Sex-Specific Evolution of the Meiotic Recombination Rate

April L. Peterson, Bret Payseur

2020-06-02

# INTRODUCTION

# MATERIALS AND METHODS

### Mice

We used a panel of wild-derived inbred strains of house mice (*Mus musculus*) and related murid species to profile natural genetic variation in recombination (Table 1). Our survey included 5 strains from *M. m. musculus*, 4 strains from *M. m. domesticus*, 2 strains from *M. m. molossinus*, 2 strains from *M. m. castaneus*, and 1 strain each from *M. spicilegus* and *M. spretus*. We subsequently denote strains by their abbreviated subspecies and name (*e.g.* *domesticusWSB*). Mice were housed at dedicated, temperature-controlled facilities in the UW-Madison School of Medicine and Public Health, with the exception of mice from Gough Island, which were housed in a temperature-controlled facility in the UW-Madison School of Veterinary Medicine. Mice from an inbred strain of Gough Island mice were sampled after XX generations of brother-sister mating. All mice were provided with *ad libitum* food and water. Procedures followed protocols approved by IACUC.

### Tissue Collection and Immunohistochemistry

The same dry-down spread technique was applied to both spermatocytes and oocytes, following Peters et al. (1997), with adjustment for volumes. Spermatocyte spreads were collected and prepared as described in Peterson et al. (2019). The majority of mice used for MLH1 counts were between 5 and 12 weeks of age. Juvenile mice between 12 and 15 days of age were used for DMC1 counts. Both ovaries were collected from embryos (16-21 embryonic days) or neonates (0-48 hours after birth). Whole testes were incubated in 3ml of hypotonic solution for 45 minutes. Decapsulated ovaries were incubated in 300ul of hypotonic solution for 45 minutes. Fifteen microliters of cell slurry (masticated gonads) were transferred to 80ul of 2% PFA solution. Cells were fixed in this solution and dried in a humid chamber at room temperature overnight. The following morning, slides were treated with a Photoflow wash (Kodak, cite). Slides were stored at -20\*C if not stained immediately. To visualize the structure of meiotic chromosomes, we used antibody markers for the centromere (CREST) and lateral element of the synaptonemal complex (SC) (SYCP3). Crossovers (COs) were visualized as MLH1 foci. Double strand breaks (DSBs) were visualized as DMC1 foci. The staining protocol followed (Anderson et al. 1999) and (Koehler et al. 2002). Antibody staining and slide blocking were performed in 1X antibody dilution buffer (ADB) (normal donkey serum (Jackson ImmunoResearch), 1X PBS, bovine serum albumin (Sigma), and Triton X-100 (Sigma)). Following a 30-minute blocking wash in ABD, each slide was incubated with 60ul of a primary antibody master mix for 48 hours at 37\*C. The master mix recipe contained polyclonal anti-rabbit anti-MLH1 (Calbiochem; diluted 1:50) or anti-rabbit anti-DMC1 (mix of DMC1), anti-goat polyclonal anti-SYCP3, (Abcam; diluted 1:50), and anti-human polyclonal antibody to CREST (Antibodies, Inc; diluted 1:200) suspended in ADB. Slides were washed twice in 50ml ADB before the first round of secondary antibody incubation for 12 hours at 37\*C. Alexa Fluor 488 donkey anti-rabbit IgG (Invitrgoen, location; diluted to 1:100) and Coumarin AMCA donkey anti-human IgG (Jackson ImmunoResearch; diluted to 1:200) were suspended in ADB. The last incubation of Alexa Fluor 568 donkey anti-goat (Invitrogen; diluted 1:100) was incubated at 1:100 for 2 hours at 37\* C. Slides were fixed with Prolong Gold Antifade (Invitrogen) for 24 hours after a final wash in 1x PBS.

### Image Processing

Images were captured using a Zeiss Axioplan 2 microscope with AxioLab camera and AxioVision software (Zeiss, Cambridge, UK). Preprocessing, including cropping, noise reduction, and histogram adjustments, was performed using Photoshop (v13.0). Image file names were anonymized before manual scoring of MLH1 foci or DMC1 foci using Photoshop.

### Analysis

To estimate the number of crossovers across the genome, we counted MLH1 foci. MLH1 foci were counted in cells with intact and complete karyotypes (19 acrocentric bivalents and XY for spermatocytes; 20 acrocentric bivalents for oocytes) and distinct MLH1 foci. A quality score ranging from 1 (best) to 5 (worst) was assigned to each cell based on visual appearance of staining and spread of bivalents. Cells with a score of 5 were excluded from the final analysis. Distributions of MLH1 count per cell were visually inspected for normality (Supplemental Figure 1). MLH1 foci located on the XY in spermatocytes were excluded from counts. In addition to MLH1 counts, we measured several traits to further characterize the recombination landscape. To estimate the number of double-strand breaks, a minority of which lead to crossovers, mean DMC1 foci per cell was quantified for a single male from each of a subset of strains (*molossinusMSM*, *musculusPWD*, *musculusMSM*, *domesticusWSB*, and *domesticusG* ). SC morphology and CREST foci number were used to stage spermatocytes as early zygotene or late zygotene.

