Cooperation of Selfish Genetic Elements in Stalk-Eyed Flies

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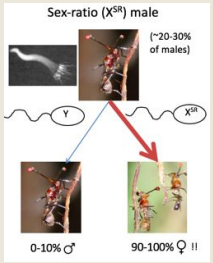
Abstract

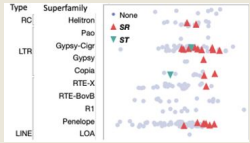
SGEs are selfish genetic elements that increase the likelihood of their own transmission regardless of the host's best interest. Transposable elements (TEs) and meiotic drivers are both types of SGEs. SGEs subsequently result in genetic conflict as they disrupt functional elements in the genome. We are working to better understand the cooperation of selfish genetic elements in Stalk-Eyed flies. Transposable elements are counteracted by small non-coding RNA molecules called piRNA. These RNA molecules work by reducing the expression of TEs by degrading TE RNA transcripts. Prior work in stalk-eyed flies has shown that TEs are expressed at a higher rate in male carriers of meiotic drive (SR males). We are comparing the expression of piRNA in SR and wild-type males using small RNA sequencing analysis software (proTRAC, PILFER) designed for this type of data. If meiotic drive and TEs cooperate, we would expect to see increased expression of piRNAs targeting TEs in SR males.

Introduction

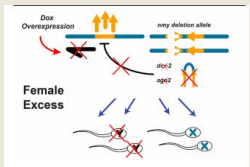
- Our goal is to understand how the expression of small RNAs that regulate TEs (piRNAs) may change due to the presence of meiotic drive
- piRNAs produce RNAi which degrade the expression of TEs



Presgraves and Wilkinson 1997 Genetics



Work by Dr. Reinhardt et. al. displayed that several TE families are overexpressed in SR males.



Work by Eric Lai and colleagues. Found that when RNAi pathway is repressed sex ratio is exhibited and there is an excess of females in the population. When RNAi is not mutated there is a 50:50 male to female ratio. Displays decrease expression of piRNA increases meiotic drive.

Methods

Collecting samples:

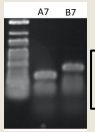
DNA (Puregene) → PCR → Gel → -SR male -ST male -SR/ST female -SR/ST female -ST/ST female

Testis or ovaries → RNA (Mirvana) → RNAseq or small RNAseq

Wilkinson and Riello 1994 PRSL; David et al 2001 Nature

Puregene protocol to create filtrate → PCR - 60x40L

Run Gel

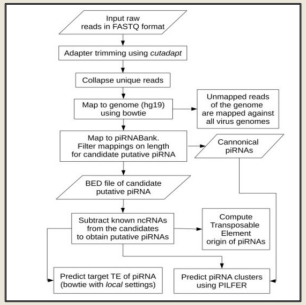


-Smaller band indicates SR -SR contain less base pairs in selected primer region

RNA of each determined genotype is sent to be sequenced

Bioinformatics:

- Pilfer
- ProTRAC

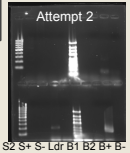


Softwares compare piRNA sequence reads to the stalk eyed fly genome. The softwares detect, visualize and analyze piRNA clusters (Ray, Rishav et. al.)

Gel Attempts-

First attempt-with control DNA (not shown)
- First attempt did not display bands —> problem in either PCR or DNA extraction

Second attempt with manufactured master mix —> see if the problem was in PCR
- DNA ladder is present however fly DNA did not display bands indicating problem in DNA extraction



Ldr S1 S2 S+ S- Ldr B1 B2 B+ B- Ldr JS JB J+ J- Ldr GS GB G+ X

Results

Hypothesis 1- Meiotic Drive (SR) → piRNA → TE's

Hypothesis 2- Meiotic Drive (SR) → TE's → piRNA

- Prior results showed that meiotic drive males express more TEs
- piRNA defend against TE expression, therefore over expression of TEs could be due to underexpressed of piRNA
- Fewer piRNAs expressed in SR males

Bioinformatics:

