The expression levels of clockwork orange in Female vs. Male *D. melanogaster*

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Summary

Study was done on gene expression in female and male *Drosophila melanogaster* (fruit flies).
- Flies were fed food that had acetone mixed in it
- RNA was extracted and used to perform qRT-PCR to measure gene expression
- The *cwo* gene expression level for females is lower than males

Introduction

Hypothesis: Female flies will have *clockwork orange* (*cwo*) expression levels within ±0.1-fold compared to males when given food with acetone for 7 days.

Flies are one of the most commonly used model organisms[1]:
- low cost and fast reproduction rate, producing many offspring at once
- ~60% fruit flies genes shared with humans, ~75% of human disease-causing genes shared[2]
- shares biological similarities with humans

Female vs. Male:
- these different sexes were tested due to males and females having different organs, hormones, and genes[4]
- all of these reasons add up to the body responding differently when exposed to certain stressors

Acetone was tested to see its effect on the neurobiological aspects in fruit flies
- vehicle control for future B[a]P exposures

Gene Expression was measured because:
- RNA encodes proteins/gene products that have different responses to external stressors[3]
- due to similar genes in flies and humans, info gained from flies on the effects of stressors could be learned from and applied to humans[2]

clockwork orange *(cwo)* chosen as the target gene
- modulates clock gene expression, identified to be closely tied to *D. melanogaster* circadian rhythm

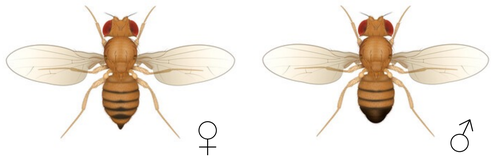

Figure 1. Female and Male Adult Fly Appearance.

Methodology

Fly Keeping:
- flies housed at room temperature and raised on cornmeal-based food
- 10 males and 10 females from the breeding population used to create F_0

Female:
- flies fed cornmeal food with acetone for 7 days
- kept with males to mate

Male:
- flies fed cornmeal food with acetone for 7 days
- kept with females to mate

RNA Extraction:
- fly tissue was homogenized using a borosilicate homogenizing method
- RNA was then extracted using the Qiagen RNeasy Mini Kit

qRT-PCR:
- performed qRT-PCR using iTaq™ Universal SYBR® Green One-Step Kit

Bioinformatics:
- obtained DNA, RNA, and protein sequences for genes through NCBI
- ran BLAST to get query cover and percent identity between orthologs
- graphed percent similarity between *D. melanogaster* and orthologs such as *H. sapiens*, *X. tropicalis*, and *D. rerio*.
- looked at predicted protein interactions through STRING

Data Analysis & Figure Generation:
qRT-PCR data:
- outliers removed during qRT-PCR data analysis
- calculated delta Ct, delta delta Ct, and fold change values
- average fold change value with females and using males as a baseline was calculated with fold change values

Results

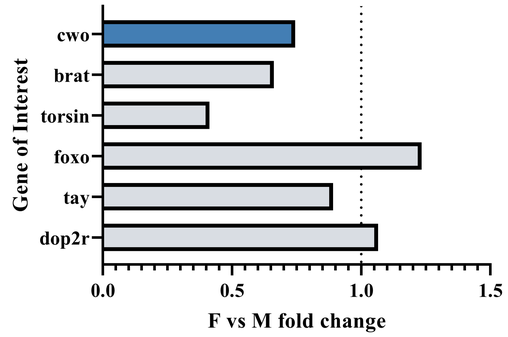

Figure 2. Bar graph showing the female vs. male fold change for *cwo* and other genes of interest. Values under one indicate that females had less expression and values over one indicate that females had more gene expression. This figure was generated on GraphPad Prism 9.

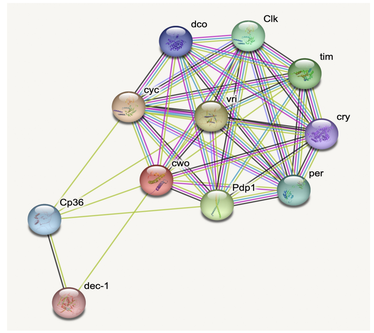

Figure 3. STRING diagram showing protein interactions with *cwo*. The figure shows the interactions between *cwo* and other proteins involved in circadian rhythm. The proteins are all first shell interactors query proteins (colored nodes) and have known 3D structures (filled nodes). All interactions except with Cp36 and dec-1 are known (teal/pink lines), while *cwo* interactions with Cp36 and dec-1 are textmining (yellow lines). The figure was created with STRING (string-db.org).

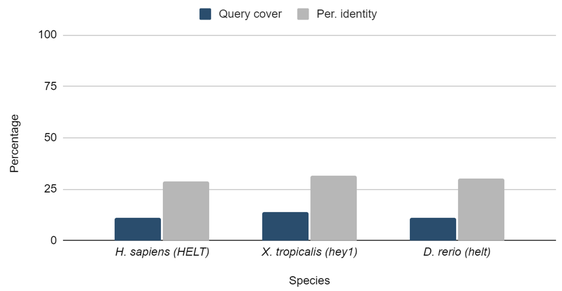

Figure 4. Gene homology graph covering *cwo* percent identity and query cover (NCBI BLAST) with orthologs. The graph depicts the similarities between the fly *cwo* gene and orthologous genes in humans (*H. sapiens*), western clawed frog (*X. tropicalis)*, and zebrafish (*D. rerio*). The graph was made with Google Sheets.

Discussion & Conclusion

- fold change value of 0.744294405 was less than 1 showing that females had higher Ct values and lower *cwo* expression (Figure 2)
  - females started the qRT-PCR with less copies of the *cwo* gene compared to males
  - means females took more cycles to pass a fluorescent signal threshold
  - does not support our hypothesis as female vs. male fold change outside ±0.1-fold range
- STRING diagram displayed predicted protein interactions with *cwo* (Figure 2)
  - due to lower expression of *cwo* in females compared to males, interacting proteins involved in circadian rhythm might be expressed differently in females
  - the protein predicted to be most associated with *cwo* is *cyc*, which is involved in the generation of biological rhythms and activates the cycling transcription of TIM and PER
- comparing homologous genes from *H. sapiens* and *D. rerio* with *D. melanogaster* showed both homologous species had low query cover and percent identity values (Figure 4)
  - results suggest that humans and zebrafish might have different *cwo* expression between the sexes
  - *X. tropicalis* had the highest query cover and percent identity out of those species, therefore will most likely have the most similar results in *cwo* expression levels with *D. melanogaster*

Limitations/Future Directions

Limitations:
- although males and females were exposed to the same amounts of acetone food, they weren't selected at the same ages
- outliers were removed during data analysis process, could have affected results

Future Directions:
- Figure 3 predicted *cwo* interactions with *Cp36*, a protein essential for fertility and endochorion integrity
  - additional tests could be run studying the effects of acetone-exposed foods on female *D. melanogaster* ability for reproduction due to the function of Cp36 and *cwo* interaction
- experiment could be run testing acetone-exposed foods on species with homologous genes in *D. melanogaster*: *X. tropicalis* and *D. rerio*, which could result in different expression levels according to the low percentages of query cover and per. identity (Figure 4)

References