To measure bivalent SC length, two image analysis algorithms were used. The first algorithm estimates the total (summed) SC length across bivalents for individual cells (Wang et al. 2019). The second algorithm estimates the SC length of individual bivalents (Peterson et al. 2019). Both algorithms apply a ‘skeletonizing’ transformation to synapsed chromosomes that produces a single, pixel-wide ‘trace’ of the bivalent shape. Total SC length per cell was quantified from pachytene cell images (Wang et al. 2019). To reduce algorithmic errors in SC isolation, outliers were visually identified at the mouse level and removed from the data set. Mouse averages were calculated from cell-wide total SC lengths in 3,719 out of 3,881 cells with MLH1 counts. SC length of individual bivalents was quantified in pachytene cell images (Peterson et al. 2019). The DNA CrossOver algorithm (Peterson et al. 2019) isolates single, straightened bivalent shapes, returning SC length, location of MLH1 foci, and location of CREST (centromere) foci. The algorithm substantially speeds the accurate measurement of bivalents, but it sometimes interprets overlapping bivalents as single bivalents. In our data set, average proportions of bivalents per cell isolated by the algorithm ranged from 0.48 (*molossinusMSM* male) to 0.72 (*musculusKAZ* female). From the total set of pachytene cell images, 10,221 bivalent objects were isolated by the algorithm. Following a manual curation, 9,576 single-bivalent observations remained. The accuracy of the algorithm is high compared to hand measures after this curation step (Peterson et al. 2019). The curated single bivalent data supplements our cell-wide MLH1 count data with MLH1 foci counts for single bivalents. Proportions of bivalents with the same number of MLH1 foci were compared across strains using a chi-square test.

To account for confounding effects of sex chromosomes from pooled samples of bivalents, we also considered a reduced data set including only bivalents with SC lengths below the 2nd quartile in cells with at least 17 of 20 single bivalent measures. This “short bivalent” data set included the four or five shortest bivalents and excluded the X bivalent in oocytes. A total of 704 short bivalents were isolated from 102 oocytes and 43 spermatocytes. Although this smaller data set has decreased power, it offers a more comparable set of single bivalents to compare between the sexes. A “long bivalent” data set was formed from those bivalents above the 4th quartile in SC lengths per cell. A total of 709 long bivalents were isolated from 102 oocytes and 43 spermatocytes.

To examine crossover interference, the distance (in SC units) between MLH1 foci (inter-focal distance; IFDraw) was measured for those single bivalents containing two MLH1 foci. A normalized measure of interference (IFDnorm) was computed by dividing IFDraw by SC length on a per-bivalent basis.

We used a series of statistical models to interpret patterns of variation in the recombination traits we measured (Table 2). We used mouse average as the dependent variable in all analyses. We first constructed a linear mixed model (M1) using lmer() from the lmer4 package (Bates et al. 2015) in R (v3.5.2) (Team 2015). In this model, strain was coded as a random effect, with significance evaluated using a likelihood ratio test (using exactRLRT() from RLRsim (Scheipl, Greven, and Kuechenhoff 2008)). Subspecies, sex, and their interaction were coded as fixed effects, with significance evaluated using a chi-square test comparing the full and reduced models (drop1() and anova()) (Bates et al. 2015). As each observation was at the level of a single mouse (mouse average), it was uniquely coded within the dataset, nesting was implicit, each strain only occurs within one strain, and was not explicitly coded in our linear and mixed models. We used the subspecies effect to quantify divergence between subspecies and the (random) strain effect to quantify variation within subspecies in a sex-specific manner. In separate analyses using model M1, we considered mouse averages as dependent variables for each of the following traits: MLH1 count per cell, total SC length per cell, single bivalent SC length per cell, IFDraw, IFDnorm, and average MLH1 position (for single-focus bivalents). Four additional linear models containing only fixed effects (M2-M5) (Table 2) were used to further investigate results obtained from model M1.

# RESULTS

## Genome-wide recombination rate evolves differently in females and males

We used counts of MLH1 foci per cell to estimate genome-wide recombination rates in 14 wild-derived inbred strains sampled from three subspecies of house mice (*M. m. domesticus*, *M. m. musculus* and *M. m. molossinus*) and three additional species of *Mus* (*M. spretus*, *M. spicilegus*, and *M. caroli*). Mean MLH1 focus counts for 185 mice were quantified from an average of 22.1 spermatocytes per male (for a total of 1,993 spermatocytes) and 18.3 oocytes per female (for a total of 1,426 oocytes).

Graphical comparisons reveal sex-specific dynamics to the evolution of genome-wide recombination rate (Figure 1A). First, MLH1 focus counts differ between females and males in most strains. Second, the difference in counts between the sexes varies among strains. Although most strains show more MLH1 foci in females, two strains (*musculusPWD* and *molossinusMSM*) exhibit higher counts in males. In females, numbers of MLH1 foci are evenly distributed around the sex-wide mean of approximately 25 (Figure 1B). In stark contrast, males largely separate into two groups of strains with high numbers (near 30) and low numbers (near 23) of foci (Figure 1C). Strain mean MLH1 focus counts from females and males are uncorrelated (Spearman’s  = 0.08; p = 0.84) across the set of strains.

