4A; Supplemental Table S17]. This indicates HEI10 dosage sensitivity, which is similar to haploinsufficiency of mouse hei10 and rnf212 mutations (Reynolds et al. 2013; Qiao et al. 2014). Consistent with our previous rQTL mapping, the progeny from rQTL1Ler crosses showed significantly fewer 420 crossovers compared...
HEI10 controls Arabidopsis crossover recombination

Figure 4. HEI10 is a dosage-sensitive regulator of Arabidopsis crossovers. (A) 420 crossovers (in centimorgans) in F1 individuals derived from crosses between HEI10Col or HEI10Ler homozygotes and wild-type (Col) or null hei10-2 mutants. Replicate individuals are shown as black dots, and mean values are shown as red dots. (B) 420 crossovers (in centimorgans) in F1 individuals derived from crosses with interfering crossover ZMM pathway mutants. (C) Schematic showing the transformation of Col-420 with additional HEI10 copies (blue triangles). (D) Diagram illustrating the HEI10 T-DNA construct used for Arabidopsis transformation via Agrobacterium. (LB) T-DNA left border sequence; (RB) T-DNA right border sequences. (E) 420 crossover frequency (in centimorgans) in empty vector, HEI10Ler::Ler, HEI10Col::Ler, HEI10Ler::Col, and HEI10Col::Col transformants compared with untransformed Col-420/Col controls. Data from individual plants are shown as black dots, and mean values are shown in red. (F) Correlation between 420 crossovers (in centimorgans) and HEI10 transcript levels measured by qRT–PCR from HEI10Col (black) and HEI10Ler (green) transformant flowers. A regression line is plotted in red.

with rQTL1Col progeny ($X^2, P < 2.2 \times 10^{-16}$) [Fig. 4A; Supplemental Table S17], which we interpret as reflecting the different activity of HEI10 Col/Ler variants, including R264G. To investigate whether haploinsufficiency is a general property of mutants in the Arabidopsis ZMM pathway, we compared Col-420 F1 crossover measurements using hei10-2, msh4-1, msh5-1, shoc1-1, and ptd1 heterozygotes [Fig. 4B; Supplemental Table S18]. Among these mutations, only hei10-2/+ heterozygotes showed significantly reduced crossovers compared with wild type ($X^2, P = 1.63 \times 10^{-38}$) [Fig. 4B; Supplemental Table S18], revealing that dosage sensitivity was specific to HEI10.

Increased HEI10 dosage elevates euchromatic crossovers genome-wide

Due to HEI10 dosage sensitivity, we next investigated whether increasing copy number would elevate recombination beyond wild type [Fig. 4C–E]. We transformed Col-420 plants with a HEI10 transgene under the control of its endogenous promoter, amplified from either Col or Ler
genomic data [referred to here as HEI10\textsuperscript{Col} and HEI10\textsuperscript{Ler}] (Fig. 4C–E). HEI10\textsuperscript{Col} and HEI10\textsuperscript{Ler} T\textsubscript{1} populations, but not empty vector T\textsubscript{1} showed significantly higher recombination than untransformed Col-420 controls [Mann-Whitney-Wilcoxon test: HEI10\textsuperscript{Col}, $P = 4.03 \times 10^{-14}$; HEI10\textsuperscript{Ler}, $P = 3.64 \times 10^{-10}$, empty, $P = 0.474$] (Fig. 4E; Supplemental Tables S2, S19). Wide variation in recombination rate was observed within T\textsubscript{1} populations [Fig. 4E], which was likely caused by varying HEI10 transgene copy numbers and position effects that influence expression level. Indeed, qRT–PCR analysis of HEI10 expression from meiotic stage flower buds [floral stages 1–12] of T\textsubscript{1} transformants revealed a positive correlation with 420 recombination [$r = 0.727; P = 7.96 \times 10^{-6}$] (Fig. 4F; Supplemental Table S20). HEI10\textsuperscript{Col} transformants showed higher recombination than HEI10\textsuperscript{Ler} transformants [Mann-Whitney-Wilcoxon test, $P = 8.93 \times 10^{-3}$] (Fig. 4E, Supplemental Table S19), which is consistent with the different recombination activity of Col/Ler HEI10 variants. To further investigate polymorphisms responsible for differences in HEI10 function, we generated Col::Ler and Ler::Col promoter swap constructs and repeated transformation [Fig. 4E; Supplemental Table S19]. The HEI10-Ler::Col transformants showed significantly higher recombination than HEI10-Col::Ler transformants [Mann-Whitney-Wilcoxon test, $P = 1.1 \times 10^{-2}$]. Furthermore, HEI10-Ler::Col were not significantly different from HEI10-Col::Col [Mann-Whitney-Wilcoxon test, $P = 0.841$], and HEI10-Col::Ler were not different from HEI10-Ler-Ler [Mann-Whitney-Wilcoxon test, $P = 0.259$]. Together, this is consistent with intragenic HEI10 polymorphisms, including R264G, causing differences in recombination activity (Fig. 4E; Supplemental Table S19).

