Effects of Meiotic Drive on Developing Eye Stalks in Stalk-Eyed Flies

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Abstract

Teleopsis dalmanni, also known as the Malaysian stalk-eyed fly, exhibits sexually dimorphic eyestalks, which means there is a difference between male and female eyestalk length. Additionally, there are noticeable differences in eyestalk length within male populations. One possible reason for these differences is that some stalk-eyed flies exhibit meiotic drive. Meiotic drive is a selfish allele on the X chromosome that violates Mendel's Law of Segregation by increasing its own transmission. Males with meiotic drive have shorter eyestalks, produce more female offspring, and have lower fitness because females preferentially mate with males that have longer eyestalks. We are working to determine what genes are differentially expressed due to the presence of meiotic drive in developing eye discs that are causing differences between standard and drive male eyestalk length. We are also attempting to determine if meiotic drive is affecting eyestalk development in the same way for males and females, and whether the same genes are impacted. PCR and gel electrophoresis are being performed using primers that indicate sex and drive status. These samples are then grouped into pools and sent for RNA sequencing. Female RNA samples are being analyzed using the differential gene expression software, Kallisto. Previous research indicates an upregulation of gene expression in standard males and a downregulation in gene expression in males with drive. Based on the data from the male flies, it's predicted that there will also be a downregulation in gene expression in eye discs in females with drive compared to standard females.

Background

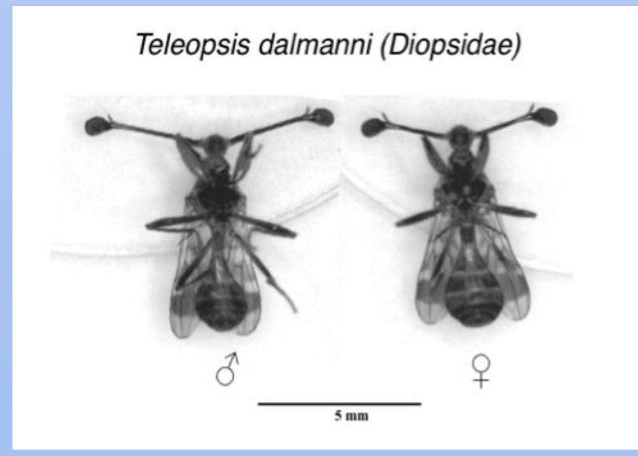


Figure 1. Teleopsis dalmanni exhibit sexually dimorphic eye stalks.

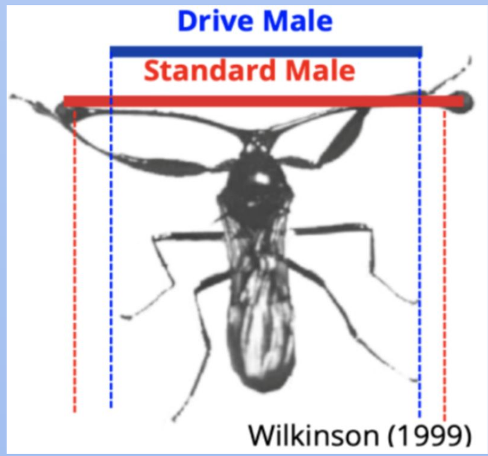


Figure 2. Males with meiotic drive have shorter eye stalks than males without meiotic drive.

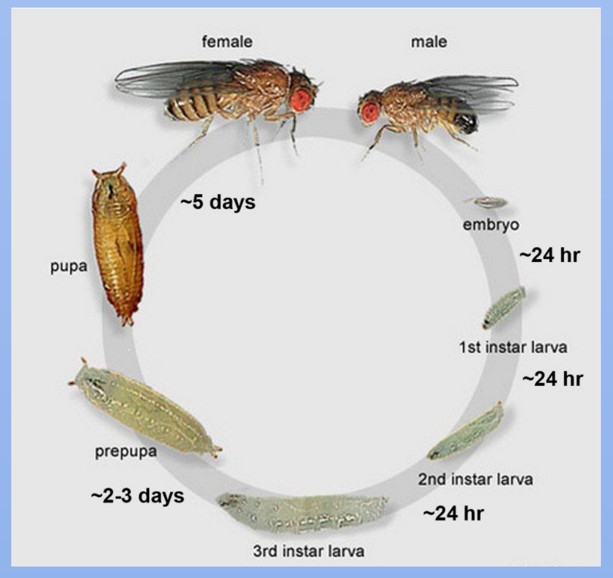


Figure 3. Life cycle of drosophila, eye discs were dissected during the 3rd instar larva stage.

