Endocrine control of mating duration in male fruit fly, *Drosophila melanogaster*

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Introduction

Evolution relies heavily on the ability of organisms to pass on their genes. This concept is referred to as fitness in Darwinian terms. In Drosophila, single-reared males and group-reared males show different phenotypes when it comes to mating duration. Their behaviour is specific to the social conditions that the flies are raised in.

Mating behaviours given different social conditions

Single-reared Naïve → **Normal (Control)**

Naïve
- No sexual experience

Group-reared Naïve → **LMD**

Longer-Mating Duration (LMD)
- By lengthening their mating duration, these individual can maximize their chance of passing on their genes and prevent rivals from stealing their sexual partner

Group-reared Experienced → **SMD**

Shorter-Mating Duration (SMD)
- By shortening their mating duration, these individuals can maximize the transmission of their genes while preserving energy

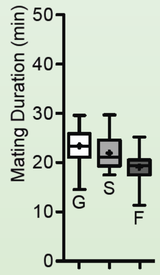

Figure 1. Example of control group mating duration. Graph representing the normal mating duration of *Drosophila melanogaster* given three different conditions for males: single-reared naïve (S), group-reared naïve (G), and group-reared with sexual experience (F).

Research Questions

- RNAi screening revealed that Ecdysis Triggering Hormone (ETH is involved in the moulting of Drosophila exoskeleton [1], is necessary for SMD.
- Ecdysis occurs in major stages of insect development so there might be a possible link to metabolic genes
- There are 8 ilps or insulin-like peptides that have been discovered in flies and relate to metabolism
- What are the links between these metabolic ilps and mating duration?
- Is there also a link between ETH and these ilps?

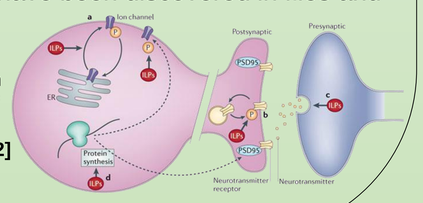

Figure 2. Expression pathway of insulin-like peptides. [2]

Results

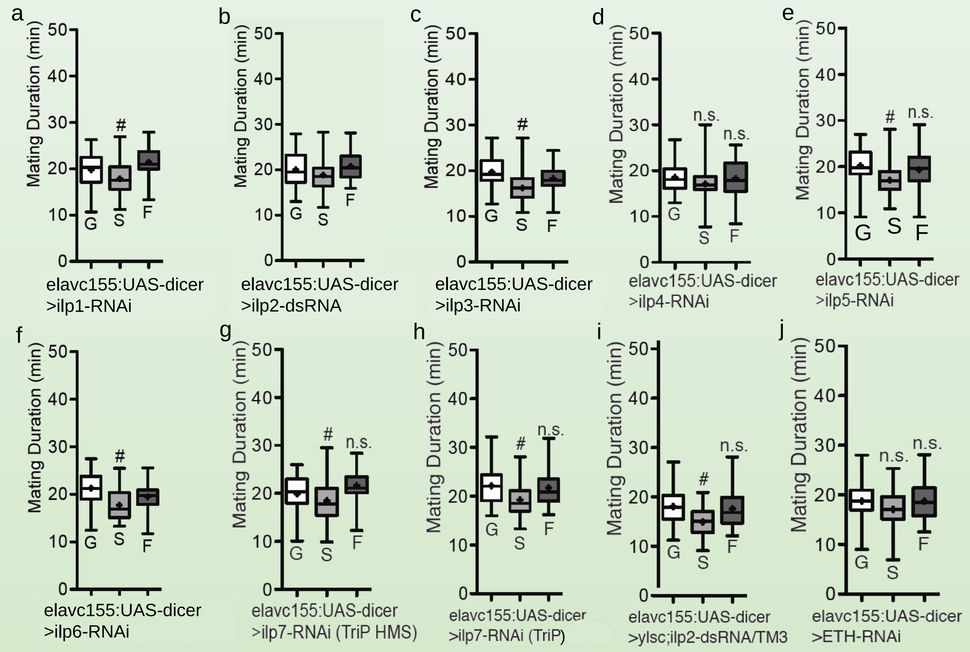

Figure 5. Mating behaviour assay results of *Drosophila melanogaster* given knockdown of different genes. Each graph represents the mating duration of *Drosophila melanogaster* given three different conditions for males: single-reared naïve (S), group-reared naïve (G), and group-reared with sexual experience (F). The box indicates the standard deviation with a line inside, indicating the mean. The bars represent the range of the data. The "#" indicates that there is a statistical significant difference, and "n.s" indicates that there was no statistical significant difference. Figures a – i represent the mating duration results for knockdown of insulin-like peptide genes 1 - 7. Figure j represents the mating duration results for knockdown of the ETH gene.

