Investigating the relationship between Dosage Compensation and sex-based aging in *D. melanogaster*

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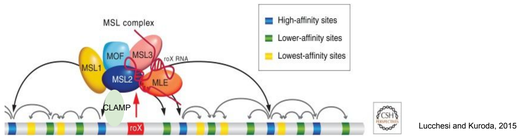
Background

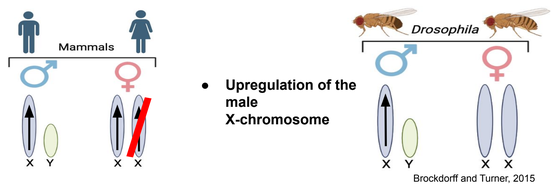
- Sex-based differences are observed during the process of aging
- Dosage Compensation (DC) is the genetic process in which a given species equilibrates the expression of sex-linked genes

The *Drosophila* Dosage Compensation Complex (DCC)



Lucchesi and Kuroda, 2015

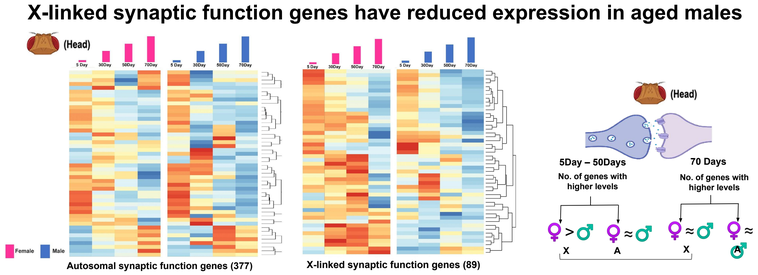
Mammalian vs. *Drosophila* Dosage Compensation

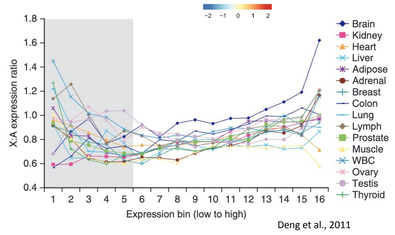


- **Upregulation of all X-chromosomes**
- **Inactivation of a single female X-chromosome**
- **Upregulation of the male X-chromosome**

Brockdorff and Turner, 2015

X-linked synaptic function genes have reduced expression in aged males





Deng et al., 2011

X:A Expression is higher than 1 in the human brain than any other tissue type (Deng et al, 2011)

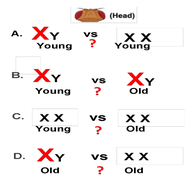
Our Question: How do X-linked genes and Dosage Compensation impact *Drosophila* aging?

Future Directions

- Continue lifespan assays for longevity data till 70 day age
- Investigate the role of individual DCC components in sex-specific longevity
- Gene ontology analysis of features and modules underlying observed sex-specific aging differences

Approaches/Methods

1. X vs A gene expression



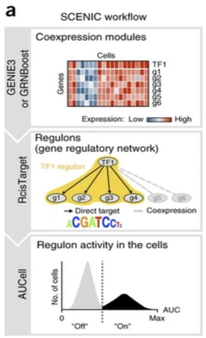
- Calculate X:A ratios per sex, age, and cell type
- Differential expression analysis of X-linked and autosomal genes between sex, age, and cell type

Aging Fly Cell Atlas

- Fruit fly snRNA-seq dataset across sexes and ages
- Detailed cell type annotations from head and body provided

