**Results Outline.v1**

**Estimates of gwRR from the house mouse species complex**

(- in order to estimate the gwRR across house mice we calculated the mean number of MLH1 foci per meiotic cell per mouse --)

- description of MLH1 dataset and measures of repeatability

- (Our results are similar to previously reported measures, and report novel measures from wild derived inbred strains in house mouse.

- Sex specific evolution is the major pattern.

-male: PWD, MSM, and SKIVE have significant strain effects. They are grouped into the ‘High Rec’ group. PWD, MSM, and SKIVE have evolved 20, 30% and 10% higher than other means respectively.

- female: G has significant strain effect. G female are 7% higher than other female means.

- The degree of hetC is pretty low there is a reversal of the female biased gwRR, for 2 of the High Rec strains. This is uncommon between closely related species.

(interpretation)

(below might need to be moved to the methods section)

**Analysis using a mixed model framework for examining patterns of heterochiasmy**

( in order to test – describe the patterns of evolution we used a mixed model, subspecies = divergence, strain = random effect – as wild derived inbred strains simulated as random samples of standing genetic variation)

(in order to understand the variance within an evolutionary framework – we fit the mouse gwRRs to a mixed model, which specified subspecies, sex and the interaction as fixed effects. The subspecies effect – is a proxy for measuring the degree of divergence across subspecies. The strains were coded as a random effect to –simulate / approximate / the standing genetic variation across subspecies )

( in order to follow up with the mixed models – we used post hoc glms to more specifically test the strain effects )

-Sex, interaction effect with subspecies were significant and the random strain effect were significant indicating the variance due to strain effect (genotype) is not 0. These results suggest there is a lack of support for uniform divergence in the trait which would be the expected pattern under a simple neutral evolution

After mixed models, we ran post hoc fixed effect models

-glm: The G strain effect is the largest effect for gwRR. The sex\*strain interaction effects for PWD and MSM are also significant. Additional glm models show that PWD\*male and MSM\*male increase the gwRR and G\*male decrease the gwRR. Qualitatively the difference between the G female and male means are greater than other strains.

1. *Predictions for ‘uniform’ patterns across all Mus musculus strains are not met; (ie. strains within subspecies didn’t diverge uniformly)*
2. *Sex is a significant effect, but not in a uniform manner, the significant fixed effects are interactions (strain \* sex).*

**Within mouse variance for MLH1 count per cell**

(in order to compare the within organism variance – we quantified the **mouse average** variance and coeffieient of variance (cV) of MLH1 foci per cell)

- The same mixed models and glms were ran with within mouse variance (and coefficient of variance) of MLH1 counts per cell as the dependent variable.

- Across models, sex had the largest effect and smallest p values. Some strains and interaction fixed effects had moderately significant values, but these varied across models.

- Models using datasets with higher quality cells had a similar pattern of the sex effect being the largest factor while some strain and interaction effects had moderately significant p values.

**CO precursors indicate a correlation with higher genome wide recombination rate in males**

( in order to better understand the recombination pathway – /

( in order to better understand the variation of CO numbers per cell – we also quantified a marker for DSB, the purcursors to COs / MLH1 foci --- we compared t.test.

- Brief Background and review of the meiotic pathway in terms of SC-AE > DSB > CO and CO:NCO decision.

- Greater range of variation in total number DSBs than total COs (cite Cole, Baier et al).

- Dataset for DMC1, subset of juvenile male samples for DMC1 staining.

- Early staged cells have significantly more DSBs than later prophase cells (leptotene cells have more DMC1 foci than zygotene).

- Musc PWD, MSM leptotene cells have more DMC1 foci than WSB and G leptotene cells.

**Genome wide recombination rate variation translates to the chromosome level**

- Review limits and comparisons of the number of COs per chromosome. Most species have a range of 1 to 3 COs per chromosome (Stapley et al 2017).

- The male high rec strains have significantly more 2CO bivalents than low rec strains. This skew in chromosome class proportions isn’t seen in female strains.

*- This is motivation to investigate more traits at the chromosome level*

**Single Bivalent Level Dataset**

- Review lit and previous findings for single bivalent measures (FISH, tetra and polar body sequencing).

- Description of the data set and brief description of the biological relevance for the following metrics:

1. SC Length,
2. Normalized 1CO position (rec landscape)
3. CO interference via interfocal distance (IFD) of 2CO bivalents

We will use this data to address 2 questions:

Q1. Which bivalent level traits will be sexually dimorphic?