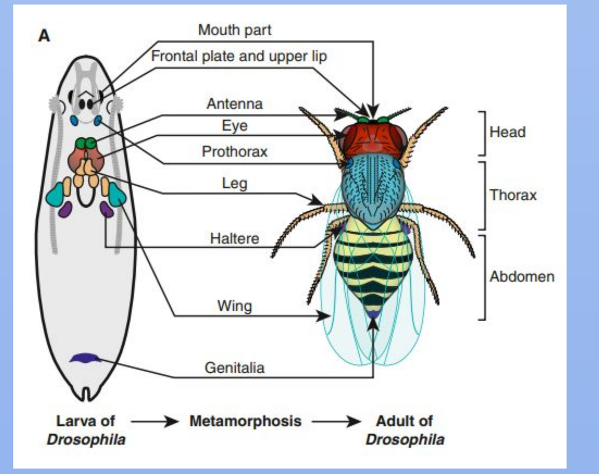


Figure 4. Imaginal discs are sac-like structures found inside the larva of insects that undergo metamorphosis and eventually turn into adult external structures, such as the eyes. The eye discs were what was dissected from the larvae.

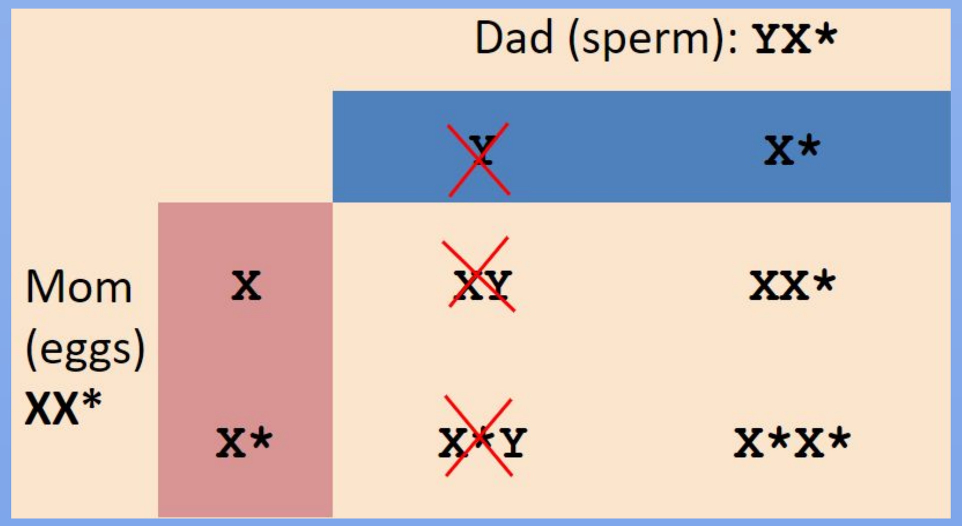


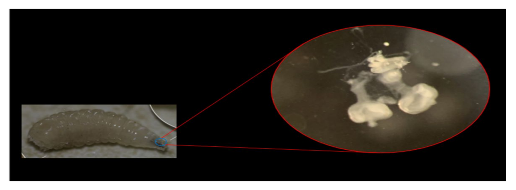
Figure 5. Meiotic drive alters the sex ratio of offspring by preventing males from making Y-bearing sperm, thus more females are produced

Goal

The goal of this project is to determine if/how meiotic drive affects gene expression in developing eye stalks of drive males, standard males, drive females, and standard females.

Project Overview

Dissections of eye discs from larvae

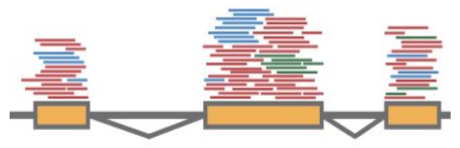


DNA extracted and PCR performed to determine sex and drive

Discs pooled together based on sex and drive status

RNA extracted and sent for sequencing

Sequencing data aligned to genome



Analyze differential expression using Kallisto and RStudio software

Conclusions

- Genes expressed at a higher level in females were enriched for chitin binding and signal peptide genes.
- Genes expressed at a higher level in males were enriched for cytoplasmic, RNA binding, and RNA recognition motif genes.
- More genes were upregulated in the eye disc among females than among males.
- There were more differences in gene expression due to meiotic drive between males than between females.
- More genes were upregulated in the eye disc in drive males compared to standard males.