To further partition variation in recombination rate, we fit a series of linear models to mean MLH1 focus counts from 127 house mice (Table 2; detailed results available in Supplemental Table X). Strain, sex, subspecies, and sex\*subspecies each affect MLH1 focus count in a linear mixed model (M1; strain (random effect): p < 10-4; sex: p = 6.2x10-4; subspecies: p = 1.1x10-3; subspecies\*sex: p = 2x10-4). The effect of subspecies is no longer significant in a model treating all factors as fixed effects (M2; p = 0.09), highlighting strain and sex as salient variables.

Two strains exhibit particularly strong effects on MLH1 focus count (M3; *domesticusG* p = ; *domesticusLEW* p =), with sex-strain interactions involving three strains (M3; *domesticusG* p = ; *molossinusMSM* p = ; *musculusPWD* p = ).

In separate analyses of males (M4; *n* = 80), three strains disproportionately shape MLH1 focus count (as observed in Figure 1C): *musculusPWD* (p = ; effect = ), *molossinusMSM* (p = ; effect = ), and *musculusSKIVE* (p = ; effect = ). These three strains point to substantial evolution in the genome-wide recombination rate in spermatocytes; we subsequently refer to them as “high-recombination” strains. In females (M4; n= 76), four strains affect MLH1 focus count: *domesticusG* (p = ; effect = ), *molossinusMSM* (p = ; effect = ), *domesticusLEW* (p = ; effect = ), and *musculusPWD* (p = ; effect = ). Strain effect sizes in females are modest in magnitude compared to those in males.

Together, these results demonstrate that the genome-wide recombination rate evolves in a highly sex-specific manner.

## Synaptonemal complexes are longer in females

The variation in sex differences in recombination we discovered provided an opportunity to determine whether sex differences in chromatin compaction, as measured by the length of the synaptonemal complex (SC), are reversed when heterochiasmy is reversed. In all strains except *musculusSKIVE*, females have longer SCs than males, whether SC length was estimated as the total length across bivalents or as the length of short bivalents (t-tests; all p < 0.05, except short bivalents in *musculusSKIVE*, p = 0.11). Among short bivalents (to which the female X bivalent does not contribute), female to male ratios of mouse mean SC length range from 1.26 (*musculusPWD*) to 1.49 (*domesticusWSB*) across strains. That females have longer SCs is further supported by models that include covariates, which identify sex as the most consistently significant effect for total SC length (M1: p = 7.16^{-20}; M2: p = 5.33^{-4}; M3: p =0.05) and short bivalent length (M1: p = 1.27^{-11}; M2: p = 1.98^{-7}; M3: p = 1.98^{-7}). The existence of some subspecies and strain effects (M1 subspecies\*sex: p = 0.03; M2: strain-*musculusSKIVE* : p=0.02; *musculusSKIVE*\*male: p = 0.08) further indicates that SC length has evolved among strains and among subspecies.

In summary, two approaches for measuring SC length demonstrate that females have longer SCs (chromosome axes), even in strains in which males recombine more. This pattern implies that in high-recombination strains, spermatocytes have less space than oocytes in which to position additional crossovers.

## Females and males differ in crossover positions and crossover interference

We used normalized positions of MLH1 foci along bivalents with a single focus to compare crossover location while controlling for differences in SC length. In all strains, MLH1 foci tend to be closer to the telomere in males (mean normalized position in males: 0.68; mean normalized position in females: 0.56; t-test; p = 3.84^{-23}). Sex is also the strongest determinant of MLH1 focus position in the models we tested (M1: p = 7.79^{-26}; M2: p = 5.39^{-8}; M3: p = 5.39^{-8}).

Males have longer normalized mean inter-focal distances (IFDnorm) than females in seven out of eight strains (t-tests; p < 0.05), with only *musculusKAZ* showing no difference (p = 0.33). Examination of IFDnorm distributions indicates that females are centered at approximately 50% and show a slight enrichment of low (<25%) values, whereas males are enriched for higher values. Models treating IFDnorm as the dependent variable support the inference of stronger interference in males, with sex being the most significant variable (M1: p = 3.11^{-12} ; M2: p = 0.01; M3: p = 0.01). In contrast, there is no clear signal of sex differences in raw mean inter-focal distances (IFDraw) across the full set of strains, whether they are considered separately or together. Visualization of normalized MLH1 foci positions on bivalents with two crossovers (Figure 4C) further suggests that interference distances vary more in females than in males, and that males display a stronger telomeric bias in the placement of the distal crossover.

In summary, controlling for differences in SC length (chromatin compaction) indicates that interference is stronger in males, whereas interference on the physical scale is similar in the sexes.

## Evolution of genome-wide recombination rate is dispersed across bivalents, associated with double-strand break number, and connected to crossover interference

We used the contrast between males from high-recombination strains and males from low-recombination strains to identify features of the recombination landscape associated with evolutionary transitions in the genome-wide recombination rate. We considered proportions of bivalents with different numbers of crossovers, double-strand break number, SC length, and crossover positioning.

