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# Material Methods Draft v1

### Mouse Husbandry

To access natural genetic variation for *Mus musculus*, wild derived inbred strains were used. The *Mus musculus* strains PWD/PhJ, PERC/Eij, WSB/EiJ, LEWE/EiJ, MSM/MsJ, MOLF/EiJ, CAST/EiJ, CZECHII/EiJ were purchased from Jackson labs (Maine, USA). The strains of KAZ/TUA, TOM/TUA, AST/TUA, and HMI/TUA were generated from frozen embryos purchased from Biological Resource Center (BRC) at Riken (Ibaraki, Japan). (<https://en.brc.riken.jp>) and rederived by the UW-Biotech centre (Madison UW). The related murid species *Mus caroli* CAROLI/EiJ and *Mus spretus* SPRET/EiJ were purchased from Jackson labs (<https://www.jax.org>) and *Mus spicilegus* SPIC/Eij from Riken. We use abriviated superscript notation for the remainder of the paper (e.g. *musculusWSB*). All mice were housed UW-Madison Biotech and MSC facilities following RARC approved protocols. A breeding colony of wild derived *Mus musculus* mice sampled from Gough Island (G) is maintained at UW Veterinary school facilities.

### Tissue Collection and Immunohistochemistry

The same dry-down spread technique was applied to both spermatocytes and oocytes based on (Peters et al. 1997), with adjustment for volumes. Spermatocyte spreads were collected and prepared as described in (Peterson, Miller, and Payseur 2019). The majority of mice used MLH1 quantification were from mice between 5 and 12 weeks, while juvillne mice ageed between 12 to 15 days were used for DMC1 quantification. Both pairs of ovaries were collected from embryo or neonate mice between 16 to 21 embyronic days and 0 to 48 hours old respctively.

Whole testes and decapsulated overaires were incubated in 3ml or 300ul of hypotonic solution for 45min respectively. 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 imeediantly\*\*

The **immunohistochemistry**

To visualize the strucutre of meiotic chromosomes we used antibody markers for the centromere (CREST) and lateral element of of the SC (SYCP3). Double strand breaks (DSB) and COs were visualized with DMC1 and MLH1 respectively. The staining protocol was based on that in (Anderson et al. 1999) and (Koehler et al. 2002). Antibodies and slide blocking was performed in 1X antibody dilution buffer (ADB) (normal donkey serum (Jackson ImmnuoResearch), 1X PBS, bovine serum albumin (Sigma), 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 degrees. 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 degrees. Slides were fixed with Prolong Gold Antifade (Invitrogen) for 24 hours after a final wash in 1x PBS.

### Image Processing

Images were capture using a Zeiss Axioplan 2 microscope with AxioLab camera and Axio Vision 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 or DMC1 foci using photoshop.

### Statistical Analysis

We tested for **effects** on mean MLH1 counts per cell; across mulitple mouse rooms, ages of mature males and differnces between embryos and neonates. No effects were found (Supplemental section).

Mean **DMC1** foci per cell for a single mouse from a subset of srains *musculusPWD*, *musculusMSM*, *domesticusWSB*, and *domesticusG* was quantified.

SC morphology and CREST/centromere number was used to stage spermatocytes **Certeria for early and late zygotene** - (bright staining - ) early zygotene defined as seperate centromere pairing – mostly complete axis signal. late zygotene judged as – low degree of centromere pairing – and more homologous pairing (but not fully synapsis). Differences in DMC1 counts per cell were tested by ttests.

**certeria** MLH1 foci were quantified from cells with intact and complete karyotypes (19 acrocentric bivalents and XY for spermatocytes or 20 acrocentric bivalents for oocytes) and distinct MLH1 foci. A quality score, between 1 to 5, was assigned to each cell based on staining quality and general spread of bivalents. With scores of 1 and 2 having the highest quality. Cells and coded as a score of 5 were excluded from the final analysis. The distributions of MLH1 counts per cell was assessed for normality (supplemental figure). MLH1 foci located on the XY in spermatocytes were excluded from this study.