A HEI10\textsuperscript{Col} T\textsubscript{1} line showing high 420 recombination (C2, 33.74 cM) was selected for cytological investigation. Immunostaining of leptotene stage meiotic nuclei for HEI10 showed a significant increase in signal intensity [Mann-Whitney-Wilcoxon test, $P = 3.90 \times 10^{-4}$], although focus numbers were not changed [Mann-Whitney-Wilcoxon test, $P = 0.5971$] (Fig. 5A–C; Supplemental Tables S21, S22). To investigate the effect of HEI10\textsuperscript{Col} transformation on crossover formation, we performed MLH1 immunostaining at the pachytene stage [Fig. 5D,E]. There were close to double the number of MLH1 foci along HEI10\textsuperscript{Col} chromosomes compared with wild type [mean = wild type 9.3, HEI10\textsuperscript{Col} 16.2; Mann-Whitney-Wilcoxon test, $P = 4.83 \times 10^{-8}$] (Fig. 5D,E; Supplemental Table S23). HEI10\textsuperscript{Col} also showed more compact bivalents at metaphase I, which is indicative of greater crossover numbers in the chromosome arms [Fig. 5D; Sanchez-Moran et al. 2002]. This provides cytological evidence that increased HEI10 dosage and expression level elevates crossovers throughout the genome.

To investigate the effect of increased HEI10 dosage on crossovers at higher resolution, we used genotyping by sequencing [Fig. 6A]. The HEI10\textsuperscript{Col} C2 line was backcrossed to Ler alongside a wild-type Col control [Fig. 6A). HEI10\textsuperscript{Col}\texttimes Ler F\textsubscript{1} plants showed highly elevated 420 recombination compared with Col/Ler F\textsubscript{1} [Supplemental Tables S19, S24], demonstrating that HEI10 increases crossovers in both hybrid [Col/Ler] and inbred [Col/Col] backgrounds. F\textsubscript{2} populations ($n = 192$) were then generated from wild-type and HEI10\textsuperscript{Col} Col/Ler F\textsubscript{1} plants and sequenced to identify crossover locations (Fig. 6A; Supplemental Table S25; Choi et al. 2016). The HEI10\textsuperscript{Col} population contained more than double the number of wild-type