Ninety-six percent of single bivalents in our pooled dataset (n = 9,576) have either one or two MLH foci (Figure 3). The proportions of single-focus (1CO) bivalents vs. double-focus (2CO) bivalents distinguish high-recombination strains from low-recombination strains (Figure 3). High-recombination strains are enriched for 2CO bivalents at the expense of 1CO bivalents: proportions of 2CO bivalents are 0.33 in *musculusSKIVE*, 0.44 in *musculusPWD* , and 0.51 in *molossinusMSM* (Supplemental Figure 3). Following patterns in the genome-wide recombination rate, male *musculusPWD* and male *molossinusMSM* have 2CO proportions that are more similar to each other than to strains from their own subspecies (chi-square tests; *musculusPWD* vs. *molossinusMSM*: p = 0.37; *musculusPWD* vs. *musculusKAZ*: p = 1.23^{-31}; *molossinusMSM* vs. *molossinusMOLF*: p = 2.65^{-6}). These results demonstrate that evolution of the genome-wide recombination rate reflects changes in crossover number across multiple bivalents.

To begin to localize evolution of genome-wide recombination rate to steps of the recombination pathway, we counted DMC1 foci in prophase spermatocytes as markers for double-strand breaks (DSBs). DMC1 foci were counted in a total of 76 early zygotene and 75 late zygotene spermatocytes from two high-recombination strains (*musculusPWD* and *molossinusMSM*) and three low-recombination strains (*musculusKAZ*, *domesticusWSB* , and *domesticusG*). High-recombination strains have significantly more DMC1 foci than low-recombination strains in early zygotene cells (t-test; p < 10^{-6}). In contrast, the two strain groups do not differ in DMC1 foci in late zygotene cells (t-test; p = 0.66). Since DSBs are repaired as either COs or non-crossovers (NCOs), the ratio of MLH1 foci to DMC1 foci can be used to estimate the proportion of DSBs designated as COs. High-recombination and low-recombination strains do not differ in the MLH1/DMC1 ratio, whether DMC1 foci were counted in early zygotene cells or late zygotene cells (t-test; p > 0.05). These results raise the possibility that the evolution of genome-wide recombination rate is primarily determined by processes that precede the CO/NCO decision, at least in house mice.

Total SC length only partially differentiates high-recombination strains from low-recombination strains (Figure 5). Whereas high-recombination strains as a group have significantly greater total SC length than low-recombination strains (t-test; p = 0.01), separate tests within subspecies show that the two strain categories differ within *M. m. molossinus* (p = 0.02) but not within *M. m. musculus* (p = 0.4). Additionally, mouse means for the reduced (short and long) bivalent datasets do not differ between high-recombination and low-recombination strains (t-test; short: p = 0.88; long: p = 0.19). In a model with total SC length as the dependent variable (?M2?), two subspecies effects are significant (*M. m. musculus* p = 2.33^{-6} , *M. m. molossinus*, p = 21^{-6}). In models with SC lengths of short and long bivalents as dependent variables, several subspecies and strain effects reach significance (p < 0.05), but they are not consistent across models. Collectively, these results reveal that evolution of SC length is not strongly associated with evolution of genome-wide recombination rate in house mice.

High-recombination and low-recombination strains show similar MLH1 focus average positions on bivalents with a single focus, in both the full dataset (t-test; p = 0.68) and in comparisons within subspecies (t-test; *M. m. musculus*: p = 0.39; *M. m. molossinus*: p = 0.22). While *domesticusWSB* and *molossinusMOLF* exhibit strain effects in a model with normalized crossover position as the dependent variable ( ), these two strains do not differ in MLH1 count. These results show that evolution of crossover position on chromosomes with single crossovers is decoupled from evolution of the genome-wide recombination rate, at least at this scale of resolution.

High-recombination strains have greater inter-focal distances than low-recombination strains (t-test; IFDnorm: p = 3.26^{-4} ; IFDraw: p = 2.06^{-4}). This pattern holds within *M. m. musculus* (t-test; IFDnorm: p = 2.04^{-5}; IFDraw: p = 2.19^{-4}), but not within *M. m. molossinus* (IFDnorm: p = 0.89; IFDraw: p = 0.23). Similar results are observed with models treating IFDraw and IFDnorm as dependent variables (?M2?): only effects associated with high-recombination strains are significant (p < 0.05). That IFDraw and IFDnorm show similar patterns eliminates variation in SC lengths (and bivalent sizes) as the primary explanation. The main distinction in IFDnorm distributions between high-recombination and low-recombination strains is an enrichment of IFDnorm values under 30% in low-recombination strains (Supplemental Figure 3). The frequency of IFDnorm values that fall below 30% ranges from 8.2% (*domesticusG*) to 16% (*musculusKAZ*) in low-recombination strains, whereas high-recombination strains show frequencies below 4% (0%, 1.3%, and 3.3% for *musculusSKIVE*, *molossinusMSM*, and *musculusPWD*, respectively). These results indicate that evolution of the genome-wide recombination rate is accompanied by the evolution of crossover interference.

