Material Metohds

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Mouse Husbandry

To access natural genetic variation for *Mus musculus*, Wild derived inbred strains strains were used. 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 (https://www.jax.org) (Maine USA). The strains of KAZ/TUA, TOM/TUA, AST/TUA, HMI/TUA and SPIC) were cryo-derived from Biological Resource Center (BRC) at RIKEN (Ibaraki, Japan) (https://en.brc.riken.jp/). All mice were housed UW-Madison Biotech and MSC facilities - following the protocols. A breeding colony of wild derived mice sampled from Gough Island (GI)is maintained at UW Veterinary school facilities. Mice were feed on dry standard breeder chow. Some strains sunflower seeds, nestlets and larger cages were added to improve fertility and litter survival. Adult mice were euthanized by CO asphyxiation. Neonate and embryonic mice were euthanized by decapitation following the protocols approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Madison.

Over the coruse of data collection some breeding colonies / mice were moved from facilities. Additionally the GI strain was kept at a seperate facility. (eviddence for environmental (room) effect on (MLH1 / SC lengths / a variety of meiotic traits was tested for. We observed no effect, Supplemental figure))

Tissue Collection and Immunohistochemistry

From males, the right teste was collected and processed as discribed in X citation. The majority of mice used were betwee 5 and 12 weeks, (supplemental table). However some strains had problems breeding, so testes from older mice were collected. We found a (small/no) age effect on MLH1 counts for male mice (Supplemental Figure)).

The vast majority of oocyte data was collected from neonate litters/mice between 5 to 48 hours. This approach maintain breeding pairing and still result in prophase oocytes (cite timeline of oocytes in neonates). (differences were assessed between embryo samples and neonates?). Presice staging of embryos by checking copulary plugs was diffcult/not feasible in many of these wild derived strains due to their behavior. Embryonic samples were collected when pregenacgy noted in females, embryos were staged based on (X table markers).

Meiocyte spreads were made following (Peters et al. 1997). For testes the tunica was removed and whole testes was incubated in 3ml of hypotonic solution for 45min. 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 to make a cell slurry which 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 (Kodak, cite) wash.

Staining / Immunohistochemistry

The same staining protocol was applied to spermatocyte and oocyte spreads. The staining protocol was bas on X with some adaptations, and same as previously discribed in Peterson etal 2019.

Image Processing

Images of cells were capture on Axio-2 microscope.

For MLH1 pachytene characterization, Cells with a full karyotype (19 acrocentric bivalents and XY for spermatocytes or 20 acrocentric bivalents for oocytes), distinct foci, and intact bivalents were included for quantification. Reprocessing in Photoshop (cite). Image file names were anonymized before manual scoring of MLH1 or DMC1 foci.

Hand measures dataset generated by using ImageJ.

total Sc was measured (Rwang et al 2019)

Bivalent level measure were done (Peterson et al 2019)

Statisical Analysis

Statistical analysis was performed in R (version and cite).

The distributions of CO counts per cell was assessed for normailty (supplemental figure). Mouse mean MLH1 count was used to get around the bad discreet nature of count data. (normality was confirmed with X distrbution, supplememtal figure).

-specific statistical tests; (means, non-parameteric kurts wallis t-test?) (correlation coeffectent (function)) linear models lm() from X package.

-specific R packages -non-parameteric (tests and measures for dealing with count data) -hypothese tested (size, DSB number)

Comparison/analysis of variation in MLH1 counts within and between groups was done within a general linear/ mixmodel frame work, using X package (cite). With X formula Co count ~ blah + blah, with random strain specific sex effect.

(we used this frame work as proxy for 'polymorphism' and 'divergence' in MLH1 counts across house mouse and closeley related species.)

References

Peters, Antoine HFM, Annemieke W. Plug, Martine J. van Vugt, and Peter De Boer. 1997. "SHORT COMMUNICATIONS A Drying-down Technique for the Spreading of Mammalian Meiocytes from the Male and Female Germline." *Chromosome Research* 5 (1): 66–68.