*hpo** and *Sid** genes are expressed 2.91-fold and 4.49-fold higher, respectively, in *D. melanogaster* male heads than female heads under standard laboratory conditions

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Summary

➔Are there differences in the expression of *hpo* and *Sid* between male and female *Drosophila Melanogaster* (fruit flies) under unstressed conditions?
➔*hpo* and *Sid* gene expression were quantified by qRT-PCR on RNA extracted from fruit fly heads.
➔*hpo* and *Sid* were expressed 2.91 and 4.49-fold higher respectivley in male fruit flies.

Abstract

This study looks at differences in gene expression of the *hpo* and *Sid* gene between males and females. Studying these genes may help explain gender differences and the importance of distinction in medicine between males and females. qRT-PCR was used to quantify gene expression levels in fruit fly heads. *hpo* and *Sid* were expressed significantly higher in male fruit flies than female flies under control conditions, with $p < 0.05$.

Introduction

Null Hypothesis

There is no statistically significant difference in gene expression levels of *hpo* and *Sid* between *D. melanogaster* genders.

hpo **Gene**

➔"Hippo" gene
➔Controls tissue growth
➔Cell growth and apoptosis, or programmed cell death
➔Encodes kinases in the Salvador-Warts-Hippo pathway (controls organ growth)[1]

Sid **Gene**

➔Protects from the toxic effects of excess DNA/RNA released by an immune response
➔Highly induced by bacterial infection and oxidative stress[2]

Why this model organism?

➔ Inexpensive to maintain and culture
➔ Morphological differences between genders easily identified
➔ Researched and well documented genome
➔ Many genes that are homologous in humans

Materials and Methods

RNA Extraction:

RNeasy Plus Mini Kit: lysate, 70% ethanol, Buffer RW1, Buffer RPE, and RNase-free water

qRT-PCR:

iTaq universal SYBR® Green reaction mix: Nuclease-free H2O, iScript reverse transcriptase; forward and reverse primers, and RNA samples

ThermoFisher QuantStudio3 used to run qRT-PCR

Primers were designed with NCBI Primer-BLAST program.

qRT-PCR Primers (5'-3')

HPO
Forward- GAGCAAGGTGTGGATGAGGG
Reverse- ATAGTGCCCAAGTTCGACTCCA

SID
Forward- GTAAACACCGTTCCACGTCCC
Reverse- CGCCCAGGTTAGTGAGCAAA

Data Analysis:

ΔΔCT Analysis of qRT-PCR data used to generate heatmap of gene expression

Significance of gene expression levels was determined using a unpaired t-test; p-values less than or equal to 0.05 were considered statistically significant.

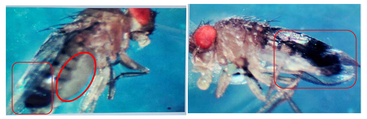
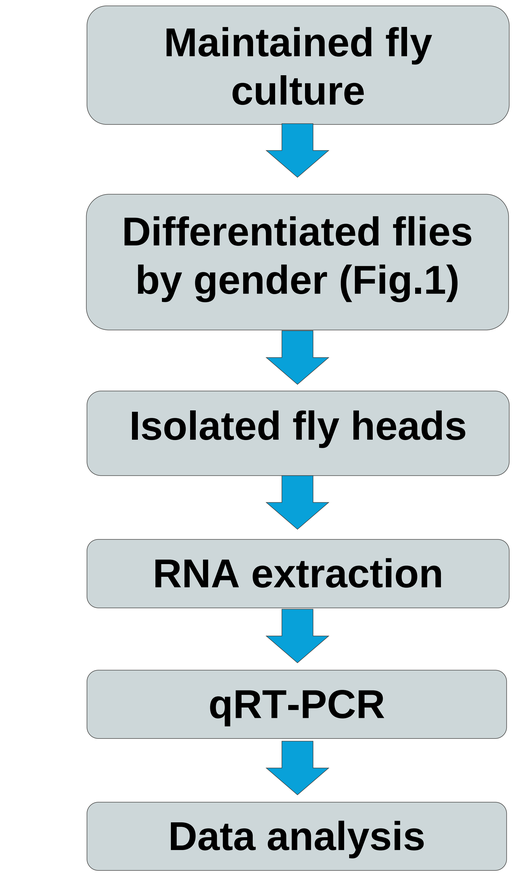


Figure 1: Female (left) and male (right) fruit fly imaged with a light microscope at 20x magnification. Gender differences include dark coloration of tergites and abdomen size indicated in red.



Results

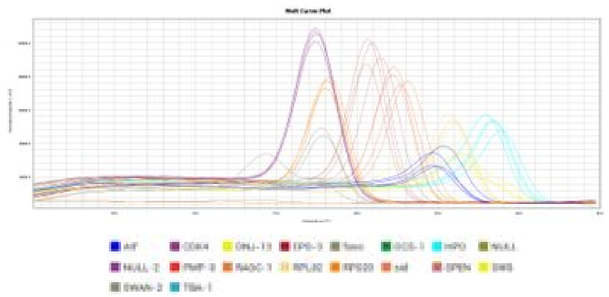


Figure 2: Melt Curve for qRT-PCR.
qRT-PCR product melting temperature indicated in light blue (*hpo)* and orange (*sid)* peaks.

Results

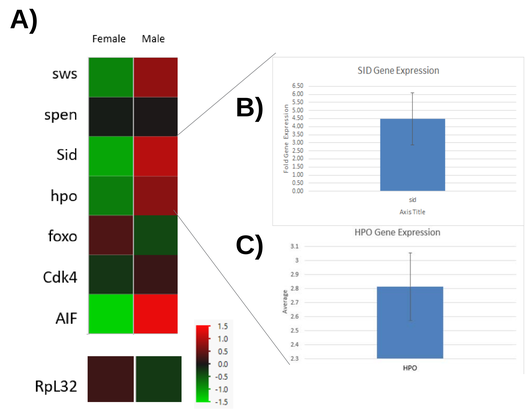


Figure 3: Heatmap and fold difference for *gene expression*
A) Higher gene expression levels are indicated in red, relatively lower expressions are indicated in green. Male and female flies were normalized to *RpL32*[3] housekeeping gene. B) There is a 4.49 fold expression difference in *Sid,* and C) a 2.91 fold expression difference in *hpo*.

Conclusion

➔Gene expression of *hpo* and *Sid* were statistically different between male and female fruit fly heads with p-values of 0.0051 and 0.0452.
➔*hpo* and *Sid* were expressed 2.91-fold and 4.49- fold higher in male fruit flies under control conditions.
➔Higher levels of expression of *hpo* and *Sid* in male fruit flies may indicate different responses to stressors, such as bacterial infection and growth between genders.

Limitation: We did not account for individual differences between fruit flies.

Future direction:

➔Exposing flies to stressors such as bacterial infection and quantifying stress-response gene expression differences of *hpo* and *Sid* in males and females.

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Bibliography