In summary, evolution of the genome-wide recombination rate in males is connected to double-strand break number and crossover interference, but not to SC length and crossover position (on single-crossover bivalents).

# DISCUSSION

By comparing recombination rates in females and males from the same diverse set of genetic backgrounds, we isolated sex as a primary factor in the evolution of this fundamental meiotic trait. Recombination rate differences are more pronounced in males than females. Because inter-strain divergence times are identical for the two sexes, this observation demonstrates that the genome-wide recombination rate evolves faster in males, at least in house mice. More generally, recombination rate divergence is decoupled in females and males. These disparities are remarkable given that recombination rates for the two sexes were measured in identical genomic backgrounds (other than the presence/absence of the Y chromosome). Our results provide the strongest evidence yet that the genome-wide recombination rate follows distinct evolutionary trajectories in males and females.

At the genetic level, the sex-specific evolution we documented indicates that some mutations responsible for divergence in recombination rate have dissimilar phenotypic effects in females and males. A subset of the genetic variants associated with genome-wide recombination rate within populations of humans (Kong et al. 2004; Kong et al. 2008; Kong et al. 2014, Halldorsson et al. 2019), Soay sheep (Johnston et al. 2016), and cattle (Ma et al. 2015; Shen et al. 2018) appear to show sex-specific properties, including opposite effects in females and males. Furthermore, inter-sexual correlations for recombination rate are weak in humans (Fledel-Alon et al. 2011) and Soay sheep (Johnston et al. 2016). Crosses between the strains we surveyed could be used to identify and characterize the genetic variants responsible for recombination rate evolution in house mice (Dumont and Payseur 2011; Wang and Payseur 2017; Wang et al. 2019). These variants could differentially affect females and males at any step in the recombination pathway. **Although our DMC1 profiling was limited to males from a small number of strains (for practical reasons), our findings suggest that mutations that determine the number of double-strand breaks contribute to sex-specific evolution in the recombination rate. A study of two classical inbred strains and one wild-derived inbred strain of house mice also found a positive association between crossover number and double-strand break number in males (Baier et al. 2014).**

Another implication of our results is that the connection between recombination rate and fitness differs between males and females. Little is known about whether and how natural selection shapes recombination rate in nature (Dapper and Payseur 2017; Ritz et al. 2017). Samuk et al. (2020) recently used a quantitative genetic test to conclude that an 8% difference in genome-wide recombination rate between females from two populations of *Drosophila pseudoobscura* was caused by natural selection. Applying similar strategies to species in which both sexes recombine, including house mice, would be a logical next step to understanding the sex-specific evolution of recombination rate.

Population genetic models have been built to explain sexual dimorphism in the number and placement of crossovers, which is a common phenomenon (Brandvain and Coop 2012; Sardell and Kirkpatrick 2020). Modifier models predicted that lower recombination rates in males will result from haploid selection (Lenormand 2003) or sexually antagonistic selection on coding and cis-regulatory regions of genes (Sardell and Kirkpatrick 2020). Another modifier model showed that meiotic drive could stimulate female-specific evolution of the recombination rate (Brandvain and Coop 2012). Although these models fit the conserved pattern of sex differences in crossover positions, they do not readily explain our observations of sex-specific evolution in the genome-wide recombination rate. In particular, the alternation across strains in which sex has more crossovers is unexpected.

We propose an alternative interpretation of our findings based on the cell biology of gametogenesis. During meiosis, achieving a stable chromosome structure requires the attachment of kinetochores to opposite poles of the cell and at least one crossover to create tension across the sister chromosome cohesion distal to chiasmata (Lane and Kauppi 2019; VanVeen and Hawley 2003). The spindle assembly checkpoint (SAC) prevents aneuploidy by ensuring that all bivalents are correctly attached to the microtubule spindle (“bi-oriented”) before starting the metaphase-to-anaphase transition via the release of the sister cohesion holding homologs together (Lane and Kauppi 2019). Hence, selection seems likely to favor mutations that optimize the process of bi-orientation and chromosome separation, thereby prohibiting the SAC from delaying the cell cycle or triggering apoptosis. Multiple lines of evidence indicate that the SAC is more effective in spermatogenesis than in oogenesis (Lane and Kauppi 2019), perhaps due to the presence of the centrosome spindle (So et al. 2019) and higher cell volume (Kyogoku and Kitajima 2017) in oocytes. The higher stringency of the SAC during spermatogenesis suggests that selection will be better at removing mutations that interfere with bi-orientation in males than in females. Therefore, faster male evolution of the genome-wide recombination rate could be driven by the more stringent SAC acting on chromosome structures at the metaphase I alignment.

Our SAC model is consistent with other features of our data. We showed that widespread sex differences in broad-scale crossover positioning (Sardell and Kirkpatrick 2020) apply across house mice, even in lineages where the direction of heterochiasmy is reversed. The number and placement of crossovers affects the area of sister chromosome cohesion distal to crossovers which needs to be released for the first reductional chromosome segregation (VanVeen and Hawley 2003; Lane and Kauppi 2019; Subramanian and Hochwagen 2014; Dumont and Desai 2012). Faster spermatogenesis may select for synchronization of the separation across all homologs within the cell (Kudo ), whereas in oogenesis, the slower cell cycle and multiple arrest stages may require chromosome structures with greater stability on the MI spindle, especially for those organisms that undergo dictyate arrest (Lee 2019).

