# ### Material and Methods

ToDo,

-fill in official names for strains (BRC)

-check example lg for re - deriving cryopreserved mice

-redo ls of the 3 rooms

# #### Mouse Husbandry

(goal, wild derived inbred strains used for natural genetic variation)The global distribution of inbred mouse strains used and genetic ancestry / species reflected in Figure X/Table X.

1. (Specific strains were obtained from X vendors, 2.
2. 3 separate rooms used (precautions taken to ensure there was limited environmental variance) b. X additional husbandry things done to help with breeding of some mouse strains. housed in X conditions (extra things done for problem breeders,
3. 3. euthanized under X protocol,

The Wild derived inbred strains PWD/PhJ, PERC/Eij, WSB/EiJ, LEWE/EiJ, MSM/MsJ, MOLF/EiJ, CAST/EiJ, CZECHII/EiJ, CORLI/EiJ and SPRET/EiJ were purchased from Jax labs (Maine USA).

The strains of KAZ/TUA, TOM/TUA, AST/TUA, HMI/TUA and SPIC) were ordered and cryo-derived from BRC at RIKEN (Japan) website). All mice were housed UW-Madison Biotech and MSC facilities – following the protocols.

A breeding colony of wild derived mice sampled from Gough Island were housed at UW Veterinary school facilities. (some of the other strains housed there – comparisons were made of strains housed across different facilities to (test if there was an effect of mouse room of MLH1 counts).

(state that mice were housed under standard husbandry procedure, but some additional things were done for mice that had trouble breeding. (Standard mouse husbandry, standard chow – but for strains with trouble breeding – additional seeds, nestlets and larger cages were used for some strains to improve fertility (litter survival).

*<Mice were euthanized by CO2 asphyxiation upon arrival at the University of Wisconsin - Madison. All work followed protocols approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Madison.*>

adult Mice were euthanized by CO asphyxiation – following the protocols approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Madison.

# #### Tissue Collection and Immunohistochemistry

1. describe how gonad tissue was collected) (right testes, both ovaries) (tunica removed decapsulated) X protocol for testes, X protocol for ovaries. X ages for males, X ages for females
2. 2. describe spread process (describe process – clearly point out the distinctions for testes and ovaries
3. 3. (describe staining process (with DMC1) (same process)

In order to make meiocytes spreads, gonads were collected from mice at the correct life stage.

the right teste was collected from mice age (X to X), 5 and 12 weeks.

Ovaries were collected from neonates less than 24 hours old or embryos between e16 to 21

Since ---most often samples were collected from neonates between 48 hours – since checking for plugs was not possible for these wild derived inbred strains.

Meiocyte spreads were made following Peters et al 2001, the volume of the hypotonic buffer incubation, was 2-3ml for whole testes and 300ul for fetal ovaries.

For testes the tunica was removed and whole testes was incubated in 3ml of hypotonic solution for 45min. X and X

for ovaries – after dissection, the pair of ovaries were 'decapsulated' in cold PBS and both were incubated in 300ul of hypotonic solution for 45 min.

After incubation, gonads were transferred to sucrose solution –for mastication?/being torn up. And cell slurry was transferred to 2% PFA solution and allowed to fix on slides overnight in a humid chamber at room temperature. (3 slides were made for each individual)

*< Spermatocyte spreads were prepared following Peters et al. (1997) with minor adjustments. The tunica albuginea was removed and whole testis was incubated in 3ml of hypotonic solution for 45 minutes. The incubated testis was transferred to 40ul of 100mM sucrose on a microscope slide and torn with fine forceps. Approximately 15ul of cell slurry added to 80ul of a 2% PFA solution was spread onto a glass slide and dried overnight in a humid chamber.*

**Staining** of meiocytes spreads was performed following X protocol.

(1:50) SCP3 primary antibody (Source), MLH1 primary antibody (Source), and centromere primary

Incubated on slides for 24 hours. Secondary antibodies ... Washes. Slides were fixed with antifade (source).

*Immunohistochemistry followed Anderson et al. (1999) and Koehler et al. (2002). Antibody work and slide blocking were conducted in 1X antibody dilution buffer (ADB), normal donkey serum (Jackson ImmnuoResearch, West Grove PA, USA), 1X PBS, and bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA), Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA). Each slide was blocked for 30 minutes in ADB before 60ul of a primary antibody mix containing anti-rabbit anti-MLH1 polyclonal antibody to* ***MLH1*** *(Calbiochem, San Diego, CA, USA; diluted 1:50), anti-goat polyclonal antibody to human SYCP3 (R&D Systems, Minneapolis, MN, USA; diluted 1:50), and anti-human polyclonal antibody to CREST (Antibodies Inc, Davis, CA, USA; diluted 1:200) in ADB was incubated for 48 hours at 37 degrees.*

**For slides/spreads for DSB characterization, DMC1 (X:100) replaced MLH1**

*Slides were washed twice in 50ml ADB between primary and secondary antibody incubations. Slides were incubated overnight at 37 degrees in Alexa Fluor 488 donkey anti-rabbit IgG (Invitrgoen, Carlsbad, CA, USA; diluted to 1:100) and Coumarin AMCA donkey anti-human IgG (Jackson ImmunoResearch, West Grove PA, USA; diluted to 1:200). Alexa Fluor 568 donkey anti-goat (Invitrogen, Carlsbad, CA, USA; diluted 1:100) was incubated at 1:100 for 2 hours at 37 degrees. Slides were washed in 1x PBS, dried, and fixed with Prolong Gold Antifade (Invitrogen, Carlsbad, CA, USA) for at least 24 hours.*

*Mice with at least 10 cells with good staining were included in our analysis. Due to variable quality of spermatocyte spreads four mice were used for quantification of either MLH1 counts or chromosome 1 SC traits instead of both (Supplemental Tables 1 and 2).*>

# #### Image Processing

1. Basic quality criteria,
2. 2. Manual quant pipeline for MLH1,,
3. 3. Quanti pipeline for DMC1 (not completely finished)

Cells were imaged on X microscope. saved as .tif files. Image Processing was done in Photoshop (source).

<*Spermatocytes were imaged 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).*

For MLH1 pachytene characterization, Cells with a full karyotype (19 autosomes and XY for spermatocyte spreads or 20 bivalents for oocyte spreads), distinct foci, and intact bivalents were included for quantification.

**(DMC1** quantification from leptotene to zygotene spreads – required distinguishing cell outlines – distinguish individual cells. (analyzed /compared within cell stage) (cell stage / timing determined following X.)

Reprocessing in Photoshop ()– Image file names were anonymized before manual scoring of MLH1 or DMC1 foci.

*Only cells with a full karyotype (23 autosomes, 1XY), intact bivalents, and clear, distinct MLH1 foci were included for quantification. Image file names were anonymized before manual scoring. We recorded the numbers of MLH1 foci, bivalents with 0 MLH1 foci, and bivalents with signs of asynapsis. We also recorded a quality score (ranging from 1 to 5, with 1 representing high quality), whether or not the X and Y were paired, and whether or not a MLH1 focus was present in the pseudo-autosomal region on the X and Y as quality control measures.*>

**DMC1 image analysis /quantification / brief description of issues and problems**

# #### Statistical Analysis

1. Software used for analysis 2. (what statistical tools are used)? (difference in analysis of MLH1 and DMC1

all analysis was done in R (cite).

Some sort of model was used for MLH1,