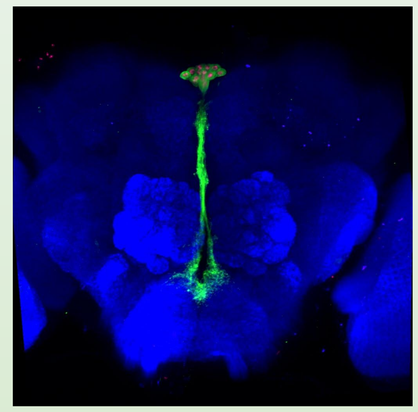

Figure 5. Immunostaining of male *Drosophila melanogaster* brain given knockdown of ilp2 gene. Using a UAS-GAL4 system via UAS-mCD8GFP and UAS-RedStinger, ilp2 gene was knocked down and green fluorescent protein (GFP) was used to identify the neural circuit. Blue represents the neurophils of brain (c82 staining), green represents mCD8GFP (labels plasma membrane), and red represents stinger (labels nucleus).

Methodology

By using the UAS-GAL4 system to drive RNAi expression in different strains of ilps, we seek to elucidate the involvement of these metabolic genes in mating duration. Resulting hits will allow us to narrow down the ilps involved in this behaviour. This is accomplished through a behaviour assay in which chambers are used to measure the mating duration of flies.

Behaviour Assay Setup

Split male flies into 2 groups:
- Single-reared
- Group-reared

4 days later

Split group-reared condition into 2 groups:
- Naïve
- Sexual experience

Leave single-reared flies alone

Running the Assay

Two-layered chamber:
- Females on bottom layer
- Males on top layer

1.5 hours later

Start mating assay:
- Dividers between male and female fly removed
- Mating duration recorded using chronometer

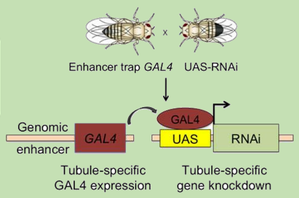

Figure 3. UAS-GAL4 system. [3] Diagram demonstrating the UAS-GAL4 system, by which an RNAi inserted downstream of a UAS sequence can be specifically translated in cells where the transcription factor GAL4 is expressed

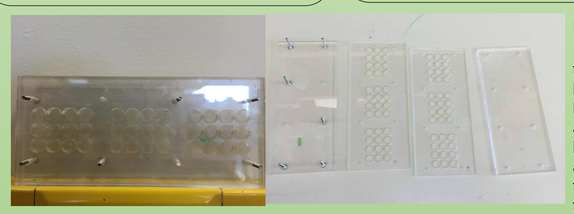

Figure 4. Two-layered chamber for mating behaviour assay. Picture showing the two-layered chamber used for mating behaviour assay. One chamber contains 36 holes in which the female and male flies were added to during assay. Females were added to bottom layer and then a plastic divider was added to separate them from males. Males were then added to the top layer and enclosed within the chamber using a hard plastic cover.

Conclusion

- The results obtained from this experiment show that ETH does indeed play a major role in SMD
- Through the UAS-GAL4 system, the knockdown of the ilp7 gene in two different mutant strains showed the disappearance of the LMD phenotype, so this behaviour can be confirmed
- Several ilps show disinhibition of SMD meaning a possible link to the SMD neural circuit
- Next steps in the project
  - Find out where in the neuronal population is the ilp7 gene affecting LMD
  - Use region specific GAL4s to knockdown the ilp7 receptor in specific regions of the brain
  - Using the results gained on knockdown of ilps, find a link to ETH and how SMD is affected

References

[1] Park, Y., 2001, "Deletion of the ecdysis-triggering hormone gene leads to lethal ecdysis deficiency", *Development*, 129, 493-503, Retrieved from http://dev.biologists.org/content/129/2/493.long
[2] Fernandez, A., & Torres-Aleman, I., 2012, "The many faces of insulin-like peptide signalling in the brain", *Nature*, 13, 225-239, Retrieved from http://www.nature.com/nrn/journal/v13/n4/fig_tab/nrn3209_F6.html
[3] Rodan, A., (n.d.). Rodan Lab Research in UT Southwestern Medical Center, Retrieved from http://www.utsouthwestern.edu/labs/rodan/research/

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