We propose that the SAC model also can explain the correlated evolution of stronger crossover interference and higher genome-wide recombination rate in male house mice. Our results show that crossovers are spaced further apart in strains enriched for double-crossover bivalents when SC length is taken into account and chromosome size effects are minimized. Assuming chromatin compaction between (prophase) pachytene and metaphase is uniform along bivalents, this increased spacing is expected to expand the area for sister cohesion to connect homologs and may improve the fidelity of chromosomal segregation. While the SAC model postulates direct fitness effects of interference, a modifier model predicted that indirect selection on recombination rate – via its modulation of offspring genotypes – can strengthen interference as well (Goldstein et al. 1993).

Regardless of the underlying mechanism, our results provide a rare demonstration that crossover interference can diverge over short evolutionary timescales. The notion that stronger interference can co-evolve with higher genome-wide recombination rate is supported by differences between breeds of cattle (Ma et al. 2015) and differences between populations of white-footed mice (Peterson et al. 2019). In contrast, mammalian species with stronger interference tend to exhibit lower genome-wide recombination rates (Segura et al. 2013; Otto and Payseur 2019). Collectively, these patterns suggest that inferences about the evolutionary dynamics of interference depend on the timescale under consideration.

Our findings further reveal that evolution of the genome-wide recombination rate does not require major changes in the degree of chromatin compaction. Female house mice consistently show longer SCs, even in strains with more recombination in males. Studies in mice (Lynn et al. 2002; Petkov et al. 2007) and humans (Gruhn et al. 2013; Tease and Hulten 2004) suggest that chromosomal axes are longer (and DNA loops are shorter) in females than males. Some authors have suggested that conserved sex differences in crossover positioning (more uniform placement in females) and interference strength (stronger interference in males) could be due to looser chromatin packing of the meiotic chromosome structure in females (Haenel et al. 2018; Petkov et al. 2007). A cellular model designed to explain interference attributes sexual dimorphism in chromatin structure to greater cell volumes and oscillatory movements of telomeres and kinetochores in oocytes (Hultén 2011). More recent work connects the sparser recombination landscape to sex differences in the crossover repair pathway (S. Wang et al. 2017).

Our conclusions are accompanied by several caveats. First, MLH1 foci only identify interfering crossovers (Holloway et al. 2008). Although most crossovers belong to this class (REF), our approach likely underestimated genome-wide recombination rates. Evolution of the number of non-interfering crossovers is a topic worth examining. A second limitation is that our investigation of crossover locations was confined to the relatively low resolution possible with immunofluorescent cytology. Positioning crossovers with higher resolution could reveal additional evolutionary patterns. Finally, the panel of inbred lines we surveyed may not be representative of recombination rate variation within and between subspecies of house mice. We considered most available wild-derived inbred lines, but house mice have a broad geographic distribution. Nevertheless, we expect our primary conclusion that recombination rate evolves in a sex-specific manner to be robust to geographic sampling because differences between females and males exist for the same set of inbred strains.

While the causes of sex differences in recombination remain mysterious (Lenormand et al. 2016), our conclusions have implications for a wide range of recombination research. For biologists uncovering the cellular and molecular determinants of recombination, our results suggest that mechanistic differences between the sexes could vary by genetic background. For researchers charting the evolutionary trajectory of recombination, our findings indicate that sex-specific comparisons are crucial. For theoreticians building evolutionary models of recombination, different fitness regimes and genetic architectures in females and males should be considered. Elevating sex as a primary determinant of recombination would be a promising step toward integrating knowledge of cellular mechanisms with evolutionary patterns to understand recombination rate variation in nature.

# REFERENCES

Anderson, Lorinda K, Aaron Reeves, Lisa M Webb, and Terry Ashley. 1999. “Distribution of Crossing over on Mouse Synaptonemal Complexes Using Immunofluorescent Localization of Mlh1 Protein.” *Genetics* 151 (4): 1569–79.

Baier, Brian, Patricia Hunt, Karl W Broman, and Terry Hassold. 2014. “Variation in Genome-Wide Levels of Meiotic Recombination Is Established at the Onset of Prophase in Mammalian Males.” *PLoS Genetics* 10 (1).

Bates, Douglas, Martin Mächler, Ben Bolker, and Steve Walker. 2015. “Fitting Linear Mixed-Effects Models Using lme4.” *Journal of Statistical Software* 67 (1): 1–48. <https://doi.org/10.18637/jss.v067.i01>.

Brandvain, Yaniv, and Graham Coop. 2012. “Scrambling Eggs: Meiotic Drive and the Evolution of Female Recombination Rates.” *Genetics* 190 (2): 709–23.

Dapper, Amy L, and Bret A Payseur. 2017. “Connecting Theory and Data to Understand Recombination Rate Evolution.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 372 (1736): 20160469.