Future work

- PCR and gel electrophoresis will be performed on more fly samples to create more pools
- More RNA pools will be sequenced to create more replicates to bolster the strength of this data

Results

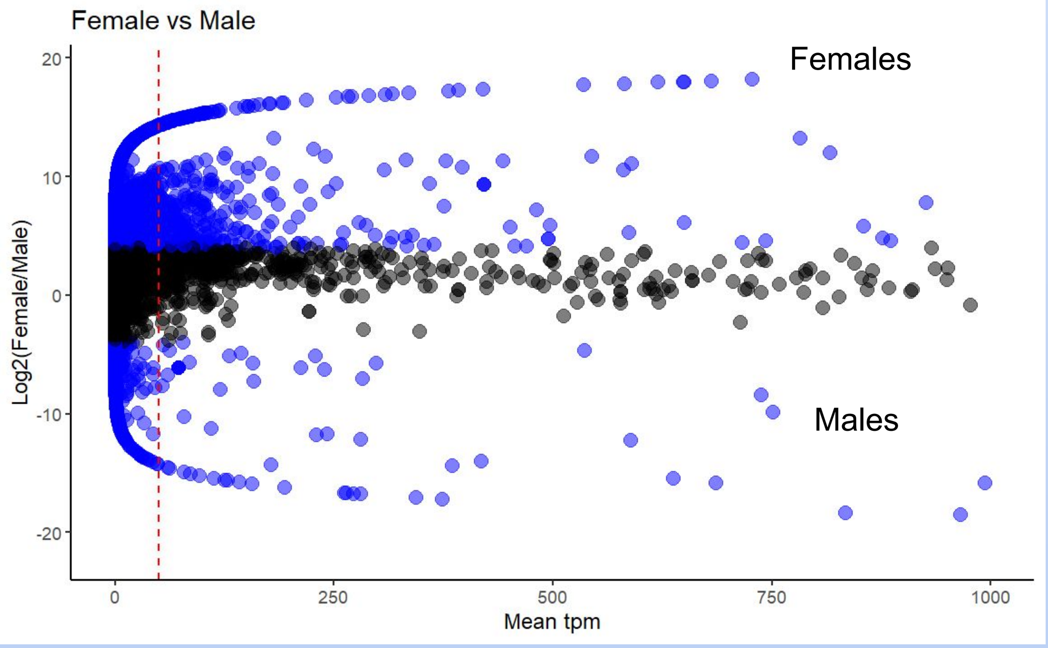


Figure 6. Differential gene expression in females compared to males, regardless of drive status

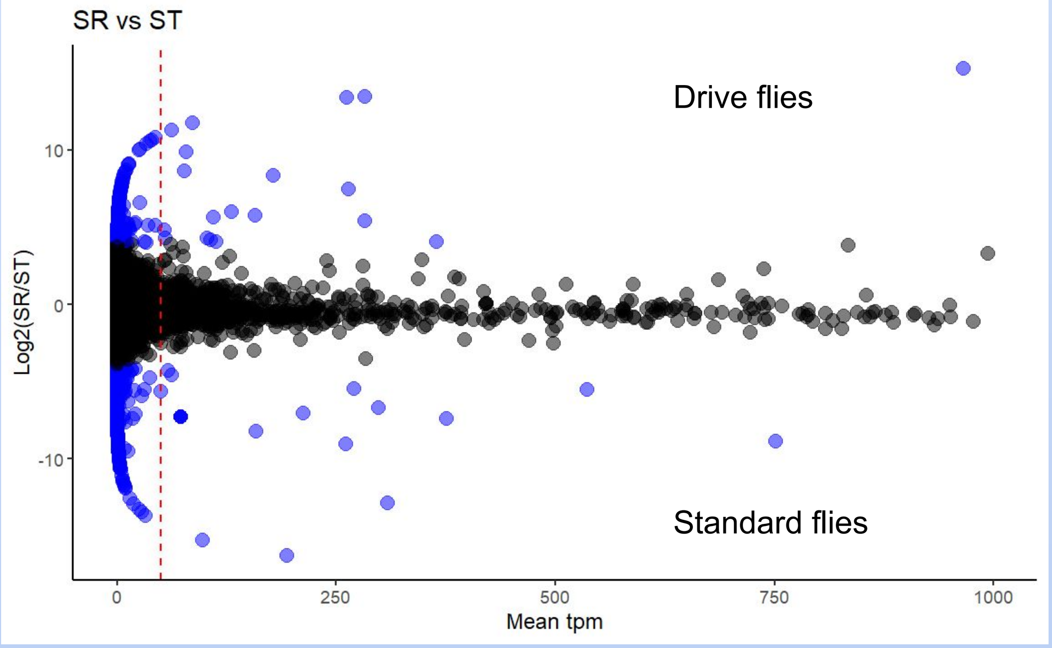


Figure 7. Differential gene expression in drive flies compared to standard flies, regardless of sex