**M1 Mixed Model**

**M2 Linear Model**

**M3 Linear Model**

**M4 Linear Model**

**M4.2 Linear Model**

Straight forward comparisons of groups (strain, sex) were done through t.tests().

We built the full mixed model (**M1**) to interpret the patterns of variation using the lmer() from the lmer4 package (cite) in R (v3.5.2)(Team 2015). Strain was coded as a random effect and tested using exactRLRT() to reflect a random sample of natural genetic variation from each subspecies. The main effects, subspecies, strain, and interaction were coded as fixed and tested by a chi-square test comparing the full and reduced models using drop1() and anova().

This model allows us to estimate and test the fixed effects (sex and subsp), and variation in mean MLH1 counts due to strain effect. We use the subspecies term as a way to quantify between group variation (or divergence) and the random strain effect to quantify within group variation (or polymorphism) in a sex specific manner. The remaining general linear models, (**M2-M3**), were used to follow up on results from the full mixed model. The general linear models, **M4** were used to analyze sex specific data sets.

Our goal was to design an analytical framework which accounts for the nested nature across categories across all the individual mice (e.g. sex, strain subspecies) and can be applied to multiple (meiotic traits) dependent variables. In addition to the MLH1 count variables for individual mice, mean MLH1 count per cell, variance and coefficient of variance for MLH1 count per cell, we analyzed SC based features: mouse average of total SC per cell, mouse average of single SC length, mouse mean IFDnorm and IFDraw for double crossover chromosomes, and the average position of crossover for single crossover bivalents.

These traits were choosen because they are markers of phenomena in the recombinaiton pathway and provide more detailed information about the recombination landscape. In order to examine crossover interference, inter-focal distance (IFD) was measured from the single bivalents containing two crossovers. Normalized measures were calculated by the position divided by the length of the SC for that single bivalent.

To characterize the SC lengths across bivalents, two image analysis algorithms were used to quantify SC area for individual cells (Wang et al. 2019) and for individual bivalents (Peterson, Miller, and Payseur 2019). Both apply a ‘skeletonizing’ transformation to the synapsed chromosomes which produces a single pixel wide ‘trace’ of the original chromosome shape. Hand measures of SC/pactyene chromosomes were performed by using ImageJ/Fiji (v1.52) (Schindelin et al. 2012).

The **total SC** per cell was quantified from the pachtyene cell images. To remove erroneous SC isolation, outliers were visually assessed at the mouse level and removed from the data set. Mouse means were calculated from cell-wide total SC lengths in 2984 out of 3680 cells with MLH1 counts (Figure X).

The **DNA CrossOver** algorithm isolates single straightened bivalents/chromosomes shapes from an image, and returns SC length, and location of green and blue signal, reflecting MLH1 foci and cetromere signal respectively. This algorithm substantially speeds the accurate measurement of bivalents, but has the limitation that not all bivalents per cell can be isolated due to overlapping bivalents. In this data set, isolation rates per cell (the number of all bivalents per cell) range from 0.5076364 (molossinusMSM male) to 0.7158857 (musculusKAZ female).

A curation step was applied to the total single bivalent data set to remove poor measures from the algorithm. From the total set of cell images, 10458 bivalent objects were isolated by the image analysis software. After a human curation step , 9829 single-bivalent observations remained. The accuracy of the algorithm is high compared to hand measures after this curation step ((Peterson, Miller, and Payseur 2019))).

From the curated single bivalent data set the **proportions** of bivalents by crossover number were quantified and tested by chi-square tests with the prop.test().

**REDUCED DATASET**

To account for confounding effects of sex chromosomes from pooled samples of bivalents, we 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. The ‘short bivalent’ data set included the four or five shortest bivalents, and excluded the X bivalent in oocytes. A total of 678 – 678 ‘short’ bivalents were isolated from 103 – 103 oocytes and 37 – 37 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 calculated as above the 4th quartille in SC lengths per cell.

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