Dumont, Beth L, and Bret A Payseur. 2011. “Genetic Analysis of Genome-Scale Recombination Rate Evolution in House Mice.” *PLoS Genetics* 7 (6).

Dumont, Julien, and Arshad Desai. 2012. “Acentrosomal Spindle Assembly and Chromosome Segregation During Oocyte Meiosis.” *Trends in Cell Biology* 22 (5): 241–49.

Fledel-Alon, Adi, Ellen Miranda Leffler, Yongtao Guan, Matthew Stephens, Graham Coop, and Molly Przeworski. 2011. “Variation in Human Recombination Rates and Its Genetic Determinants.” *PloS One* 6 (6).

Goldstein, David B, Aviv Bergman, and Marcus W Feldman. 1993. “The Evolution of Interference: Reduction of Recombination Among Three Loci.” *Theoretical Population Biology* 44 (2): 246–59.

Gruhn, Jennifer R, Carmen Rubio, Karl W Broman, Patricia A Hunt, and Terry Hassold. 2013. “Cytological Studies of Human Meiosis: Sex-Specific Differences in Recombination Originate at, or Prior to, Establishment of Double-Strand Breaks.” *PloS One* 8 (12).

Haenel, Quiterie, Telma G Laurentino, Marius Roesti, and Daniel Berner. 2018. “Meta-Analysis of Chromosome-Scale Crossover Rate Variation in Eukaryotes and Its Significance to Evolutionary Genomics.” *Molecular Ecology* 27 (11): 2477–97.

Halldorsson, Bjarni V, Gunnar Palsson, Olafur A Stefansson, Hakon Jonsson, Marteinn T Hardarson, Hannes P Eggertsson, Bjarni Gunnarsson, et al. 2019. “Characterizing Mutagenic Effects of Recombination Through a Sequence-Level Genetic Map.” *Science* 363 (6425): eaau1043.

Holloway, J Kim, James Booth, Winfried Edelmann, Clare H McGowan, and Paula E Cohen. 2008. “MUS81 Generates a Subset of Mlh1-Mlh3–Independent Crossovers in Mammalian Meiosis.” *PLoS Genetics* 4 (9).

Hultén, Maj A. 2011. “On the Origin of Crossover Interference: A Chromosome Oscillatory Movement (Com) Model.” *Molecular Cytogenetics* 4 (1): 10.

Johnston, Susan E, Camillo Bérénos, Jon Slate, and Josephine M Pemberton. 2016. “Conserved Genetic Architecture Underlying Individual Recombination Rate Variation in a Wild Population of Soay Sheep (Ovis Aries).” *Genetics* 203 (1): 583–98.

Koehler, Kara E, Jonathan P Cherry, Audrey Lynn, Patricia A Hunt, and Terry J Hassold. 2002. “Genetic Control of Mammalian Meiotic Recombination. I. Variation in Exchange Frequencies Among Males from Inbred Mouse Strains.” *Genetics* 162 (1): 297–306.

Kong, Augustine, John Barnard, Daniel F Gudbjartsson, Gudmar Thorleifsson, Gudrun Jonsdottir, Sigrun Sigurdardottir, Bjorgvin Richardsson, et al. 2004. “Recombination Rate and Reproductive Success in Humans.” *Nature Genetics* 36 (11): 1203–6.

Kong, Augustine, Gudmar Thorleifsson, Michael L Frigge, Gisli Masson, Daniel F Gudbjartsson, Rasmus Villemoes, Erna Magnusdottir, Stefania B Olafsdottir, Unnur Thorsteinsdottir, and Kari Stefansson. 2014. “Common and Low-Frequency Variants Associated with Genome-Wide Recombination Rate.” *Nature Genetics* 46 (1): 11.

Kong, Augustine, Gudmar Thorleifsson, Hreinn Stefansson, Gisli Masson, Agnar Helgason, Daniel F Gudbjartsson, Gudrun M Jonsdottir, et al. 2008. “Sequence Variants in the Rnf212 Gene Associate with Genome-Wide Recombination Rate.” *Science* 319 (5868): 1398–1401.

Kyogoku, Hirohisa, and Tomoya S Kitajima. 2017. “Large Cytoplasm Is Linked to the Error-Prone Nature of Oocytes.” *Developmental Cell* 41 (3): 287–98.

Lane, Simon, and Liisa Kauppi. 2019. “Meiotic Spindle Assembly Checkpoint and Aneuploidy in Males Versus Females.” *Cellular and Molecular Life Sciences* 76 (6): 1135–50.

Lee, Jibak. 2019. “Is Age-Related Increase of Chromosome Segregation Errors in Mammalian Oocytes Caused by Cohesin Deterioration?” *Reproductive Medicine and Biology*.

Lenormand, Thomas. 2003. “The Evolution of Sex Dimorphism in Recombination.” *Genetics* 163 (2): 811–22.

Lenormand, Thomas, Jan Engelstädter, Susan E Johnston, Erik Wijnker, and Christoph R Haag. 2016. “Evolutionary Mysteries in Meiosis.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 371 (1706): 20160001.

