Higher Intercellular Variation in Genome-Wide Recombination Rate in Female Mice

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Keywords

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Abstract

Meiotic recombination affects fertility, shuffles genomes, and modulates the effectiveness of natural selection. Despite conservation of the recombination pathway, the rate of recombination varies among individuals and along chromosomes. Recombination rate also differs among cells from the same organism, but this form of variation has received less attention. To identify patterns that characterize intercellular variation in the genome-wide recombination rate, we counted foci of the MLH1 recombination-associated protein in oocytes and spermatocytes from a panel of wild-derived inbred strains of house mice. Females show higher intercellular variation in MLH1 focus count than males from the same inbred strains. This pattern is consistent across strains from multiple subspecies, including 2 strains in which the average MLH1 focus count is higher in males. The sex difference in genome-wide recombination rate we report suggests that selection targeting recombination rate will be more efficient in males than in females.

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Introduction

Crossovers between homologous chromosomes result from a tightly regulated and coordinated series of steps during meiosis [Bergero et al., 2021]. Cells orchestrate the complex processes of replication, double-strand break generation, homology searching, synapsis, and DNA repair to achieve reciprocal exchanges among homologs [Lenormand et al., 2016; Bergero et al., 2021]. Despite cellular constraints on recombination, including a requirement of at least one crossover per bivalent [Bergero et al., 2021], the number of crossovers an organism produces during meiosis varies from one cell to the next [Ottolini et al., 2015; Wang et al., 2017, 2019; Bell et al., 2020]. This intercellular variation in the genome-wide recombination rate persists in inbred organisms with homozygous genomes [Koehler et al., 2002; Baier et al., 2014].

Sex is one biological factor expected to shape intercellular variation in recombination rate. In many species, males show higher recombination rates near telomeres, whereas crossover locations in females tend to be more uniform [Sardell and Kirkpatrick, 2020]. Some comparisons indicate that crossover interference is stronger in males [Otto and Payseur, 2019]. The relatively terminal

placement of crossovers and stronger interference effectively homogenize crossover number across chromosomes of different sizes [Petkov et al., 2007], suggesting that spermatocytes should vary less than oocytes in recombination rate.

Another potential cause of higher intercellular variation in crossover number in females is relaxed constraints on aneuploidy during oogenesis [Morelli and Cohen, 2005]. The 2 main sources of aneuploid gametes are the generation of chromosome pairs with a higher propensity for mis-segregation and the failure to detect these chromosomes via checkpoints [Lane and Kauppi, 2019]. In mammalian oogenesis, meiotic arrest and age-related loss of sister cohesion at the centromeres or chromosome arms increases the risk of mis-segregation of chromosomes at metaphase I [Lane and Kauppi, 2019]. The number and placement of crossovers determines the amount of continuous cohesion between sister chromatids once the crossovers resolve to chiasmata [Maguire, 1980]. As a result, certain crossover configurations can generate “vulnerable” chromosomes within oogenesis.

Although sex differences in the average recombination rate are well-documented [Lenormand and Dutheil, 2005; Brandvain and Coop, 2012], sex differences in intercellular variation in recombination rate within individuals have garnered less attention [Ottolini et al., 2015; Wang et al., 2017, 2019]. Cytological studies show that oocytes vary more than spermatocytes in crossover number in humans [Sun et al., 2006; Cheng et al., 2009; Gruhn et al., 2013] and mice [Lynn et al., 2005]. Analyses of human pedigrees reveal higher variation in the number of maternal crossovers than in the number of paternal crossovers [Broman et al., 1998]. Wider confidence intervals for autosomal map lengths in female humans [Coop et al., 2008] and female mice [Liu et al., 2014] are also consistent with higher intercellular variation among oocytes.

Despite this progress, the hypothesis that genome-wide recombination rates vary more in oogenesis than in spermatogenesis has yet to be evaluated across a common set of diverse genetic backgrounds. To fill this gap, we measured within-individual recombination rate variation among oocytes and among spermatocytes from wild-derived inbred strains from multiple subspecies of house mice.

Materials and Methods

To compare intercellular variation in genome-wide recombination rate between the sexes, we used immunohistochemistry to count foci of the MLH1 crossover-associated protein [Anderson

et al., 1999; Koehler et al., 2002] along the synaptonemal complex in oocytes and spermatocytes from a common set of inbred strains. We surveyed house mice from wild-derived inbred strains, including 3 strains of *Mus musculus domesticus* (WSB, LEWES, and Gough Island), 3 strains of *M. m. musculus* (PWD, SKIVE, and KAZ), and 1 strain of *M. m. molossinus* (MSM) (subsequently abbreviated as *subspecies*^{STRAIN}). Collectively, these strains represent diverse genomic backgrounds, sampling variation within and between subspecies over a timespan of approximately 500,000 years of evolution [Gerald et al., 2008]. All data analyzed for this paper were collected by Peterson and Payseur [2021]. Briefly, pachytene nuclei were staged based on morphology of the lateral element of the synaptonemal complex (SYCP3). Nuclei with the correct complement of fully synapsed bivalents were chosen for MLH1 quantification. MLH1 foci on the XY were not included in this dataset. A quality score (1–4) was assigned to each cell based on uniformity of antibody staining and evenness of bivalent spreading. Details about mice, tissue collection, immunohistochemistry, and imaging can be found in Peterson and Payseur [2021].

