qRT-PCR reveals female *Drosophila melanogaster* express 8.43-fold higher *FoxP* and 33.2-fold lower *cwo* relative to males

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Summary

- Are female and male brains functionally different?
- The activity of genes *FoxP* and *cwo* were quantified.
- Females express more *FoxP* and less *cwo* than males, suggesting sex-specific differences in communication development and circadian rhythms.

Abstract

This experiment focuses on sex-specific gene expression of *FoxP* and *cwo* in female and male *Drosophila melanogaster* (fruit fly) brains. *FoxP* is associated with cognition in humans and male courtship in flies, and *cwo* is a circadian rhythm regulator in both species. qRT-PCR revealed 8.43-fold higher levels of *FoxP* mRNA and a 33.2-fold lower levels of *cwo* mRNA in females compared to males. Results indicate sexual dimorphism in brain function.

Introduction

Hypothesis: *FoxP* and *cwo* will be higher in male fruit flies relative to female fruit flies.

Fruit Flies

- Share many behavioral patterns with humans such as sleeping, aggression, mating, and circadian rhythm[1].
- Easily observable developmental stages, quick reproduction cycle, many offspring[1].
- Good model organism for genetic disease studies; share about 75% of human disease-related genes.

Target genes

FoxP (Forkhead box P)

- Encodes a transcription factor expressed in the nervous system involved in locomotion, male courtship behavior, veined wing generated song production, and behavioral conditioning[3].
- Human *FoxP2* regulates expression of genes involved in cognitive development, mainly communication[4].
- Mutations cause cognitive disabilities[5].

cwo (Clockwork Orange Gene)

- Regulates rhythmic gene expression within a transcriptional circadian feedback loop[6].
- *cwo* is expressed more in male wasps[6].
- *cwo* is similar to human *Dec1* and *Dec2*[6].

Methodology

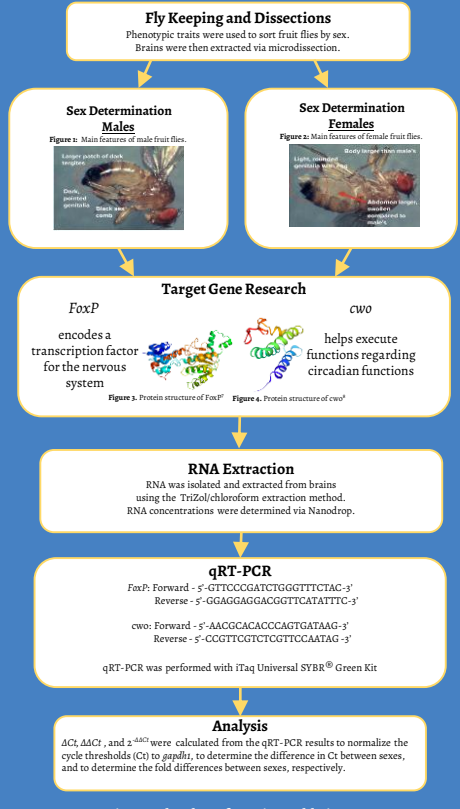
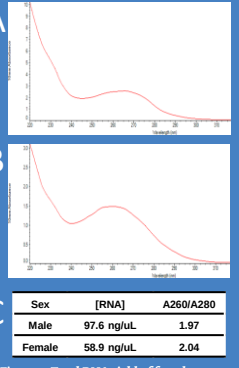


Figure 5. Flowchart of experimental design.

Results



Sex	[RNA]	A260/A280
Male	97.6 ng/uL	1.97
Female	58.9 ng/uL	2.04

Figure 6. Total RNA yield of female and male brain samples: (A) Total RNA yield from female brain samples, (B) total RNA yield isolated from male fly brains, and (C) total RNA concentrations for male and female pooled samples. Readings taken from Nanodrop 2000.

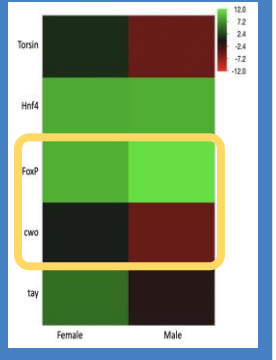


Figure 7. *FoxP* expression in female flies is 8.43-fold higher than male flies; *cwo* expression in female flies is 33.2-fold lower than male flies. *FoxP* and *cwo* gene expression normalized to *gapdh1*. Red indicates a higher expression level while green indicates a lower expression level. The heatmap generated using JMP Pro.

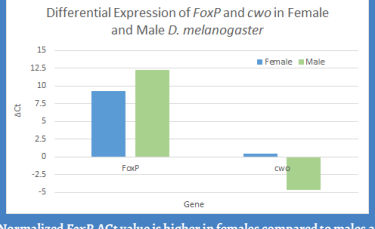


Figure 8. Normalized *FoxP* ΔCt value is higher in females compared to males and lower for *cwo* : For *FoxP*, females had a ΔCt value of 9.244 and males had a ΔCt value of 12.319. For *cwo*, females had a ΔCt value of 0.428, and males had a ΔCt value of -4.623. A smaller positive ΔCt value correlates to higher target mRNA levels as does a larger negative ΔCt value. Both genes are normalized to *gapdh1*.

Discussion/Conclusion

FoxP and *cwo* are differentially expressed in female and male fruit flies.

FoxP

- Females express 8.43-fold higher levels of *FoxP* mRNA compared to males.
- Differential expression in fruit flies suggests sex-specific differences in locomotion.
- *FoxP2* expression differences in humans could indicate sex-specific cognitive development.

cwo

- Females express 33.2-fold lower levels of *cwo* mRNA compared to males, suggesting females and males have different circadian rhythm regulating mechanisms.
- Higher levels of *cwo* in male fruit flies is in agreement with previous studies showing higher expression of *cwo* in male wasps[6].

Differences in gene expression between sexes were quantified, establishing a baseline for observing sexually dimorphic expression of *FoxP* and *cwo* induced by stressors.

Study Limitations

- No statistical analysis.
- Gene expressions were quantified using only mRNA.
- Fruit flies were studied post-eclosion.
- Fruit flies are evolutionarily distant from humans.

Future Directions

- Increase the number of samples.
- Measure protein levels.
- Perform study in different life stages.
- Perform this experiment in a higher organism.

References