Lynn, Audrey, Kara E Koehler, LuAnn Judis, Ernest R Chan, Jonathan P Cherry, Stuart Schwartz, Allen Seftel, Patricia A Hunt, and Terry J Hassold. 2002. “Covariation of Synaptonemal Complex Length and Mammalian Meiotic Exchange Rates.” *Science* 296 (5576): 2222–5.

Ma, Li, Jeffrey R O’Connell, Paul M VanRaden, Botong Shen, Abinash Padhi, Chuanyu Sun, Derek M Bickhart, et al. 2015. “Cattle Sex-Specific Recombination and Genetic Control from a Large Pedigree Analysis.” *PLoS Genetics* 11 (11).

Otto, Sarah P, and Bret A Payseur. 2019. “Crossover Interference: Shedding Light on the Evolution of Recombination.” *Annual Review of Genetics* 53: 19–44.

Peters, Antoine HFM, Annemieke W. Plug, Martine J. van Vugt, and Peter De Boer. 1997. “SHORT COMMUNICATIONS A Drying-down Technique for the Spreading of Mammalian Meiocytes from the Male and Female Germline.” *Chromosome Research* 5 (1): 66–68.

Peterson, April L, Nathan D Miller, and Bret A Payseur. 2019. “Conservation of the Genome-Wide Recombination Rate in White-Footed Mice.” *Heredity* 123 (4): 442–57.

Petkov, Petko M, Karl W Broman, Jin P Szatkiewicz, and Kenneth Paigen. 2007. “Crossover Interference Underlies Sex Differences in Recombination Rates.” *Trends in Genetics* 23 (11): 539–42.

Ritz, Kathryn R, Mohamed AF Noor, and Nadia D Singh. 2017. “Variation in Recombination Rate: Adaptive or Not?” *Trends in Genetics* 33 (5): 364–74.

Samuk, Kieran, Brenda Manzano-Winkler, Kathryn R Ritz, and Mohamed AF Noor. 2020. “Natural Selection Shapes Variation in Genome-Wide Recombination Rate in Drosophila Pseudoobscura.” *Current Biology*.

Sardell, Jason M., and Mark Kirkpatrick. 2020. “Sex Differences in the Recombination Landscape.” *The American Naturalist* 195 (2): 361–79. <https://doi.org/10.1086/704943>.

Scheipl, Fabian, Sonja Greven, and Helmut Kuechenhoff. 2008. “Size and Power of Tests for a Zero Random Effect Variance or Polynomial Regression in Additive and Linear Mixed Models.” *Computational Statistics & Data Analysis* 52 (7): 3283–99.

Segura, Joana, Luca Ferretti, Sebastián Ramos-Onsins, Laia Capilla, Marta Farré, Fernanda Reis, Maria Oliver-Bonet, et al. 2013. “Evolution of Recombination in Eutherian Mammals: Insights into Mechanisms That Affect Recombination Rates and Crossover Interference.” *Proceedings of the Royal Society B: Biological Sciences* 280 (1771): 20131945.

Shen, Botong, Jicai Jiang, Eyal Seroussi, George E Liu, and Li Ma. 2018. “Characterization of Recombination Features and the Genetic Basis in Multiple Cattle Breeds.” *BMC Genomics* 19 (1): 304.

So, Chun, K Bianka Seres, Anna M Steyer, Eike Mönnich, Dean Clift, Anastasija Pejkovska, Wiebke Möbius, and Melina Schuh. 2019. “A Liquid-Like Spindle Domain Promotes Acentrosomal Spindle Assembly in Mammalian Oocytes.” *Science* 364 (6447): eaat9557.

Subramanian, Vijayalakshmi V, and Andreas Hochwagen. 2014. “The Meiotic Checkpoint Network: Step-by-Step Through Meiotic Prophase.” *Cold Spring Harbor Perspectives in Biology* 6 (10): a016675.

Team, RStudio. 2015. “RStudio: Integrated Development Environment for R.” Boston, MA. <http://www.rstudio.com>.

Tease, C, and MA Hulten. 2004. “Inter-Sex Variation in Synaptonemal Complex Lengths Largely Determine the Different Recombination Rates in Male and Female Germ Cells.” *Cytogenetic and Genome Research* 107 (3-4): 208–15.

VanVeen, J Edward, and R Scott Hawley. 2003. “Meiosis: When Even Two Is a Crowd.” *Current Biology* 13 (21): R831–R833.

Wang, Richard J, Beth L Dumont, Peicheng Jing, and Bret A Payseur. 2019. “A First Genetic Portrait of Synaptonemal Complex Variation.” *PLoS Genetics* 15 (8): e1008337.

Wang, Richard J, and Bret A Payseur. 2017. “Genetics of Genome-Wide Recombination Rate Evolution in Mice from an Isolated Island.” *Genetics* 206 (4): 1841–52.

Wang, Shunxin, Terry Hassold, Patricia Hunt, Martin A White, Denise Zickler, Nancy Kleckner, and Liangran Zhang. 2017. “Inefficient Crossover Maturation Underlies Elevated Aneuploidy in Human Female Meiosis.” *Cell* 168 (6): 977–89.