To quantify variation among cells in the genome-wide recombination rate, we computed the standard deviation (SD) of MLH1 focus count among cells for each mouse. To account for differences in mean genome-wide recombination rate among mice, we also computed the coefficient of variation (CV) in MLH1 focus count among cells for each mouse.

We used 2 approaches to analyze intercellular variation in MLH1 focus count. First, we conducted separate permutation tests for each strain comparing the observed sex difference in SD (or CV) within a strain to the distribution of sex difference in SD (or CV) across 10,000 permuted datasets in which sex was randomized. We conducted separate permutation tests that either computed SD (or CV) among pooled cells across mice or computed average SD (or CV) among mice from the same strain and sex. Second, we constructed linear mixed models in which the SD (or CV) of MLH1 focus count for individual mice was treated as the dependent variable, sex was treated as a fixed effect, subspecies was treated as a fixed effect, and strain was treated as a random effect.

To examine the potential effects of experimental error, we constructed a “filtered” data subset that passed thresholds for cell image quality and sample size. We assigned each cell image a quality score between 1 (best) and 4 (worst) based on the spread of bivalents and the brightness of immunohistochemistry staining. We retained cells with quality scores of 1 or 2 and we removed mice with fewer than 7 cells that met this criterion (the *musculus*^{SKIVE} strain was removed from these analyses as a result). The full, unfiltered dataset contained MLH1 focus counts from 1,412 oocytes taken from 73 females and 1,696 spermatocytes taken from 74 males across 7 strains representing 3 subspecies. The filtered dataset contained MLH1 focus counts from 562 oocytes taken from 40 females and 838 spermatocytes taken from 52 males across 6 strains representing 3 subspecies.

Data files, analysis scripts, and other supplemental materials are available in FigShare and in a public github repository (<https://github.com/petersoapes/MeioticRecombSexualDimorphism>). Because of their large sizes, image files are hosted on the NSF CyVerse (<https://de.cyverse.org/de/>) and can be shared upon request.

Fig. 1. Within-mouse standard deviation in MLH1 focus count. **A** Full dataset. **B** Filtered dataset. Each point represents a mouse, with circles denoting females and triangles denoting males. Point size is scaled by the number of cells sampled per mouse. Horizontal lines show mean standard deviation.

Results

Females vary more than males in MLH1 focus count in all 7 strains (Fig. 1a). The two-tailed binomial probability of observing this result if females and males are equally likely to vary more is 0.016. Separate permutation tests pooling cells across mice show significantly higher female SD and CV in every strain (Fig. 2). Permutation tests at the mouse level show significantly higher female SD in all strains except *domesticus*^{WSB} and significantly higher female CV in all strains except *musculus*^{PWD} (Fig. 2).

To examine the contribution of technical error to this pattern, we repeated the permutation tests on a dataset filtered for quality (see Materials and Methods). Filtering reduces the SD of MLH1 focus count for most sex-by-strain combinations (Fig. 3). Filtering increases the difference between female SD and male SD in 3 strains (*domesticus*^{WSB}, *domesticus*^{LEW}, and *musculus*^{SKIVE}) and decreases the difference in 3 strains (*domesticus*^G, *musculus*^{KAZ}, and *musculus*^{PWD}) (Fig. 3). Nevertheless, females retain higher SD and higher CV in all strains in the filtered dataset (Fig. 1b), with permutation tests showing significant differences in most comparisons (online suppl. Table 1; for online suppl. material, see www.karger.com/doi/10.1159/516998). The few cases in which sex differences are not significant in the filtered dataset may be attributed to reduced sample sizes (cells and/or mice) or mouse effects on quality scores.

In mixed models analyzing the full dataset, sex is the only variable showing a significant effect on SD (and

CV) of MLH1 focus count (Table 1). In mixed models considering the filtered dataset, sex, strain, and subspecies significantly affect the SD (and CV). There is also a significant sex-by-subspecies interaction in the filtered dataset, suggesting that sex affects intercellular variation in recombination rate differently in the 3 subspecies.

