The role of Alternative Splicing in Fruit Fly Thermal Tolerance

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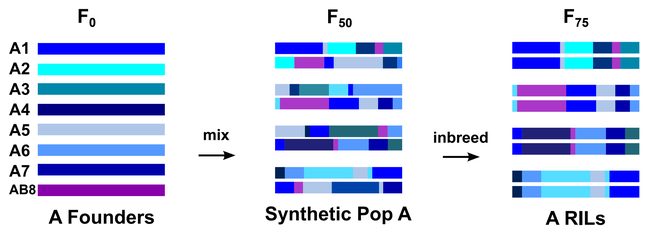
Objectives

- To Identify alternatively spliced genes that influence thermal tolerance in fruit flies
- Hypothesis: **There are alternatively spliced genes that contribute to phenotypic variation in thermal tolerance**

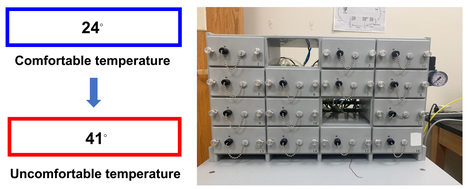
Background

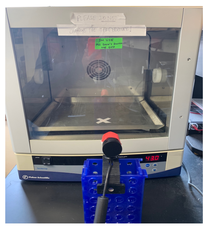


- The **Drosophila Synthetic Population Resource (DSPR)** is multi-parental (8 founders) intercrossed panel of *D. melanogaster* (fruit flies).
- Population was mixed for 50 generations, which created a population each with a genome consisting of a mosaic of the 8 founder genomes.
- After another 25 generations of full sibling mating, a panel of 800 (population A) **Recombinant Inbred Lines (RILs)** were generated. Each line is homozygous, allowing for multiple measurements using the same genotype (RIL).

Thermal Tolerance Assays

1.



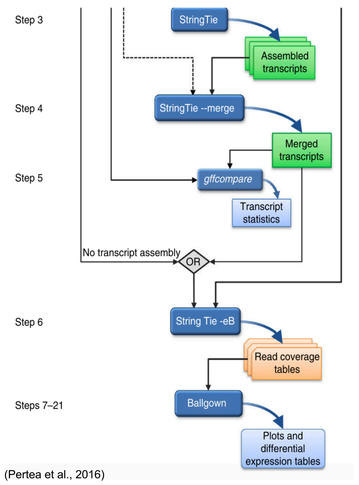


- **Figure 1** – Phenotyping for thermal tolerance in adult fruit flies. Two apparatuses were used to measure thermal tolerance. Thermal tolerance is defined as the time is takes an individual to become incapacitated.14 of the highest and lowest thermal tolerant RILs were used for RNA extraction and sequencing.

Experimental Design

2.

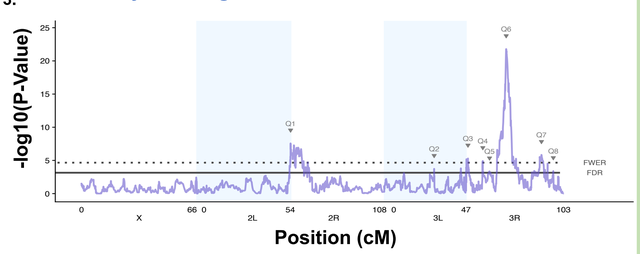
Tuxedo Protocol



(Pertea et al., 2016)

3.

QTL Analysis of High and Low Thermal Tolerant Fruit Flies



- **Figure 2** – Partial outline of the Tuxedo Package Protocol . Stringtie groups RNA sequenced reads into distinct gene loci and assembles transcripts, which creates multiple isoforms. Ballgown is then used to visualize the transcripts and expression levels found in Stringtie. Separated data into differentially expressed groups; Incubator and Incubator control, and High and Low thermal Tolerance groups.
- **Figure 3** – Quantitative Trait Loci (QTL) of thermal tolerance of 741 RILs. Eight QTLs were identified as having significant QTL's and influencing thermal tolerance in fruit flies.