**Figure 5.** Increased HEI10 dosage promotes formation of meiotic MLH1 foci. (A) Representative images showing leptotene stage male meiocytes from Col or HEI10\textsuperscript{Col} [line C2] immunostained for HEI10 [red] and ASY1 [green] and counterstained with DAPI [blue]. Bars, 10 μm. (B) Quantification of HEI10 expression level via immunostaining of Col and HEI10\textsuperscript{Col} [line C2]. (C) As for B but showing quantification of HEI10 foci. (D) Representative images of DAPI-stained bivalents at metaphase I in wild type [Col] [top left panel] and HEI10\textsuperscript{Col} [line C2] [bottom left panel]. Bars, 5 μm. [Top middle panel] A magnified view of a wild-type ring bivalent is shown with homologs outlined in red and blue. [Bottom middle panel] The inferred chiasmata sites are marked with an “X.” A magnified view of a HEI10\textsuperscript{Col} ring bivalent is shown. Representative images showing leptotene stage male meiocytes from Col [top right panel] or HEI10\textsuperscript{Col} [line C2] [bottom right panel] stained for MLH1 [red], ASY1 [green], and DAPI [DNA; blue]. Bars, 10 μm. (E) Quantification of MLH1 foci on pachytene stage meiotic chromosomes in wild type and HEI10\textsuperscript{Col} [line C2].
crossovers (1230 vs. 2928 crossovers; mean per F2, 6.41 vs. 15.25; Mann-Whitney-Wilcoxon test, \( P = 1.07 \times 10^{-59} \)) (Fig. 6B; Supplemental Fig. S5; Supplemental Table S1), consistent with our MLH1 focus analysis (Fig. 5D,E). Eu- chromatic chromosome arms showed the greatest increase in HEI10Col crossovers (2.6×, Mann-Whitney-Wilcoxon test, \( P = 1.98 \times 10^{-8} \)), with the largest effects in the subtelomeric regions (Fig. 6C,D; Supplemental Tables S26, S27). A lower yet significant crossover increase was observed in the pericentromeres (1.6×, Mann-Whitney-Wilcoxon test, \( P = 2.34 \times 10^{-9} \)) (Fig. 6C; Supplemental Fig. S6; Supplemental Tables S26, S27). In contrast, centromeric suppression of crossovers was observed in both populations (Fig. 6C; Supplemental Fig. S6; Supplemental Tables S26, S27; Copenhaver et al. 1999; Yelina et al. 2015).

Crossovers were mapped using ∼1×–2× depth sequencing data and the TIGER analysis pipeline [Supplemental Table S25; Rowan et al. 2015], which resolved events to a mean width of 976 base pairs (bp). To analyze the fine-scale distribution of wild type versus HEI10 crossovers, we overlapped them with gene and transposon annotations and compared them with matched sets of randomly chosen intervals [Supplemental Fig. S6A; Supplemental Table S28]. Both wild-type and HEI10 crossovers show increased intergenic and decreased transposon overlap compared with random [Supplemental Fig. S6A; Supplemental Table S28), which is consistent with Arabidopsis crossover hot spots associating with euchromatic gene promoters and terminators (Choi et al. 2013; Mercier et al. 2015). We also compared DNA methylation levels and observed that crossovers from both populations were hypomethylated in CG, CHG, and CHH sequence contexts compared with random [Supplemental Fig. S6B; Stroud et al. 2013]. This is further consistent with both wild-type and HEI10 crossovers being enriched within euchromatic regions along the chromosome arms.

Discussion

Plants, fungi, and invertebrates possess a single HEI10/RNF212 ortholog [Bhalla et al. 2008; Chelysheva et al. 2012; Wang et al. 2012; Serrentino et al. 2013; De Muyt et al. 2014; Lake et al. 2015], whereas vertebrates encode separate RNF212 and HEI10 proteins that function as antagonistic SUMO and ubiquitin E3 ligases [Ward et al. 2007; Reynolds et al. 2013; Qiao et al. 2014; Gray and Cohen 2016; Rao et al. 2017]. In mice, HEI10 and RNF212...
that increased in diverse eukaryotic lineages. It is interesting to note disposables acting as efficiency and dosage sensitivity of (Kong et al. 2008; Fledel-Alon et al. 2011; Sandor et al. 2014). We propose that haploinsufficiency (Reynolds et al. 2013; Qiao et al. 2015). Therefore, we speculate that differences in HEI10/RNF212 expression or regulation have the potential to contribute to sex differences in recombination. We also note that increasing HEI10 copy number may be an attractive mechanism to elevate crossover numbers during breeding of crop species.