The strains we examined exhibit evolved differences in the mean genome-wide recombination rate, including greater recombination in males (an unusual pattern for house mice) in *musculus*^{PWD} and *molossinus*^{MSM} [Peterson and Payseur, 2021]. If the number of crossovers follows a Poisson distribution, we would expect mice and strains with higher means to show higher intercellular variation. To consider the contribution of variation in the mean genome-wide recombination rate to differences in intercellular variation in the genome-wide recombination rate, we compared the SD of MLH1 focus count and the mean of MLH1 focus count across mice. These quantities are positively correlated (Fig. 4). The correlation is stronger in females (Spearman's $r = 0.562$; $p = 2.63E-7$) than in males ($r = 0.369$; $p = 0.001$), perhaps because of a wider female range in intercellular SD. Like males from the other strains, males belonging to strains *musculus*^{PWD} and *molossinus*^{MSM} retain lower levels of intercellular variation for genome-wide recombination rate compared to females, suggesting that mean genome-wide recombination rate is not the primary driver of sex differences in intercellular variation.

Fig. 2. Permutation tests for 4 sets of analyses. For each strain, results for the standard deviation (top) and coefficient of variation (bottom) are shown. Red vertical line reflects the observed sex difference. Black shows the distribution of the sex difference in 10,000 datasets with sex randomly permuted.

Fig. 3. Differences in standard deviations between datasets. Strain average standard deviation in both datasets (full and filtered) for females (**A**) and males (**B**). **C** Sex differences in standard deviation in both datasets.

Table 1. Results from linear mixed models

Dataset	Metric	Subspecies	Sex	Subspecies*Sex	Strain
Full	SD	0.4521	1.44e-10	0.4763	0.0914
Full	CV	0.8425	6.80e-09	0.6134	0.0932
Filtered	SD	0.0143	1.41e-16	0.0058	0.0050
Filtered	CV	0.2685	3.46e-14	0.3995	0.0170

Standard deviation (SD) and coefficient of variation (CV) served as the dependent variables. *p* values are listed for sex (fixed effect), subspecies (fixed effect), subspecies*sex (fixed effect), and strain (random effect).

Discussion

Our results indicate that higher intercellular variation in genome-wide recombination rate among females is a conserved pattern across house mice from diverse genetic backgrounds. Males and females from the same inbred strain have identical genomes (other than the set of sex chromosomes), suggesting that this sex difference is caused by non-genetic factors. In mammals, each step of the meiotic program varies more during oogenesis than spermatogenesis [Morelli and Cohen, 2005]. Our results could be explained by higher variation among oocytes in any of the processes that lead to crossovers. Reduced

stringency of the meiotic checkpoint in females compared to males [Powers et al., 2020] could be an important contributor.

Over-dispersion of crossover number among cells has been attributed to co-variation between axis length and crossover count across chromosomes in the same cell and cell-wide regulation of axis length [Wang et al., 2017; Bell et al., 2020]. In one study, women and men differed in intercellular variation in crossover number, but not in variation in compaction of the synaptonemal complex nor the density of double-strand breaks [Wang et al., 2017]. This pattern suggests that a lower maturation rate of crossovers in females could be the source of higher in-

Fig. 4. Correlation between standard deviation of MLH1 focus count and mean of MLH1 focus count. Each point denotes a mouse, with strain indicated by colour. r = Spearman's correlation.

tercellular variation in recombination rate [Wang et al., 2017], at least in humans. Data from more species are needed to establish the generality of this interpretation.

Although we attempted to reduce technical error by filtering for cell quality and repeating analyses, caveats accompany our findings. In particular, heterogeneity in MLH1 loading extends for a longer period in oogenesis than in spermatogenesis [Lipkin et al., 2002]. This phenomenon could inflate intercellular variation in females relative to males. We note that pedigree-based studies that do not rely on MLH1 foci also suggest higher intercellular variation in recombination rate in females [Broman et al., 1998; Coop et al., 2008], in support of our main conclusion.

Sex differences in intercellular variation could affect the evolution of recombination rate. If selection targets recombination in a sex-specific manner, selection would shift recombination rate more effectively in the sex with lower intercellular variation. Although the degree to which the recombination rate is shaped by selection is unclear [Ritz et al., 2017], the lower intercellular variation in males we found matches the pattern of comparatively rapid evolution of recombination rate in males we inferred from the same strains [Peterson and Payseur, 2021].

Our results also might have implications for inheritance. Simulations based on human sex-specific maps

show that genomic regions that are identical-by-descent (IBD) are expected to be more numerous and shorter in maternal half-siblings than in paternal half-siblings [Cabrero et al., 2019]. Higher intercellular variation in recombination rate among oocytes than among spermatoocytes has the potential to affect patterns of IBD sharing among relatives as well.

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Statement of Ethics

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee in the School of Medicine and Public Health at the University of Wisconsin-Madison (approval number M005388-R01).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.L.P. and B.A.P. designed the study, A.L.P. conducted the analyses, and A.L.P. and B.A.P. wrote the manuscript.

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