Characterizing Candidate Genes for Sperm and Seminal Receptacle Length in *Drosophila melanogaster*

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Background

- **The genetics of spermatogenesis has been well-studied for 20 years, but little is known about genetics of variation in sperm length.**
- **Sperm length is important in sperm competition, which occurs when females mate with multiple males and can evolve rapidly and contribute to new species formation via reproductive isolation[1].**
- **Sperm length coevolves with the length of the seminal receptacle (SR), a female sperm storage organ[2].**
- **Fruit fly is a model organism because of its small size, large family size, short life cycle, genetic manipulation methods and genes homologous to humans.**
- **In this research two genes were investigated: *crossveinless c* (*cv-c*) and *tenectin (tnc*).**

Methods

- **Mutant lines were obtained from the Bloomington Stock Center[3], Bloomington, Indiana.**
- **Each mutant line was crossed with its original genetic background to generate a genetically similar control.**
- **Virgin males were dissected at 5 days old, and virgin females were frozen at 5 days old and dissected at a later time.**
- **Seminal vesicles from males were dissected into 1X phosphate buffered saline (PBS), and sperm were spread on a slide, dried, fixed and stained, imaged on a Nikon upright microscope, and measured using ImageJ software.**
- **SR's were dissected into PBS, secured under a coverslip with clay on the corners to achieve optimal compression, imaged and measured as above.**
- **A t-test was used to compare the mutant lines to the control.**

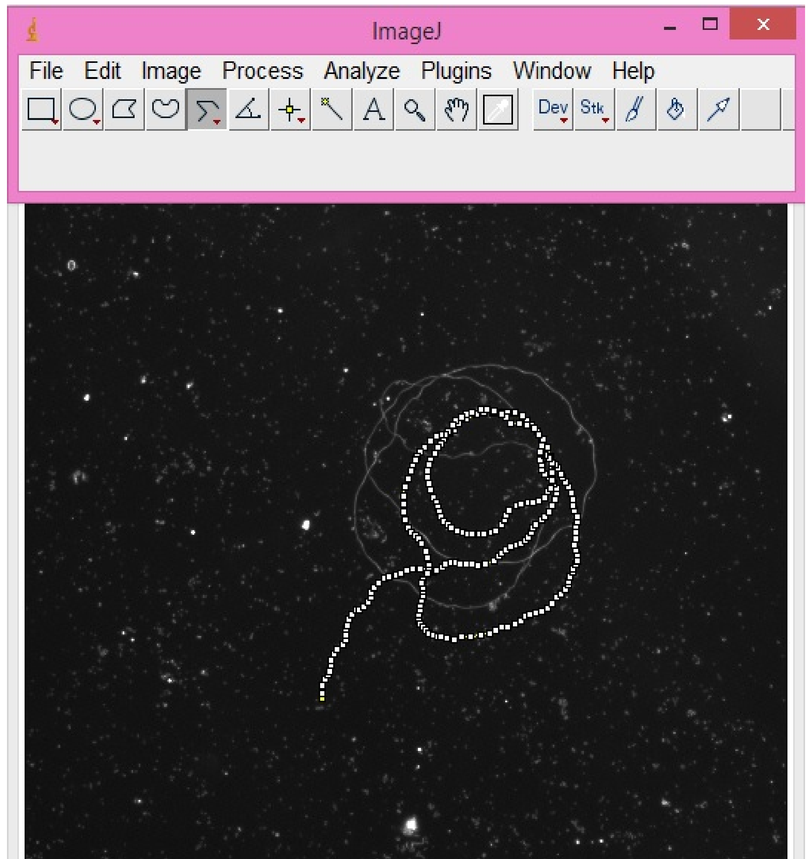

Fig. 4. Wild-type sperm being measured using ImageJ (darkfield, 400X magnification).

Results

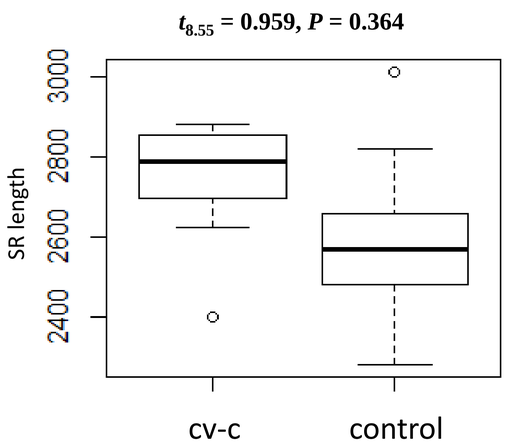

Fig. 1. SR lengths of *cv-c* mutants are significantly longer than control wild-type.

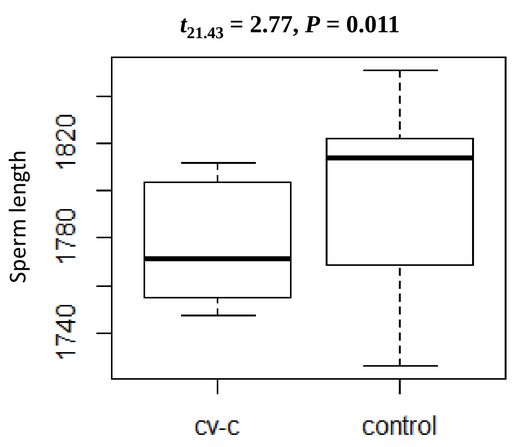

Fig. 2. Sperm lengths of *cv-c* mutants are not significantly different from control wild-type.

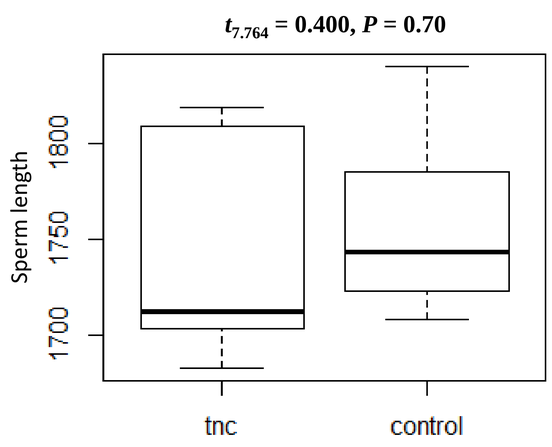

Fig. 3. Sperm lengths of *tnc* mutants are not significantly different from control wild-type.

- **There was no change in sperm length in both mutant lines. This result could mean that either these genes do not play a role in sperm length, or a functionally gene or set of genes is taking over the role of the knocked out gene. Alternatively, the knockout may not have been effective in *tnc*; future efforts will validate this using qPCR.**
- **However, longer SR's were observed in *crossveinless c*. This result suggests that this gene plays a role in negatively regulating SR length and may have pleiotropic effects on sperm length.**
- **Pleiotropy acting on *crossveinless c* to control both sperm length and SR length is a possible mechanism for coevolution of these two traits across the *Drosophila* phylogeny.**

Future Directions

Future studies will investigate:

- **The roles of these genes during spermatogenesis.**
- **The role of more genes using mutant lines.**

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Works Cited

1. Manier, M.K. et al. 2013. Postcopulatory sexual selection generates speciation phenotypes in *Drosophila*. Current Biology 23: 1853-1862.

2. Miller, G.T., S. Pitnick. 2002. Sperm-female coevolution in *Drosophila*. Science 298:1230-1233.

3. http://flystocks.bio.indiana.edu/

4. http://imagej.nih.gov/ij/