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

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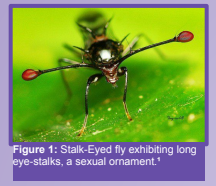
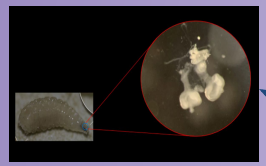


Figure 1: Stalk-Eyed fly exhibiting long eye-stalks, a sexual ornament.[1]

Abstract

Teleopsis dalmanni, commonly known as Stalk-Eyed flies, are known for their sexually dimorphic eye-stalks which females use to pick mates. In the wild, some individuals contain meiotic drive which is known to influence eye-stalk length and sex ratios. Our goal was to identify genes in developing eye tissue that are affected by meiotic drive. We are studying males with drive because they tend to have shorter eye stalks, then we will determine what genes are being disrupted or changed due to meiotic drive being present in that individual. We want to study this because males with shorter eye stalks have a lower fitness. Since eye stalks are a sexual ornamentation females prefer to mate with males with larger eye stalks. To identify these candidate genes, we dissected eye antennal discs from 3rd instar larvae, froze them in liquid nitrogen for later use, and retained carcasses. We extracted DNA from the carcasses, and performed PCR for markers diagnosing the sex and meiotic drive status of individuals, then sent them for fragment analysis. We identified 31 male and 66 female Stalk-Eyed larvae and approximately one third of males and females within the population had meiotic drive. Based on this information, we are using the larvae' eye-antennal imaginal discs from our dissections to measure differential gene expression using RNA from meiotic and non-meiotic drive individuals of each sex. After pooling tissues together by drive, and sex we were able to do RNA extractions using RNeasy RNA extraction kit. We will send these samples for RNA sequence analysis to determine which genes are being affected by meiotic drive.



Location of the two eye-antennal imaginal discs within a Stalk-Eyed fly larva.

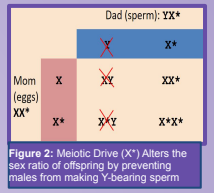


Figure 2: Meiotic Drive (X*) Alters the sex ratio of offspring by preventing males from making Y-bearing sperm

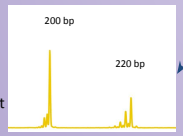
Terminal base pair data, we need to align these RNA sequencing "reads" to the genome to measure gene expression

Collection: Larvae picked from corn food → Dissection: Larval discs removed & frozen, carcass retained → DNA analysis: DNA extracted from carcass, PCR to determine sex and Drive status → Pool flies together based on sex and whether or not they contain drive → Extract RNA using Qiagen RNeasy protocol → Sequence illumina RNAseq → Align Sequencing reads to genome and quantify RNA expression using Kallisto → Differential Expression - Deseq2 Statistical analysis

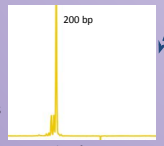
T. dalmanni (figure 1) are insects native to Malaysia and belong to the fly family Diopsidae (stalk-eyed flies). They are characterized by their sexual ornamentation: long eyestalks protruding from their head with an eye attached to the end. Studies show that females favor males with longer eye-stalks—whose length is influenced by meiotic drive[3]. Meiotic drive—carried on the X chromosome—violates the law of segregation since in male meiotic drive flies they will always pass on their X chromosome and not their Y chromosome (figure 2). This could cause a population to go extinct because this results in not enough male progeny to sustain the population [5].

To study the effect of meiotic drive on developing eye-stalks, we looked at the larvae' imaginal discs—tissues that develop into parts of an adult fly—specifically the eye-antennal discs. We plan to first genotype individual flies for their sex and meiotic drive status, then look at their RNA to study gene expression in the eye-antennal discs.

Fragment analysis data: Female Heterozygous for meiotic drive. Marker above 220 means that chromosome has drive.

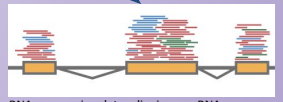


Male without meiotic drive. Male has one X chromosome so there is only one peak and it is below 220 so it does not contain drive.



DNA Extraction Method	Puregene	DNeasy	Total	Population
Females Heterozygous for Meiotic Drive	9	4	13	13.40%
Females Homozygous for Meiotic Drive	1	1	2	2.06%
Females without Meiotic Drive	20	6	26	26.80%
Males with Meiotic Drive	4	7	11	11.34%
Males without Meiotic Drive	13	7	20	20.62%
Inconclusive Data	19	6	25	25.80%
Total	66	31	97	
Success Rate	71.20%	80.60%		

Number of larvae of each genotype- we enough discs for 6 total pools
(1) female heterozygous for drive: 13 larvae = 1 pool of 13 discs
(2) female homozygous for drive: 2 larvae = not enough!
(3) males with meiotic drive: 11 larvae = 1 pool of 11 discs
(4) males without drive: 20 larvae = 2 pools of 10 discs each
(5) female without drive: 26 larvae = 2 pools of 13 discs each



RNA sequencing data, aligning our RNA sequence with the genome will show us what genes are similar and different (rnaseqclopedia.com)

Sample #	ng/ul	A260/A280	A260/A230
1	7.2	1.62	0.13
2	3.4	1.41	0.06

We found that we were able to extract RNA from test larval tissues- next we will be extracting from the pooled eye-antennal discs

So far, we have dissected and genotyped 97 larvae, and performed test RNA extractions. We have enough samples to create 6 pools of Eye-antennal discs for RNAseq analysis. We did not obtain enough homozygous drive females so future directions include more dissections.

Going forward, We will compare differences in gene expression between non-meiotic and meiotic drive individuals, leading to possible gene differences that are affected by meiotic drive. Sample RNA extractions were done and we will now run gel electrophoresis on the extracted RNA samples to see if it was successful in extracting RNA. Once we are able to do RNA extractions on the larvae eye-antennal discs, we will send out the samples for RNA sequence analysis and be able to conclude which genes exactly are being affected by meiotic drive. Currently, we are not able to do RNA extractions so we are analyzing sample RNA sequences in a software called kallisto.

References

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