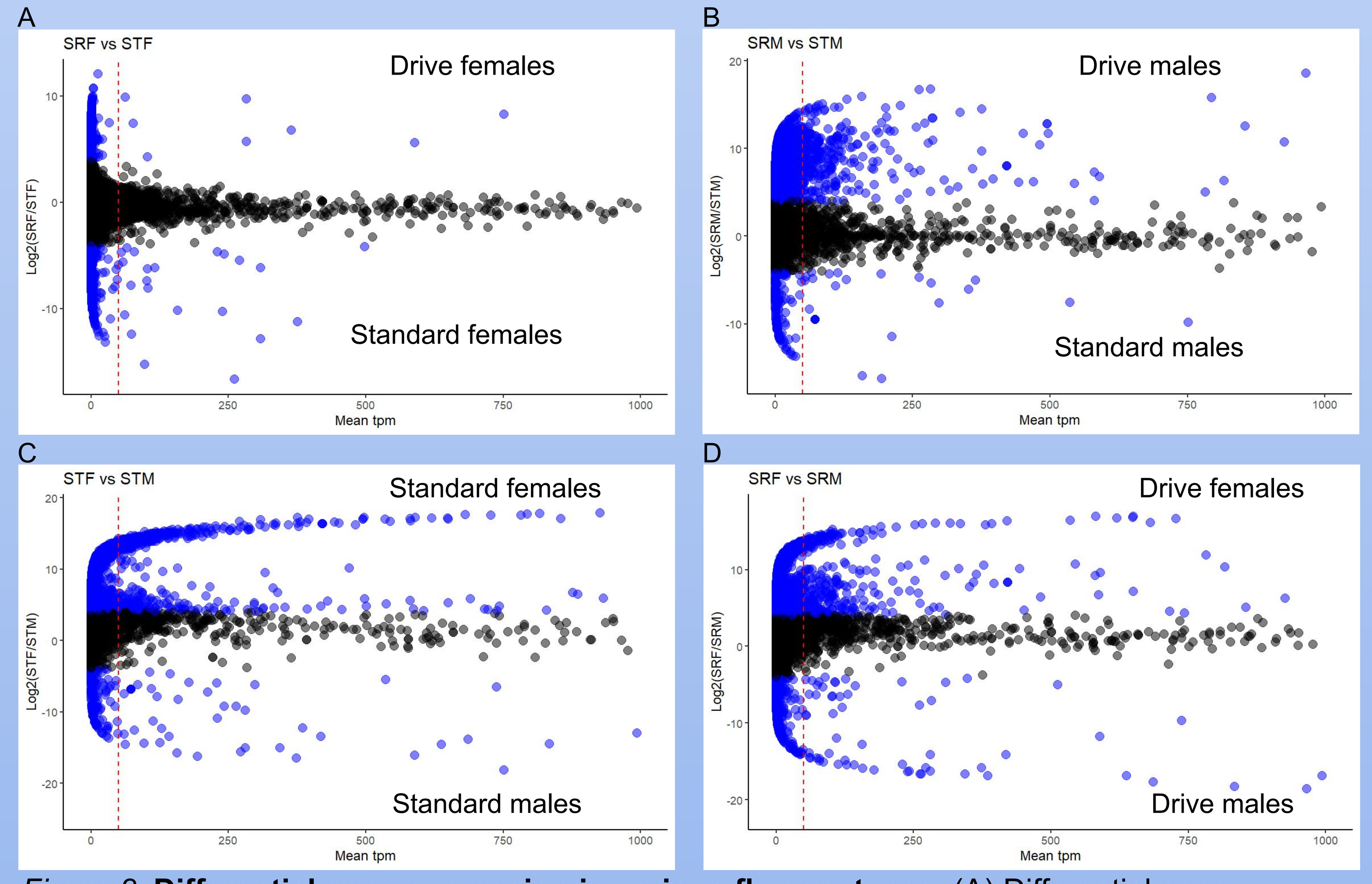


Figure 8. **Differential gene expression in various fly genotypes.** (A) Differential gene expression in drive females compared to standard females. (B) Differential gene expression in drive males compared to standard males. (C) Differential gene expression in standard females compared to standard males. (D) Differential gene expression in drive females compared to drive males.

