Assessing a Possible Regulatory Network of *Obp56h* in *Drosophila Melanogaster*

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Background and Motivation

The odorant binding protein ***OBP56h*** affects mating performance: lower expression of the gene *Obp56h* expression decreases the time it takes for male flies to successfully mate with a female (Shorter et al., 2016).

Experiments from the Meisel Lab in the house fly identified five genes whose expression is negatively correlated with *Obp56h*. This suggests that these five genes may be regulating *Obp56h* expression or, conversely, *Obp56h* may be regulating the expression of the genes. One of the genes is ***CG2120***.

I performed a genetic experiment to up-regulate expression of *CG2120* to see if it affects male mating, possibly via expression of *Obp56h*.

Prediction

I predict that higher *CG2120* expression will lower *Obp56h* expression and thus shorten the time it takes for males to mate.

Methods

Experimental groups:

- The Gal4-UAS system includes an activator protein and enhancer sequence to recruit transcription factors such as nuclease-dead Cas9 (dCas9).
- CRISPR is an effective tool for specific genetic upregulation. It uses dCas9 protein, which activates transcription but only in the presence of guide RNA as shown in **Figure 1**. Because the guide RNA is complementary to the target gene's DNA sequence, the dCas9 protein will only activate transcription at the target sequence.
- I used the Gal4-UAS-dCas9 system to upregulate *CG2120* in specific tissues of males.
- I used two different *Gal4* drivers: *Elav* and *Apolpp*, expressed in nervous system and fat body, respectively.
- *CG2120* upregulation was accomplished by crossing *CG2120* sgRNA males to females with *Elav or Apolpp-Gal4* and dCas9.
- Control males were created using sgRNA that does not target any gene.

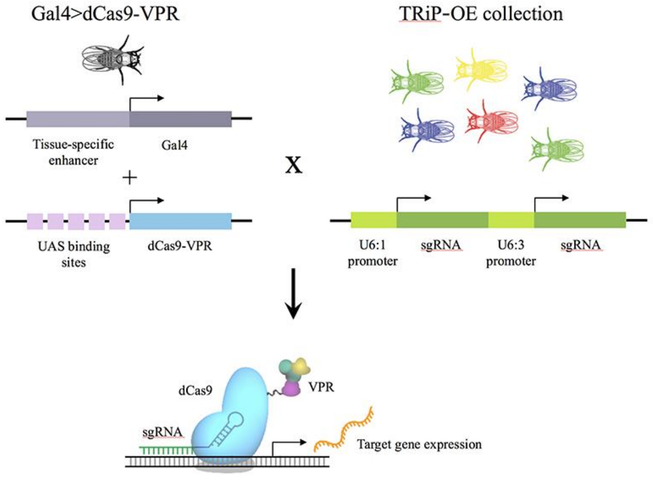
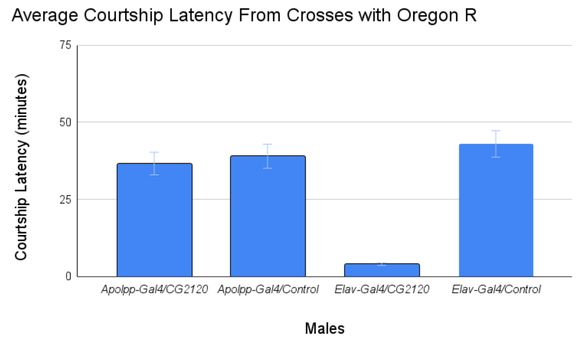


Figure 1. CRISPR Overexpression Mechanism
Image was taken from the Perrimon lab to show how overexpression by CRISPR/Cas9 can be achieved. (fgr.hms.harvard.edu/trip-overexpression-stocks)

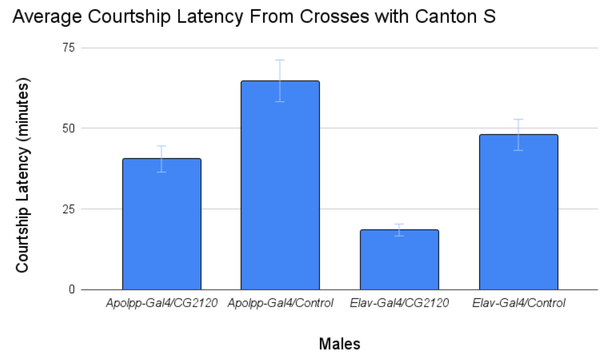
Mating Assay:

- Males were mated with virgin females from two strains, *Canton S* and *Oregon R*, at 25°C
- The time the male and female were put in together to the start of mating were recorded

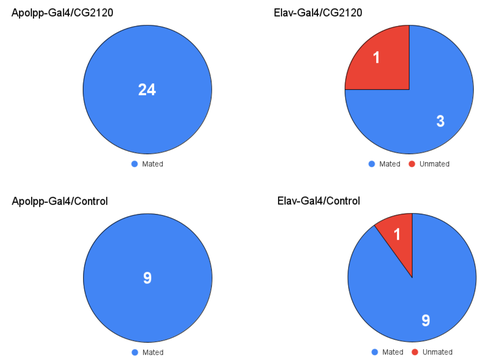
Results



Graph 1. Courtship Latency From Crosses with *Oregon R*
2 - 7 day old males were mated with 2 - 4 day old *Oregon R* virgin females. Sample size used in graph from left to right is 14, 4, 1, and 4.



Graph 2. Courtship Latency From Crosses with *Canton S*
2 - 7 day old males were mated with 2 - 4 day old *Canton S* virgin females. Sample size used in graph from left to right is 10, 5, 2, and 5.



Graph 3. Percentage of Mated Males in Allotted Time Interval
No mating occurred in 1 male from *Elav-Gal4/CG2120* and *Elav-Gal4/Control.*

Discussion

The current data supports the hypothesis that *CG2120* is affecting *Obp56h* and therefore the time it takes for males to mate but only in the crosses with *Canton S*. This is supported by Graph 2 where courtship latency decreased in *CG2120* upregulated groups in comparison to the controls. Still, this conclusion is limited because of the low sample size. Further data are required to test whether *CG2120* is indeed regulating *Obp56h.* I plan to use qRT-PCR to test for changes in *Obp56h* expression at the molecular level. As an additional insight into the study, F1 progeny are being scored to test whether *CG2120* and *Obp56h* expression affects fertility.

References

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Lin S, Ewen-Campen B, Ni X, Housden BE, Perrimon N. In Vivo Transcriptional Activation Using CRISPR/Cas9 in Drosophila. Genetics. 2015 Oct;201(2):433-42. doi: 10.1534/genetics.115.181065. Epub2015 Aug 5. Erratum in: Genetics. 2015 Dec;201(4):1615. PMID: 26245833; PMCID: PMC4596659.

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