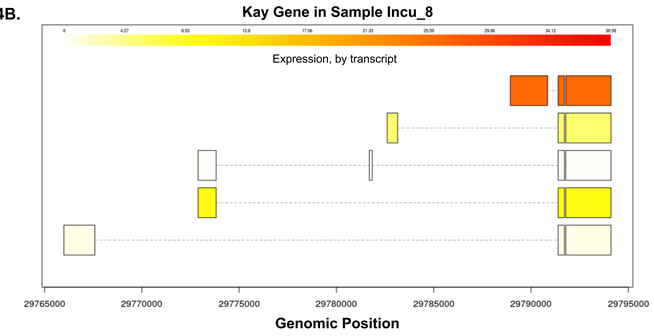
Results

Alternative Splicing under previously identified QTLs

4A.

Gene Name	Data Set	Number of Isoforms	QTL
asRNA:CR43481	Incubator	3	2
CCT3	Incubator	4	4
CG11035	Incubator	2	3
Hsp22	Incubator	2	2
Hsp27	Incubator	2	2
kay	Incubator	5	8

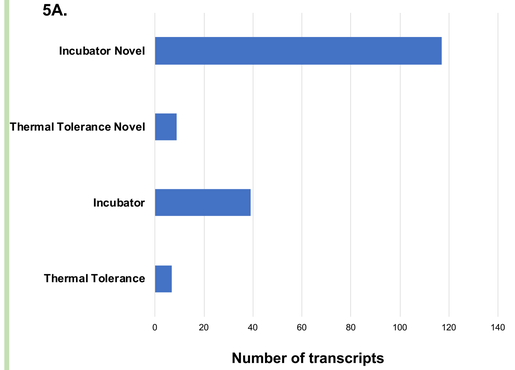
4B.



- **Figure 4** – Alternatively spliced genes found under previously identified QTLs. **A)** Table showing genes that were alternatively spliced under the QTLs. Table shows the dataset the gene identified in, the number of isoforms, and which QTL the gene was identified under. **B)** Structure and expression levels of five isoforms of the *kay* gene in incubator sample 8. Expression levels are shown in varying shades of yellow, with darkest color showing the highest level of expression.

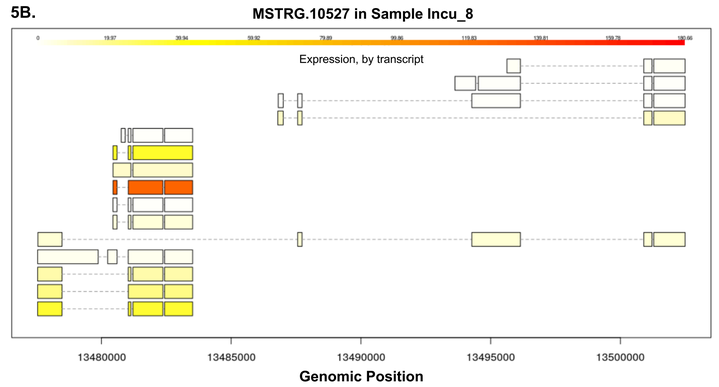
Alternatively Spliced genes throughout known genome

5A.



Novel transcripts within unannotated genome

5B.



- **Figure 5** – Alternatively spliced genes found throughout entire genome. **A)** Table showing the number of transcripts within each of our samples. Thermal Tolerance and Incubator also had novel groups which were shown to have more transcripts and alternatively spliced genes that were not annotated. **B)** Structure and expression levels of an alternatively spliced novel gene in incubator sample 8. Expression levels are shown in varying shades of yellow, with the darkest having the highest level of expression.

Conclusions

- **There are multiple genes identified to be alternatively spliced within the eight previously identified quantitative trait loci (QTL)**
- **There are numerous novel splice variants that are influential in thermal tolerance**

Future Directions

- **Perform RNA-seq on additional DSPR strains to confirm associations with thermal tolerance**
- **Validate candidate genes by altering their expression and observing effects on thermal tolerance**

Acknowledgments

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References

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