Q2. Which traits distinguish high and low recombining strains in males?

**Heterochiasmy starts at the recombination landscape of single bivalents (SC, rec landscape, and interference)**

**1. SC length (Chromatin Compaction Differences)**

- Brief review of how SC length and rec landscape sexual differences are supported in the literature.

-For almost all models, sex is the only significant effect for mouse averages of SC length.

The exception is in the Musc strains, where the SKIVE strain effect is also significant, because both male and female bivalents are shorter compared to PWD and KAZ.

-Female SC are longer than male even despite the XX. i) all bivalents are longer within cells (there isn’t a single longer bivalent), ii) shortest bivalents within cells are also longer in females.

**2. Rec landscape differences**

**-**The sex effect is highly significant for glms of the normalized foci 1 position. In models for the full dataset, LEW, MOLF, and KAZ had significant strain effects, which MOLF being the largest. The LEW and the MOLF strain effects are replicated in models sub setting the subspecies.

-Males have more telomeric positions of single foci while female single foci are closer positioned to 50% of the total length.

-Some strains MOLF and LEW, have significant strain effects, indicating both male and female have positions closer to the middle of the bivalent than the total average.

- *SC length and the normalized 1CO position are conserved chromosome level traits of heterochiasmy in house mouse (and other species).*

**3. Sex differences in cytological measures of CO interference**

- Review of CO interference measures (approaches and species). Example tiger vs elephant shrew show a clear negative correlation between interference strength and genome wide recombination rate (Segura et al).

– Note that sex specific comparisons of IFD / interference in the physical scale (SC-AE) are very rare, De Boer et al 2006 (maybe Hassold).

- Review genetic map measures sex differences in COI and how this is also connected to more uniform REC in females and localized REC in males.

- Female normalized IFDs are an average of 45 to 50% of the length of the SC-AE across strains. Male normalized IFDs are on average 51 to 60% the length of the SC-AE across strains.

- Generally all females have enrichment of short normalized IFD (except KAZ). This enrichment is most pronounced in strains with high rec males, (PWD, MSM, SKIVE) there is a cut off of low normalized IFDs ~30%. In the remaining strains the normalized IFD ranges overlap between males and females.

- *Females have weaker interference as indicated by normalized IFD.*

*- High rec males have stronger interference, in terms of raw and normalized IFD measures. They also have a lower threshold for IFDs, 30%, this could indicate stricter control over the REC landscape to enrich for more 2CO bivalents.*

**Variation in gwRR across house mouse males, Framework for Q2**

Which single bivalents traits distinguish high and low recombining males in Musc strains?

- Review dataset of male, additional strains which didn’t have female observations

- General predictions based on the gwRR results:

I. Dom strain will not have significant effect (WSB = LEW = G)

II. Musc strains (PWD > SKIVE > KAZ, CZECHII )

III. Mol strains (MSM > Mol)

-glms and logistic regression models

**High rec strains have longer SC lengths, but depends on how you measure it**

- All strains effects are significant

-When all chrm classes are pooled, the general pattern is that the higher rec strains have longer bivalent SC lengths (except SKIVE).

- Unlike the sex differences in SC lengths where all female SCs are longer across chromosome class, mean 1CO SC are shorter in high rec strains compared to low strains. While the mean 2CO SC lengths are on average longer.

*-Shaper clustering of SC lengths across chromosome classes in the high rec males. If the physical length is long enough, a bivalent will move from 1CO to 2CO class in the high rec males. Where as in the low rec males, long chrms are more likely to remain in the 1CO class.*

**Weak correlation with lower gwRR and terminal CO landscape**

-Review why only 1CO bivalents and mouse average normalized measure is used.

-WSB has the most terminal 1CO rec landscape and MOLF has the most central.

-High rec strains have more central normalized Foci1 pos, but so do other strains: G, **PWD**, **MOLF**, **SKIVE** and KAZ, are significant strain effects in the full model.

-High and low strains are not clearly predicted by logistic regression modes (for mouse average normalized Foci1).

**Higher rec strains have stronger interference**

- PWD, MSM, and SKIVE are significant strain effects in glm for raw IFD (mouse averages).

-PWD and MSM are significant strain effects for normalized IFD.

-Mouse average for normalized IFD is significant in predicting high and low rec strains in the model where all strains are pooled.

*-High Rec strains have stronger interference measured via longer normalized IFDs.*