Crossover modifier loci are able to alter population responses to selection (Feldman et al. 1996). For example, recombination can mitigate the effects of Hill-Robertson interference when linked loci are under selection (Hill and Robertson 1966; Barton and Charlesworth 1998). Therefore, loci that modify crossover frequency may influence genetic adaptation to diverse environments and conditions. Interestingly, total recombination levels compared across eukaryotes are generally low, typically with one or two crossovers per chromosome per meiosis, despite wide variation in physical genome size (Mercier et al. 2015). It is possible that high recombination levels might cause infertility and be selected against. However, Arabidopsis anti-crossover pathway mutants show normal fertility despite greatly elevated crossover frequency, at least in the short term (Girard et al. 2015; Mercier et al. 2015). Therefore, we propose that Arabidopsis recombination modifiers may act to maintain relatively low crossover numbers. As rQTL1Col and rQTL4Col alleles show opposite effects on crossover frequency, this example is consistent with antagonistic modifiers acting to balance recombination. It is also important to note that the effect of modifiers will be highly dependent on genome architecture and outcrossing levels. Crossover modifiers may be especially common in plants, where frequent polyploidization causes challenges for balanced meiotic genome transmission (Bombly et al. 2016). Indeed, meiotic axis proteins [ASY1, ASY3, PDS5, ZYP1a, ZYP1b, SMCI, and REC8] have been strongly selected during polyploid evolution in Arabidopsis arenosa (Yant et al. 2013), and the Ph1 locus is required for promotion of homologous versus homeologous recombination in hexaploid bread wheat (Martin et al. 2014). Therefore, further study of plant meiotic modifier loci is likely to reveal insights into the control of recombination and how this interacts with selection during evolution.

**Materials and methods**

**Arabidopsis strains**

Crossover frequency was measured using fluorescent reporters in seeds (Col-420) and pollen (Col-I29) (Emmanuel et al. 2006;
Benchowitz and Copenhaver 2008; Yelina et al. 2013; Ziolkowski et al. 2015). In F2 populations derived from FTL hemizygotes, only a subset of progeny will contain the fluorescent protein-encoding transgenes also in a hemizygous state, which is necessary for crossover measurement. When using the seed-based 420 line, it is possible to enrich for FTL hemizygous F2 plants by examining seed under a fluorescence microscope prior to sowing and separating nonfluorescent, hemizygous, and homozygous seeds based on eGFP and dsRed fluorescence intensities. CSLs were kindly provided by Erik Wijnker, Jose van der Belt, and Joost Keurentjes (University of Wageningen) [Wijnker et al. 2012], with the exception of LC CCC, which was obtained from an esd7-1 backcross line [de Olmo et al. 2010]. Mt-0, Ct-1, and Cvi-0 accessions were obtained from the Nottingham Arabidopsis Stock Centre. The ZMM mutant alleles used were hei10-2 (Salk_014624), mshd-1 (Salk_136296), msh5-1 (Salk_110240), ptd1 (Salk_127447), and shoc-1-1 (Salk_057589). Genotyping primer sequences for these mutations used with the Lbh1.3 T-DNA left border primer are in Supplemental Table S29.

rQTL mapping

Genomic DNA was extracted using CTAB and genotyped using PCR amplification of Col/Ler SSLP, CAPS, or dCAPS markers [Supplemental Tables S30, S31]. We performed one- and two-dimensional QTL mapping using the R statistical package rQTL. We implemented the Haley-Knott regression algorithm using 2.5-cM steps across the genome and 0.1-cM steps for rQTL fine mapping. To fit models with multiple QTLs, we used the fitqtl function with Haley-Knott regression. We used 10,000 permutations for each mapping population to empirically calculate genome-wide LOD score significance thresholds.

HEI10 transformation

HEI10 was amplified from Col or Ler genomic DNA using primers HEI10-XbaI and HEI10-BamHI [Supplemental Table S29]. Amplification products were cloned into the pGREEN-0029 binary vector using XbaI and BamHI restriction enzymes. Promoter swap constructs were generated using XbaI/PacI digestion and Amplification products were cloned into the pGREEN-0029 binary vector religation. These vectors were transformed into Col or Ler genomic DNA using primers HEI10-XbaI was amplified from Col or Ler genomic DNA using primers HEI10-qPCR2 and HEI10-qPCR1, and the metiosis-specific gene DMC1 was amplified using primers DMC1-PCR1 and DMC1-PCR2 as a control for qPCR calculations [Supplemental Table S29]. For HEI10 T1 analysis, the 2^-ΔΔCt method was used to quantify relative transcript levels in comparison with untransformed plants.