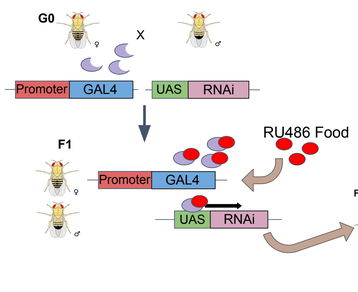
Tzu-Chiao Lu et al., 2023

2. Inferring Sex-/Age- Specific Gene Regulatory Networks with SCENIC



Aibar, S., González-Blas, C., Moerman, T. *et al.*, 2017

3. Disrupting Dosage Compensation in adult flies using the Geneswitch (GS) system

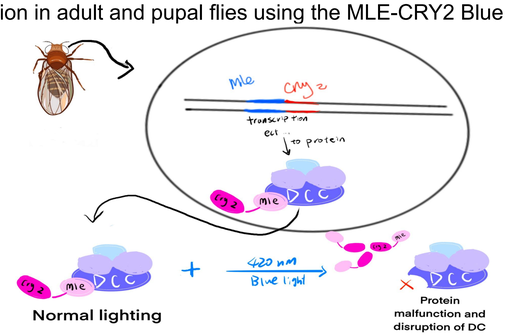


1. Validating the efficacy of the GS system with qPCR and Western Blotting
2. Lifespan Assay to quantify longevity
3. RNAseq and ATACseq analysis for specific X-linked genes

RNAi's:
- GFP (control)
- CLAMP
- MLE

4. Disrupting Dosage Compensation in adult and pupal flies using the MLE-CRY2 Blue light system

- Under blue light CRY2-tagged MLE activate protein aggregation and disrupt mle function



- Lifespan Assay
- qPCR to quantify mle gene expression for both control and treatment group
- Study exactly how mle affects aging

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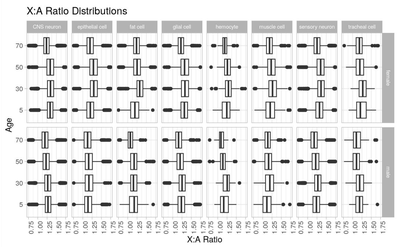
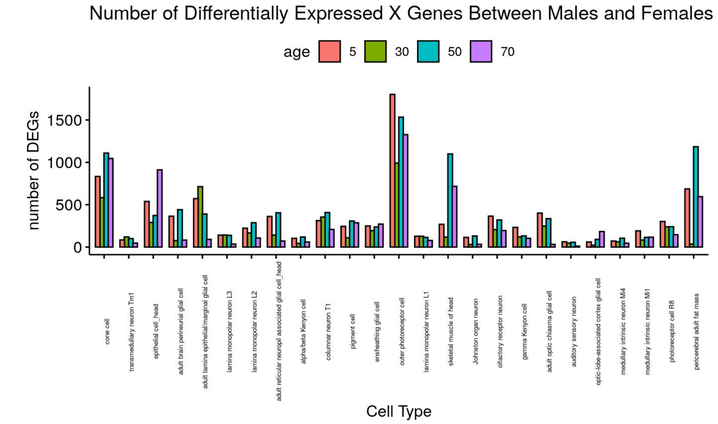
Results

1. **X:A Ratio analysis in different cell types from head**



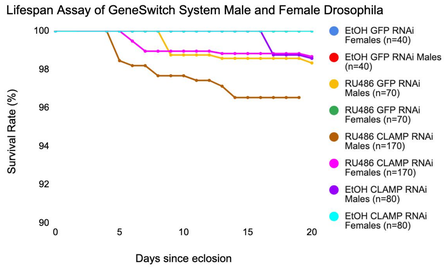
- X:A ratio shows small changes at different ages
- Several cell types show decrease at 70d
- No apparent consistent difference between sexes

2. **Neural cell types generally show a decrease in differential expression at 70d**

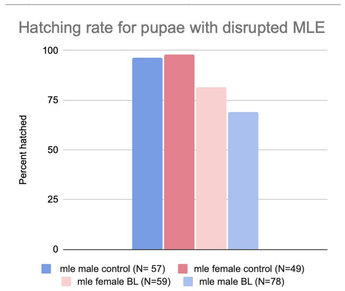


3. **Loss of CLAMP results in reduced longevity**

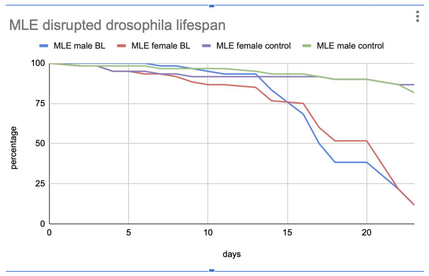
GeneSwitch-induced CLAMP RNAi expression causes a lower survival rate in males



4. **Blue light causes non-sex-specific decrease in MLE-CRY2 adult flies longevity**



- Significant reduction in hatching rate



- Significant reduction in the lifespan of both sexes under blue light.