Female *D. melanogaster* up-regulation in *Cyp18a1* gene in controlled conditions

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Summary

-Biological differences in males and females do affect their ways of regulation in biological pathways.
-Female vs. male up-regulation in CYP2 Clan genes were compared and represented by log2 fold changes.
-*Cyp18a1* is up-regulated in females compared to males indicating that this gene is associated with activities that especially take place in females..
-Data on gene homology in H. sapiens gene orthologs of *D. melanogaster* CYP2 clan were collected.
-Data on proteins related to Cyp18a1 were also collected.
-In future studies, the effects of B[a]P on *Cyp18a1* could be studied through multiple generations.

Introduction

Hypothesis

Cyp18a1, Spo*ok (spo)* and *Phantom* (*phm*) in the CYP2 Clan is up-regulated in females compared to male *Drosophila melanogaster.*

Background

Drosophila melanogaster

*A m*odel organism that share biological similarities with humans, are cost efficient, and have short maturation periods. 1,2

Female Male

Adult Oregon R wild-type *D. melanogaster* Female and Male

Males versus Females

Different reproductive organs, hormones, and other physiological differences in males and females may result in different expression of genes or maturation rates.

RNA

Sequencing and analyzing RNA gives us information on genes that are up-regulated or down-regulated. 3

Cytochrome P450- CYP2 Clan

Members in this clan have many roles in environmental response genes, such as their involvement in the ecdysteroid biosynthetic pathway and metabolism. 4

Future Experiments

Epigenetics

Gene expression changes due to the environment that may be passed down through generations. 5

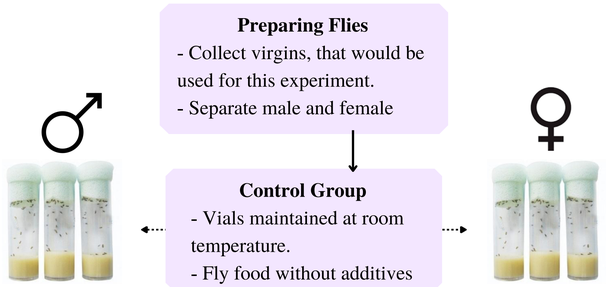
PAHs

Pollutants that can cause both short and long term effects for humans. 6

B[a]P (Benzo[a]pyrene)

A type of PAH that is carcinogenic, and is prevalent in today's world. Many data can be accessed since on B[a]P since it is oftentimes used in research. 7, 8

Methodology



Materials

D. melanogaster, Vials, Fly Plugs, Fly Food, EtOH, Silicon Beads, Centrifuge, Chloroform, RNase-free water, TriZol, Sequencing adapters, Spectrophotometer, Illumina NextSeq 2000

Results

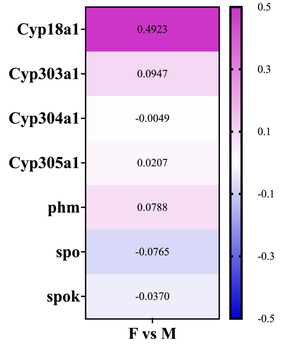


Figure 1: Heatmap of CYP2 Clan genes. Difference in expression levels in females versus males is represented by log2 fold change values. This figure was generated in GraphPad Prism 9.

***D. melanogaster* gene**	***H. sapiens* gene**
Cyp18a1	CYP2J2
Cyp303a1	CYP2B6
Cyp304a1	CYP2F1
Cyp305a1	CYP2J2
phm	CYP2A6
spo	CYP1B1
spok	CYP2C8

Table 1. *D. melanogaster* vs. *H. sapiens* CYP2 Clan orthologs. List of *D. melanogaster* and *H. sapiens* genes found in FlyBase that were used to create our gene homology graph.

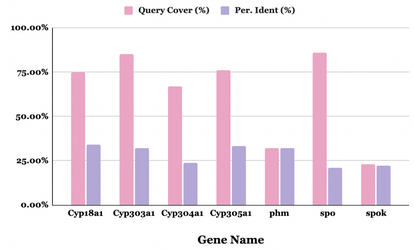


Figure 2: Bar chart of *D. melanogaster* Cytochrome P450 CYP2 Clan *H. sapiens* gene homology. Query cover and % identity are compared between each CYP2 Clan genes. These results were found by using NCBI BLAST. This graph was generated using Google Sheets.

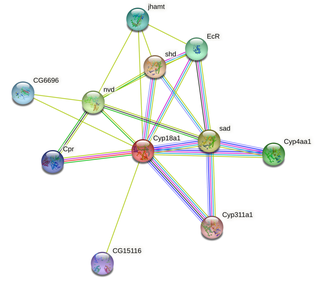


Figure 3: Search Tool for the Retrieval of Interacting Genes (STRING) Map showing proteins that interact with *Cyp18a1*. Proteins connected to *Cyp18a1* are involved in breakdown of synthetic insecticides and metabolism. This figure was generated in STRING database, https://string-db.org/.

Discussion and Conclusion

Figure 1

-*Cyp18a1* has the highest upregulation (1.407-fold) in females out of the CYP2 Clan genes.
-*Cyp303a1*, *Cyp305a1*, and *phm* are also up-regulated in females (1.014 to 1.068-fold).
-*spo* was the most downregulated in females (0.9484-fold)
-also put the other downregulated genes?

Figure 2

-*Cyp18a1* had the highest total score generated from NCBI BLAST.
-Orthologous to *CYP2J2* in *H. sapiens*
-*CYP2J2* in *H. sapiens*, is an ortholog to two of the CYP2 Clan gene, *Cyp18a1* and *Cyp305a1* (Table 1).
-*CYP2J2* might be involved in processes that *Cyp18a1* is also involved in, potentially those that are female specific.

Figure 3

-Proteins Sad and Shd showed strong interactions with Cyp18a1 in the STRING could be involved in functions that *Cyp18a1* holds.
-Cyp18a1 and Shd are negatively associated to each other
-Cyp18a1 inactivates 20 hydroxyecdysone (20E), which Shd produces
-Sad produces Ecdysone (E) which is used by Shd to make 20E.

Conclusion

-*Cyp18a1* in the CYP2 Clan, that belongs to the cytochrome P450 gene family is involved in oogenesis.
-*CYP2J2* in *H. sapiens* that were homologous to *Cyp18a1* and *Cyp305a1* in *D. melanogaster*, could also be involved in oogenesis.
-*Cyp18a1* gene is relevant to humans due to its high score in NCBI BLAST
-Through NCBI, it is seen that *H. sapiens* gene is involved in drug metabolism and synthesis of cholesterol.
-Proteins Sad and Shd showed strong interactions to *Cyp18a1* in the STRING map. These proteins associate with functions such as molting and metamorphism, which is a function that *Cyp18a1* also holds.

Future Directions

-*Cyp18a1*, which showed high up-regulation in females compared to males, may be the target gene in future studies.

-By using B[a]P as a positive control in our future studies, we may be able to study both long and short term effects caused in *Cyp18a1*.

-Have multigenerational settings for the experiment, to see effects that are passed down through generations from an epigenetic standpoint.

References→