Quantitative gene expression analysis

RNA was extracted from ~40 mg of immature flower buds (closed buds up to stage 12, which contain all meiotic stages) using TRI reagent (Sigma-Aldrich). Reverse transcription was performed with SuperScript II reverse transcriptase (ThermoFisher Scientific). Relative HEI10 expression was measured by qPCR using primers HEI10-qPCR1 and HEI10-qPCR2, and the metiosis-specific gene DMC1 was amplified using primers DMC1-PCR1 and DMC1-PCR2 as a control for qPCR calculations [Supplemental Table S29]. For HEI10 T1 analysis, the 2^-ΔΔCt method was used to quantify relative transcript levels in comparison with untransformed plants.

Meiotic cytology and immunostaining

Chromosome spreads of Arabidopsis pollen mother cells and rDNA in situ hybridization were performed as described using fixed buds (Sanchez-Moran et al. 2007). Pachytene stage meiocytes were immunostained for ASY1, ZYP1, MLH1, and HEI10 using fresh buds as described [Armstrong et al. 2002; Sanchez-Moran et al. 2007; with the following antibodies: a-ASY1 (rabbit; 1:500 dilution), a-MLH1 (rabbit, IgG-purified; 1:200 dilution), a-ZYP1 (rat; 1:500 dilution), and a-HEI10 (rabbit; 1:200 dilution) [Armstrong et al. 2002; Sanchez-Moran et al. 2007; Chelysheva et al. 2012; Lambing et al. 2015]. HEI10-immunostained slides within experiments were prepared side by side, and images were captured using the same exposure times. HEI10-immunostained cell images were acquired as Z-stacks of 10-μm x 0.4-μm optical sections, and maximum intensity projections were reconstructed using ImageJ [Lambing et al. 2015]. Cell boundaries were defined manually, and total signal intensity within cells was measured. An adjacent image region was used to measure background intensity, and this value was subtracted from the cell intensity. The fluorescence signal from an adjacent Inspeck Red microsphere (ThermoFisher Scientific) was also used to normalize HEI10 signal intensity. Microscopy was conducted using a DeltaVision personal DV microscope [Applied Precision/GE Healthcare] equipped with a CDD CoolSnap HQ2 camera (Photometrics). Image capture was performed using SoftWoRx software version 5.5 [Applied precision/GE Healthcare]. For MLH1 and ZYP1 coimmunostaining of pachytene nuclei, individual cell images were acquired as Z-stacks of 0.1-μm optical sections, and the maximum intensity projection for each cell was rendered using ImageJ. Numbers of MLH1 foci associated with the synaptonemal complex were scored. DAPI staining of chromosomes from metaphase I nuclei and chiasma counting were performed as described [Sanchez-Moran et al. 2002]. Image capture was conducted using a Nikon 90i fluorescence microscope. Images were analyzed with NIS-Elements-F software and ImageJ.

Mapping crossovers via genotyping by sequencing

DNA was extracted using CTAB and used to generate sequencing libraries as described [Rowan et al. 2015; Yelina et al. 2015] with the following modifications. DNA was extracted from three rosette leaves of 5-wk-old plants, and 150 ng of DNA was used as input for each library. DNA was sheared for 20 min at 37°C with 0.4 U of DNA Shearase (Zymo research). Each set of 96 libraries was sequenced on one lane of an Illumina NextSeq500 instrument (300-cycle Mid Output run). FastQ sequencing data files are available from ArrayExpress accessions E-MTAB-4657 (wild type) [Choi et al. 2016] and E-MTAB-4967 [HEI10]. Sequencing data were analyzed to identify crossovers as reported previously using the TIGER pipeline [Rowan et al. 2015; Yelina et al. 2015; Choi et al. 2016]. Crossovers were tallied in 10-kb windows and plotted along chromosomes after smoothing using the R function “filter.” Crossovers were counted and compared between populations using 2 x 2 contingency tables and \( \chi^2 \) tests.

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