Category	Term	Genes in category	Benjamini significance value
GOTERM_CC_DIRECT	cytoplasm	FBgn0020238, FBgn0000044, FBgn0011742, FBgn0029943, FBgn0260859, FBgn0001149, FBgn0050000, Fbgn004838, FBgn0027108, FBgn0037637, FBgn0053126, FBgn0020513, FBgn0044020, FBgn0053113, FBgn0014020, FBgn0013325, FBgn0026320, FBgn0010288, FBgn0004436, FBgn0050476, FBgn0031114, FBgn0032421, FBgn0011760, FBgn0061515, FBgn0000618, FBgn0015391, FBgn0263391, FBgn0051363, FBgn0263396, FBgn0033378, FBgn0037465, FBgn0039869, FBgn0039790, FBgn0031668, FBgn0035025, FBgn0046214	3.6E-4
EUP_KW_MOLECULAR_FUNCTION	RNA-binding	FBgn003926, FBgn0004838, FBgn0004903, FBgn0013325, FBgn0015298, FBgn0035827, FBgn0035692, FBgn0263396, FBgn0033378, FBgn0035271, FBgn0034564	2.1E-4
UP_KW_CELLULAR_COMPONENT	Cytoplasm	FBgn0000044, FBgn0011742, FBgn0029943, FBgn0001091, FBgn0061198, FBgn0004838, FBgn0027108, FBgn0030894, FBgn0014020, FBgn0013325, FBgn0015298, FBgn0035827, FBgn0050476, FBgn0031114, FBgn0011760, FBgn0061515, FBgn0000618, FBgn0263391, FBgn0051363, FBgn0263396, FBgn0033378, FBgn0039869, FBgn0035025	3.3E-4
GOTERM_MF_DIRECT	RNA binding	FBgn0004838, FBgn0261068, FBgn0004903, FBgn0013325, FBgn0035692, FBgn0031114, FBgn0035423, FBgn0263396, FBgn0033378, FBgn0035271, FBgn0038989, FBgn0034564, FBgn0046214	4.2E-2
SMART	RRM (RNA recognition motif)	FBgn0004838, FBgn0004903, FBgn0035692, FBgn0263396, FBgn0033378, FBgn0038989	2.7E-2

Table 2. A GO analysis was performed on genes that were differentially expressed in drive males compared to standard males. Functional categories that are significantly enriched in the gene list are shown.

# of genes	Genetic expression
51	Upregulation in drive females compared to drive males
49	Upregulation in drive males compared to drive females
99	Upregulation in drive males compared to standard males
1	Upregulation in standard males compared to drive males
217	Upregulation in standard females compared to standard males
40	Upregulation in standard males compared to standard females
5	Upregulation in drive females compared to standard females
6	Upregulation in standard females compared to drive females
106	Upregulation in females compared to males, regardless of drive status
43	Upregulation in males compared to females, regardless of drive status
5	Upregulation in drive flies compared to standard flies, regardless of sex
0	Upregulation in standard flies compared to drive flies, regardless of sex

Table 1. The number of significantly differentially expressed genes based on the TPM analysis

Category	Term	Genes in category	Benjamini significance value
UP_KW_DOMAIN	Signal	FBgn0002578, FBgn0002533, FBgn0002564, FBgn0020642, FBgn0036228, FBgn0030050, FBgn0033788, FBgn0038643, FBgn0030929, FBgn0054026, FBgn0085311	4.5E-4
GOTERM_MF_DIRECT	Chitin binding	FBgn0036228, FBgn0038643, FBgn0085311	1.9E-2

Table 3. A GO analysis was performed on genes that were differentially expressed in drive females compared to standard females. Functional categories that are significantly enriched in the gene list are shown.

References

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Johns, Philip M, et al. "Genetic Linkage between a Sexually Selected Trait and X Chromosome Meiotic Drive." *Proceedings of the Royal Society B: Biological Sciences*, vol. 272, no. 1576, 23 Aug. 2005, pp. 2097–2103., doi:10.1098/rspb.2005.3183.

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Wilkinson, Gerald S., et al. "Sex-Biased Gene Expression during Head Development in a Sexually Dimorphic Stalk-Eyed Fly." *PLoS ONE*, vol. 8, no. 3, 19 Mar. 2013, doi:10.1371/journal.pone.0059826.