THE GEORGE WASHINGTON UNIVERSITY
WASHINGTON, DC

Control Genes for Genetic Analysis of Sperm Length in *Drosophila melanogaster*

Abby Pepin and Mollie Manier

Introduction

- Spermatozoal morphology is a prime system to study the evolution of complex traits because of its rapid evolution. *Drosophila* represents the most dramatic variation in sperm average sperm lengths ranging from 0.32 mm to 5,800 mm
- In isolines that were artificially selected for sperm length, candidate genes for sperm length were identified using RAD QTL and Illumina sequencing
- This project identified appropriate control genes, sequenced coding regions of the genes, and compared the sequence data with the previously generated sequences for the candidate sperm genes
- **Hypothesis: Any variation in the sequence data for control genes should be due to random mutation and should not segregate based on sperm length in lines that have been artificially selected for sperm length**

Methods

- Examined all the genes located on chromosome 4 and standard reference genes with those genes that were **moderately highly** expressed in all tissues in FlyBase
- Identified Rad 23, ND-49, EF1 and Rpl32 as candidate genes
- Obtained the sequence data from the largest exon of the candidate gene using GEP UCSC Genome Browser
- DNA from the largest exon was inputted into the Primer3 tool base
- The primer sequences were then blasted in the NCBI BLAST tool against *D. melanogaster* to confirm specificity for target regions
- Each set of the primers for the control genes were tested via PCR amplification on DNA isolated from flies in a high selection line and low selection line
- Products were then sequenced by Macrogen and analyzed in Geneious

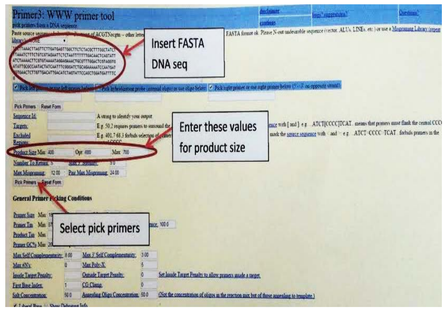

Figure 1. Primer3 tool base used to design primer sequences

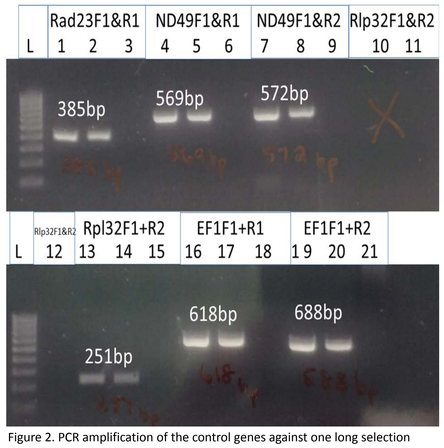

Figure 2. PCR amplification of the control genes against one long selection line, one short selection lines and water

Results & Conclusions

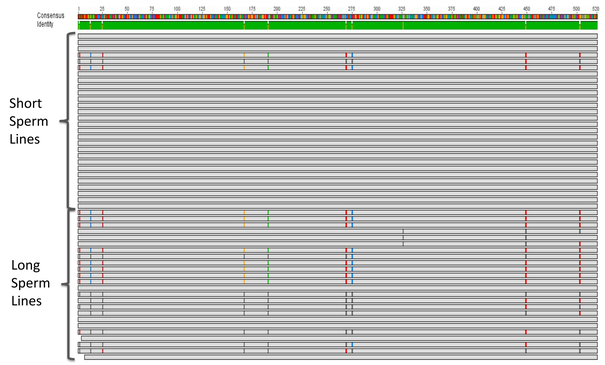

Figure 3. Geneious alignment of *cdi* showing 9 segregating SNPs.

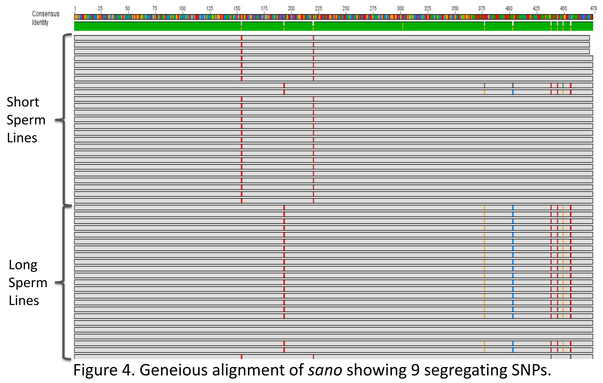

Figure 4. Geneious alignment of *sano* showing 9 segregating SNPs.

Figure 5. Geneious alignment of ND-49 showing no SNPs.

Figure 6. Geneious alignment of Ef1 showing no SNPs.

- Previous work confirmed that SNPs for candidate genes for sperm length, *sano* and *cdi*, segregated among the long and short sperm selection lines (Figs. 3 and 4)
- Control genes ND-49 and Ef1 exhibited no SNPS along the sperm selection lines (Figs. 5 and 6)
- Based on the results, ND-49 and Ef1 are appropriate control genes to use in a broader experiment identifying genes involved in sperm length
- These results support our hypothesis

Future Directions

The Manier lab will transform the long- or short-sperm line with the reciprocal allele and look for predicted changes in sperm length

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References

B.P. Matta, B.C. Bitner-Mathe, M. Alves-Ferreira, "Getting Real with Real-Time qPCR: A Case Study of Reference Gene Selection for Morphological Variation in *Drosophila melanogaster* Wings," *Development Genes and Evolution*. Vol 221, no. 1, pp. 49-57, 2011.
GEP UCSC Genome Browser http://gander.wustl.edu/cgi-bin/hgTracks (Accessed July 2015)
NCBI Blast Tool http://blast.ncbi.nlm.nih.gov/Blast.cgi (Accessed July 2015)
Ponton F, Chapuis MP, Pernice M, Sword GA, Simpson SJ (2011) Evaluation of Potential Reference Genes for Reverse Trasncription-qPCR studies of Physiological Responses in *Drosophila melanogaster*. Journal of Insect Physiology. 57: 840-850
Primer3: WWW Primer Tool http://biotools.umassmed.edu/bioapps/primer3_www.cgi (Accessed July 2015)