Fastq piRNA reads:

```
[reinlab@gs70n122 shared]$ head RSB257-345.trimmed.fastq
@NB551536:167:HY3LMBGXF:1:11101:19411:1043 1:N:0:TTAGGCAT+AGAGAACA
TGCTNGGACTACATATGGTTGAGGGTTGTA
+
AAAA#EAEEEEEEEEEEEEEEEEEEEE
@NB551536:167:HY3LMBGXF:1:11101:18201:1043 1:N:0:TTAGCCAT+AGAGAACA
TGCTNGGACTACATATGGTTGAGGGTTGTA
+
AAAA#EEEEEEEEEEEEEEEEEEEEE
```

Fasta (.fa) of the fly genome (beginning of Chromosome 1):

```
>PGA_scaffold0__9314_contigs__length_23434265
ACACAGCTGTTCTTTTTAAATTTCAAGGTGCTGTTTTTGCATCCAAGAATCACGTCAGTTCAAAAGCAGCAAAACCGAAGAAAAATGCGAAAGCAGACAAACTTATGTTGATTAACTGCTGAAAAAACGAATTTT
GCAATTGCTTTTTTTAAAATTTCAAGGTGCTGTTTTTGCATCCAAGAATCACGTCAGTTCAAAAGCAGCAAAACCGAAGAAAAATGCGAAAGCAGACAAACTTATGTTGATTAACTGCTGAAAAAACGAATTTT
GCAATTGCTTTTTTAAACAATTTGAGTCCTGTTTTTGCATCCAAGAATCACGTCAGTTCAAAAGCAGCAAAACCGAAGAAAAATGCGAAAGCAGACAAACTTATGTTGATTAACTGCTGAAAAAACGAATTTT
ATTTGAGTCTGTTTTTGACCAATTTGAGTCCTGTTTTTGCATCCAAGAATCACGTCAGTTCAAAAGCAGCAAAACCGAAGAAAAATGCGAAAGCAGACAAACTTATGTTGATTAACTGCTGAAAAAACGAATTTT
GTAGTTTGACCAATTTGAGTCCTGTTTTTGCATCCAAGAATCACGTCAGTTCAAAAGCAGCAAAACCGAAGAAAAATGCGAAAGCAGACAAACTTATGTTGATTAACTGCTGAAAAAACGAATTTTGTAGTTT
GTAGTTTGACCAATTTGAGTCCTGTTTTTGCATCCAAGAATCACGTCAGTTCAAAAGCAGCAAAACCGAAGAAAAATGCGAAAGCAGACAAACTTATGTTGATTAACTGCTGAAAAAACGAATTTTGTAGTTT
GTAGTTTGACCAATTTGAGTCCTGTTTTTGCATCCAAGAATCACGTCAGTTCAAAAGCAGCAAAACCGAAGAAAAATGCGAAAGCAGACAAACTTATGTTGATTAACTGCTGAAAAAACGAATTTTGTAGTTT
GTAGTTTGACCAATTTGAGTCCTGTTTTTGCATCCAAGAATCACGTCAGTTCAAAAGCAGCAAAACCGAAGAAAAATGCGAAAGCAGACAAACTTATGTTGATTAACTGCTGAAAAAACGAATTTTGTAGTTT
GTAGTTTGACCAATTTGAGTCCTGTTTTTGCATCCAAGAATCACGTCAGTTCAAAAGCAGCAAAACCGAAGAAAAATGCGAAAGCAGACAAACTTATGTTGATTAACTGCTGAAAAAACGAATTTTGTAGTTT
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Sam example (alignment of reads to genome):

piRNA cluster- using proTrac:

(Rosenkranz, David. 2019.)

Future Directions

- Complete editing pipeline + successfully annotate piRNA clusters in SR male and standard
- Use SAM alignment files in Protrac to get better visual images of clusters
- Also sequence female genomes as well-->see how they differ
- Eventually determine the level of expression of the piRNAs

References

- Lai, Eric. *Sex-Ratio Distortion is Suppressed by RNAi.*
- Ray, Rishav, and Priyanka Pandey. Genomics (2017). "piRNA analysis framework from small RNA-Seq data by a novel cluster prediction tool-PILFER."
- Reinhardt, Baker, Zimin, Ladias, Paczolt, Werren, Hayashi, and Gerald Wilkinson. 2020. *Transposable element control disrupted by meiotic drive in stalk-eyed fly genome.*
- Rosenkranz, David. 2019. *ProTRAC.*