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# Material Methods Draft v1

ToDo; Bret’s suggestion for moving thing to Mat and Methods

-House mouse used as the model system for studying heterochiasmy and recombination rate evolution -- both types of gametes looked at in different animals in the same genetic background (individual average (across many cells)

(gwRR = MLH1 number per cell is a good estimate of gwRR)

### Mouse Husbandry

-Highlight the 3 sbspecies (evo history?)

**-separate the list by subspecies**

**-not all strains rendered oocyte data**

To access natural genetic variation for *Mus musculus* a panel of 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 center (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 abbreviated 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

Combine the age information for both sexes

~~Spermatocyte spreads were collected and prepared as described in (another citation?) (Peterson, Miller, and Payseur 2019)~~

The majority of male mice used were between 5 and 12 weeks for MLH1 and juvenile mice (12 to 15 days) for DMC1 spreads. The majority of oocyte tissue was collected from neonate mice between 5 to 48 hours old.

Meiocyte spreads were made following (Peters et al. 1997) - and was similar for both gonads – adjusted for volumes. Whole testes and decapsulated overaires were incubated in 3ml or 300ul of hypotonic solution for 45min respectively. After incubation, gonads were transferred to sucrose solution for mastication. 15ul of cell slurry was transferred to 80ul of 2% PFA solution. Cells were fixed in this solution and dried in a humid chamber at room temperature overnight. The next morning, slides were treated with a photoflow wash (Kodak, cite).

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

**~~Staining / Immunohistochemistry~~**

Meiocyte spread slides were incubated following the antibody protocol / Immunohistochemistry ~~was based on that~~ in Anderson et al. (1999) and Koehler et al. (2002). To visualize chromosomes in the meiocytes we used markers for the centromere (CREST) and lateral element of of the SC (SYCP3). Double strand breaks (DSB) or COs were visualized with DMC1 and MLH1 respectively.

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) ).

Each slide was blocked for 30 minutes in ADB then incubated with 60ul of primary antibody master mix for 48 hours at 37 degrees. For each master mix 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) were 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 of cells 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 (cite).

### Analysis

### (DMC1)

The mean of DMC1 for spermatocytes from 2 prophase stages – was quantified from X strains

The means of **DMC1** foci per cell from a single mouse from a subset of strains *musculusPWD*, *musculusMSM*, *domesticusWSB*, and *domesticusG* were tested by t-tests. SC morphology was used to stage spermatocytes in zygotene, leptotene, or pachtyene (following X).

### (MLH1) / general analytical framework (for mouse averages)

For **MLH1** pachtyene quantification, cells with a full karyotype (in males/spermatocytes; 19 acrocentric and XY bivalent and in oocytes 20 acrocentric bivalents), distinct foci, and intact bivalents were included for quantification. A Quality score 1 to 5 was assigned based on foci brightness /staining and spreading of bivalents. The distributions of MLH1 counts per cell was assessed for normality (supplemental figure).

**Background for setting up/organizing models --** (heiarchal nature of multiple strains within subspecies -- ) – hierarchical nature of categories for inviual mouse averages ()

(primary focus is mean MLH1 count per cell – at the mouse observation level – apply to more meiocyte traits)

Our goal -- design an utilize a mixed model for our analytical framework – which leverages / accounts for the hierarchical/nested nature of categories across all the individual mice (e.g. sex, strain subspecies).

~~We built our model to predict the strain average MLH1 count per cell. The~~ main effects and their interaction of subspecies and sex were coded as fixed. While, strain (or genetic background) was coded as a random effect to reflect a random sample of natural genetic variation from each subspecies. A strains to have a specific sex effect was also included.

We applied the following models to analyze several dependent variables: mouse average mlh1 count per cell, the mouse average of variance in crossover count per cell, mouse mean SC length, mouse mean IFDnorm and IFDraw, and X.

**M1. Mixed Model**

**M2. Linear Model**

**M3. Linear Model**

**M4. Linear Model**

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). From the mixed model the estimates of heterochiasmy (sexual dimorphism) are a combination of subspecies and strain specific sex effects.

### Single bivalent charaecterization

To quantify the SC area of indiviual cells and single chromosomes we applied the approaches discribed in (Wang et al. 2019) and (Peterson, Miller, and Payseur 2019) respectively. Both which apply a ‘skeletonizing’ transformation to the synapsed chromosomes, which transforms the elongatde chromosome space into a single pixel wide ‘trace’ of the originial chromosom shape. Hand measures of SC/pactyene / meiotic chromosomes generated by using ImageJ/Fiji (v1.52) (Schindelin et al. 2012).

##### total SC - Wang

As an additional metric of chromatin compaction, we computed the total (summed) SC length of all bivalents for single cells, using the image analysis algorithm of (Wang et al. 2019). Mouse means were calculated from cell-wide total SC lengths in 2,984 – 2984 out of 3,680 – 3680 cells with MLH1 counts (Figure X). the mouse average total sc was applied to the model framework.

##### DNACrossover

The DNACrossover algorithm is hosted on CyVerse, more detailed description in Peterson, Miller, and Payseur (2019). The DNA CrossOver algorithm isolated single chromosomes shapes from an image, in addition to returning the SC length and also measured the location of (green and blue signal along the single SC - 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 dataset, isolation rates per cell range from 0.5076364 – 0.51 (molossinusMSM male) to 0.7158857– 0.72 (musculusKAZ female).

**curation step** From the total set of cell images, 10458 – 10,458 bivalent objects were isolated by the image analysis software. After a human curation step ((Peterson, Miller, and Payseur 2019)), 9829 – 9,829 single-bivalent observations remained.

To better understand the whole genome rec landscap – we charecterizaed the number of crossover/MLH1 foci on single chromosomes (from the curated bivalent dataset).

We defined/classified each bivalent into a ‘class’ based on the number of MLH1 foci (range X to X). The **chromosome class proportions** different chromosome classes per strains by sex were tested with chi-sqaure tests with the prop.test function.

# main traits

chrm chrteriztions – SC length (chromatin compaction), foci position(raw and normalized), and interfocal distance, these traits were fit into the main models (as mentioned above) … t.tests were used to test between sexes and groups

**CO Interferecne** – Our primary mode of examining interference is to compared the interfocal disatnces (IFDs) (raw and normalized by SC length.) – Gamma parameter v has historically been used to charaecterize interference and was calculated using .

**SC Length model**

* **Linear model M1**
* **Linear model M2**

**IFD, Interference**

In order to examine interference, Interfocal distance was measured from the single bivalent data sets. - This metric is closer to measures of interference from linkage maps which are also removed from physical scales in that they measure frequencies of crossovers. Crossover interference is stronger in male specific linkage maps compared to female maps in a variety of species (ref). **IFDnorm**

**REDUCED DATASET**

To account for confounding effects of sex chromosomes from pooled samples of bivalents, we considered a reduced dataset including only bivalents with SC lengths below the 2nd quartile (for SC length) in each cell. This dataset 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 dataset has decreased power, it offers a more comparable set of single bivalents to compare between the sexes.

* The long bivalent and short bivalent data sets were compiled by calculating the 1st and 4th quartille of SC lengths for each cell with at least 16 bivalent measures. Bivalents from each cell were assigned into the long and short bins based on these SC Length cut offs.

Using the single bivalent dataset, the within cell distribution of bivalent SC lengths from cells with at least 17 isolated chromosome measures. The longest and shortest 4 to 5 chromosomes within each cell were identified by qualqulating the fourth and second quartille. (these comprised the short and long bivalent datasets).

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